Water-use adaption via the barley circadian clock

Growth and water-up take physiology of barley circadian clock mutants in response to osmotic stress in warm and cold night temperatures.

Kayla McCarthy

PhD

University of York

Biology

February 2022

Abstract

This thesis compromises work related to measuring physiological growth and water uptake traits in four early maturing mutants and the parental line of *Hordeum vulgar* cultivar Bowman alongside a winter cultivar Antonella using simply hydroponic systems on young plants. The aim of the project was to evaluate evidence of difference water regulation in response to artificially limited water availability. Plants were tested under warm night conditions and cold night conditions with cold nights substantially improving responses to osmotic stress by decreasing the severity of responses in severe osmotic stress but inhibiting growth. In all barley lines, cold night temperatures (4°C) reduced growth and water uptake. Results are inconclusive if mutations in evening complex circadian-clock genes influence water regulation in barley; however there were small differences in physiology, likely related to early maturing plants accelerated growth. Cold nights reduced growth and development in all barley lines while spring cultivar growth and water uptake physiology become more like winter cultivars. Environmental influences of water availability and night temperatures have strong effects on barley physiology and this work is further evidence that the circadian clock in barley has less of an influence on the plants survival fitness compared to Arabidopsis.

I declare that this thesis is a presentation of original work and I am the sole author. This work has not previously been presented for an award at this, or any other, University. All sources are acknowledged as References.

Acknowledgements

This work would have been impossible without Professor Seth Davis and Professor Sue Hartley who lay the foundation for the research and were attentive, supportive and guiding supervisors throughout. The donation of seeds and advice for RNA work from and Professor Maria von Korff and her lab in Köln (at the time of this project) and especially Kirsten Luxa were also essential to the progress made throughout the work achieved and kindly allowed me time with them to improve on RNA extractions.
As part of the University of York's TAP system the additional instruction, suggestions and feedback from Professor Ian Bankcroft and Dr. Sangeeta Chawla aided the new directions taken in this research and attempts to improve the writing and presentation.
This work was also possible thanks to the help and friendship of the evolving Davis Lab, including the advice and help of Amanda Davis (keeping the laboratory functional and enabling much of the work), Dr.Takayuki Shindo (an excellent teacher), Dr. Rachael Oakenfall (extremely generous with her time and advice), Dr. Jack Munns (he put up with so much with me), Dr. Manuela Lovinella (a joyous presence with her Galderia), Sarah Lock (much needed maths expertise) and Dr. James Roland (very generous with offers to help) who endured my presence while offering and give assistance and patience generously despite their own troubles.
A special thank you to York Biology Horticulture team, notably Alison Fenwick, Chris Lancaster, Paul Scott who accommodated and cared for my plants in the green houses and the constant changing of growth cabinet and their settings, even allowing me to hang big sheets of plastic around their equipment and pretty much taking up large amounts of space all over their facilities over the years.
Dr. Sally James, Dr. Lesley Gilbert and Dr. Peter Ashton in the University of York's Technology facility were very helpful regarding qPCR learning and testing primers.
Alison Fellgett is largely responsible for the breakthrough in barley RNA extractions and Dr. Samantha Donninger was very accommodating and helpful in the beginnings of adapting 96 well qPCR for this project.
The Stomata work referred to and much more left out was possible thanks to the advice of Professor Julie Grey and her lab group, who kindly allowed me to spend a day with their lab and demonstrate their techniques. Additional thanks to Dr. Michael Schultze, Prof. Frans Maathuis and Dr Will Brackenbury for their help and use of their equipment during the project.

Thank you to my parents Norman and Val McCarthy whose attentiveness, support and care has been vital to me even making this far in life, especially academically. Jan Hampson for helping me through the rough patches near the end. To Julia Gills and her cat Yoda who rehomed himself with me, providing the companionship and validation only a cat can.

Rest in Peace to Elvera Marshall my birth mum, and my childhood cats and support system, Kira, Tara, Zoya and Suki who all passed on over the course of this research.

Table of Contents

1 CHAPTER 1: INTRODUCTION....1

1.1 Introduction to barley 1

1.2 Economic importance of barley 2

1.3 Origin and geographical distribution of barley 3

1.4 Barley develolment, physiology and morphology 6

1.5 Barley production and breeding technologies 16

1.6 The gene architecture and behavioural properties of the circadian clock in in model plants 17

1.7 Associations between the plant circadian clock and water regulation (influencing other physiology, photosynethsis and metabolism) 23

1.8 Barley as a model for Monocot circadian clock research 25

1.9 Barley lines in this thesis 26

1.10 Aims and objectives....27

2. CHAPTER 2: PRELIMINARY STUDIES, GENERAL METHODS AND RECIPES....29

2.1 Introduction 29
2.1.1 Previous work with the Bowman Barley lines 29
2.1.3 Areas of focus.... 32
2.1.3.2 Development stages 32
2.1.3.3 Tiller numbers.... 33
2.1.3.5 Stomata 35
2.1.3.6 Chlorophyll.... 36
2.1.2 Aims and Objectives.... 36

2.2 General methods, recipes and lists 36
2.2.0 Barley line materials 36
2.2.2 Hydroponic solutions 38
Low nutrient 'Hoagland' solution 39
2.2.3 Simulating osmotic stress 40
2.2.4 Growth Cabinet and temperature conditions 43
2.2.5 Method of growing in 50ml centrifuge tubes 43
2.2.6 Method of measuring and calculating water-uptake 45
2.2.7 Methods for collecting growth performance data 45
2.2.8 Protocols to extract RNA from barley 47

2.3 Preliminary studies....53
2.3.2 Germination 53

Methods 53
Results and discussion 54
2.3.3 Primary root measurements on Agar 58
Methods 58
Results and discussion 59
2.3.4 Green house measurements: Relative development rate, tillers, height and yield 61
Methods 62
Results and discussion 65
2.3.5 Leaf and growth traits in response to osmotic treatments 70
Methods 71
Results and discussion 71
2.3.6 Stomata density 77
Methods 78
Results 80
Discussion 84
2.3.7 Preliminary work Using lowering water levels as an indicator of water uptake to investigate differences between mutant and wild type of young plants 85
Aims and Hypotheses 85
Methods 86
Results 87
Discussion 88
Conclusions 89
2.3.8 Effect of osmotic stress on growth performance and morphology in wild type and circadian mutants from a spring background. 90
Results 91
Growth traits 94
Water traits 96
Resource allocation 98
Root characteristics 101
Water-used relative to the dry biomass of the plant as an estimate of water-use efficiency. 102
2.3.9 Leaf mass area 103
Methods 103
Results and discussion 104
2.3.10 Chlorophyll 105
Methods 106
Results and discussion 107

2.4 Discussion and conclusions 112

CHAPTER 3 115

3.1 Introduction 115
3.1.1 Circadian clock and water regulation 115
3.1.3 PHYC and temperature regulation 117

3.2 Methods 127
3.2.1 Plant material 127
3.2.2 Growing conditions 127

3.3 Results 133

Comparison of barley lines grown in two different osmotic stress treatments in warm and cold nights. 133

3.4 Conclusions 135

CHAPTER 4 DISCUSSION 140

4. 2 Expanding on the work in this project 140

4.3 Molecular work examining the gene expression 140

4.4 Molecular work examining sugars 141

4.5 Physiological measurements in larger plants and longer term water-stress 141

4.6 Thermal imaging 142

4.7 Stomata measurements 143

4.8 Fluorescence 144

4.9 Final conclusion 145

REFERENCES I

APPENDICES 1

Table of figures

Figure 1.3.1....4
Figure 1.3.2....5
Figure 1.3.3....6
Figure 1.4.1....8
Figure 1.4.2....12
Figure 1.4.3....14
Figure 1.4.4....16
Figure 1.6.2 a....19
Figure 1.7.1....25
Figure 2.2.3.1a....41
Figure 2.2.3.1b: 41
Figure 2.2.3.2....43
Figure 2.2.5.1:....44
Figure 2.3.2.1....54
Figure 2.3.2.2....55
Figure 2.3.3....61
Figure 2.3.4.1....65
Figure 2.3.4.2....67
Figure 2.3.4.3....68
Figure 2.3.4.4....70
Figure 2.3.5a....74
Figure 2.3.5b....76
Figure 2.3.6b....83
Figure 2.3.7a....88
Figure 2.2.8.3....96
Figure 2.2.8.4....100
Figure 2.3.9:....104
Figure 2.3.10....106
Figure 2.3.10a....110
Figure 2.3.10b....111
Figure 3.1.1....117
Figure 3.1.2....123
Figure 3.2.1....128
Figure 3.3.3.1....134

List of tables

Table 1.6.1 21
Table 2.2.2: 39
Table 2.3.2.1: 55
Table 2.3.4.1 64
Table 2.3.6a: 79
Table2.3.6b 81
Table 2.3.7 86
Table 2.2.8.3 92
Table 2.3.10 107
Table 3.2.1 130
Supplementary table 3.3.1a: 2
Supplementary table 3.3.1b: 31
Supplementary table 3.3.2a: 40
Tables 3.3.2b 57
Supplementary table 3.3.3a: 67
Tables 3.3.3b 91
Supplementary table 3.3.3c: 105
Tables 3.3.3d 126
Supplementary table 3.3.4a: 139
Tables 3.3.4b 173

Abbreviations

BOA	BROTHER OF LUX ARRHYTHMO
$C_{10}H_{12}FeN_2NaO_8$	EDTA ferric sodium salt
$Ca(NO_3)_2 \cdot 4H_2O$	Calcium Nitrate Tetrahydrate
CCA1	CIRCADIAN CLOCK-ASSOCIATED 1
CFW	Shoot fresh weight (g) post treatment
CHE	CCA1 HIKING EXPEDITION
$CuSO_4.5H_2O$	Copper(II) sulphate pentahydrate
ddH_2O	Double distilled water
eam10.m	Bowman Hvlux (B284), spring early maturing mutant
eam5.x	Bowman HvPHYC (B285), spring early maturing mutant
eam8.k	Bowman Hvlelf3 (B289), spring early maturing mutant
eam8.w	Bowman Hvelf3 (B2910), spring early maturing mutant
EC	Evening complex
ELF	EARLY FLOWERING
FFW	Total fresh weight (g) post treatment
GI	GIGANTEA
H_3BO_3	Boric acid
IFW	Total fresh weight (g) pre treatment
$K_2HPO_4 \cdot 3H_2O$	Potassium phosphate dibasic trihydrate
KNO_3	Potassium nitrate
LHY	LATE ELONGATED HYPOCOTYL
LNK	LIGHT-INDUCIBLE AND CLOCK-REGULATED
LUX	LUX ARRHYTHMO
$MgSO_4.7H_2O$	Magnesium SulfateHeptahydrate
$MnCl_2 \cdot 4H_2O$	Manganese(II) Chloride Tetrahydrate
N(LNK)	NIGHT LIGHT-INDUCIBLE AND CLOCK-REGULATED
$Na_2MoO_4 \cdot 2H_2O$	Sodium molybdatedihydrate
NOX	BROTHER OF LUX ARRHYTHMO (Night version of LUX)
PEG	Polyethylene glycol
PRR	PSEUDO-RESPONSE REGULATOR
RAD	Average diameter (mm)
RDW	Root dry biomass (g)
RDWC	Root: Shoot water content ratio
RFW	Change in fresh weight (g)
RL	Accumulated root length (cm)
RSA	Total surface area (cm^3)
RSDW	Root: Shoot Dry Biomass ratio
RT	Total number of root tips

RV	Average root volume (cm^3)
RVE8	REVEILLE8
RWC	Root water content (g)
SDW	Shoot dry biomass (g)
SFW	Root fresh weight (g) post treatment
SWC	Shoot water content (g)
TDW	Total dry biomass (g)
TOC1	TIMING OF CAB EXPRESSION 1
TWC	Total water content (g)
W%	Percentage of water as total biomass (%)
WU	Water uptake (ml)
WU/DW	Water uptake per total dry biomass (ml/g)
WU/WC	Water uptake per total water content stored (ml/g)
ZnSO4.7H2O	Zinc SulfateHeptahydrate
ZT	Zeitgeber time
ZTL	ZEITLUPE
Ppd-h1	Photoperiod-H1 (PRR37)
Rfd_Lss	Relative Fluorescence decline ratio in steady-state (Fp − F')/F'
NPQ_Lss	Non-photochemical quenching is measured by the quenching of chlorophyll fluorescence (Fm/Fm') – 1
Fv/Fm_Lss	Maximum quantum yield of PSII photochemistry measured in the dark-adapted state

1 Chapter 1: Introduction

1.1 Introduction to barley

Barley is a monocotyledon (monocots) named after the single embryonic leaf of the young plant common to grass and grass-like flowering plants. The Barley of this research has the scientific name is *Hordeum vulgare* L. Genetically, *Hordeum vulgare* L. is diploid (2*n* = 2*x* = 14) with 14 chromosomes and a genome size of 5.1 Gb, can be regarded as comparatively small at least to other known allopolyploid genera and species such as the Triticeae (the genus of wheat) which are tetraploids (4n) or hexaploids (6n) (The International Barley Genome Sequencing Consortium et al., 2012). This made *Hordeum vulgare* an attractive candidate to become a model species for monocot cereals since its genome was sequenced in 2012 with the cultivar Morex (The International Barley Genome Sequencing Consortium et al., 2012) and more cultivars continue to have their genomes sequenced and assembled (Schreiber et al., 2020) as its scientific value and importance is recognised (Pourkheirandish and Komatsuda, 2007; Saisho and Takeda, 2011).

Monocot cereals needing a representative model for scienfific research can be understood when considering that *Arabidopsis thaliana*, the main model species for plants is a dicotyledon (two embryonic leafs in young plants) and monocotyledons are estimated (with large degrees of error) to have diverged from their earliest common ancestor in other angiosperms around 120-140 million years ago (MYA) (Bremer, 2002, 2000; Sanderson et al., 2004) while some estimates have extended this to 150 MYA (the Jurassic period) (Zeng et al., 2014). Other grain models such as Oryza sativa (rice) (Eckardt, 2000) and Zea mays L. (maize) (Dolgin, 2009) had their genomes mapped earlier but what made barley special was its similiarity to wheat while also being comparatively simplicer as a diploid genome. In 2014 the wheat genome was still in a draft form (Consortium et al., 2014), it would not be until 2018 that it would be complete (Appels et al., 2018; Guan et al., 2020). Despite the wheat genomes completion barley as a model and aid in research is still attractive (Dawson et al., 2015).

Barley is recognised as one of the first domesticated cereal crop plants and is the only species of the thirty in the Hordeum genus to be domesticated (*Hordeum vulgare* L. is a subspecies under *H. vulgare*, as the wild relative *Hordeum spontaneum* C. *Koch* and *Hordeum agriocrithon Åberg* are considered distinct sister subspecies by modern taxonomy(Bothmer et al., 1985)). *H. vulgare*'s form reflects it cultivated history with its broader leaves, shorter stem and awns, tough ear rachis, a shorter and thicker spike, and larger grains than its wild relative *Hordeum spontaneum* - but is otherwise morphologically similar (Zohary, 1969).

1.2 Economic importance of barley

Barley has made up 6%-7% of total global grain production over the last 13 years (2008-2021) according to data collected by the US department of Agriculture (USDA) and the Food and Agriculture organisation of the United Nations (ADM Germany, 2021; FAO, 2021; FAO and USDA, 2021). This constitutes a range of 122.7 million metric tonnes (MMT) to 159.74 MMT annually within this period, with the highest production being between 2019-2021, the highest it has been since 2008-2009 as all previous years had lower yields particularly the period of 2001-2013. Regionally or nationally annual trends vary. This may appear modest relative to Wheat contributing 29% to 36% (655MMT to 772.64 MMT) of global crop production in the same years (USDA Foreign Agricultural Service, 2021), but this production has kept barley as the most 4thmost produced grain by MMT worldwide. In 2021 the top five cereal crops by global production where.), maize (*Zea mays L*.) (1125 MMT), wheat (*Triticum aestivum L*.) (775.8 MMT), milled rice (*Oryza sativa* L.) (505 MMT), Barley (159.74 MMT) and Sorghum (*Sorghum bicolor)* (62.05 MMT) (FAO and USDA, 2021). According to the FAO, global area dedicated to barley production in 2019 was 51.15 million hectares. Compiled data was not available for Wheat or milled rice but area used to grow corn totalled to 197.2 million in the same year (FAO, 2021). Therefore per hectare approximately 3 tons of barley can be grown (corn in comparison is roughly closer to 6 tons and sorghum an estimate of 1.5 ton based off dividing total yields from 2021 by the area used in 2019).

According to FAO and USDA the European Union (EU) is the highest barley producing region (>55MMT), unsurprising given half of EU farms grow cereals, and cereal crops occupy one-third of the EU's agricultural area (European Commision, 2013). This is followed by Russia (20 MMT) meaning these regions produce over half the total global harvested, followed by Australia (13 MMT), Canada (>10 MMT), Turkey (8.1 MMT) and Ukraine (7.95 MMT), where all produce more than 5MMT. This reflects the economic importance of barley to these regions and countries (Agriculture, 2021). In the United Kingdom total barley production is more than 8 million tonnes with an increased production of spring barley (38%) and decrease in winter barley (46%) in 2020 as the area used to grow spring barley increased (52%) up to 1.1 million hectares (Clark and Thompson, 2020). Scotland makes up 12% of the UK cereal crop area and its major cereal crop is barley. In 2018 NFU Scotland reported >250 thousand ha of Spring barley sown in March or April and >37 thousand ha of Winter Barley sown in autumn, of this 35% goes into malting and 55% to animal feed (Nfus.org, 2018).

Since the 1980s, crop area dedicated to barley has declined from an estimated 80 million hectares (ha) to around 55 million(Zhou, 2009). Despite this, barley still ranks the fourth highest cereal crop in terms of annual yield. Countries such as USSR, USA, India and China have notably produced less barley in the last decade. In contrast, the EU, (ADM Germany, 2021; FAO and USDA, 2021) Turkey, Iran and Australia have seen

increasing production (Zhou, 2009). This reflects the global consumption of barley where the majority of commercial barley is sold for animal feed, with the second largest commercial use in malting. Other uses of barley include human consumption, chemical industries, starch production, and straw used for roofing and animal bedding. Barley is considered a resilient crop noted for its ability to adapt to multiple biotic and abiotic stresses, making it particularly valuable in developing areas where production is hindered by these factorsas well as a model in understanding plant stress responses therefore barley is looked to for future exploitation in crop development due to expected environmental change in the near future (Newton et al., 2011). Molecular research in barley has advanced to bring practical function to barley breeding, with methods including marker-assisted selection using known quantitative trait loci (QTL) and genes or gene-editing technology (clustered regularly interspaced short palindromic repeats/CRISPR-associated protein 9 (CRISPR/Cas9), clustered regularly interspaced short palindromic repeats from Prevotella and Francisella 1 (CRISPR-Cpf1), and multiplexed accurate genome editing with short, trackable, integrated cellular barcodes (MAGESTIC) as a means to efficiently speed up plant breeding, with barley being among the most well studied and fairly successful with these technologies (Friedt et al., 2010; Riaz et al., 2021). When it comes to drought tolerance (a stress important for all crops but has been an area of considerable focus with barley), genes of interest and important traits that improve performance in response to limited water-availability (early vigor, plant height, spike waxiness, low stomatal density)(Kebede et al., 2019). However, work on barley has also shown modern bottle-necks that reduce elite varieties adaptability to expected climate change, fortunately its selection as a model for research (due to being similar to Wheat but comparatively genetically more manageable due to its smaller genome size) has meant well-curated and commodious collections of landraces, wild barley accessions (H. *vulgare ssp. spontaneum*) and other Hordeum species exist to become new allele sources (Dawson et al., 2015).

1.3 Origin and geographical distribution of barley

Approximately 10,000 years ago, around 8000 B.C., wild *Hordeum spontaneum* was domesticated. This is based on remains found in various archaeological sites in the Fertile Crescent(Badr et al., 2000; Zohary and Hopf, 2000) making Barley (Hordeum vulgare L.) one of the founder crops of Old World agriculture.

The Fertile Crescent is a large crescent shaped region spanning the Persian Gulf and modern day Israel, Palestine, Jordan, Lebanon, northern Egypt, Southern Iraq, Israel and Syria. The main rivers in the region are the Tigris and the Euphrates that rise from the Taurus Mountains of south-eastern Turkey to terminate in the Persian Gulf. The soil around the rivers is enriched by regular floods. In parts the Nile River also run through it. Together the availability of fresh water from rivers and the associated fertile soil, irrigation and agriculture developed. The Fertile Crescent, intersected ancient,

Egyptian, Sumerian, Babylonian, Assyrian, Phoenicians and other civilisations of Mesopotamia, Egypt and Levant, is also known as the "Cradle of Civilization", credited with the advancement of many aspects of human cultural development including, science, technology, agricultural techniques and (fittingly) the concept of time (Mark, 2018).

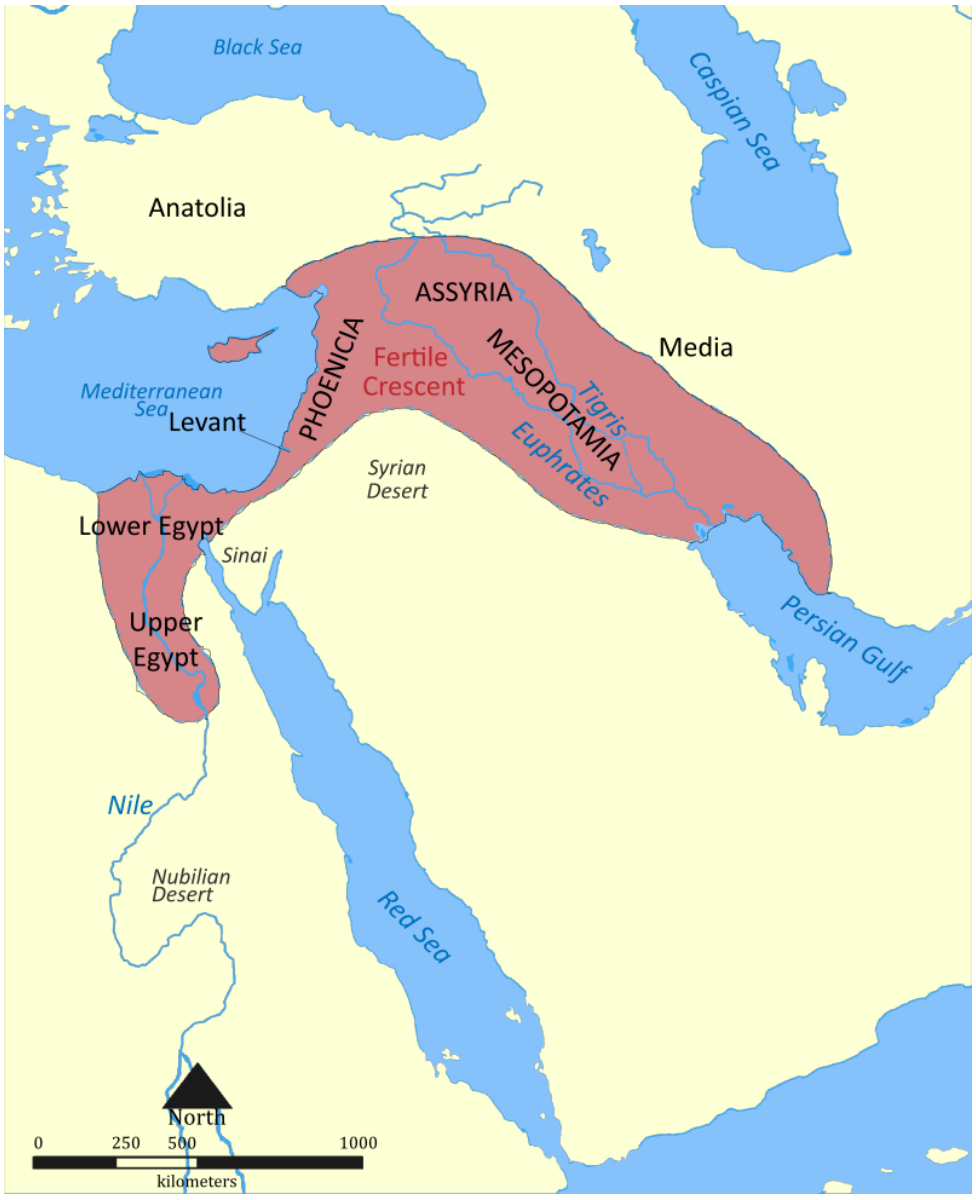


Figure 1.3.1 Graphic Image of the Fertile Crescent, showing the extent of the area from the northeast of the Sinai Peninsula, to the Mediterranean coast and the Jordan River, and the curve to the southeast, following along the Tigris and Euphrates valleys and regions in ancient Egypt. Source: Colt .55 (CC-BY-SA Version 1.3, 3 November 2008 http://www.gnu.org/copyleft/fdl.html)(Colt.55, 2006)

Unlike other domesticated crop plants, which have been traced back to a centric origin (Zohary, 1999), barley's domestication source has been less clear, and as of 2020, it still

debated. Many studies have described cultivated barely as having monophyletic origins (modern cultivars all share a single common ancestor). This resembles most crops but different studies have nominated distinctive regions as the starting place (Badr et al., 2000; Dai et al., 2012; Haas et al., 2019; Molina-Cano and Conde, 1980; Ruberti et al., 2012). The central origin view has been challenged with the aid of single nucleotide polymorphisms (SNP) technologies (Allaby, 2015) and some studies believe evidence shows domestication appeared to have emerged from a number of different populations spanning across the Fertile Crescent (Poets et al., 2015), a pattern described as mosaic ancestry. Pankin *et, al.* used 'Illumina enrichment resequencing ' technology, to identify ancestral neutral loci that could be used to trace back the origins of domesticated barley(Pankin et al., 2018). Their conclusions suggested candidates for domesticated barley origins where wild barley from the Levantine (West) and Zagros (East) regions of the Fertile Crescent, with wild and proto-domesticated lineages barley populations grew all over the Fertile Crescent, and that there was continued gene flow across populations.

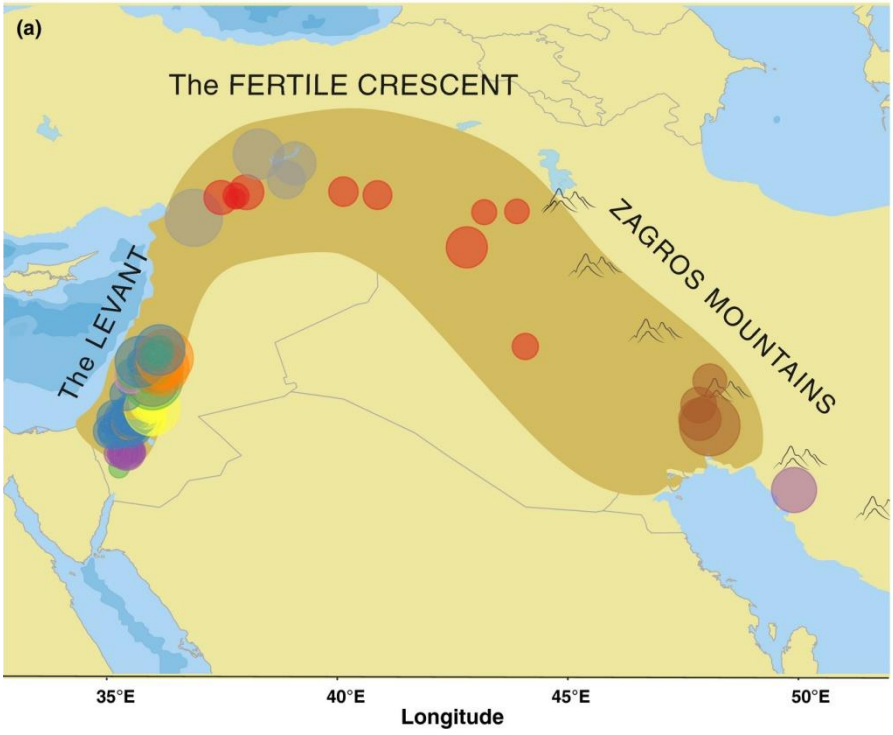


Figure 1.3.2 Edited image from (Pankin et al., 2018) figure 4a, their graphic map of the geographic distribution of wild barley accessions that carry the ancestral haplotypes of the domesticated barley loci within the Fertile Crescent. Nine colours corresponding to nine wild barley populations, Carmel & Galilee (pink); Golan Heights (orange); Hula Valley & Galilee (green); Judean Desert & Jordan Valley (yellow); Lower Mesopotamia (brown); Negev Mountains (magenta/purple); North Levant (gray); Sharon, Coastal Plain & Judean Lowlands (blue); and Upper Mesopotamia (red).

In modern day the geographic distribution of barley is almost global and can be found on almost every continent, mainly as cultivated varieties, a testament to *H. vulgare's* adaptability and hardiness. Today, the majority of barley is produced in high latitude regions with cooler environments.

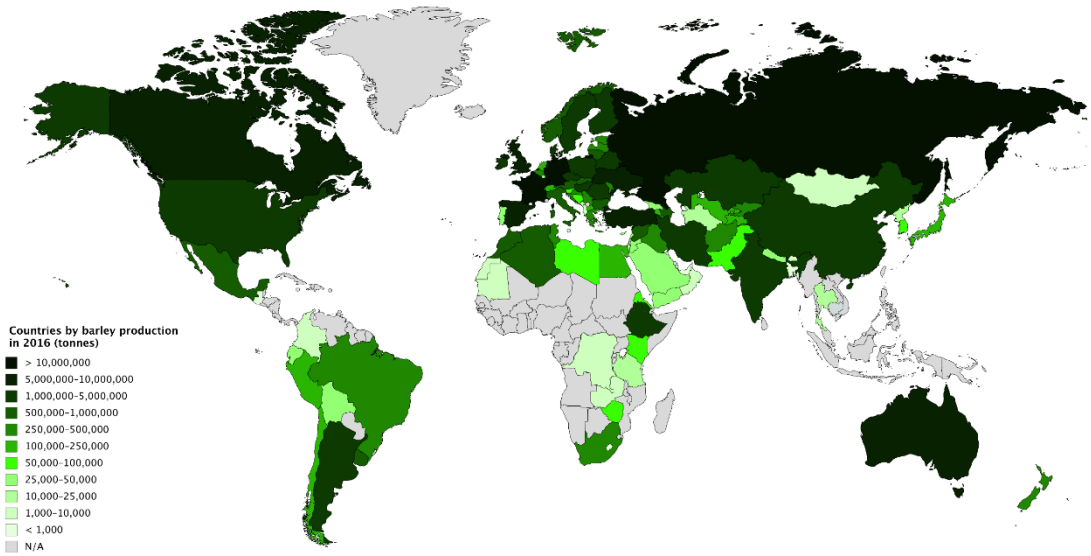


Figure 1.3.3 Choropleth map of barley production in tonnes by country, based on 2016 data from the Food and Agriculture Organization Corporate Statistical Database (FAOSTAT). Source: JackintheBox (CC BY-SA 4.0), (commons.wikimedia.org/w/index.php?curid=71552744, Accessed on 16th June 2018).

Meanwhile H. spontaneum (wild or spontaneous) still grows primarily in the Fertile Crescent and shows feats of hardiness by colonising secondary marginal habitats including open Mediterranean marquis (shrub land biome), abandoned fields, and roadsides, areas that can be more competitive, low in nutrients, degraded or polluted environments. Naturally, wild barley's geographical range has been expanding. Appearing in the Aegean region, South-eastern Iran, areas of central Asia such as Afghanistan(Harlan and Zohary, 1966; Nevo and Shewry, 1992; Zohary et al., 2012) Greece, Egypt, South-western Asia, and eastward into southern Tajikistan and the Himalayas(Bothmer et al., 2003).

1.4 Barley develolment, physiology and morphology

Plant structure is determined around the need to conserve water, assimilate CO_2 and aspects of their environment. Morphology comes to reflect strategies of water management for environments plants have adapted to.

The key morphological distinguishing feature of the monocot is their single (mono) cotyledon in their seed. Additionally the more uniform organisation of shoots, leaf structures and floral configurations than other angiosperms have been useful morphological taxonomic characteristics(Vogel, 1998) in (Kubitzki et al., 2010). Some traits, like vein organisation and floret number, can be found outside the Monocot lineage. However the atactostele arrangement of vascular tissue is defining. Unlike dicots, which have concentrated rings of vascular tissue, vascular bundles in monocots are scattered. Monocots lack Collenchyma, long cells with thickened cell walls usually found around the vascular cambium where they add additional structural support. Like other plants under the commelinid subclade barley cells walls contain the antioxidant UV-fluorescent ferulic acid (Fincher, 1976) protecting against radiation. Under the order Poales, defined by the small flowers enclosed by bracts (modified leafs or scales) arranged in inflorescences and typically starch containing seeds. Barley is a flowering grass under the family Poaceae, having hollow stems with nodes and narrow alternate leaves forming in two ranks with the lower part of the leaf forming a leaf-sheath around the stem. Barley uses the C3 photosynthetic pathway, and like many other Pooideae is more of a cool-season grass thriving in temperate climates (Soreng et al., 2017).

Barley stems are made of hollow cyndrical internodes (5 to 7) separated by nodes from which leaves grow (Teagasc, 2017). The complex aromatic polymer lignin in the secondary cell wall of grasses (including barley) is important for mechanical strength of the stem while also facilitating impermeability of the cell wall of the vascular tissues for water and nutrient transport (Boerjan et al., 2003; Vogel, 2008). The length of the internodes, stem thickness and stem cortex determine stem strength (van den Berg and Labuschagne, 2012). Lignin content and deposition varies among barley genotypes (Begović et al., 2015) and lignification changes as barley plants mature leading to structural and strength changes. Weaker stems may be indicative of lower lignin and more permeability (Peng et al., 2014). Barley stems grow, between nodes and internodes1 from their intercalary meristems. The development of nodes may differ across the plants nodes, with base internodes being fully developed while upper nodes may be elongating and lignifying until the anthesis stage (Jung, 2003). From observation, early maturing lines tend to have more bend at the nodes suggesting possible less lignin in the stem structures (figure 1.4.1).

The shape of barley leaves are long, linear lanceolate blades, comprised of a sheath which wrap around the base of the stem at the node where leaves originate and species characteristic auricle and ligule. Leaves grow sequentially from the nodes and at the opposite side from the previous developing leaf. Leaves are established quickly and protected throughout the growing season, typically growing in an upwards direction close to the stem, with mature leaves falling outwards as the sheath loosens

from the stem. Leaf establishment rates are responsive to nutrient quality and temperature, happening faster under higher conditions. Nodes are detectable after stem elongation, after the tillering stage (Briggs, 1978).

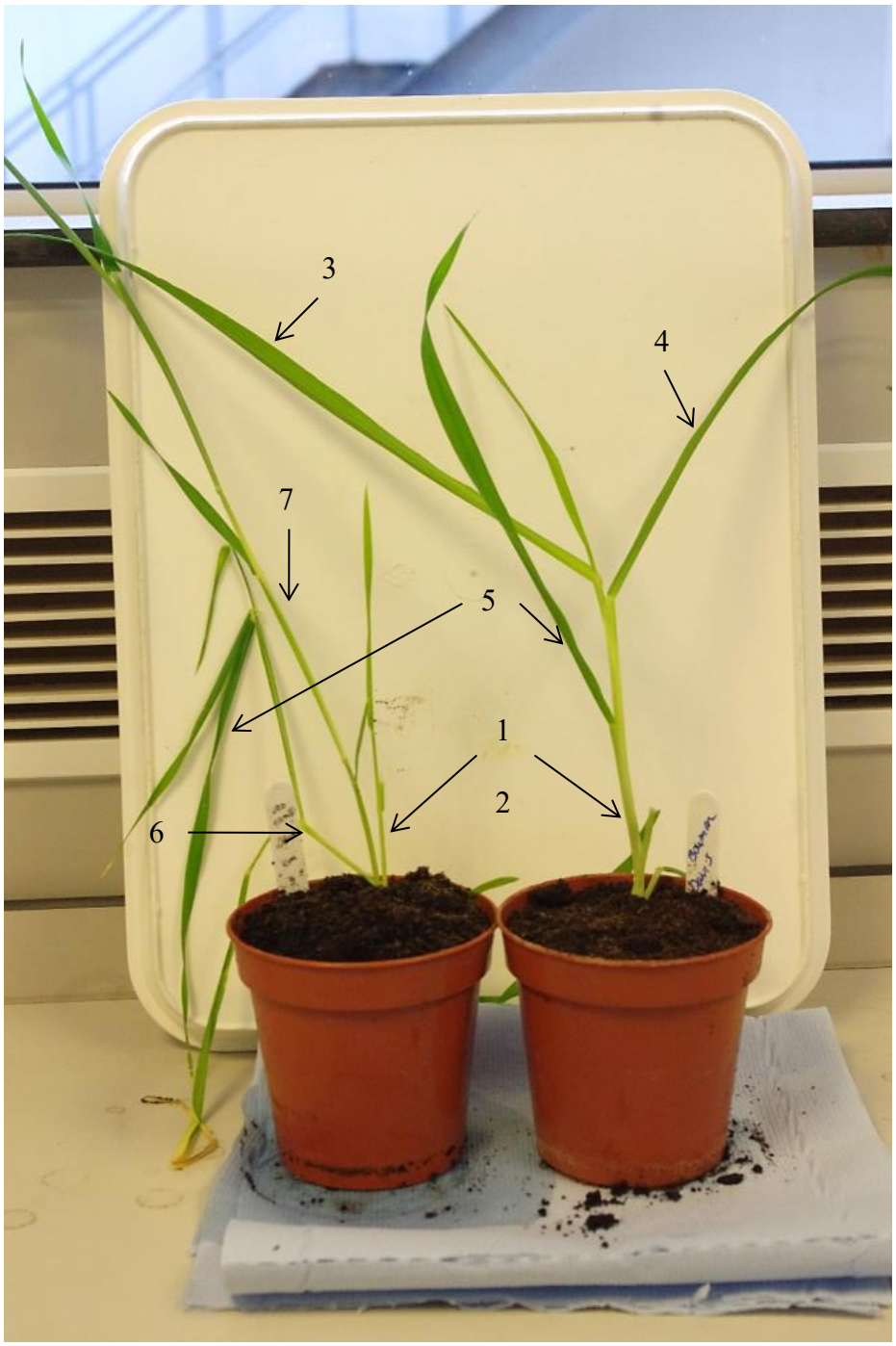


Figure 1.4.1 Photograph comparing Eam8.w (left), Bowman parent line (right). Same age plants approximately 6 weeks old, in the same soil (Levingtons f2+soil), same pot size, same watering, light and temperature conditions grown in a growth room (16/8 light and dark cycles, 20°C/16°C temperature cycles) demonstrating differences in, 1)tiller number, 2) stem thickness, 3)leaf broadness, 4) typical lengths, 5) angles , 6)stem bending at nodes and 7) stem strength. Many differences in stem width disappeared when bowman lines entered booting phase and seed maturation. Both have 5 matured leaves on the main stem and a 6th developing.

Different cultivars may vary but typically once three leaves have developed, tillers may start to emerge. Tillers are additional stems, developing leaves and their own spikes with seed. Tillers are a means for barley plants to adapt to changing environmental conditions with tiller numbers increasing under favourable conditions or to compensate for reduced plant density. In rice it has recently been shown that tiller-bud and panicle (much-branched inflorescence) development is also under circadian regulation via sugar and strigolactone pathway with Rice *CIRCADIAN CLOCK ASSOCIATED1* (OsCCA1) and *PSEUDORESPONSE REGULATOR1* (OsPPR1) genes having key roles {Wang, 2020 #802}.
When tillers develop the roots begin to develop more adventurous roots, which has consequences for anchorage and water management. For spring barley cultivars tillers are typically produced over a two-week span, winter cultivars in a prolonged vegetative phase may produce more tillers. Tiller numbers have been noted to be sensitive to seed planting depth, early seasonal temperatures producing more in cooler conditions, plant populations and soil nitrogen availability (Minnesota, 2019a). Tillers do not always mature to produce seed and often there is premature senescence for a number of tillers after four weeks with the extent varying depending on genotype and environmental conditions (del Moral and del Moral, 1995; Kirby and Faris, 1972; Kirby and Jones, 1977; Leopold, 1949). In this research, it was observed in barley that early maturing lines are more prone to produce tillers than the parental lines and Early maturing lines tended to continue developing tillers beyond four weeks with less premature death for the same conditions, perhaps indicating disrupted regulation in plants with imperfect clocks, as will be described later.

The morphology of the plant changes as it develops. One of the most common ways to monitor barley plant development is to divide morphological and develop changes into groups called stages and these stages are coded. This method is useful as growth rates, even in the same genetic backgrounds can vary with annual environmental conditions. However more than one coding system has been developed but one of the most highly recognised across disciplines is the Zodaks scale(Zadoks et al., 1974), a two digit coding system implement since the 70s. It divides cereal growth into nine principle stages as the first digit and subdivides each of those with a second digit with the number 5 indicating the midpoint. The nine main stages are:

- 0 - Germination
- 1 - Seeding growth
- 2 - Tillering
- 3 - Stem elongation
- 4 - Booting
- 5 - Awn emergence
- 6 - Flowering (anthesis)
- 7 - Milk development

- 8 - Dough development
- 9 - Ripening

When tillering is occurring the plants are also developing microscopic spikes (also referred to a ‘heads’ which is the part that will form floral structures and kernels). Once spike have completely formed the plant enter the stem elongation stage. As part of stem elongation jointing is when nodes become detectable above the soil surface. Before the jointing stage the plant apex (from where the plant is growing) is below the soil, this protects the delicate node from mechanical damage and harsh weather. When the stem’s upper internodes elongates the apex is moved above the soil surface and the small spike begins to rapidly grow under the surrounding leaf sheaths (Liller et al., 2015; Simmons et al., 1982).

The barley plant's first three internodes usually remain short and the fouth is the first to elongate followed by the later internodes which get progressively longer. This seprates out the leaves to form a tall plant with a segmented stem. In agriculture growth regulators may be applied at this stage to counter lodging, by limiting elongation and producing shorter and stiffer internodes (Daniels et al., 1982; Patil et al., 2019; Tavakoli et al., 2009).

The bolting stage is when the spike becomes prominent with the flag leaf sheath, the final leaf after bolting, approximately six to seven weeks since emergence, flowering and pollination occur. Barley is self-pollinating beneath the leaf sheath(Starling, 1980; Zohary, 2017). Pollination is one of the most sensitive times to stresses, which can affect pollen quality and cause sterility (Abiko et al., 2005; Rehman et al., 2004; Rieu et al., 2017; Yakir et al., 2007). High temperatures and dry conditions are particularly degrading resulting in lower kernel production and yields. By the time of heading, maximum leaf area has usually been reached, which determines the maximum photosynthetic capacity of the plant. Sowing dates are often determined in consideration of heading dates to avoid warmer, drier months for these reasons with earlier sowing in colder months typically advised to farmers (AHDB, 2018). As such cold stress tolerance in seedlings is important.

The duration of leaf function is important for grain yield. Lower leaves tend to die first, increasing the importance of upper leaf blades as the photosynthetic sources for grain filling, with the last two leaf blades being regarded as the most important at this stage. Grain quality is affected by the time and capacity for plants to mobilise substances produced and stored in the leaves and stems into the grains, which can be influenced from photosynthate production the top two leaves providing the energy to enable the necessary active transport (Anderson et al., 1985; Bonnett and Incoll, 1993).

After flowering the length of barley kernels is established, then their widths, with the latter being more stress sensitive. Under stress kernels are narrower even if kernels are the same length as unstressed plants (Dodig et al., 2018). By observation in this reserach, early maturing lines tended to have shorter and narrower kernels than

parental lines even when grown in similar conditions. The first kernel development stages typically last about ten days and determines the number of cells that will go on to be storing starch over the following ten days there is rapid kernel growth and starch storage in the form of a milky fluid that becomes more concentrated (AHDB, 2018; Druka et al., 2006; Ellis and Pieta Filho, 1992). The stage when the grains are filled is called the milk stage. Starch accumulated in the leaves and produced in the flag leaf is relocated into fertilised grains. This stage can be vulnerable to infection as the grains are softer. Desiccation at early kernel development will reduce viable seed yields. After the kernels are filled they approach maturity, losing water and eventually their green colour. After plants become physiologically mature and stop producing food, the milky contents of the fully filled grains begins to harden, this is the dough stage (AHDB, 2018).

Beneath the soil, primary roots emerge first from the seed, making the crown roots, followed by seminal roots from which adventitious lateral roots are produced, after plants produce their third leaf. Roots anchor plants into the soil but their main function is water and nutrient uptake. Leaf and stem number (anatomical structures), growth conditions and plant age affect uptake and are thus important considerations in regards to plant water management (Minnesota, 2019b).

The composite transport model figure 1.4.2 (Kim et al., 2018) describes how roots take up water and nutrients via three major pathways, the apoplastic (cell walls), symplastic and transcellular pathways. The last two are also known as the cell-to-cell pathways. The apoplastic pathway can be blocked by Casparian bands and suberin lamellae in endodermal and exodermal cell walls. The cell-to-cell pathway can additionally be regulated by aquaporins (Peterson and Cholewa, 1998; Steudle, 2000a, b, 2002; Steudle and Jeschke, 1983). It led to the belief that a majority of water transport went via the apoplastic route. However, it appears that there is substantial transcellular and symplasmic transport of water in barley roots(Steudle and Jeschke, 1983) , meaning a greater involvement in transport pores which can be genetically and hormonally regulated. It has been shown that using polyethylene glycol (PEG, m.w.8000) to induce osmotic stress to simulate water deficit (to percentages as high as 50%), there are changes in the suberized barrier development in barley roots and canages in global gene expression patterns, in particular an increase in suberin in barrer tissues like the casperian strip help seal roots and prevent uncontrolled passive water loss by backflow via the nonselective apopplastic pathway while the cell-to-cell pathway maintains water-uptake(Kreszies et al., 2019).

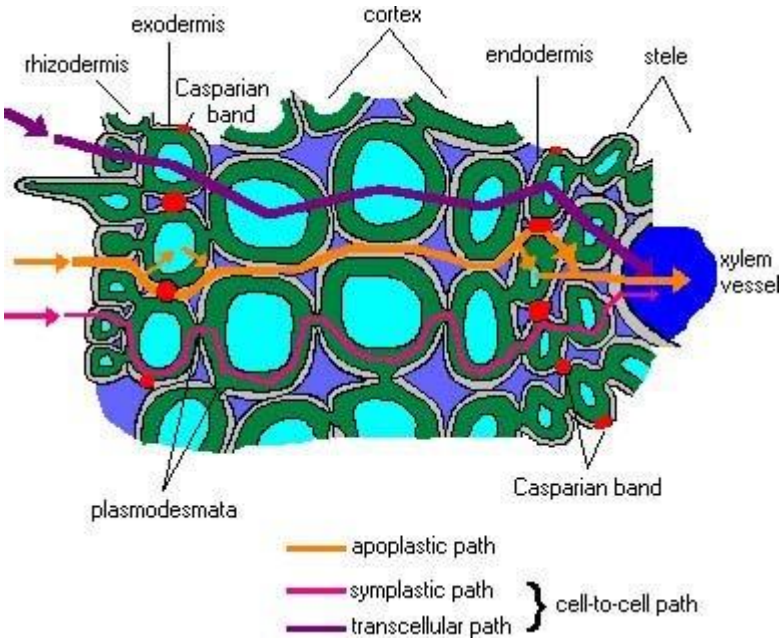


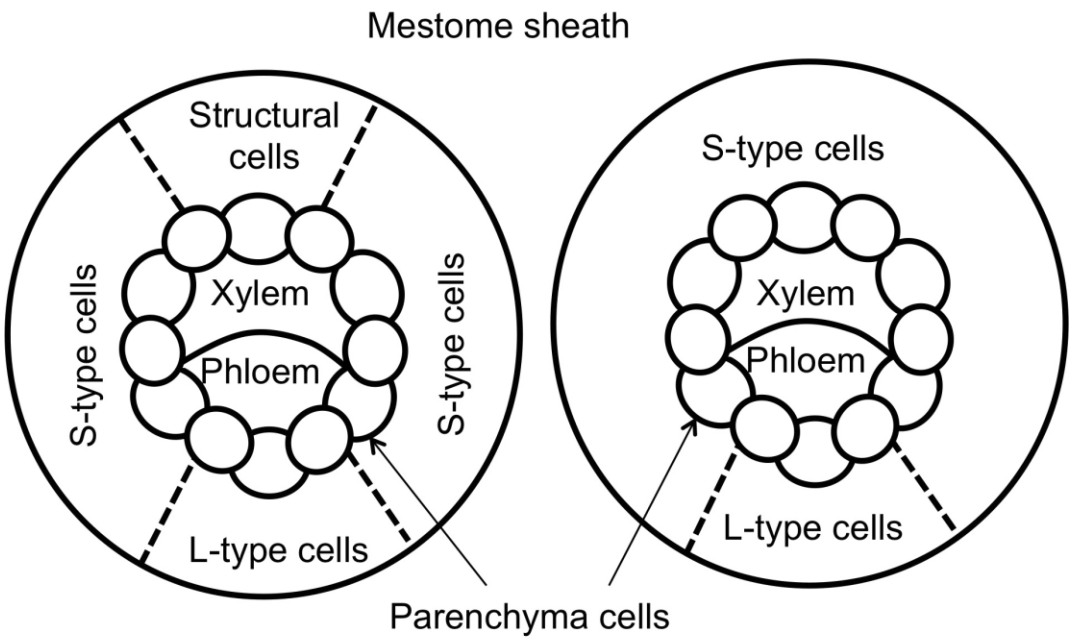
Figure 1.4.2 Water and solute transport pathways in plant roots from (Steudle, 2000a) showing the apoplastic pathway (orange) moving water via cell walls and occasionally intothe cell. The symplastic pathways (pink) moving water through the cytosol in the cytoplasm and using plasmodesmata canal between cells to navigate through the roots. The transcellular pathway (purple) moves water through the symplast (interconnected cytoplasms), apoplast (cell walls and spaces in between cells) and occasionally the tonoplast (entering the central vacuole) as it crosses plasma membranes through the root.

Through the roots, stem and leaves runs the vascular system (the xylem, phloem and associated parenchyma cells). These are the main 'highways' by which water and nutrients, as well as solutes, hormones and RNA are transported around the plant, less hindered by cell walls (Lucas et al., 2013). Barley vascular tissue is made up of vascular bundles and contiguous (adjacent) tissues called bundle sheaths. Bundle sheaths in barley are among the best-studied for C3 plants. Barley like many grasses, have two cell layers surrounding the vascular structures, an outer layer called the parenchyma sheath which provide energy to the xylem and phloem cells, and an inner layer, with a thickened inner walls (typical of Pooideae) called the mestome sheath (Arber, 2010) adjacent to the (parenchymatous) bundle sheath cells (Williams et al., 1989)(figure 1.4.3B). The bundle sheath is a compact layer of parenchymna cells (generalised plant cell types with thin walls they usually make up photosynthetic tissue). The bundle sheath is an essential channel between the vascular and mesophyll cells (the main photosynthetic cells that have two types, palisade and spongey), it conducts fluxes of compounds and water that must pass through these cells. In C3 plants bundle sheaths prevents air entering the xylem and can store water maintaining hydraulic integrity and buffering transpiration surges as well as potentially more functions {Griffiths, 2013 #804}. There are no airspaces at the bundle sheath–mestome sheath cell interface allowing for a potential symplastic association between the two sheaths. Bundle sheath cells are large, vacuolate, approximately cylindrical in shape, and have volumes about four times that of mesophyll cells. The bundles are distinguished as S-type and L-type

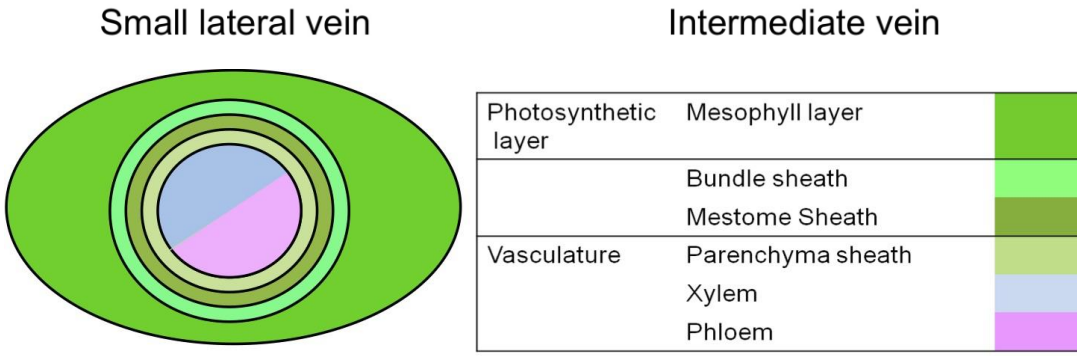
differentiated by their size and size of their chloroplasts, S-type mean small with chloroplast a third of the volume of mesophyll chloroplasts while L-type refer to large with chloroplast similar in volume and number per number to mesophyll cells , (Leegood, 2008). The majority of cells are S-type (figure 1.4.3A).

Like other plants, arrangement of vascular tissues in barley is organ specific. In roots the vascular tissue is arranged in a ring. In stems, here is an atactostele(scattered)arrangement within the organ (e.g. Leaf, Stem, root) unlike many dicots that have uniformed ring organisation classified as a eustele arrangement.In leaves they form major and minor veins running in parallel lines with the thickness of the leaf blade determining vascular physiology(Trivett and Evert, 1998).

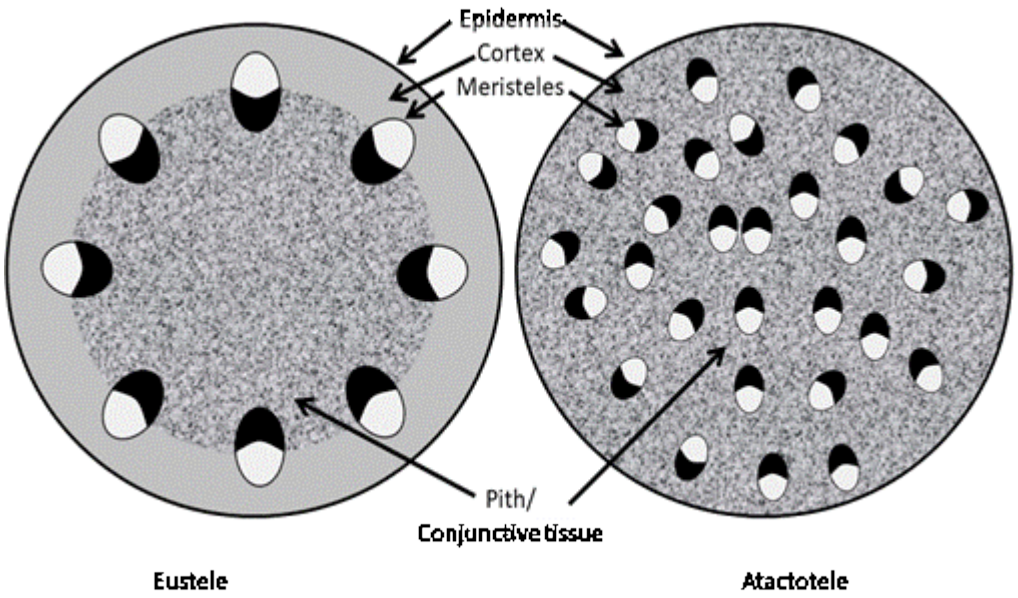
A



B



C



D

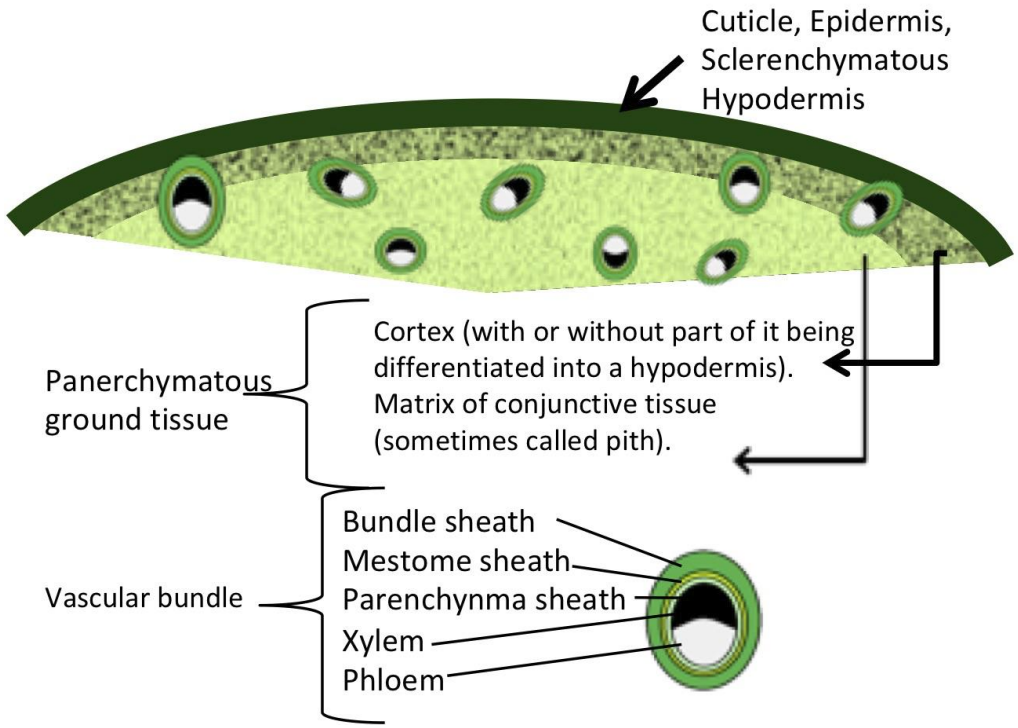


Figure 1.4.3. Illustrations of bundle and mestome sheath arrangements in barley showing (A) the positioning of S-type and L-type bundle shealth cells on the mestome for different leaf veins redrawn from (Leegood, 2008)from their redraw of Williams (1989), (B) shows the arrangement of the 2 layer sheaths between the photosynthetic mesophyll layer and the vascular structures. (C) Diagrams Comparing of two stele arrangements, eustele typical of dicots (left) and Atactoctele (Right) found in monocots that are differentiated by the distribution and pattern of the meristeles (vascular tissue units), Xylem Black; Phloem white; pith, stippled grey. The diagram indictates the typical location of the epidermis (outer most layer of surface cells), cortex tissue of unspecialized cells between the epidermis and the vascular, or conducting, tissues, and Pith (medulla) the soft and spongy parenchyma cells or conjunctive tissue as it is called

in monocots(Morrow and Algiers)(accessed June 2020). (D) Diagram of how vascular bundles could be arranged in a monocot leaf including the sheath layers.

The vascular tissues lead to the stomata with mesophyll cells between them. Stomatal development in grasses is constrained to the leaf base and Stomata form in specified cell files adjacent to veins. This results in an organised patterning of almost parallel lines of intermittent stomata along the length of the leaf (Hepworth et al., 2018; Rudall et al., 2013; Stebbins and Shah, 1960).

Stomata in grass leaves are linear 'dumbbell'-shaped and have accompanying subsidiary cells making a stomatal complex (Rudall et al., 2017) rather than the kidney-shaped stomata without subsidiary cells in Eudicots. It has been shown that smaller changes in volume are required for stomatal opening in the dumbbell shape guard cells, making higher diffusible pore area more readily than kidney-shaped stomata is able to (Hetherington and Woodward, 2003). The subsidiary cells create a structural limit and accommodate guard cell movement to create a mechanical advantage (Franks and Farquhar, 2007) while also providing a fast supply of ions to guard cells. This facilitates reciprocal changes in turgor pressure by osmotic flux, enabling large and rapid stomata responses (Franks and Farquhar, 2007; Raschke and Fellows, 1971; Schäfer et al., 2018). Studies that have compared opening and closing behaviour in the dumbbell-shaped of grasses to kidney-shaped stomata in other plants, have shown that the stomata in grasses have greater speed and efficiency of stomatal regulation and adaptive advantages over other species. This may explain why grasses are often some of the first pioneering plant species in harsh environments (Grantz and Zeiger, 1986; Haworth et al., 2018; McAusland et al., 2016; Merilo et al., 2014; Vico et al., 2011).

On the external surface of the plant, there is the cuticle. This is composed of cutin embedded in wax (cuticular wax embedded within the intracuticular wax (cutin polymer matrix) and epicuticular wax (outer surface)). The Cuticular waxes are made of complex organic solvent-extractable mixtures of monomeric long carbon chains (C-20 to C-60) aliphatics that may include triterpenoids, phenylpropanoids, and flavonoids (Samuels et al., 2008). Cutin is made of saturated and unsaturated carbon chain hydroxyl and epoxy fatty acids covalently cross linked. In Barley the Epicuticular wax forms three-dimensional crystallites (Bargel et al., 2004; Jetter et al., 2000; Kunst and Samuels, 2003). Prior to leaf emergence the cutin is deposited as the leaf cells elongate and the wax is deposited once elongation ceases (Richardson et al., 2005) the rate of elongation prior to emergence influences deposition and thus thickness. The composition of these waxes can be highly variable (Bargel et al., 2004; Post-Beittenmiller, 1996) across the length of the leaves {Richardson, 2005 #805} and in different barley genotypes {Febrero, 1998 #806}. The purpose of the wax is to further protect against water loss. Drought-tolerance and better water use efficiency (WUE) is associated with more cuticle wax {Xue, 2017 #808}. The Bowman cultivar parental line has a waxy layer and even after standard acetone treatment to strip the epidermis of

waxes for surface electron microscopy there is still wax on the leaf surface (figure 1.4.4).

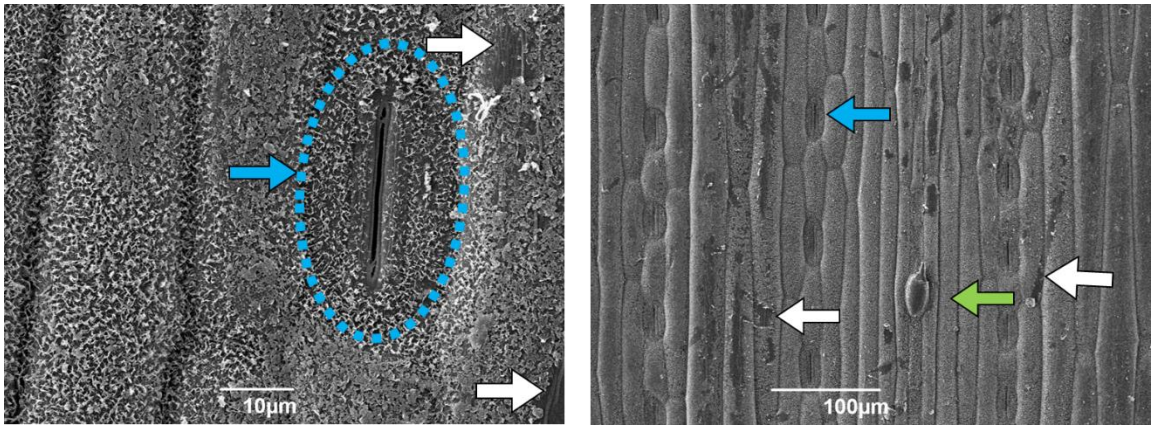


Figure 1.4.4 SEM image of barley zoomed in 1.7k magnification (A) and 250 magnification (B) showing a typical stomata of Bowman parental line, the cracked and jagged structures are waxes which remained even after treatment in ethanol and acetone with arrows showing some places where waxes had fully been removed and the under epidermis is darker shown by white arrows. Blue arrow show examples of stomata and green arrows show a trichome, scale bars shown at the bottom of the image.

1.5 Barley production and breeding technologies

In exploring new ways to tackle old challenges the discovery of genetic timekeeping has provided more than a literal example for the term "A blind watchmaker", but unlocked a new field of research, circadian biology, relevant across the "tree of life". It was not long before new insights turned into tinkering as the master regulator circadian genes were deemed candidates for biological innovation and targets for breeding technologies.

The International Barley Genome Sequencing Consortium (IBSC) assembled the first functional, map-based reference genome sequence of barley cv. Morex using hierarchical shotgun sequencing and published in 2012 (The International Barley Genome Sequencing Consortium et al., 2012). Since then work continues to improve annotation of genes within the genome (Beier et al., 2017) and the creation of analytical tools (Mascher et al., 2017) bring these advancements to be broadly accessible and even virtual resources like those available for *Arabidopsis thaliana*. Barley is regarded as the model organism for Triticeae (Muñoz-Amatriaín et al., 2014) although research and resources in wheat are quickly catching up.

1.6 The gene architecture and behavioural properties of the circadian clock in in model plants

Most of our knowledge about the circadian clock mechanism in plants, for example how all the genes and regulatory components interact, has been based in the model plant *Arabidopsis thaliana (*Arabidopsis*)*. The current model is often described by dividing the 'clock' into three loops, interconnected mainly by negative feedback interactions which together sustain an oscillation which lasts approximately 24 hours although the exact period often varies (McClung, C R, 2011; McClung, 2014; McClung, 2019; McClung, 2010; McClung, 2006; McClung, 2009). Familiarisation with the model system can be the basis for developing ideas of what to possibly expect of similar genes in barley. The circadian oscillator often descripted as being composed of the morning, evening and central parts.

The morning loop includes the MYB-like transcription factors CIRCADIAN CLOCK-ASSOCIATED 1 (CCA1), LATE ELONGATED HYPOCOTYL (LHY),REVEILLE (RVE)8 (also known as LHY and CCA1-like 5, LCL5), PSEUDO-RESPONSE REGULATOR (PRR)9, PRR7, and the proteins NIGHT LIGHT-INDUCIBLE AND CLOCK-REGULATED 1 (LNK)1, and LNK2. The evening loop includes TIMING OF CAB EXPRESSION 1 (TOC1)(also known as PRR1 for it is a homolog to the other PRRs), GIGANTEA (GI), ZEITLUPE (ZTL), BROTHER OF LUX ARRHYTHMO (BOA) (also known as NOX, for 'night' in Latin), CCA1 HIKING EXPEDITION (CHE), PRR5, PRR3, LUX ARRHYTHMO (LUX), EARLY FLOWERING 3 (ELF3), and ELF4 (Greenham and McClung, 2015; McClung, C. Robertson, 2011; McClung, 2014; McClung, 2009).

The central loop is the core oscillation considing of *CCA1/ LHY* and *TOC1* feedback loop, which is slowed by the additional feedback loops from the other phases. Once the clock is reset, normally by light of sufficient intensity after a period of darkness, *CCA1/LHY* become activated and together activate components of the morning-phase. The morning components in sequence repress expression of *CCA1/LHY*, repress each and are repressed by *LHY* and the evening expressed components, once they activate (Deng et al., 2015; Ding et al., 2007).

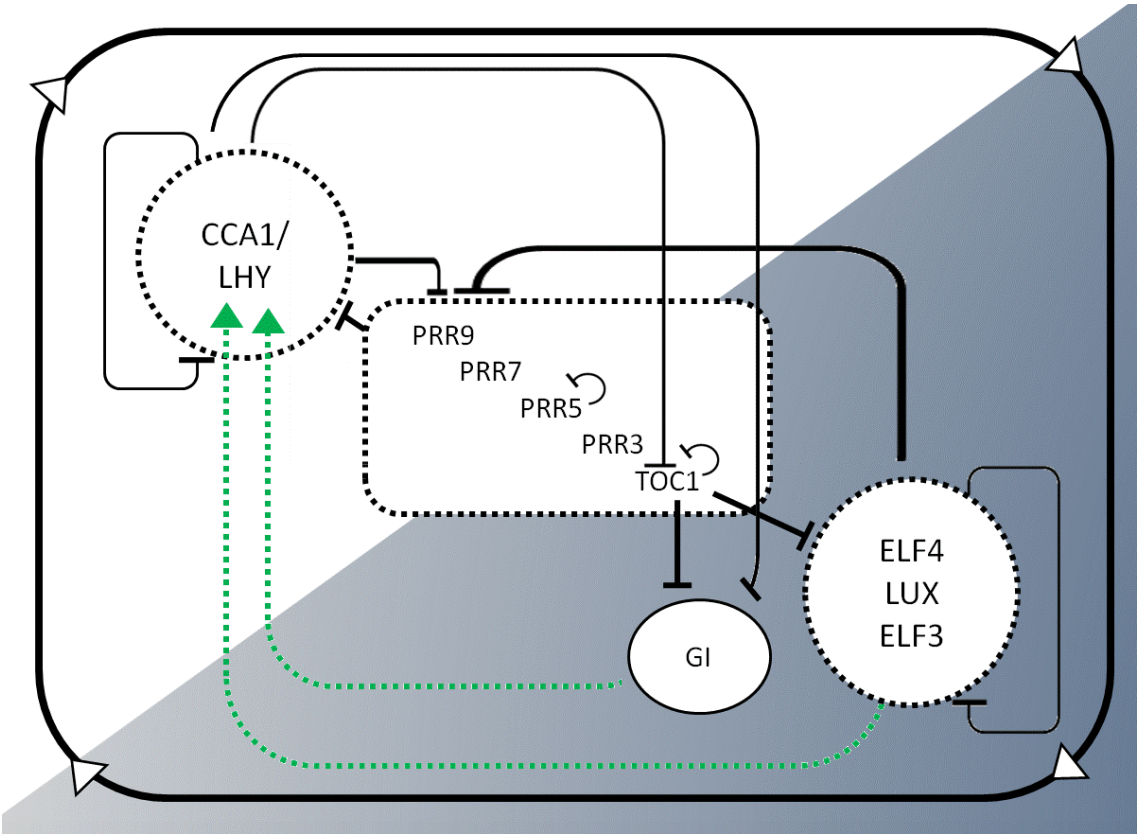


Figure 1.6.1 A simplified model of the Arabidopsis circadian clock, this diagram does not show the transcriptional activators of the clock genes but focuses on the feedback relationship of Arabidopsis circadian genes with discovered orthologs in Barley. In *Arabidopsis thaliana* the clock's core components are multiple interlocked transcriptional feedback loops.The day/night cycle is shown from left to right by black border line with white directional arrow, the morning is represented by a white background and the night represented by a grey gradient background. Black bar indicate repression and green arrows indicate activation of transcription, and dashed lined indicate indirect relationships. Enclosed dashed lines represent protein complexes, the complex of *ELF4/LUX/ELF3* is known as the Evening complex (EC). At dawn expression of the *PRR* genes, *TOC1*, *GI*, and the EC from the night before are repressed by *CCA1* and LHY. Over the course of the morning *PRR9*, *PRR7*, *PRR5*, and *TOC1* are sequentially expressed and repress the transcription of *CCA1* and *LHY*, as well as their own transcription. In the evening TOC1 represses all of the daytime components as well as evening expressed *GI, LUX* and *ELF4* and the EC represses *GI, PRR9,* and *PRR7.* As the night ends GI and the EC are required for the transcriptional activation of *CCA1 and LHY.* This diagram was adapted from(McClung, 2019).

Part of the evening- phase is the interaction of nuclear proteins *ELF3* and *ELF4* with MYB-like transcription factor *LUX* to form the 'evening complex' which represses *PRR9* (Dixon et al., 2016; Helfer et al., 2011; Herrero et al., 2012; Nusinow et al., 2011). Meanwhile, the homolog of *LUX, BOA* is thought to also interact with both *ELF3* and ELF4 into a complex which promotes *CCA1* (Dai et al., 2011; Nusinow et al., 2011). Any loss of function mutation to the evening complex components results in a arrhythmic

plant in Arabidopsis (Nusinow et al., 2011; Zagotta et al., 1996).

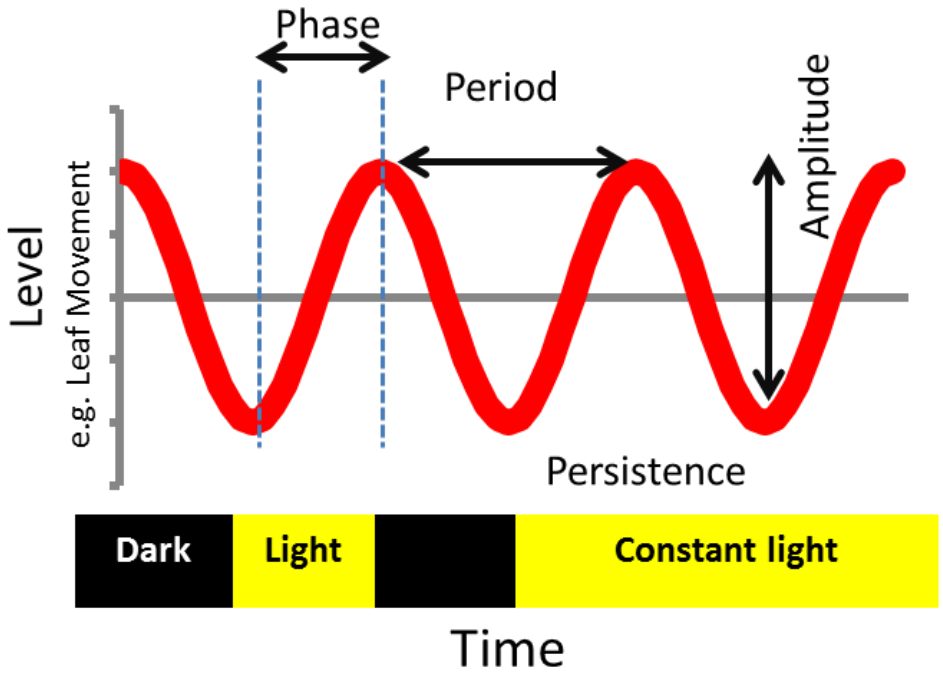


Figure 1.6.2 a Representation of a circadian rhythmcircadian rhythms refer to endogenous rhythms which follow diurnal cycles, usually lasting approximately 24 hours although they can be shorter or longer of an organism. Important characteristics of a rhythm included the Amplitude, how much something, which is measured rises and falls in a rhythm; Phase, the time between a fixed event and a reference point in the rhythm; Period, The time between an events reoccurring in the rhythm. The definitive characteristic is the Persistence, when the environment is constant (or changes) the rhythm carries on almost the same for a length oftime after other stimulus cease (McClung, 2006).

The circadian clock of plants provides a fitness advantage (Dodd et al., 2005; Green et al., 2002; Michael et al., 2003) by inducing rhythmic behaviours on many key processes to match diurnal cycles in the environment (as reviews, (Baldwin and Meldau, 2013; Harmer, 2009; Kinmonth-Schultz et al., 2013; Nagel and Kay, 2012) (fig.1.6.2). The clock has been shown to be a master regulator of a high percentage (around 30%) of plant gene expression in Arabidopsis, rice, papaya, maize, soybean and popular (Covington et al., 2008; Filichkin et al., 2011; Harmer et al., 2000; Hayes et al., 2010; Khan et al., 2010; Marcolino-Gomes et al., 2014; Michael et al., 2008; Zdepski et al., 2008). This includes genes involved in flowering time, hormone synthesis and signalling, metabolism, growth control, biotic and abiotic stress responses (Covington and Harmer, 2007; Goodspeed et al., 2012; Graf et al., 2010; Legnaioli et al., 2009; Ni et al., 2009; Nozue et al., 2007; Wang et al., 2011; Yu et al., 2008). Notably, alleles of clock

genes have been shown to influence key crop traits (Bendix et al., 2015). For example, in barley the genes *PSEUDO-RESPONSE REGULATOR 37* (*PRR37*), *GIGANTEA (GI)*, *EARLY MATURING 8* and *10* (*EAM8 and EAM10*) have been linked to vernalisation, photoperiod sensitivity and flowering time. My thesis study aims to investigate the possible water stress related fitness advantages of the circadian clock and specific clock genes of barley.

In plants the circadian clock can be considered to act as a co-ordinating mechanism, taking in input signals and modulating a response that balances the current and future needs in a given time. One way this occurs is by gating, a process which regulates the timing of sensitivity to a given stimulus and then the scope of the response depends on another factor in this case, the stage in the genetic circadian oscillator. An example in plants is stomata aperture and Abscisic acid (ABA) sensitivity, which is gated by the circadian clock (Yoshida et al., 2019; Yoshida et al., 2014) resulting in distinct rhythmic patterns in stomata activity. Regulated sensitivity to inputs enables almost instantaneous acclimation and adaptation to changes in the environment. Responding to photoperiod is useful for seasonal acclimation and adaptation during range shifts into new latitudes while still maintaining the endogenous rhythms. Circadian clocks balance an almost contradictory role, evolving from anticipating predictability while aiding survival and adaption for the unpredictable.

The fitness benefits which have led to the wide spread evolution of circadian clocks (Dodd et al., 2005) likely depend on local adaptation, synchronising with the immediate environment. Over time it might be expected that the biological processes become increasing gated and linked to the circadian clock becoming more robustly rhythmic to correlate with the most predictable phenomena (McClung, 2010). Moving from established ranges, especially across latitudes, maintaining these rhythms would cause the organism to be 'out of time' with external events, in which case deconstructing the 'old clock', reducing its gating effects to redundant rhythms may be more advantageous. Altering pathways which feed into the clock or dampening the clock routes to such a strategy (Faure et al., 2012).

Conserved orthologues and paralogues of core circadian clock and clock-associated genes in Arabidopsis have been identified in a number of domesticated plants, including barley (Campoli et al., 2012c; Takata et al., 2010). Previous reports noted the high degree of conserved genomic sequences and functional protein domains when comparing gene in barley to Arabidopsis (Campoli et al., 2012c; Murakami et al., 2003; Song et al., 2013). Similar associated orthologous sequences in barley and Arabidopsis are likewise named, Hv*GI* is At*GI*, Hv*TOC1* is At*TOC1* (sometimes called At*PRR1*), Hv*CCA1* is At*LHY*/At*CCA1*, Hv*PRR37* and Hv*PRR73* are At*PRR3* and At*PRR7*, Hv*PRR59* and Hv*PRR95* are At*PRR5* and At*PRR9, HvEAM8 is AtELF3, HvEAM10 is AtLUX* (Campoli

et al., 2012a; Campoli et al., 2013; Campoli et al., 2012c; Faure et al., 2012). The conservation of gene sequences between the Arabidopsis clock and barley leads to suggest that the clock composition in monocots and dicots is potentially similar(Campoli et al., 2012a).

Table 1.6.1: **Circadian clock genes in both Arabidopsis (*Arabidopsis thaliana (At)*) and Barley (*Hordeum vulgare* (Hv))**

Gene	Arabidopsis	Barley
CIRCADIAN CLOCK ASSOCIATED 1	*AtCCA1*	*HvCCA1*
LATE ELONGATED HYPOCOTYL[1]	*AtLHY*	-
PSEUDO RESPONSE REGULATOR 3	*AtPRR3*	*HvPRR37/HvPpd-H1* *HvPRR73*
PSEUDO RESPONSE REGULATOR 7	*AtPRR7*	
PSEUDO RESPONSE REGULATOR 5	*AtPRR5*	*HvPRR59* *HvPRR95*
PSEUDO RESPONSE REGULATOR 9	*AtPRR9*	
TIMING OF CAB EXPRESSION 1	*AtTOC1 /AtPRR1*	*HvTOC1*
GIGANTEA	*AtGI*	*HvGI*
EARLY FLOWERING	*AtELF3* *AtELF4*	*HvELF3/ HvEAM8*
LUX ARRHYTHMO	*AtLUX*	*HvLUX/HvEAM10*
BROTHER OF LUX ARRHYTHMO	*AtNOX /AtBOA*	
REVEILLE	*AtRVE*	*HvRVE*

[1]***LHY* and *CCA1* are partially functionally redundant in Arabidopsis** (Green and Tobin, 1999)**. *HvCCA1* is more closely related to *AtCCA1*** *(Boxall et al., 2005)* **however it is sometimes referred to as *HvLHY.***

The phylogeny *PRR* gene family contain paralogs in both barley and Arabidopsis, TOC1 (PRR1) clades separately to PRR3/7 and PRR5/9, which then clade separately to each other. Within these latter clades monocots and dicots diverged before independent gene duplication events led to paralogous PRR genes within them. In barley this would be PRR37 and PRR73 in the PRR3/7 clade and *PRR59* and *PRR95* in the *PRR5/9* clade

(Campoli et al., 2012c; Murakami et al., 2003; Takata et al., 2010). Therefore, despite the similarities there is evidence that the Arabidopsis and barley clocks are not identical.

Along with divergence in gene sequence, there is evidence that the function of some clock genes have changed between species. The transcripts of the PRRs in barley have broad peaks over the day in the order *PRR37/73*, then *PRR59/95* followed by *TOC1 (Campoli et al., 2012a; Murakami et al., 2007a, b)* whereas in Arabidopsis, peak expression is in the sequential order *PRR9, 7,5,3, TOC1* with less overlap (Matsushika et al., 2000). Additionally, in Arabidopsis AtCCA1 and AtLHY, have both evolved to be important for circadian rhythms (Calixto et al., 2015), but in other plants there is one ortholog for both (Campoli et al., 2012c). Furthermore *PRR*37, also named *Ppd-H1*, has evolved novel flowering time functions in barley, mediating the acceleration of development under long days, independently of the clock (Campoli et al., 2012a). Both the circadian clock and *Ppd-H1* have direct and indirect regulation of *Vrn-H1* (Campoli et al., 2012a), whereas the circadian clock in Arabidopsis has not been shown to regulate the *Vrn-H1* orthologs *APETALA1 or FRUITFUL (AP1 /FUL).* Unlike Arabidopsis, barley during early development requires initial photoperiod cues before showing robust rhythmic gene expression. Additionally the barley circadian oscillation response to photoperiod cues was much faster than other plants tested (Deng et al., 2015). Therefore it is open to the possibility that circadian clock genes in monocots have physiological functions not observed in Arabidopsis.

The circadian clock network of genes mediate plant responses to many environment signals. Other signal transduction pathways connect to circadian genes as both inputs and responses. The circadian system can act like a gating mechanism modulating plants development until the right combination of environment signals align. Classic examples include the flowering time pathway (Hubbard and Webb, 2011), shade avoidance pathway (Salter et al., 2003), Absisic acid (ABA) signalling pathway (Seung et al., 2012) and seasonal modulation (Song et al., 2015). Rigorous studies have explored the circadian clock in Arabidopsis results found in dicot species cannot be assumed to translate exactly onto other species. Calixto et al. (2015) proposed that two thirds of the clock components found in Arabidipsis may have been present in the last common ancestor prior to the monocot/dicot separation and since then there have been independent gene duplication events and other evolutionary trajectory (Calixto et al., 2015).

Noteable differences are in the “Morning loop” of the Arabidopsis circadian clock, which has semi-redundant *CCA1* and *LHY* genes that derive from a gene duplication, whereas barley only has one gene ortholog. The pseundo response regulator genes

which include *TIME FOR COFFEE / PSEUNDO RESPONSE REGULATOR (TOC1*/*PRR1*) and *PRR37* (*PHOTOPERIOD SENSITIVE -1*, *HvPpd-h1*) were also present in the ancestral plant but the monocots and dicots had independent duplication events so there are more differences in this group of related genes between the two clades. The *EARLY FLOWERING* (*HvELF3*) and *LUX ARRHYTHMO (HvLUX*) genes do have true orthologs to the equivalent genes in Arabidopsis. Although, in Monocots there was a duplication of the *ELF3* genes (ELF3a and ELF3b) and different families subsequently lost one, for example temperature grasses (Pooideae) kept *ELFa* and lost *ELF3b* while rice kept *ELF3b* and lost *ELFa (Calixto et al., 2015)*.

To further support the idea that different evolutionary history has potentially influenced the role of the circadian clock components there is increasing evidence showing domestication has impacted the circadian clock of crop plants (as reviewed in (Shor and Green, 2016)). One effect of domestication in crop plants has been to change the characteristics of circadian rhythms, such as increasing period length in tomato (Muller et al., 2016). The majority of allelic variation found in domesticated crops is changes to photoperiodic flowering (Cockram et al., 2007; Faure et al., 2012; Nakamichi, 2015) likely a reflection of range shifts, artificial growing conditions or selection pressure on performance phenotypes which in some crops resulted in selecting for circadian clock mutants. This seems to be the case for European spring varieties (Faure et al., 2012) selecting for recessive *PHOTOPERIOD RESPONSE* gene (*Ppd-H1*) which is actually a *PRR* gene, *PRR37* and Scandinavian spring varieties having an additional recessive circadian mutation in EAM8, a orthologue to the Arabidopsis EARLY FOWERING 3 (*ELF3*) of the evening complex (EC), the result of which causes arrhythmia but is better adapted to light and dark cycles of 6 months rather than 24 hours. Additionally, the growth and morphological shoot phenotype differences between arrhythmic Arabidopsis *elf3* plants to their respective rhythmic wildtypes is comparatively greater than those observed between barley wild type and *eam8* lines (Dodd et al., 2005; Faure et al., 2012; Green et al., 2002; Müller et al., 2014; Zagotta et al., 1996). Further differences include that barley grows continuously rather than cyclically. While both Arabidopsis and barley appear to be able to store and adjust rate of consumption of their sugar stores throughout the night to avoid periods of starvation before dawn, Arabidopsis stores sugar as starch to be used through the night, while barley stores sugar as sucrose(Müller et al., 2014). The biology and ecology of a laboratory model and agricultural cereal are likely to be different enough to warrant deeper investigation in the latter, particularly concerning stress tolerance.

1.7 Associations between the plant circadian clock and water regulation (influencing other physiology, photosynethsis and metabolism)

Movement and storage of water influences many properties of an organism, such as the physical structure and properties; the biochemical reactions of proteins, membranes, nucleic acids and other cell constitutes; metabolic processes such as photosynthesis and respiration as well as having regulatory value, in particular in the case of temperature (Taiz and Zeiger, 2003). Despite its abundance water is one of the most limiting resources needed for plants to grow and function. Therefore understanding mechanisms behind the regulation of uptake, loss and distribution of water is of particular interest. Water stress, the deficit of water relative to the plants needs thus applying a disadvantageous influence on the organism, is of great interest for economically valuable plants due to the negative impact on yield and quality. Even mild stress can lead to great physiological, metabolic and developmental responses (Clauw et al., 2015) and often interrelates with other stresses such as salinity, anoxia, biotic, freezing and heat.

The circadian clock plays a part in stress acclimation and adaptation. In Arabidopsis water regulation is at least partially modulated by the circadian clock via aquaporins in the roots (Takase et al., 2011) and stomata in the leaves (Legnaioli et al., 2009) demonstrating endogenous control over water in anticipation of heat. During warmer times of the day transpiration rates increase. It has been observed stomata close before the hottest time of day likely to preserve water. Furthermore, the conductance have circadian rhythms which are preserved in constant light conditions (Holmes and Klein, 1986) even when the epidermis is separated from the leaf (Gorton et al., 1989).

Involvement of the circadian clock in the abscisic acid (ABA) signalling pathway in responses to water shortage by gating (Covington et al., 2008; Hanano et al., 2006; Sanchez et al., 2011). Plant sensitivity to ABA may be fine-tuned through the reciprocal regulation of *TIME FOR COFFEE 1* (*TOC1*) and a putative ABA receptor (ABAR). *TOC1* negatively regulates the expression of ABAR by binding directly to the promoter of the receptor in a periodic manner that has a gating effect locally adapting water sensitivity to local diurnal patterns (Legnaioli et al., 2009). In turn, water shortages triggering ABA expression can influence the clock by inducing *TOC1* transcription (Legnaioli et al., 2009) as the ABAR receptor can positively regulate *TOC1* transcription creating a feedback loop which can fine tune the speed of the generated oscillations of the circadian gene (Hanano et al., 2006). Changes in water availability can feed back into the clock demonstrated by osmotic stress applied to the roots of barley plants altering the gene expression of clock genes measures in the shoot tissues (Habte et al., 2014a). Clock control in stress response has been observed in monocotyledonous plants when expression of water stress response genes (including ABA) was affected by the expression of clock genes *Ppd-H1* and *HvELF3*. This was demonstrated by the expression of water stress genes being different in plants with mutations in these clock genes (Habte et al., 2014a). Recently, water use efficiency has been shown to be effected by circadian oscillator components in Arabidopsis (Simon et al., 2019).

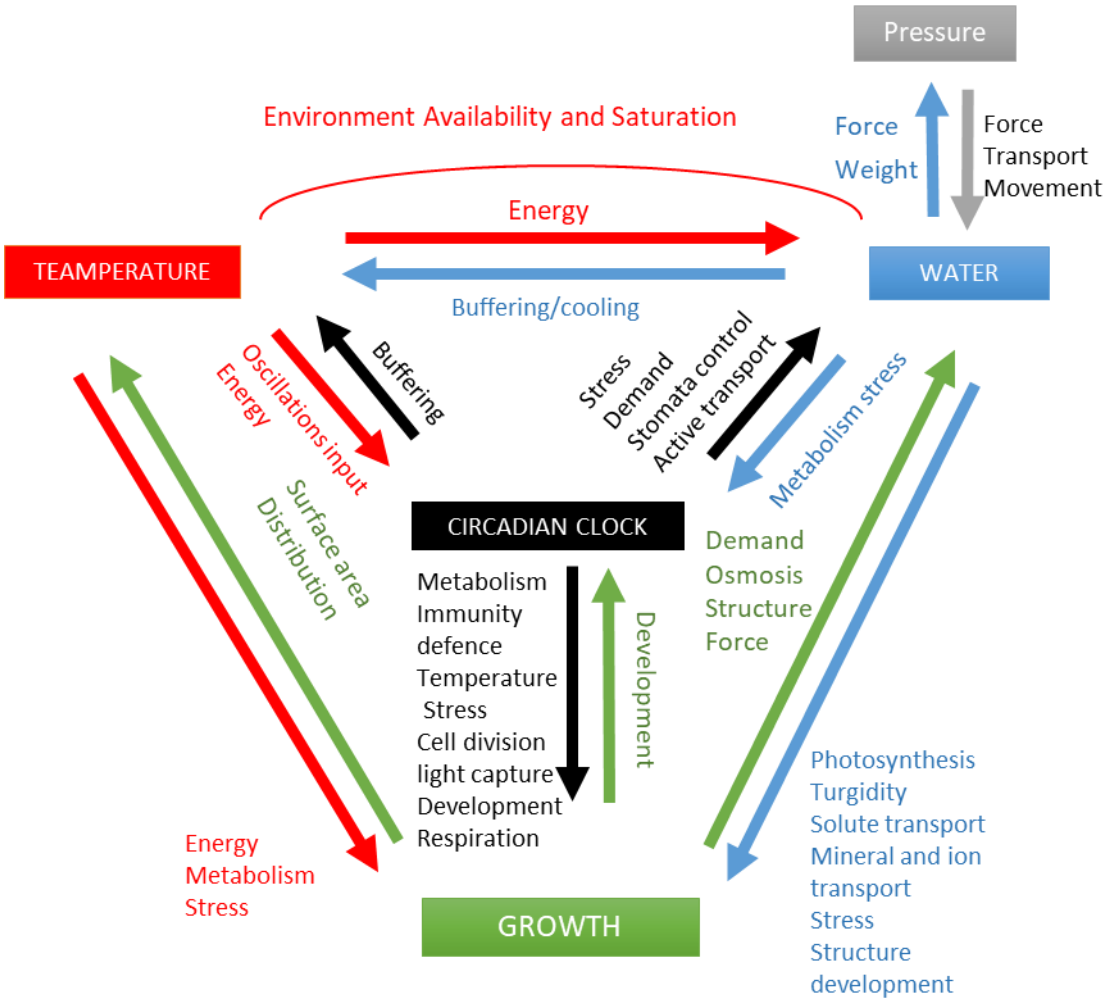


Figure 1.7.1 Diagram of ways temperature, water, pressure, growth and circadian clock can interact and influence each other.

1.8 Barley as a model for Monocot circadian clock research

Barley is considered the most stress tolerant cereal, particularly to drought making it a more favourable crop in developing countries with arid soils as well as an ideal model for water related studies. In barley abiotic stress-responsive genes, including drought responsive genes, have rhythmic expression (Habte et al., 2014a). Most reported circadian clock genes rapidly respond to temperature changes. However, for *GI* and *PRR* genes this is dependent on a functional *EAM8 (ELF3)(Ford et al., 2016)*. Through barley cultivars more insights about how the circadian clock may contribute to acclimation in the monocots can be understood.

Spring Barley is more often used in research than winter because its relatively short growing period, 3 months instead of 6 without a vernalisation period, is more convenient. As such there are some establish circadian mutant lines documented (Druka et al., 2010). In this study lines Bowman 280 (Faure et al., 2007) and Bowman 289 (Zakhrabekova et al., 2012) have loss of function in the *EAM8* gene resulting in a recessive *eam8* caused by different insertions into the sequence. Bowman 284 is a loss of function for *EAM10* resulting in a recessive *eam10* also caused by a random insertion (Campoli et al., 2013) and Bowman 285 over expresses the gene coding phytochrome C (*PHYC*) (Pankin et al., 2014) possessing is a dominant, gain-of-function allele for EAM5, which is presumed to repress the evening complex of which EAM8 would be a component and possible EAM10 if they behave as in Arabidopsis.

Under most climate models the predictions expect the hydrological cycle to intensify, increasing drought and flood risks. The concern is this will occur at a faster rate than ecosystems can adapt. Furthermore extreme weather events are expected to rise. UK temperatures are expected to increase to generally warmer winters and drier summers. Precipitation in winter is expected to be greater, while in summer rainfall is expected to occur in small but more intense clusters in the season possibly causing drought and flood events (Watts et al., 2015). To prepare for the challenges of changing water regimes in the interests of protecting important crops, there are benefits in understanding the mechanisms which control resistance to water stress, one being the circadian clock.

1.9 Barley lines in this thesis

This project uses the mutant lines that contain allelic variation in genes coding for the ELF3 and LUX homologs in barley called *EARLY MATURITY 8 (EAM8)* and *10 (EAM10)*. As the names suggest they mature early despite photoperiod cues which normally regulate flowering with long day photoperiods.

The expectation is, based on conserved sequences, that these interactions will be repeated in most species. However, plants have not evolved the same way. Not all plants have clock component LHY, (Calixto et al., 2015). PRR genes vary in their phylogeny (Campoli et al., 2012c) and clock genes may have novel physiological roles depending on what is relevant for the plant to synchronise within its environment.

1.10 Aims and objectives

Barley is one of the most highly produced cereal crops globally and favoured for its relatively higher stress tolerance and adaptability compared to other cereal crops (Nevo et al., 2012), particularly in poorer countries (Grando and Macpherson, 2005). Even for this hardy grass global rises in temperature, expected changes to regional climates and resulting changes in water distribution and availability will be a challenge. In attempts to keep up with the predicted climate trajectories, agricultural technology and breeding is constantly looking for ways to safe guard food security. Fortunately, for barley large germplasm collections of wild accessions, land races and elite varieties from diverse geographic locations have been amassed in seed banks internationally and are readily available (Ullrich, 2010). Since the early 20th century investigations into the effects of mutagens for crop improvements have added rigorously maintained and characterised mutant collections regarded as containing all the species development and morphological variation have expanded the resources available for barley research. From the Bowman mutant collection created in the 1980s by Franckowiak et al (Franckowiak et al., 1985) and the genotyping carried out in the 2010s (Druka et al., 2010) a number of early maturing lines were characterised B284/eam10.m (Campoli et al., 2013), B289/eam8.k (Faure et al., 2012), B290/eam8.w (Zakhrabekova et al., 2012) as having mutations in clock genes and B285/eam5.x (Pankin et al., 2014) has a mutation in Phytochrome C (HvPHYC), which interacts with the circadian clock via clock gene PRR37 (Ppd-H1) (Turner et al., 2005)and acts on the same pathway as evening complex genes HvELF3 and HvLUX accelerating flowering in short days (Campoli et al., 2013; Pankin et al., 2018). Additionally, the same single point mutation was found by Nishida et al (2013) in the early flowering allele of HvPHYC in the Japanese winter cultivar Hayakiso 2 (Nishida et al., 2013). The listed early maturing mutants all upregulate FLOWERING LOCUS (HvFT1) in long and short photoperiods, in Europe (Faure et al., 2007) and Japan (Nishida et al., 2013) these early maturing mutants appear to have been selected by farmers and could explain geographic range expansions of barley. Alongside the value of changing seasonal growing periods and latitudinal shifts, the connection to the circadian clock may have additional adaption values. The circadian clock is a genetic master regulator with growing evidence that it plays an active role in water regulation in the model plant Arabidopsis. Although current research is showing differences in the functions of circadian genes and the circadian clock in other plants species compared to Arabidopsis, research by Ermias Habte (2014) showed that osmotic conditions at the root influenced circadian rhymes in the leaf. This demonstration of the circadian clock being responsive to osmotic stress lead to the speculation that the early maturing lines could have different water regulatory physiology (Habte et al., 2014b).

Therefore the first main aim of this research project was to determine whether there were differences in water-use. To achieve this work was carried out to investigate if morphological traits, which would influence water regulation were different between

barley lines and to make a low technology system to compare multiple barley lines simultaneously for water-use.

The second main aim of this research project was to explore the effect of cold nights on water-use in early maturing mutants on the basis of strengthen the temperature oscillation signal for the plants and simulating spring-like conditions in high latitudes and altitudes when most sowing of Spring barley often occurs.

To achieve this plants were grown in controlled growth cabinets with regulated light and temperature cycles to compare physiological responses when plants had 4°C or 18°C during dark cycles while having 20°C during the light cycle and water regulation was tested with osmotic stress.

The work presented in this thesis divides across two research chapters. For the first research chapter the objective are to identify physiological and morphological traits that were defining of the early maturing lines compared to the parental line which be associated to potential causes of difference in water-regulation and contains more explorative and preliminary investigations in the mutants plants physiology and identify traits which would be reliable measurements for comparisons when plants were tested for their water-regulation.

For the second research chapter the objectives are (a) to investigate differences in water-use regulation and water-availability sensitivity across the spring barley mutants plants to plants with the parent background genotype and a winter cultivar, (b) explore the barley lines physiological traits with and without sever, chronic osmotic stress, (c) investigate the influence of cold night temperatures on water use and osmotic stress response, (d) examine the effect of osmotic stress at different development points in plants grown in cold nights.

2. Chapter 2: Preliminary studies, general methods and recipes

2.1 Introduction

Preliminary work for this research embarked to establish physiological difference between barley lines with circadian clock gene mutations, which could then be further examined to attribute changes in performance to the mutations in the circadian genes of these lines. A challenge of acquiring repeatable, robust results generated a number of attempted small studies that generated interesting results but due to various difficulties were not taken further. This chapter highlights some of the data and methods that were discontinued as well as methods and materials taken forward.

Work described in this chapter focuses on performance traits relating to growth (root and leaf measurements, tiller numbers and seed numbers) or water-use (water uptake and stomata numbers), as traits that could be varied across the circadian mutants and thus an area of more in-depth focus.

2.1.1 Previous work with the Bowman Barley lines

The barley lines were a small selection of early maturing mutant lines in Barley cultivar Bowman with mutations in genes related to circadian clock evening complex genes *HvELF3 (eam8)(Faure et al., 2012; Zakhrabekova et al., 2012)* and *HvLUX (eam10) (Campoli et al., 2013)*, as well as a mutation in *PHYTOCHROME C* gene *PHYC (eam5) (Pankin et al., 2014)* have been previously identified and described. Notably the barley lines are in the background of the two-row spring cultivar Bowman, which has a natural occurring change in the *PSEUDO-RESPONSE REGULATOR* (*Ppd-H1*) gene and has the *ppd-H1* allelle (Turner et al., 2005)) This allele changes the photoperiod sensitivity of barley and is responsible its adaptation to high latitudes after migrating from the Fertile Crescent and growing in more temperate biomes that have longer and milder summers that extent the spring to summer growing season.

The origin of these barley Bowman lines traces back to the 1980s from Franckowiak et al. work (Franckowiak et al., 1985) when the two-row spring cultivar was first described. This cultivar was selected to be the recurrent female parent to backcross genetic stocks in spring barley that contained multiple morphological markers as described in Wolfe and Franckowiak's research (Wolfe and Franckowiak, 1990). It was Druka et al. subsequent work in 2011 that generated a extensive catalogue of backcrossed lines into Bowman (Druka et al., 2010) and they work used single-nucleotide polymorphisms to identify the mutant donor introgression regions of these near-isogenic lines. By using the same genetic background, Bowman, this makes comparisons between subsequent mutant phenotypes easier. Bowman carries the a recessive mutation in the CCT domain of *Ppd-H1* a ortholog to Arabidopsis clock genes PRR3 and PRR7 campoli (Campoli et al., 2012c) on chromosome 2H (Dunford et al., 2002), the mutation causes a amino acid G to a T change resulting in reduced

photoperiod sensitivity in long days and late flowering due to reduced expression of barley *FLOWERING TIME-1* (*HvFT 1) (Turner et al., 2005)*, properties advantageous to take advantage of longer growing seasons.

From Druka et al.'s work, four Bowman lines are of interest to this work, described as B290, B289, B284 and B285 (Druka et al., 2010). These lines had early maturing phenotypes and were named and numbered from this mutate phenotype as EAM mutants for EARLY MATURING becoming eam8.k, eam8.w, eam10.m and eam5.x, respectively. Barley early maturity mutants have reduced response to no response to photoperiod (either long or short days) and have been used in breeding programs around the world to adapt cultivars to short growing seasons.

The allele for HvELF3 is located on the end of the long arm of barley chromosome 1H. B289 or eam8.k is a complex mutation involving insertions and deletions (Zakhrabekova et al., 2012). Specifically, relative to the wild type allele, it has an insertion of AGCTGCATGGCG at position 1,189 from the start of the allele followed by a deletion of 2,466 bp at position 1,189–3,656, directly followed by an inversion of bps 3,657–4,697, immediately followed by an insertion of CCGTCTCCTCCGCCTCCGCACCGTT and a deletion of 147 bp at position 4,698–4,845. These changes causes a substantial change in the gene and contribute to the loss of function in the *Hvelf3* allele. B290 or eam8.w is a naturally occurring allele in the cultivar Early Russian as a single nucleotide T to C point mutation at position 2109 or in the cultivar Mona as a 4-bp deletion, both in exon 2 of the wild type allele and both result in premature stop codons causing a recessive *Hvelf3 (Faure et al., 2012)*. Early maturity occurs due to the mutation in *HvELF3* causing up-regulation of *Ppd-H1* and the downstream *HvFT1* under noninductive short day conditions (Faure et al., 2012). The alleles for HvLUX1 are located on the long arm of chromosome 3H below the marker ABC166 (Campoli et al., 2013), B284 or eam10.m is a nonsynonymous single nucleotide polymorphism within the exon region encoding the GARP family MYB domain, changing the wild type A nucleotide to a T in the mutant resulting in an encoded S to C amino acid change in the highly conserved SHLQKY(R/Q) motif in Hvlux1 resulting in similar phenotypes to that observed in eam8. Under short days mutation in *HvLUX* causes up-regulation of *Ppd-H1* and *HvPRR1* during light hours (diurnal or constant) and causes strong down regulation in HcCCA1 expression in subjective days. Unlike mutations in *HvELF3* that cause compromised expression of clock oscillator and output genes other clock *HvPRR* genes were not as strongly affected by the Hvlux allele (Campoli et al., 2012a).

The HvPHYC locus was mapped on to chromosome 5H (Pankin et al., 2014), the eam5 mutation is caused by a nonsynonymous single nucleotide mutation (T/C) in exon 1 of the HvPHYC gene. The change results in a missense substitution when the encoded F (hydrophobic phenylalanine) amino acid is replaced by a S (hydrophilic serine) within a highly conserved HHTSPRFVP (F/S)PLRYA motif in the GAF domain of phytochromes. Similar to *Hvelf3* and *Hvlux1* alleles the HvPHYC mutants displays photoperiod insensitivity as it interacts with Ppd-H1 and causes early maturity under long and short days, it was also found to act on the same pathway as HvELF3 and HvLUX1 disrupting circadian clock genes (Pankin et al., 2014).

All lines are less photoperiod sensitive compared to barley cultivars with the Ppd-H1 allele (for example the cultivar Igri). Previous research has shown that the mutations in

these barley lines cause additional differences from the parental line, such as increased photoperiod insensitivity as flowering occurs in both long or short photoperiods, up regulation of floral activator barley *HvFT1* that is downstream of Ppd-H1 and circadian defects, were clock genes do not express as they do in the parental line (Campoli et al., 2012b; Campoli et al., 2013; Faure et al., 2007; Faure et al., 2012).

Furthermore, *HvELF3* is required for induction of transcriptional oscillations by a single light pulse (Deng et al., 2015) as well as rapid response to temperature (Ford et al., 2016). *Hvelf3* alleles cause greater changes in stress response and circadian clock gene expression than variation in the *Ppd-h1* alleles does alone (Habte et al., 2014a). *HvPHYC* likely transmits light signals to the circadian clock thus contributing to light-dependent processes including photoperiodic regulation of flowering (Pankin et al., 2014). *HvLUX* interacts with *Ppd-h1*. *Hvlux* in a *Ppd-h1* background accelerates flowering in long and short days. *Hvlux* in a *ppd-h1* background still has accelerated flowering in long and short photoperiods but not as fast in short days compared to the Ppd-h1 background. Additionally *Hvlux* plants also have circadian defects in *HvCCA1* and *HvPRR1* genes including *Ppd-H1* expression in diurnal and light pulse conditions (Campoli et al., 2013).

2.1.2 Barley in comparison to Arabidopsis and current knowledge on circadian control ingrowth performance and water regulation.

In Arabidopsis growth and water regulation have been shown to have circadian rhythms. The *ztl - 1* mutant of Arabidopsis has Independent circadian regulation of assimilation and stomata conductance (Dodd et al., 2004). Physiological and molecular aspect of stomata were shown to have circadian rhythms (Hubbard and Webb, 2015), including stomata aperture is under circadian control via *CCA1* (Hassidim et al., 2017), aquaporin expression in the Arabidopsis roots is rhythmic and overall the circadian clock influences long-term water-use efficiency Arabidopsis (Simon et al., 2019).

Regardless of the strong evidence showing the circadian clocks' importance in water regulation in the small Arabidopsis, the role of circadian genes in water regulation could be species specific. Species-specific effects have been shown to be the case for connections between circadian clocks and carbon metabolism comparing Arabidopsis to barley (Müller et al., 2014), Arabidopsis carbon metabolism is regulated by the circadian clock, meanwhile temperature has a greater influence on diurnal patterns of carbohydrate utility and growth than the clock in barley in warm (Müller et al., 2018)} and cold (Barros et al., 2020a)} night temperatures. Part of the difference between the species is due to carbohydrate energy sources, Arabidopsis using starch as a carbon store and the circadian clock has a role in pacing the break-down of the complex starch molecules enabling the plants to avoid starvation during the non photosynthetic periods at night and thus regulating the plants growth rate. In contrast barley main carbon source is sucrose instead of starch and nocturnal supply of carbon is regulated by enzyme kinetics and temperature (Müller et al., 2018). Temperature also influences what carbons are synthesised, in warmer night conditions starch will be synthesised and then be consumed but this is inhibited in cold nights and other sugars like malate become more important (Barros et al., 2020a; Barros et al., 2020b). While the clock has less influence there is some evidence of *HVELF3* having a role has Hexoses, malate, and

sucrose mobilization and starch accumulation was observed to be slightly affected in *Hvelf3* mainly due to slightly impaired cold compensated starch degradation. Additional to temperature light intensity influences Barley carbon metabolism (Barros et al., 2020b).

Temperature and light quality are known direct influencers on plant performance and growth. As part of a plants performance water regulation is critical for survival and temperature directly affects water molecule movement while light intensity influences photosynthesis which in turn interacts with water regulation as water movement through the plant is regulated by stomata activity, which also affects carbon dioxide entering the plant and both carbon dioxide and water are used in the photosynthesis reaction and creation of sugars. The relationships between water use, photosynthesis and growth can be examined by calculating plants water-use efficiency, (for a measured amount of water taken in and/or lost by the plant relative to the amount of carbon assimilated and biomass accumulated). The rates of development and growth of barley circadian mutants vary, as such it may be expected that water-use also varies between plants. Water is highly interconnected to growth performance and likely as affected by the same drivers but the influence of the circadian clock on water regulation in barley is still relatively unclear.

2.1.3 Areas of focus

2.1.3.1 Root and Leaves

Roots and leaves are important entrance and exit points of water in plants, their development and morphology can influence transpiration, photosynthesis and respiration rates. There is evidence to support Ppd-H1 controlling variation in width and length of barley leaves, linking a clock and photoperiod gene to morphological traits (Digel et al., 2016) and circadian regulation of auxin signalling in Arabidopsis has been reported to affect lateral root development (Vosz et al., 2015), suggesting that the altered circadian rhythms and gene expression reported in early maturing lines may affect auxin to influence physiology. The importance of roots and shoots in the control of water regulation and the possible influence of circadian regulation via hormone signalling in development justifies examining their morphological traits to compare difference across lines to determine the potential influence of clock genes on root and leaf development and activity. In this chapter, leaf and root growth traits were examined, including leaf mass per area, chlorophyll content and stomata traits and compared across barley lines to provide more general information on typical physiology of these lines that could be considered when evaluating their water regulation.

2.1.3.2 Development stages

As described in chapter 1 section 1.4 barley transitions through a number of defined development stages with distinct morphology and physiology. Different development stages can be more water sensitive that others, for barley soil moisture is important for seed germination while water availability and air humidity are important during flowering and seed development, as pollen and water transport are affected. Difference in yield, particularly seed weight, under equivalent conditions may also be indicative of differences in water regulation as the milk and dough filling stages are very water sensitive when transport of water, minerals and starch up to the top of the plant is vital to seed quality. The flag leaf and second to last leaf developed by barley are considered the most important leaves at this stage as transpiration from them drive much of this important transport.

2.1.3.3 Tiller numbers

Tiller number can be highly influenced by environmental conditions, reducing in unfavourable growing conditions as plants conserve resources while increasing in favourable conditions to capitalise on opportunity. More tillers generally result in more roots and leaves, sometimes maturing to flowering and seed production. Tillers can be a way of sectioning and safe guarding resources or expanding the canopy. The tillering stage of development can be an important time for a plant to accumulate resources and adapt to the local conditions e.g. growing more tiller away from shade or neighbouring plants, to be in a strong position in terms of accumulated resources and options before elongation and flowering. Typically in later stages of development, not all tillers develop flowers and seeds, but tillers provide more options for where the plant may invest. This can be advantageous if some tillers have acquired an infection or are on the side of the plant that received less light, which may lead to the plant pulling resources from these tillers and reallocating to more beneficial areas. Studies have shown that plants carrying reduced photoperiod sensitivity (ppd-H1) grow significantly more total tillers per plant at all developmental stages compared to photoperiod sensitive plants (Ppd-H1), but also more variation in tiller number and productivity between plants with ppd-H1. The productivity potentially being linked to the pre-anthesis phase (before the flowers are fully open and functional) as tillers developed post-anthesis are less likely to make seed. The increased number could also be linked to ppd-H1 plants delaying development and spending longer in the pre-anthesis phase (Alqudah et al., 2016). As Bowman lines are two- rowed and carry the *ppd-H1* variation in tiller number can be expected in these plants, but importantly comparing them to the early maturing lines with a faster development was a point of interest to see if tiller numbers would be reduced.

2.1.3.4 Height

Height can also affect a plant's relationship with water as the greater the height the more structure and force is needed to pull water to the highest parts of the canopy. Additionally height affects a plants interaction with its environment being more exposed to air temperatures, wind and different humidity that influence transpiration rates. Height can also give plants advantages over neighbouring plants by raising their canopy above surrounding plants, but at the risk of lodging (the permanent displacement of crop stems from their vertical position usually near ground level that makes harvest difficult and reduces yields) and potentially wasted resources. Height is a strong phenotype under genetic selection with genes specifically influencing height, such as *SEMI-DWARF 1 (SDW1)* that can have dramatic adaptation consequences (Wang et al., 2010). There is a link between the genetic control of row-type, heading time, tillering, and plant height via associated QTLs (Alqudah et al., 2016). Relevant to Bowman, plant accessions carrying *ppd-H1* were found to be generally shorter than accessions carry *Ppd-H1*. Association with the photoperiod sensitive and plant height is likely linked to heading time as other flower developing genes *HvCMF10* (a CCT motif family protein) and *HvCONSTANS8* (*HvCO8*), and circadian genes *CIRCADIAN CLOCK ASSOCIATED1* (*HvCCA1*) and *HvGIGANTIA* (*HvGI*) were closely associated with plant height.

Observations of height variation when bulking up seeds in the greenhouses (figure 2.1.3.4a) inspired taking more measurements of height to confirm this as a consistent observation that barley circadian mutants are shorter than the parental lines plants which could be advantageous against lodging and influence water use.

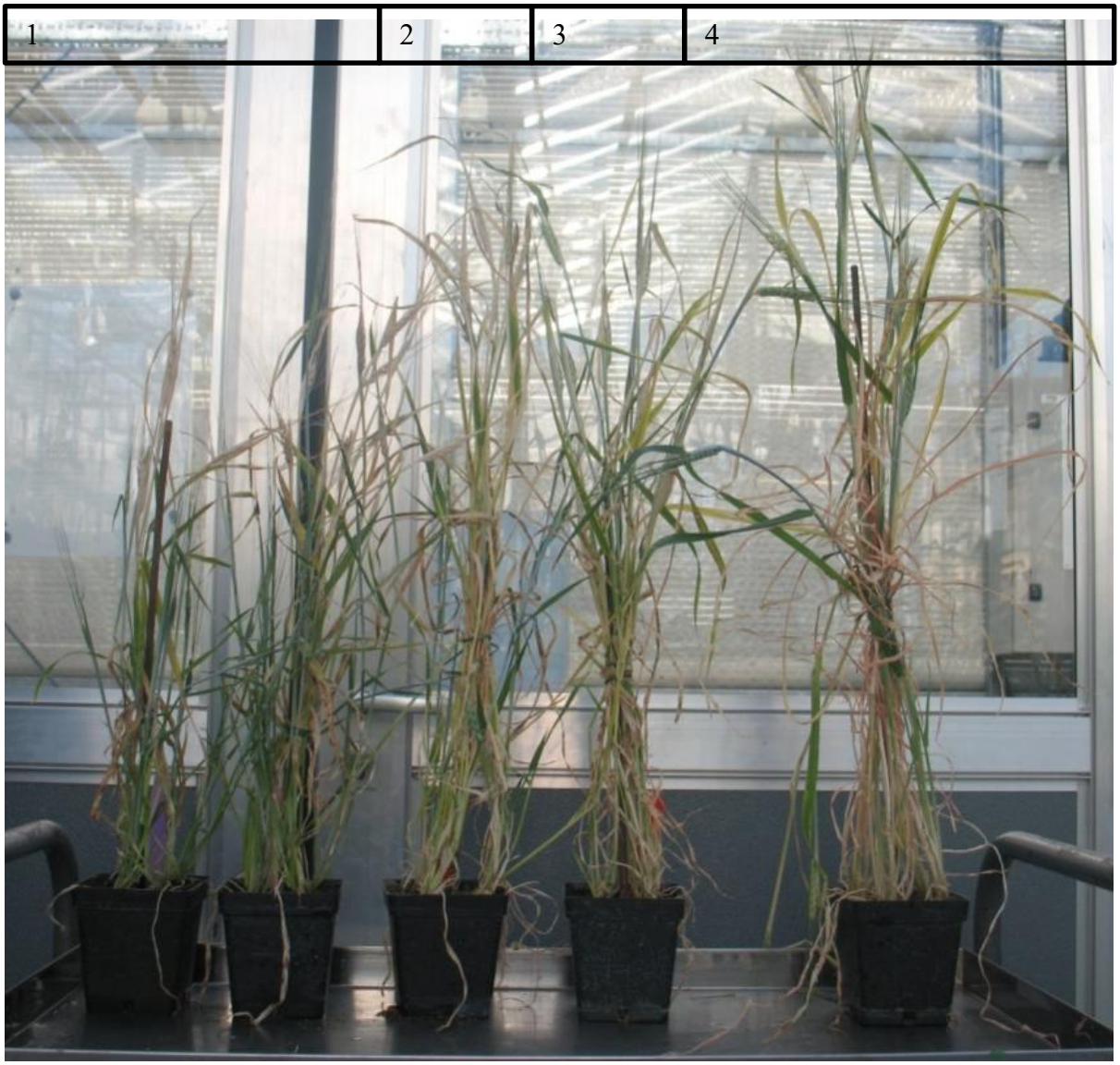


Figure 2.1.3.4a: Height variation in Bowman lines post heading stage (seed development). A Photograph of Bowman lines grown in a temperature regulated (20°C) green house taken when bulking up seed for experiments. Plants shown have 2 to 3 plants per pot, grown with the same ambient watering conditions (generally twice daily, watering from the bottom in trays), same soil (Levington F2) and pot size (15cm2), numbers indicate Bowman line from left to right: 1) eam8 lines, eam8.k and ea8.w (Hvelf3), 2) eam5.x (HvPHYC), 3) eam10.m (Hvlux) and 4) Bowman.

2.1.3.5 Stomata

Circadian regulation in stomata is well documented in Arabidopsis with a number of studies finding circadian rhythms in stomata physiology to be rhythmic (Hubbard and Webb, 2015) . The circadian regulated abscisic acid (ABA) signaling pathway responds to shortages in water by inducing stomata closure to conserve water (Legnaioli et al., 2009). Additional to stomata closure and rate of opening being crucial in active water regulation (Haworth et al., 2018; Hepworth et al., 2018; Hetherington and Woodward, 2003), natural variation in stomata density may passively influence a plants water-

useas well as a active response to temperature sensitivity (Hill et al., 2014). When comparing barley lines a consistent difference in stomata density between them would suggest a link between the circadian clock and stomata development which would impact the plants water regulation capabilities.

2.1.3.6 Chlorophyll

Photosynthesis uses water. Rate of photosynthesis impacts water use and subsequent biomass accumulation. Chlorophyll pigment in the chloroplast thylakoids is vital to converting light energy into chemical energy and the more a plant has theoretically the more photosynthesis the plant is capable of doing (and the more efficient the plant is as using the water in its system). Variation in chlorophyll would potentially indicate a circadian role in chlorophyll development that would impact water-use.

2.1.3.7 Hydroponics

Hydroponics can be a useful system to homogenise growing conditions in a way that is difficult to control with soil. Soil is a complex environment that naturally has. Finally, water demand can be determined by comparing how much water plants take up through the roots when plants are grown hydroponically.

2.1.2 Aims and Objectives

The aim of the work in this chapter was to compare water-uptake in plants with different circadian phenotypes. To accomplish this aim, a number of preliminary investigations were conducted to test methodology to build a robust system to compare plants. This chapter will include the results and observations of some of this cursory research and methods. .

2.2 General methods, recipes and lists

2.2.0 Barley line materials

Bowman barley lines and Antonella seeds were kindly provided from stocks at the Max Planck Institute for Plant Breeding in Koln from Dr Maria von Korff's lab. Antonella seed was bulked up in the walled gardens on York University grounds in 2014. Bowman seeds and Bowman mutant lines were periodically bulked in York greenhouses over 2017-2018 in all seasons. Bowman seeds were bulked up in varying soil substrate, from John Innes number 2 (Clover Peat, Co. Tyrone, N. Ireland) to Levington F2 pluc sand

(ICL, Ipswich,UK) and finally a Levington F2 and sand, terra green (clay), and industrial sand in a ratio of 3:2:1 which work best with the twice daily watering regime. Variation in growing conditions (substrate and time of year) could have lead to variation in seed quality with consequences in growing performance. To mitigate the potential interference of seed quality variation impacting experiment repeats, different batches of bulked up seeds (of the same type) were mixed together which could increase variation within each genotype with each experiment but would prefer seed quality variation influencing results across repeated experiments.

Bowman and the described Bowman circadian mutants could be used to investigate circadian regulation in water-uptake and growth performance traits. Antonella was included because as part of the initial study, 200 µM N -ethyl-N-nitrosourea (ENU) treated Antonella seeds were also provided by Dr Maria von Korff's lab to perform a genetic screen looking for early maturing winter cultivars, which could then be studied in parallel with the spring type. Two attempts were made to screen the seeds; the first attempt was in 2015 planting seeds in the ground in the walled gardens. However, flooding and a very wet winter resulted in a loss of a number of plots the plants that survived into spring were severely affected by disease that rotted the heads. The second screen done in green houses in the walled garden (which have lower quality temperature regulation than Green houses connected to the Biology department allowing seedlings to experience vernalisation). Planting seeds in pots also did not produce any plants displaying a strong early maturing phenotype. Despite this Antonella has been used in the physiology experiments in the meantime to collect data on a winter cultivar's performance and was analyzed alongside the Bowman lines to see if the circadian mutants' performance was more or less like a winter cultivar with a Pph-H1 allele comparatively more similar genetically to a wild barley, although both are elite cultivars.

2.2.1 Method of cleaning seeds for germination

Infection and mould reduced germination of seeds and influence the performance of plants. To reduce the effects and keep treatment standardised all seeds were cleaned before planting unless otherwise stated.

Prior to storage, seeds collected from the greenhouses were removed from the spikes and sprayed with 70% ethanol in a sieve until fully covered then allowed to dry. Seeds were stored in macro-centrifuge tubes with loose lids to allow any moist air to escape the tube and prevent potential mould developing. This method was later changed in 2017 where seeds were still sieved to remove spikes but without spraying in ethanol and stored in paper or glassine bags. Although barley seeds were hardier against the effects of ethanol, there was still a possibility that any ethaol entering cracks in the husk could harm the seeds.

Prior to 2017 before use, seeds were again sprayed or soaked for a few seconds in 70% ethanol, padded down with paper towel, allowed to air dry then rinsed in distilled water (dH_2O) three times then soaked in dH_2O for 2-3 hours, dried and then soak in 33% bleach and 200µL/L triton for 20-30 minutes, vortexed for a few seconds, rinse at least three times in distilled water (dH_2O), then put on petri plates with wet tissue paper and put in 4°C for 1 week.

Using ethanol negatively affected germination, so the cleaning protocol was changed. Dry seeds were rehydrated for at least 2-3 hours, although usually longer, in distilled water, distilled water was drained and then soak in 33% bleach and 200µL/L triton for 1 hour, vortexed for a few seconds, bleach was drained of and replaced with fresh bleach and vortexed for 10 seconds, this was repeated twice more for 30 minutes, before finally draining the bleach and covering seed in dH_2O (up to 50ml) for another hour, then put on petri plates. Depending on the sterility requirement seeds were either, drained then transferred to geminate on 1.2% phytoagar (DuchefaBiochemie, Germany, CAS number 9002-18-0) in sterile conditions or, for nonsterile conditions seeds were placed onto Whatman paper or paper towel with the water (to moisten the paper). Traces of bleach were left in the solution and this did not cause germination problems. Seeds were put in 4°C for at least 5 days.

This process was gradually simplified to be more efficient by putting seeds into a centrifuge tube, covering them with 70% ethanol for a few seconds, pouring the ethanol away, covering seed with 33% bleach and 200µL/L triton for 20-30 minutes, pouring away the bleach and triton solution, then covering in dH_2O for 2-3 hours and then transferring the water and the seeds on a paper towel in a square petri-dish and storing in the dark in 4°C

(Germination improved if seeds were left to root in the cold, possibly because pathogens remaining after sterlisation caused mortality when seedlings moved into the warmth).

2.2.2 Hydroponic solutions

The ‘Hoaglands’ solution referred to in the Methods for measuring physiology traits refers to a modified recipe based on the Hoagland and Aaron nutrient solution (Hoagland and Arnon, 1950) with concentrations slightly changed. Nutrient salts were first dissolved in Millipore water to make up 500mL to 2L stock solutions stored in glass bottles and kept on the bench top out of direct sunlight. 10L solution were made by diluting measured volumes of the stock solutions indistilled water from the labratory tap into 10L plastic containers.

A low nutrient solution was used in preliminary work ((1mM KNO_3, 1mM $K_2HPO_4 \cdot 3H2O$, 1mM $Ca(NO3)2 \cdot 4H2O$, 1mM $MgSO_4.7H_2O$, 0.46mM H3BO3, 0.05M $MnCl2 \cdot 4H2O$, 0.2mM $ZnSO_4.7H_2O$, 0.1mM Na_2MoO_4, 0.2mM $CuSO_4$, 0.45mM $C_{10}H_{12}FeN_2NaO_8$). This was made from diluting 1M stock solutions (the same stock solutions described in table 2.2.2) as 10mL to 10L.

High nutrient 'Hoagland' solution

A higher nutrient solution was used that made a modified half strength 'Hoaglands' solution (0.65M KNO_3, 0.2M $K_2HPO_4 \cdot 3H_2O$, 0.4M $Ca(NO3)2 \cdot 4H2O$, 0.2M $MgSO_4.7H_2O$, 0.46mM H3BO3, 0.05M $MnCl2 \cdot 4H2O$, 0.2mM $ZnSO_4.7H_2O$, 0.1mM Na_2MoO_4, 0.2mM $CuSO_4$, 0.45m $MC_{10}H_{12}FeN_2NaO_8$, (Table 2.2.2 and described in (Habte et al., 2014a)), by making 1M stocks of macronutrients to dilute as different volumes (ml) into 10L and a mixed micronutrient solutions to dilute as 10ml into 10L.

Initially when lower nutrient solutions were used young plants were grown with their seed still attached up to three weeks of age of the experiment period. To reduce pathogen infections from the seeds, the seeds were removed from young plants when transferred to hydroponics. Additional potential benefits to removing seeds include reducing nutrient variation within the experiement that seedlings could still be acquiring from the seed.

Table 2.2.2: solutes and concentrations to make half strength 'Hoaglands' solution.

Macronutrients	Formula	Molar Weight	Stock concentration M/L	Volume (ml) diluted in 10L
Potassium nitrate	KNO_3	101.10	1	65
Potassium phosphate dibasic trihydrate	$K_2HPO_4 \cdot 3H_2O$	228.22	1	20
Calcium Nitrate Tetrahydrate	$Ca(NO_3)_2 \cdot 4H_2O$	236.149	1	40
Magnesium SulfateHeptahydrate	$MgSO_4.7H_2O$	246.48	1	20

Micronutrients*			Stock concentration mM/L	
Boric acid	H_3BO_3	61.83	4.6	
Manganese(II) Chloride Tetrahydrate	$MnCl_2 \cdot 4H_2O$	143.86	0.5	
Zinc SulfateHeptahydrate	ZnSO4.7H2O	287.55	0.2	10
Sodium molybdatedihydrate	$Na_2MoO_4 \cdot 2H_2O$	241.95	0.1	
Copper(II) sulfate pentahydrate	$CuSO_4.5H_2O$	249.68	0.2	
* Combined in solution to make up one stock of micronutrients				
EDTA ferric sodium salt	$C_{10}H_{12}FeN_2NaO_8$	367.05	45	10

2.2.3 Simulating osmotic stress

Osmotic stress conditions were created by using polyethylene glycol (PEG) of approximately 8000 molecular weight dissolved in the 'Hoagland's solution. The percentages of PEG were usually dissolved to make up a volume of 5L or 10L, pH adjustment to pH 7.2-7.5 using KOH or HCL tended to cause precipitation and thus was discontinued, with higher nutrient solutions having a range of pH 5.1-6.2.

Early work including calculating the Osmolarity and early experiments testing different PEG percentages (0%, 5%, 10%, 15% PEG solutions) described in chapter 2 used low nutrient 'Hoagland' solution. Experiments described in chapter 3 used in high nutrient 'Hoagland' solution. Plants grown hydroponically in section 2.3.5 and in trial with 20%, to 50% PEG concentrations also usehigh strength nutrient solution.

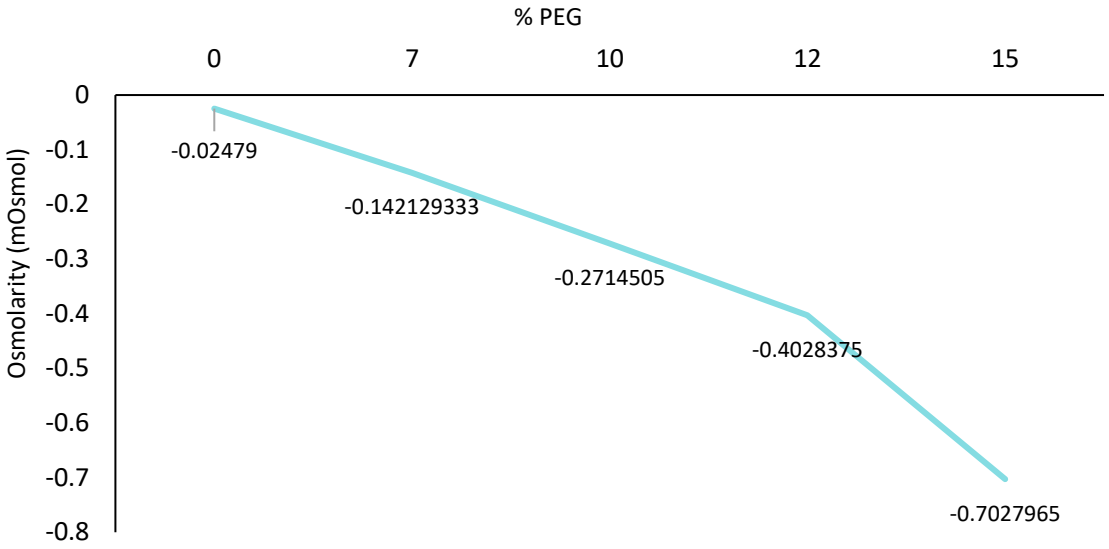


Figure 2.2.3.1a: Osmolarity correlated to % PEG using PEG 8000 in low strength Hoagland solutions, osmolarity measured using an Osmometer.

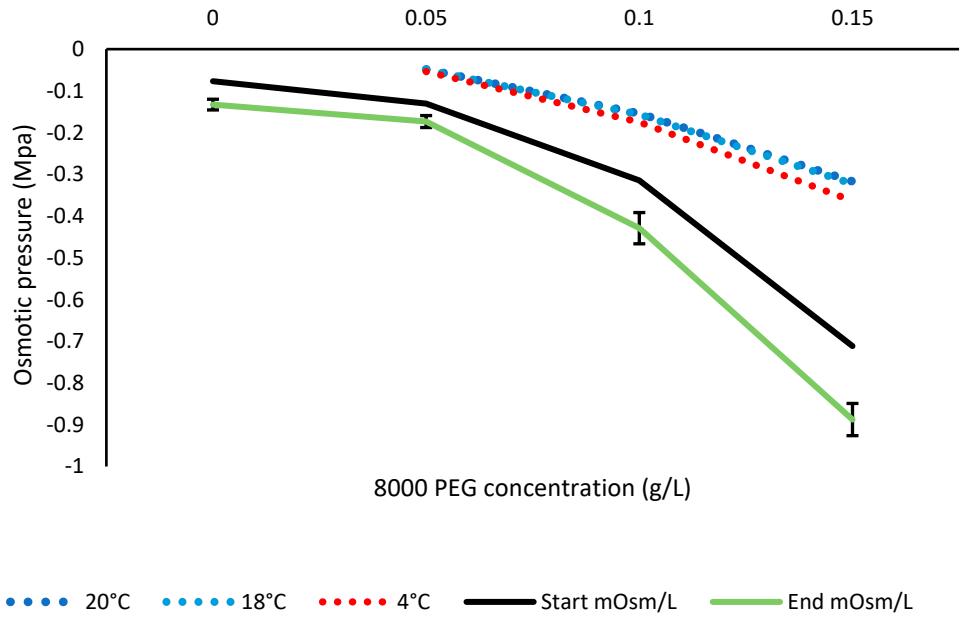


Figure 2.2.3.1b: Calculated osmotic pressure in MPa for given concentrations of PEG at the experimental temperatures in this study (dashed lines) (osmotic pressure= 1.29xC2xT − 140xC2 − 4.0xC, C= concentration, T=Temperature) as calculated from Michel (1983), alongside preliminary measurements of low concentration Hoagland solutions with PEG (solid lines) calculated from measured milliosmole converted to Mpa (1 Osmol Kg-1 = 2.279 Mpa at 25°C, () before and after plants were grown in the solution for 7 days, the error bars on the "after" line are the standard error for each PEG treatment across all plant lines.

A disadvantage with using PEG to simulate osmotic stress is that prolonged exposure even with high molecular weights of PEG, such as 8000, can eventually be taken up by plants and result in blockage in the vascular tissue (Jacomini et al., 1988; Janes, 1974;

Yaniv and Werker, 1983). However, the higher the molecular weight of the PEG the less likely this is to occur providing plants are not left in the PEG for longer than a week as PEG can eventually breakdown and smaller particles may begin to be absorbed by the roots or PEG may accumulate on the outer surface of the roots also causing blockage. Osmolarity and pH was tested before and after plants were grown for one week in the same PEG solution and results showed only minor changes after one week suggesting there had not been notable changes in solutions containing PEG over the experiment time period.

Currently, drought stress models rely on presumably non-permeable high-molecular-weight osmolyte polyethylene glycol (PEG) with an average molecular weight of 6000 Da or more (Hohl and Schopfer, 1991; Zhong et al., 2018). It is well documented that PEG effectively decreases medium potential (Ψw), thereby disrupting absorption of water by plant roots (Chutia and Borah, 2012). In terms of this approach, 5 – 20% (w/v) or even 40% (w/v) PEG in growth medium enables a stabile decrease of Ψw during any desired period of time (Bressan et al., 1981). Importantly, PEG-based aqueous models allow the setup of recovery experiments by transfer of stressed plants to PEG-free nutrient solution or exchange of the PEG solution (Verslues et al., 2006). Therefore, PEG-based models of drought stress represent the method of choice in molecular biology and plant protectant studies and screening experiments (Rao and Jabeen, 2013).

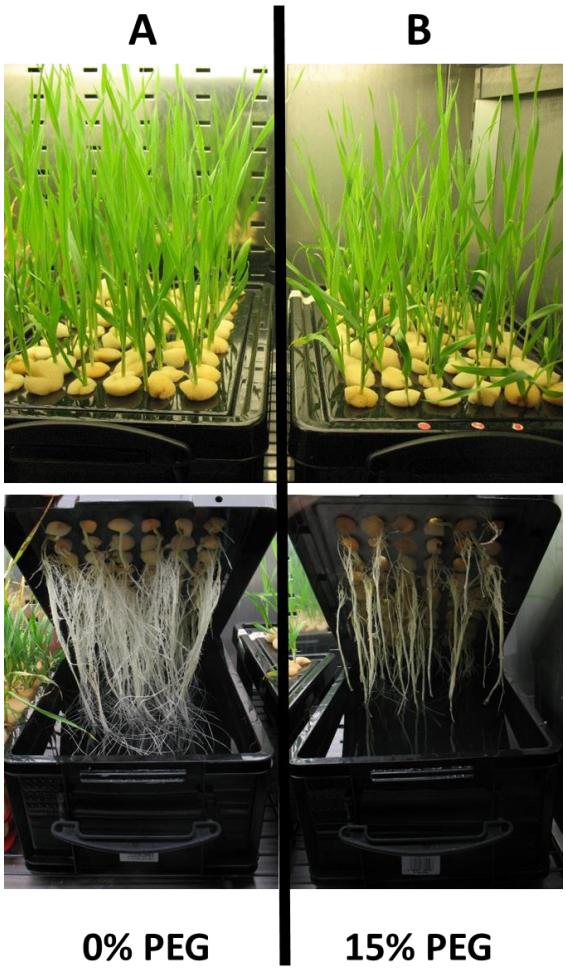


Figure 2.2.3.2 visible effects of 15% PEG (B) on barley roots in 4°C dark: 20°C light conditions after 8 days of treatment. PEG collected around the roots making them darker and causing roots to stick together when removed from solution. Roots grown in PEG noticeably shorter.

2.2.4 Growth Cabinet and temperature conditions

Plants were grown in control growth cabinets (Weiss Fitotron® SGC 120) in 12 hours 300 µmolm^{-2} s^{-1} light and dark cycles, with 60%-70% relative humidity in 20°C – light and 18 °C – dark diurnal cycles (warm night) or 20°C – light and 4 °C – dark diurnal cycles (cold night).

2.2.5 Method of growing in 50ml centrifuge tubes

Once a majority of seedlings growing on petri plates had shoots of approximately 3cm or more (with root adding to the total length, Figure 2.2.4.1 step 4) they were transferred to 50ml centrifuge tubes filled with 'Hoaglands' solution, ensuring the roots were immersed in solution with the shoot secured at the top with a sponge. The first 7 days post transfer the plants were left undisturbed to grow. After this 7 days nutrient solution was replaced more frequently, changing every 4 to 5 days. Typically were grown for approximately 2 to 3 weeks post germination before being used in experiments. Figure 2.2.4.1 shows this process with images from step numbers 3 to 5, with photographs showing the system of growing plants in centrifuge tubes supported in racks.

When applying treatment, the nutrient solution was replaced and replenished with the treatment solution in the same tube. This system was suitable for young plants (grown up to 3 or 5 weeks). Limited space limits root growth. Another disadvantage is the impracticality of oxygenating the system continuously (which was as difficult to accomplish in the growth cabinets) so there was some amount of hypoxia, under higher percentage PEG conditions, that should be factored into results (Verslues et al., 1998). Additionally plants grown in centrifuge tubes did not grow as large (in the same amount of time) as plants grown in 10L hydroponics. This may be due to effects of pot size on plant growth and physiology (Huang et al., 1996; Poorter et al., 2012; Ray and Sinclair, 1998). However, the advantage of the hydroponic system in centrifuge tube is keeping roots separate. This system is practical for smaller plantslike Arabidopsis (Simon et al., 2019).

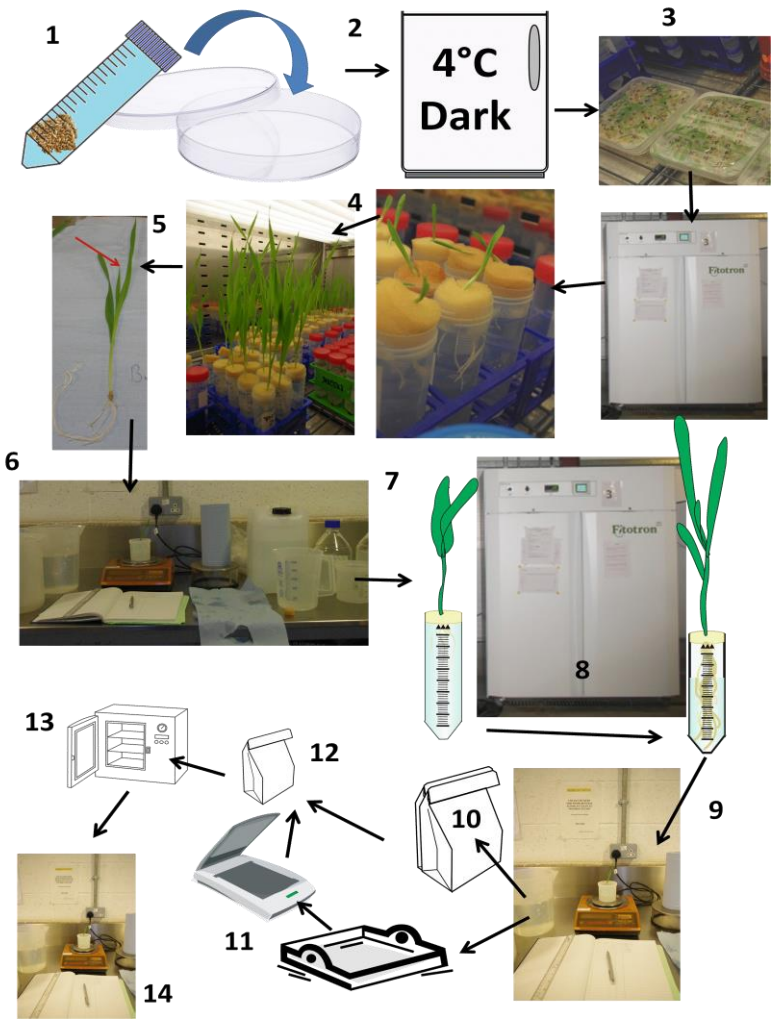


Figure 2.2.5.1: A diagram illustrating the steps followed to grow barley plants in centrifuge tubes and collecting water-uptake and growth performance data**.** Numbers denote the step described 1) Cleaning: Chemically clean seed. 2) Stratifying: Place seeds 4°C fridge in the dark for 5-7 days. 3) Germination: Transfer seeds in petri dishes into growth chambers configured and stabilised to the light and temperature conditions the plants will grow keeping them constant from germination to the end of treatment. 4) Growing pre-treatment: seedlings ~5cm total length (root and shoot), remove seeds husks and transfer to 50 ml centrifuge tubes containing nutrient solution, securing them with a sponge. Change water after 7 days then after 4 days. 5) At ~2-3 weeks since germination 2 leaves have emerged and a third is starting to emerge (red arrow) prepare plants for PEG treatment. 6) Remove plant from tube, dry root, weigh (total fresh mass). 7) Fill tube up with treatment solution to 50 mL, transfer plant back to tube securing with sponge. 8) Plants returned to theigrowing cabinets for a 7 day treatment. [Plants can be monitored daily for water uptake or leaf measurements, disturbing the plants in

confided conditions risks damaging the plants and vibrations can affect the plants]. 9) After treatment, plants remaining water volume in the tube is recorded, fresh weights (total and root and shoot separately) was recorded. 10) Shoot material was transferred to individual paper bags, 11) Root material was returned to solution to be photo scanned within 24 hours for root measurements taken using Win RHIZO®, 12) then transferred to the same paper bag as the shoot samples. 13) dried at 70°C+ degrees for at least 3 days 14) dry weight measurements for calculations and analysis. Photographs in diagragm are of images taken of the plants, equipment and method as the project was running. Clip art images representing steps were found from Google® under free use and edited.

2.2.6 Method of measuring and calculating water-uptake

The Volume (ml) of liquid in the 50 ml centrifuge tube was recorded daily based on the manufactures scale printed on the side of the tube starting from 50 ml at the beginning of treatment. Sponges would allow water to evaporate so three controls without plants were included for each treatment solution to estimate the average daily evaporation volume.

When measurements were taken daily, the average daily change in liqid volume (volume of previous day - volume of day) by evaporation from the controls was calculated for each treatment and then added onto the recorded value for each measurement.

Water uptake over time by the plant was calculated as the difference in liquid volume from the start of treatment (50 ml) and the volume recorded each day with the evaporation volume added on.

2.2.7 Methods for collecting growth performance data

Preparing plants for treatment, they were removed from the nutrient solution as described in section 2.2.4 and dried with paper towel to removed external moisture and weighed just before they were put into treatment solutions. Post-treatment provided initial and final fresh mass (g), respectively. Difference in total fresh weight was calculated by subtracting the initial fresh weight by the final fresh weight of each plant.

Post-treatment plants were cut in half to collect shoot and root fresh biomass (g) seperately.

Dry weight was measured after root and shoot tissue was dried at 70°C for >72 hours as illustrated in figure 2.2.4.1 in steps 9 to 13.

Water content (g) was calculated by subtracting the dry weight values from the final fresh weight values for each tissue and total water content was calculated by adding the root and shoot dry weights and subtracting them from the final total fresh weight.

The root:shoot ratios for dry biomass and water content were calculated by dividing the values for roots by the values for shoots. A value over 1 indicates greater resource allocation in the roots, whereas less than 1 indicates more in the shoots.

Before drying roots, roots were transported in dH_2O water to be imaged by 3D photo-scanner (Epson, perfection v850, Pro) as illustrated in figure 2.2.4.1 step 11. Roots were loaded onto the scanner by placing a transparent, plastic, shallow tray with flate sides into the scanner, filling the tray with distilled water dH_2O and roots suspended in the water allowing the roots to be spread with toothpicks so that the image scanned could distinguish more of the fine roots and reduce overlap.

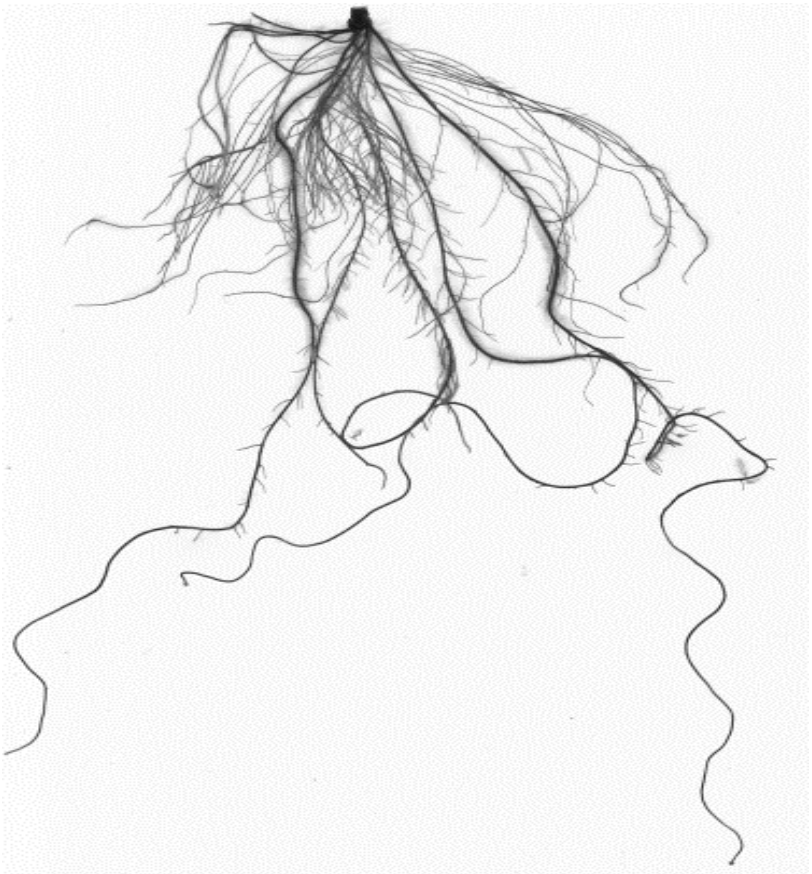


Figure 2.2.7.1 A scanned image of a barley root grown in nutrient solution with 0% PEG, images were scanned in grey scale and 800 pixel quality WinRHIZO softeware traces the dark pigments in the image and measures the length and thickness of the branches as well as counting the number of branches and fork (were roots branch out). This means it is important to scan the roots in clear water as debris will be detected and counted as part of the root system. The images were processed in the WinRhizo® software to collect data on global root morphology traits, total root length (cm): all the root length measurements added together, total surface area (cm^2): calculated from the length and diameter measurements, average

diameter (mm): from the thickness of all the roots, total root volume (cm^3): calculated based on the previous measurements, and number of tips (number of roots).

2.2.8 Protocols to extract RNA from barley

Gene expression of circadian clock genes in barley and water stress genes was to be collected and analysed to examine the circadian rhythms in clock and stress tolerance genes in the barley lines, unfortunately this was unable to be completed due to difficultly in making standards for the qPCR. A reason for focusing on optimizing the hydroponic system despite it being small in space for a large growing plant such as barley was because hydroponics in control growth chambers provides a much cleaner and controlled environment to extract plant tissue, including root tissue. Following work showing osmotic stress in roots causes changes in circadian genes in the shoot (Habte et al., 2014a), this inspired using similar methods to collect root and leaf material for qPCR analysis and the physiological study expanded in chapter 3 was meant to compliment the molecular data collected.

2.2.8.1 Sampling for gene expression

Plants were grown in controlled growth chambers (different models were used over the course of the project) and all external sources of light blocked out (e.g. using black plastic sheeting over the chamber) to prevent ambient light from the room entering when sampling during dark hours and using a green safe-light as a flash-light torch or desk lamp as a light source. Plants were germinated and grown hydroponically as described in previous sections. For tissue extractions growing plants in 10L boxes was more efficient and reduced accidentally damaging other plants when sampling.

Over the course of the project, prior to sampling plants were grown in controlled growth chambers with long day 12:12 light:dark cycles and 20°C: 18°C or 20°C: 4°C temperature cycles simulating warm or cold nights respectively for 2 week post-germination. Timing of sampling was consistent. First the osmotic treatments using 8000 PEG in the nutrient solutions was applied 48 hours before the first sampling would begin. Plants acclimated to the treatment for 48 hours in diurnal conditions before sampling started at zeitgeber time 0 (ZT0) usually set to be at 8AM and continued every 2 hours for 24 hours until the following ZT0. The first 24 hour sampling occurred in diurnal conditions with the aim of collecting data that would show gene-expression when environmental inputs (light and temperature) could entrain the clock. Sampling was destructive, taking a ~2 cm^2 base segment of the third leave of a plant and most of the root tip material (anticipating less RNA could be extracted from the roots). Following the first sampling day at ZT0, growth chambers were switched to constant light and warm conditions removing environment signals to indicate time

passing. Plants had another 48 hours to acclimate to these conditions while still in the treatment solutions. When the second sampling day occurred following the same schedule as the first day, plants would have been in the treatment solution for 6 days by the time sampling finished. The second day sampling happening in constant light and temperature conditions to capture changes in circadian rhythms compared to the first day and between plants and treatments.

Samples were collected by taking a plant from the tube or box it was growing in, quickly cutting the leaf tissue (from the base of the third leaf) and immediately placing in a prepared tube filled with liquid nitrogen to avoid cross contamination. Following tissue sampling the plant was removed from the solution, the roots dried with paper towel and cutting root branches ~2-3cm from the root tip to collect the most active tissue and placing this material in a separate liquid nitrogen filled tube, lids were loose attached to allow gas to escape safetly. Utensils (tweezers and scissors) were cleaned in between each sample, Tubes rested in a rack sitting in ice or liquid nitrogen to keep samples as cold and prevent RNA degradation before being transferred to a -18°C freezer and lids were pushed on more firmly when all the liquid nitrogen had evaporated. Samples were stored here until RNA was extracted.

The aim was to sample a similar amount of material from each plant to avoid over-representation individuals by over sampling. This was difficult under time constraints and in the dark, and variation would likely need to be accounted for when generating cDNA.

2.2.8.2 RNA extractions

Two protocols were used over the course of the project. Below are the protocols in their final form as used to successfully produce high yield of RNA that could be used for making cDNA. Before doing any RNA extractions the working area was sterilized.

2.2.8.2.1 Modified QIAGEN RNeasy Plant mini Kit protocol for barley

This method uses the QIAGEN Plant mini Kit (cat. 74904) and samples are processed in individual tubes. This protocol was modified due to low yield of RNA following the protcol with the kit. After trying the Omega bio-tek E.Z.N.A.® Plant RNA kit which uses warm temperatures, the protocol with the QIAGEN kit was adapted to do the same. This method was mostly used on a open lab bench designated for RNA work, it was cleaned, sterilised and environmental RNA was treated with RNAseZap® on the work surfaces and pipettes.

Preparing sampled tissue to be used with the kit

Plant tissue needed to be ground to a fine powder with clean tools keeping samples frozen. The method below uses autoclaved plastic drill tips and a modified electric bench top drill to grind the plant tissue inside its tube.

Remove the samples from- 80°C straight into Liquid Nitrogen (L. Nitrogen). Individually, grind material using L. Nitrogen to keep samples frozen and a sterile plastic drill tip dipped in L. Nitrogen to mechanically break open the tissue, being careful to not let frozen material thaw, until extraction samples are kept frozen after grinding.

Kit extraction protocol

Prepare all the solution in the kit and equipment prior to starting extractions to ensure steps are carried out at the exact times. To each sample add warmed (29°C) 450µl of RLC buffer with 2M dithiothreitol (DTT)[+ 1µl RNAse* (SUPERase• In™ RNase Inhibitor, thermos fisher scientific, UK (cat. AM2696))]. Vortex vigoursly- put on vortexing platform (thermomixer) for 5 minutes. Meanwhile prepare DNAse I10:70µl by first cooling required amount of buffer on ice. Transfer the lysate (by tipping) to labelled QIshredder spin column (lilac) placed in a 2ml collection tube centrifuge for 5 min at full speed. Add DNase I to cold buffer, vortex and centrifuge with balance for 6s. Put back on ice. Add 400 µL 70% ethanol to microcentrifudge tubes. Carefully transfer 400µLof the supernatant flow-through to the ethanol filled microcentrifuge tubes without disturbing pellet in the collection tube. Vortex on mixer at max speed for 1 minute. Transfer the sample (usually 800µl), including any precipitate that may have formed, to an RNeasy spin column (pink) placed in a 2 ml collection tube (supplied). Close the lid gently, and centrifuge for 1 minuteat ≥8000 x g (≥10,000 rpm). Discard the flow-through.*Reuse the collection tube in step 7.If the sample volume exceeds 700 µl, centrifuge successive aliquots in the same RNeasy spin column. Discard the flow-through after each centrifugation.

On column DNA digest using QIAGEN RNase-Free DNase (cat.79254)

Prepare DNase I stock solution before using the RNase-Free DNase Set for the first time, dissolve the lyophilized DNase I (1500 Kunitz units) in 550 µl of the RNasefreewater provided. Procedure: Prepare and load samples onto the RNeasy spin column as indicated in the individual protocols. Add 350 µl Buffer RW1 to the RNeasy spin column. Close the lid gently, and centrifuge for 30 s at ≥8000 x g (≥8,000 rpm) to wash the spin column membrane. Discard the flow-through but resuse the collection tubes. Add 80µl DNase I stock solution directly to the column. Place on the mixer (29°C) for 20mins. No shaking.Prepare RNAase free water for elution step in new tube and warm to 65°C in oven or 29°C on shaker. Add 350 µl Buffer RW1 to the RNeasy spin column in mixer.Let sit for 5 minutesat 29 °C. Close the lid gently, and centrifuge for 1 minuteat ≥8000 x g (≥8,000 rpm). Change the collection tube.

Continue with the first Buffer RPE wash step.

Add 400 µl Buffer RPE to the RNeasy spin column. Close the lid gently, and centrifuge for 1 minute at ≥8000 x g (≥10,000 rpm) to wash the spin column membrane. Discard the flow-through. Reuse the collection tube. Add 400 µl Buffer RPE to the RNeasy spin column. Close the lid gently, and centrifuge for 1min at ≥8000 x g (≥10,000 rpm) to wash the spin columnmembrane. Centrifuge at full speed for 2 min. Place the RNeasy spin column in a new 1.5 ml collection tube (supplied). Add50µl RNase-free water directly to the spin column membrane. Sit on membrane for 4 minutes at room temperature. Close the lid gently, and centrifuge for 3 min max speed to elute the RNA. Centrifuge 50µl for 10 minutes at maximum speed, transfer (40)µl of the supernatant to a fresh tube. Check RNA quality by NanoDrop™ (Thermo scientific™) spectrophotometer or 1-1.5% agarose gel. Store purified RNA at -80°C

This method takes longer to do fewer samples, however the incorporation of the DNAse I step, cleaner RNA was prodiced that was more efficient for subsequent steps. RNA yield with this method was usually more than 100ng/uL with leaf tissue. Root tissue tended to be less successful using this method as the grinding step could struggle if there was any frozen PEG attached to the roots.

2.2.8.2.2 Up scaling extractions using a 96-rack and TRIzol™

The method described here is adapted to work in the Davis laboratory, but is following the reliable protocol used in the Max Planck Institute for Plant Breeding in Koln developed in Dr Maria von Korff group. The procotol makes use of Quiagen 96 well block (cat.19560) to do 96 extractions at once. The DNase I used in this method is the same as the RNeasy method. The DNase I step is optional as it is possible to have very clean extractions with this method. This method must be done in a working fume cupboard as it is a guanidinium thiocyanate-phenol-chloroform extraction. TRIzol™ is referenced to as Trizol in the protocol.

Samples must be collected in the Quiagen 96 well block. Before sampling in it works better to add 1 sterile steel ball to each microtube in the rack. Collect samples as normal, however for this method two biological replicates (of the same developed leaf) were added to the same tube instead of a single sample per tube as this was advised as getting better yield and a more even and representative sample if all yields are the sum of two different plants.

Make DEPC treated water, add DEPC 0.1% to water, put at 37°C overnight, autoclave.

75% ethanol has to be prepared with DEPC treated water to autoclave together.

Sampling:

1. Place one stainless steel bead in each collection microtube and, store racks in -80°C before sampling.
2. When sampling pour L. Nitrogen into microtubes and rack, collect you samples directly into the collection micro tubes
3. Loosle close tubes with the strip caps
4. Store plant material at -80°C until needed securing caps when L.Nitrogen has evaporated

Beating using Mill shaker:

5. Keep everything frozen before beating
6. Cool Mil (Retsch-Mill) adapter at -80°C at least 30 minutes before use or freeze in liquid nitrogen (make sure Mill is balanced)
7. (make sure caps are firmly on the microtubes) Grind samples 2x 15 seconds at maximum frequency (change orientation of rack in-between)
8. On ice, Take to fume cupboard, place hand on caps to flaw
9. Add 500µl of **Trizol** to the frozen samples and **mix vigorously** until all the material is melted (96 well shaker **or by hand**)
10. Leave samples for 5 minutes at room temperature
11. Briefly spin down samples (to clean caps)<-centrifuge to spin 96 racks
12. Add 100µl of **chloroform** and shake tubes vigorously by hand
13. Leave at room temperature for 2-3 minutes (**to shake**)
14. **Centrifuge** samples at maximum speed for 40 minutes at **2-8**°C (4000rpm plate centrifuge, Eppendorf 5810R)
    Transfer the colourless, aqueous, upper phase in fresh collection tubes by using 8 channel pipette (~250µl)
15. Add 250µl of **isopropyl alcohol**, mix (gentle invert tubes) and incubate for 10 minutes at room temperature.
    Optional overnight -20C incubate in isopropyl alcohol
16. Centrifuge the samples at maximum speed (not more than 12000g) for 40 minutes at 2-8°C
17. Remove supernatant by inverting the tubes
18. Wash the pellet with 1ml of the **75% ethanol (gently invert by hand x3 to wash the sides)**and centrifuge for 5 minutes at 2-8°C
19. Remove the supernatant and invert tubes on paper
20. Wash the pellet with 1ml of the 75% ethanol (gently invert by hand x3 to wash the sides) and centrifuge for 5 minutes at 2-8°C
21. Remove the supernatant and invert tubes on paper
22. Briefly spin down the samples and remove the last drop with a pipette
23. Dry RNA pellet (8-10 minutes under the flow cabinet) -pellet shouldn't dry out otherwise it won't re-suspend.**(Make sure all the ethanol has evaporated)**
24. Resuspend the RNA in 86µl of **RNAse free water** by pipetting up and down (add a 10 minute 56°C incubation step.)

DNAase Step– 24 samples at a time (3x8). (Repeat this step 4 times per rack)

25. Taking 3 tubes of 8, put the rest on ice in 4°C
26. Transfer RNA into RNeasy spin columns and spin for 1 minute at ≥8000 x *g* (≥10,000 rpm).
27. Discard flow-through
28. On column DNA digest
    - **a.** Prepare DNase I stock solution before using the RNase-Free DNase Set for the first time:
        - **i.** Dissolve the lyophilized DNase I (1500 Kunitz units) in 550 µl of the RNase free water provided.
    - b. Procedure
        - **i.** Add 350 µl Buffer RW1 to the RNeasy spin column. Close the lid gently, and centrifuge for 30 s at ≥8000 x *g* (≥**8**,000 rpm) to wash the spin column membrane. Discard the flow-through.
        - **ii.** Add **80**µl DNase I stock solution directly to the column.
        - iii. Place on the mixer (29°C) for 20mins. No shaking.
        - iv. Prepare RNAase free water for elution 56°C in oven.
        - **v.** Add 350 µl Buffer RW1 to the RNeasy spin column in mixer. Let sit for 5 minutes at 29 °C. Close the lid gently, and centrifuge for 1 minute at ≥8000 x *g* (≥8,000 rpm). Change the collection tube.
29. Add **400**µl Buffer RPE to the RNeasy spin column. Close the lid gently, and centrifuge for 1 minute at ≥8000 x *g* (≥10,000 rpm) to wash the spin column membrane. Discard the flow-through. Reuse the collection tube.
30. Add **400**µl Buffer RPE to the RNeasy spin column. Close the lid gently, and centrifuge for **1min** at ≥8000 x *g* (≥10,000 rpm) to wash the spin column membrane.
31. Centrifuge at full speed for 2 min.
32. Place the RNeasy spin column in a new 1.5 ml collection tube (supplied). Add 86µl RNase-free water directly to the spin column membrane. Sit on membrane for **5** minutes at room temperature.
33. Close the lid gently, and centrifuge for **3** min max speed to elute the RNA.
34. Centrifuge **86**µl for 10 minutes at maximum speed, transfer **supernatant to new microtube rack for storage.**

Check RNA quality by NanoDrop™ (Thermo scientific™) spectrophotometer, and/or 1-1.5% Agarose gel

The additional DNase I step can slow this method down and severely reduce yield but can reassure that DNA is not present in the extractions if the quality checking step detect possible DNA contamination.

From both protocol cDNA was generation from the RNA extracted however problems in

making the qPCR assay including using working primers, finding stable housekeeping genes under drought stress for calculating relative gene expression, developing clones to quantify total gene expression, gene-expression data was not successfully analyzed by the end of this project.

However the hydroponics developed to cleanly extract root tissue of plants was an easy system to measure water-uptake, apply controlled osmotic stress and take root-measurements without having to filter through soil.

2.3 Preliminary studies

2.3.2 Germination

Seed germination is the first stage of the plants life and in barley soil moisture is the main environmental signal that triggers germination, alongside temperature.

In response the problem of low seed germination, poor stratification and infections developing before across the six barley lines which began to occur, this preliminary work was designed to help improve germination synchrony and rates by altering the length of time seeds were kept in cold (in 4°C) dark conditions, while also documenting if there were consistent difference in germination success and rate between the barley lines.

Methods

Seeds (n=210) were cleaned as follows, 30 seconds in 70% ethanol to remove surface bacteria, rinsed with double distilled water (ddH_2O) thrice, then left to soak in ddH_2O for 2 hours to hydrate the seeds, followed by a soak in 33% bleach with 200µL/L trition for 30 minutes, followed by rinsing in ddH_2O thrice, repeating the bleach and ddH_2O rinsing two more times. Seeds were laid on paper towel and sorted into groups of 30 seeds, making seven groups for each barley line. Seeds were placed on 12cm^2 square shaped petri dishes with autoclaved Phyto agar 1.2% (6g/500mlddH_2O, DUCHEFA) with sterilised tweezers. For each barley line there were seven petri dishes, one for each day they would spend in the cold before being moved to light conditions (figure Figure 2.3.2a**)**. Plates were sealed with parafilm and put into 4°C.

The first group of seeds were left for 24 hours before being transferred into a Weiss control growth cabinet 12hour light/dark and 20/18°C temperature , 300µmol$m^{-2}s^{-1}$, 65% relative humidity (RH). Any seeds that showed signs of emergence during the first

transfer were measured as emerging at day 0. Every subsequent 24 hours a new petri dish for each barley line was moved to the growth cabinet. Each day, every petri dish was monitored for germinating seeds for seven days.

Figure 2.3.2.1 Seeds being scored for germination in petri dishes in growth cabinets.

Results and discussion

The effect of length of time in day in 4°C dark conditions on germination was compared between barley lines in the parental cultivar Bowman. Seven days after transfer to warm and light cyclic conditions the germination in Bowman parental line and *eam8.w* was high regardless of length of time in the cold, *eam10.m* and *eam8.k* lines benefited from longer periods in the cold, at least when seed had been incubated for three to four days. *Eam5.x* germination was greatly reduced relative to other lines in all cold treatments with seemingly higher seed germination if seeds were given four to five days in 4°C however germination was still below 20% (figure 2.3.2)

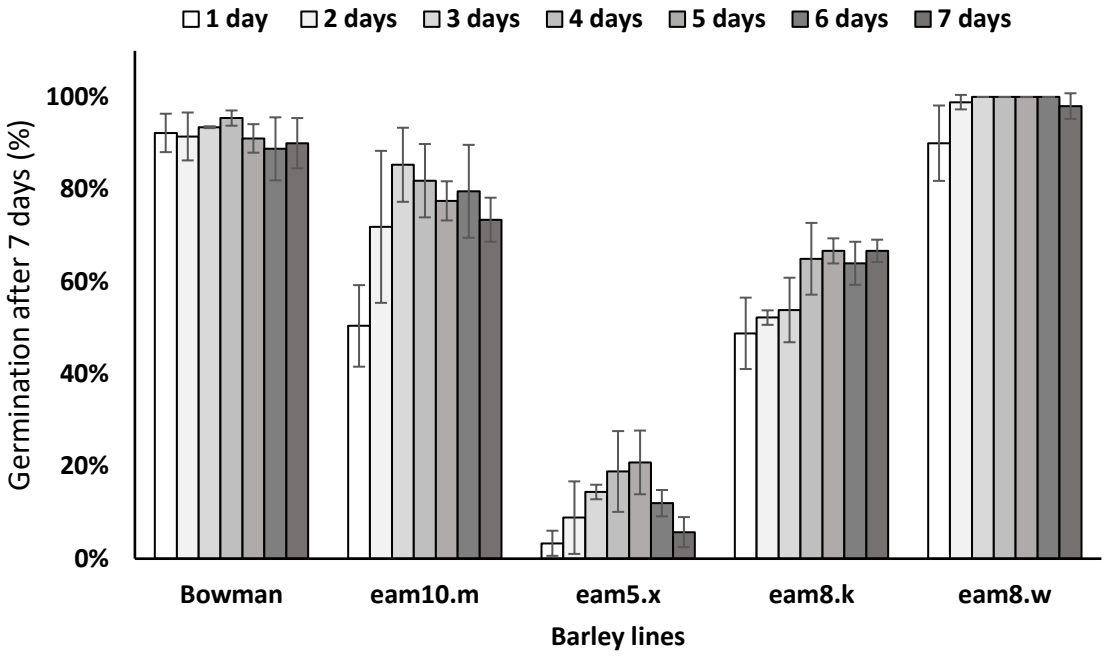


Figure 2.3.2.2 Average final percentages of seeds that germinated from a potential 30 seeds for Bowman lines, error bars are standard deviation across three experiment repeats.

The longer plants were in the cold, the more seeds germinated while in the cold. This was reflected in the percentage germination scored on day 0, which is expected because as so long as seeds have moisture, they can germinated in 1-2°C if the seeds are viable. Once seeds moved into light conditions the majority of germination happened by the first two to three days for the majority of barley lines and cold treatments (table 2.3.2.1)

Table 2.3.2.1: *Average percentage of germinated seeds by day for each day after leaving the fridge, up to 7 days.*

		Number of days after leaving the							
Number of Days in 4°C : dark	Barley line	0	1	2	3	4	5	6	7
1	Bowman	29%	89%	93%	93%	93%	93%	93%	92%
1	eam10.m	10%	27%	38%	43%	45%	47%	49%	50%
1	eam5.x	0%	1%	1%	3%	3%	3%	3%	3%

1	eam8.k	1%	19%	40%	44%	45%	47%	47%	49%
1	eam8.w	12%	43%	76%	88%	89%	90%	90%	90%
2	Bowman	24%	81%	89%	89%	89%	90%	90%	91%
2	eam10.m	7%	46%	61%	63%	64%	66%	69%	72%
2	eam5.x	0%	0%	4%	4%	6%	8%	9%	9%
2	eam8.k	3%	28%	39%	43%	44%	46%	47%	52%
2	eam8.w	21%	80%	96%	99%	99%	99%	99%	99%
3	Bowman	29%	85%	91%	91%	92%	92%	93%	93%
3	eam10.m	17%	62%	67%	70%	74%	80%	84%	85%
3	eam5.x	1%	10%	11%	12%	12%	12%	13%	14%
3	eam8.k	7%	45%	47%	48%	49%	52%	53%	54%
3	eam8.w	33%	100%	100%	100%	100%	100%	100%	100%
4	Bowman	60%	91%	91%	93%	94%	95%	95%	95%
4	eam10.m	37%	69%	72%	76%	77%	81%	81%	82%
4	eam5.x	3%	9%	13%	16%	17%	17%	19%	19%
4	eam8.k	30%	53%	59%	62%	63%	64%	65%	65%
4	eam8.w	67%	100%	100%	100%	100%	100%	100%	100%
5	Bowman	59%	90%	90%	90%	90%	90%	91%	91%

5	eam10.m	43%	69%	72%	74%	74%	75%	78%	78%
5	eam5.x	6%	15%	18%	20%	21%	21%	21%	21%
5	eam8.k	40%	61%	62%	62%	62%	63%	67%	67%
5	eam8.w	67%	100%	100%	100%	100%	100%	100%	100%
6	Bowman	83%	86%	86%	89%	89%	89%	89%	89%
6	eam10.m	54%	64%	72%	76%	76%	77%	80%	80%
6	eam5.x	10%	11%	11%	12%	12%	12%	12%	12%
6	eam8.k	56%	60%	60%	63%	63%	63%	64%	64%
6	eam8.w	100%	94%	100%	100%	100%	100%	100%	100%
7	Bowman	87%	88%	90%	90%	90%	90%	90%	90%
7	eam10.m	61%	66%	68%	68%	71%	72%	72%	73%
7	eam5.x	3%	5%	5%	5%	6%	6%	6%	6%
7	eam8.k	60%	63%	64%	66%	66%	66%	67%	67%
7	eam8.w	98%	98%	98%	98%	98%	98%	98%	98%

.

Following the seed germination testing, the protocol for cleaning seeds was changed (as described in 2.2.1) with the ethanol cleaning step removed as advise warned that alcohols should not be used to sterilize seeds as it kills faster than bleach and is less effective at removing spores. Without using ethanol and incubating seeds for at least 3 to 4 days in the cold, germination improved in all barley lines, including eam5.x. Improvement in germination allowed following experiments to move forward more efficiently.

However, batch dependent variation in germination was still observed in different barley lines after the improvements, there may be several causes for this. Greenhouse conditions were variable and barley was grown to bulk seed for experiments across seasons from 2015 to 2018. Although greenhouse temperatures were meant to be controlled to 20°C- 22°C in light hours, the plants were still susceptible to much higher or lower temperatures during their flowering periods, with additional variation in monthly light quality affecting seed quality. Additionally, since bulking seed, the greenhouses were fumigated against pests and diseases of which any could have infected the plants while they were growing. The low germination percentage of eam5.x seeds cleaned with ethanol may suggest some sensitivity to the chemical by this genotype, but that was not further investigated in this research. In conclusion keeping seeds at 4°C for six days before being moved to growth chambers optimized germination and this approach was used in subsequent experiments.

2.3.3 Primary root measurements on Agar

At germination barley seedling roots emerge before the cotyledon. Until approximately three weeks old, barley plants can potentially rely on their seeds for nutrients, but not for water. The emergent primary roots are the plants first interaction with the environment to seek moisture. The following measurements looked for differences between primary roots at emergence to consider if some genotypes have immediate advantages upon emerging based on root numbers produced and length of roots.

Methods

1.2% phyto agar to total volume of low strength Hoagland's solution was sterilised and 40ml was poured into pre-labelled square petri dishes (12cm^2), using a ruler mark a division onto the plate and a line ~1cm from one edge. Seeds were surfaced cleaned with 70% ethanol only (these measurements pre-dated using more rigorous cleaning methods). Two seeds of the same genotype were put onto a plate, then sealed with tape to keep lid on. Plates were stores vertically in rack with seeds at the top for seven days before being measured. Roots number was counted and root and shoot lengths recorded with a ruler. For each experiment, 15 seeds were used for each barley line.

The root lengths for each individual plant was averaged, totalled and the variance calculated to be a single value for each plant. These values were then averaged across all the biological replicates.

Barley lines were compared for the average number of primary roots and lengths of these roots as indicators of plants potential advantages in scouting out water in their available terrain at germination. Barley genotypes had consistently different primary root characteristics suggesting potential advantages during emergence and establishment in some lines.

The number of primary roots were counted as an indication of barley lines initiative to increase their probability of detecting water by growing more at emergence. The number of primary roots varied from as low as 4 to a high as 8 (Figure 2.3.3A). The root average lengths were calculated to give an impression of which barley lines had longer or shorter roots (Figure 2.3.3B+C) showing most lines averaged around 6 to 7 roots including the both non mutant lines. The total sum of the root lengths was calculated in consideration of plants with a single long root and many short ones and the resulting skew in the root averages. The average sum of the root lengths could be amass to over 30cm and near 40cm after 7 days in most genotypes (Figure 2.3.3A).Early maturing lines, besides eam10.m tended to have lower total root lengths compared to Antonella and Bowman.

Observation of root lengths suggested some seeds having one or two roots, which were much longer than the rest while other plants had fairly similar length roots with potentially one much shorter root. Such differences would not be immediately clear from the sum of the lengths. Therefore the variance was used to represent this "strategy". Large average variance indicates that a number plants of that genotype had long and short root lengths, while lower variances indicate more even lengths within the barley line. There is a general positive correlation with high variance and more roots (Figure 2.3.3B). eam5.k and eam10.m plants showed high variance in root lengths, whereas average variance in other lines was relatively low, more so in eam8.w and Antonella lines. eam8.w seeds had the poorest germination of the barley lines only 18 seeds out of a potential 30 seeds germinated. The low eam8.w values are represented from relatively few biological samples; therefore the findings could be genuine differences between lines or an artefact of some low quality seed. The average and total root lengths were also the lowest relative to the other barley lines. Both eam8 lines have the lowest root averages of the barley lines although eam8.k lines have a total summate of their root lengths closer to the Bowman parental lines (Figure 2.3.3A). Bowman parental line and eam10.m both had a higher average number of roots, and average root lengths, than other lines but the early maturing mutant had higher variance than the bowman line suggesting greater unevenness across root growth (Figure 2.3.3B). Antonella roots showed lower average root number emerging per seed compared to Bowman parental line, but higher root lengths per seed and

overall root lengths with low variance across roots suggesting greater investment per root in the winter barley (Figure 2.3.3C). eam5.x and eam8.k had similar average root number to Antonella, less than the spring parental line. Unlike Antonella these lines did not have greater total root lengths than the parental lines (Figure 2.3.3A). Overall early maturing lines differ from the bowman parental line in at least one aspect of their primary root characteristics, either with lower average number of roots (eam8.k, eam5.x and eam8.w), lower average root lengths (eam8.k and eam8.w), much higher variance in root lengths (eam10.m and eam5.x) or lower total root lengths (eam8.k, eam5.x and eam8.w), while Antonella lines may have had lower average number of roots but greater evenness in root lengths and overall total root lengths.

Over the seven-day growing period, cotyledons did emerge and their lengths were also recorded despite their growth being adversely affected by the limited growing conditions. By comparing cotyledon length with root measurements possible patterns between more roots with longer cotyledons or if there was a negative correlation suggesting a trade-off in tissue investment were evaluated. The result show positive association there was a positive association between more roots and longer cotyledons (Figure 2.3.3C). The average cotyledons, despite having higher standard errors in all barley lines and short average lengths may present a reason for the difference between early maturing lines and the parental line as differences between lines are less clear in shoot tissue regardless of clear differences in roots. Early maturing lines may invest more in shoot growth over root growth.

In conclusion, results of primary roots at emergence suggest *Hvelf3* lines have reduced root number and lengths at emergence, while *Hvlux*lines have slightly higher average number of roots and lengths relative to the parental line. In chapter 3, root measurements were taken using different methods that examined more root morphological characteristics from root scans of older plants.

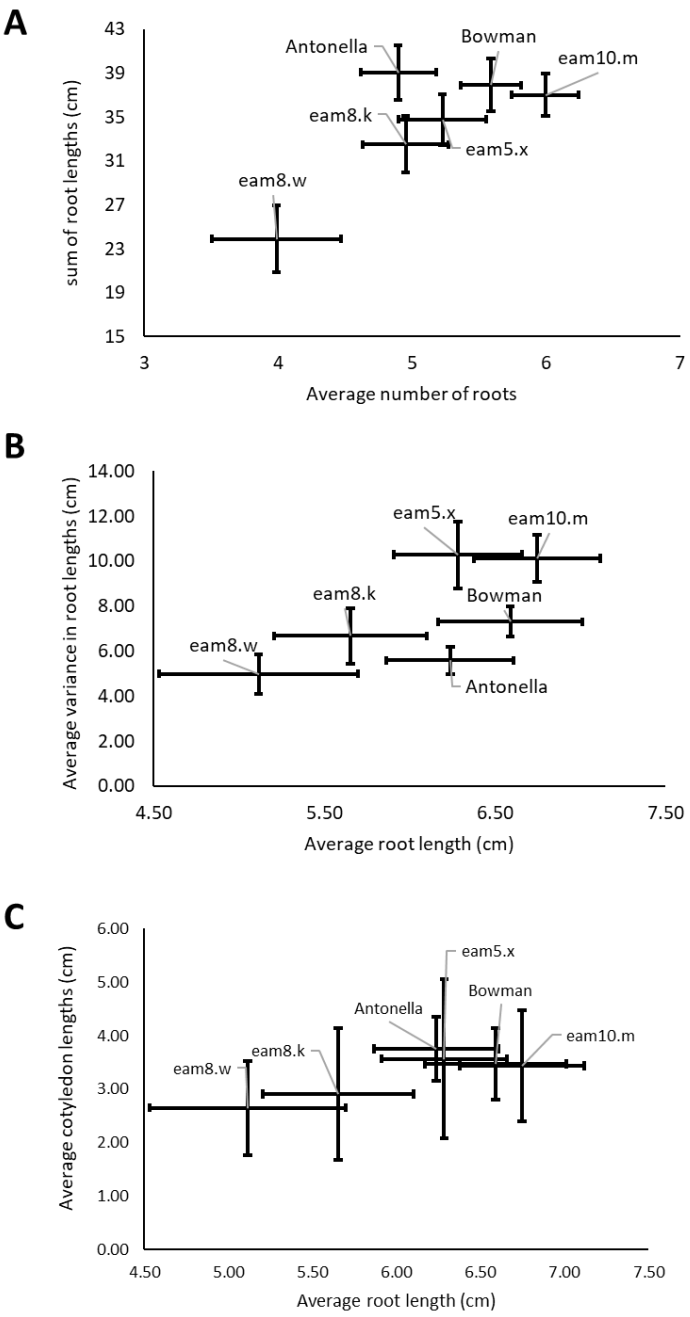


Figure 2.3.3: Relative characteristics of primary roots of barley lines, the sum of the root lengths (cm) to the average number of roots (A), and average root lengths (cm) relative to the average variance in root length (B) and average cotyledon lengths (C), error bars show the standard error. Average represent the sample numbers of n= 23 (Antonella), 28 (Bowman), 22 (eam10.m), 23 (eam5.x), 23 (eam8.k), 18 (eam8.w), which were pooled from two experiment repeats.

2.3.4 Green house measurements: Relative development rate, tillers, height and yield

Maturing early was the developmental trait that distinguished the barley circadian mutants in this project. Development maturity in barley at the tillering stage, before stem elongation. During the delay barley will be relatively dormant until adequate environmental requirements have been experienced to trigger transition to the next developmental stage(Ibrahim, 2016). Delays in development evolved to protect the plant from seasonal changes, which could bring upon conditions such as drought or high temperatures that would be detrimental to survival and fitness for a given

geographical region. As such, genes which gate development conditions are under higher selection pressure when plants enter areas with different climates.

In barley genes which regulate vernalisation (Vrn- H1, Vrn-H2 and Vrn-H3) (Cockram et al., 2007; Karsai et al., 2005; Sasani et al., 2009), photoperiod (Ppd-H1 (also known as PRR37) and Ppd-H2) and the duration of the vegetative phase (Eps) are important for flowering and heading date. The three loci for the vernalisation genes have an epistatic relationship. The Bowman barley circadian mutants in this research were identified as they flowered earlier than their parental line, a spring cultivar that already by-passes the vernalisation which can be caused by different combinations of changes to the three Vrn loci that change the sensitivity to vernalisation, dormancy period that typifies winter cultivar types and can reach maturity in the same year they are sown rather than overwintering. In Bowman, as a spring cultivar plants have the recessive *ppd-H1*allele, also annotated as *prr37*, as the gene is part of the photosensitivity pathway and circadian clock network in barley. The Ppd-H1 locus on chromosome 2(2H) regulates flowering time under long days. Ppd-H2 on 2H regulates phenology under short day length. Maturing faster can therefore manifest at relatively different points. The result of the recessive allele improves adaptation to milder, longer European growing seasons because plants are less sensitive to changes in photoperiod as a signal to delay development. Over expression of light signalling photochrome C causes similar accelerate development rate in barley plants. As Barley in a drought and cold tolerant cultivar this accelerated development has been capitalised by farmers already in high artic latitudes where extended summer months of almost continuous light for half the year is not a preventative of sowing more than one crop of barley per year.

Methods

Plants were grown in the York University (UK) Green Houses, temperatures were set to 20-22°C day time temperatures and 16-18°C night for 16 hour day lengths with supplementary flourscent lighting over winter months. Temperatures, humidity and light intensity were variable depending on weather conditions. Plants were sown three seeds per pot in $10cm^3$ pots with soil substrate composed of Levington F2+sand soil, terra-green and industrial sand in a ratio of (3:2:1) which had been found to be a good substrate for barley plants (the sand providing plenty of silicone, which is also good for water retention and protection from herbivores). Pots were placed in growing trays, 6 pots per tray and watered twice daily by filling the bottom of the tray up to 2-3cm of water which was typically absorbed in between watering. Data in this section was recorded from plants grown to 60 days since sowing and 72 days since sowing. For each barley line there were 10 pots, except for in the 60 day old group the Bowman parent lines there were 9 and the eam8.w lines there were 11.

Zadok growth stage data was recorded using Zadok growth stage chart (Table 2.3.4.1). The Zadoks scale was developed for cereals to describe the phonological development of the plant to aid management and is widely used in cereal research and agriculture. It breaks the growing stages down in a number scale from 0 to 99, including the germination stage. It has inspired other similar growth guides and numbered scales. For each plant two recordings were made one for the lowest Zadok growth stage and highest Zadok growth stage of the tillers. The total of times a plant had a tiller at each Zadok development stage from 2 to 7 for each barley line was summarised. At 60 days and 72 days old for each plant the number of tillers was recorded as well as the height from the base of each tiller (at the soil surface) to the top of the stem and beneath the head. From these measurements average tiller number and tiller height was calculated for each barley line. Seed yield measurements were taken from 72 days old seed number was recorded as seeds, which were developing. However at this stage seeds had not fully developed so were not weighed. To collect seed weight data seeds were counted from plants that were 90 days old and 130 days old after growing in the same green house conditions for bulking seed. The main difference to plants used to collect height data was that barley plants had been sown with five plants per pot and representation was more uneven, Bowman (90 days n= 17, 130 days n=14), eam10.m (90 days n= 10, 130 days n=14), eam5.x (90 days n= 10, 130 days n=6), eam8.k (90 days n= 11, 130 days n=5) and eam8.w (90 days n= 14, 130 days n=1). Seed number and weight was recorded per pot as the crowding of five plants per pot likely introduced a competitive factor on the plants potential. Only seeds that had been filled were counted as not all flowers developed into seed.

Table 2.3.4.1: A simplified chart of Zadok's scale adapted from the AHDB Barley growth guide and Tottman's illustrated reproduction of the original Zadok's scale (AHDB, 2018; Tottman, 1987; Zadoks et al., 1974). The Numbers and description in bold and highlighted in green refer to the principal development /growth stages and what was used to group plants by development stage in my study.

Zadok scale	Description	Zadok scale	Description
G0	Germination	G4	Booting
0	Dry seed	40	-
1	Start of imbibition	41	Flag leaf sheath extending
3	Imbibition complete	45	Boots just swollen
5	Radicle emerged from seed	47	Flag leaf sheath opening
7	Coleoptile emerged from seed	49	First awns visible
9	Leaf just at coleoptile tip	G5	Inflorescence emergence
G1	Seedling growth	50	First spikelet of inflorescence visible
10	First leaf through coleoptile	53	1/4 of inflorescence emerged
11	First leaf unfolded	55	1/2 of inflorescence emerged
12	2 leaves unfolded	57	3/4 of inflorescence emerged
13	3 leaves unfolded	59	Emergence of inflorescence completed
14	4 leaves unfolded	G6	Anthesis
15	5 leaves unfolded	60	Beginning on anthesis
16	6 leaves unfolded	65	Anthesis half-way
17	7 leaves unfolded	69	Anthesis completed
18	8 leaves unfolded	G7	Milk development
19	9 or more leaves unfolded	70	-
G2	Tillering	71	Kernel watery ripe
20	Main shoot only	73	Early milk
21	Main shoot and 1 tiller	75	Medium milk
22	Main shoot and 2 tillers	77	Late milk
23	Main shoot and 3 tillers	G8	Dough development
24	Main shoot and 4 tillers	80	-
25	Main shoot and 5 tillers	83	Early dough
26	Main shoot and 6 tillers	85	Soft dough
27	Main shoot and 7 tillers	87	Hard dough
28	Main shoot and 8 tillers	G9	Ripening
29	Main shoot and 9 or more tillers	90	-
G3	Stem Elongation	91	Kernel hard (difficult to divide with thumbnail)
30	Pseudo stem erection	92	Kernel hard (no longer dented with thumbnail)
31	1st node detectable	93	Kernel loosening in daytime
32	2nd node detectable	94	Overripe, straw dead and collapsing
33	3rd node detectable	95	Seed dormant
34	4th node detectable	96	Viable seed giving 50% germination
35	5th node detectable	97	Seed not dormant
36	6th node detectable	98	Secondary dormancy induced
37	Flag leaf just visible	99	Secondary dormancy lost
39	Flag leaf ligule/collar just visible		

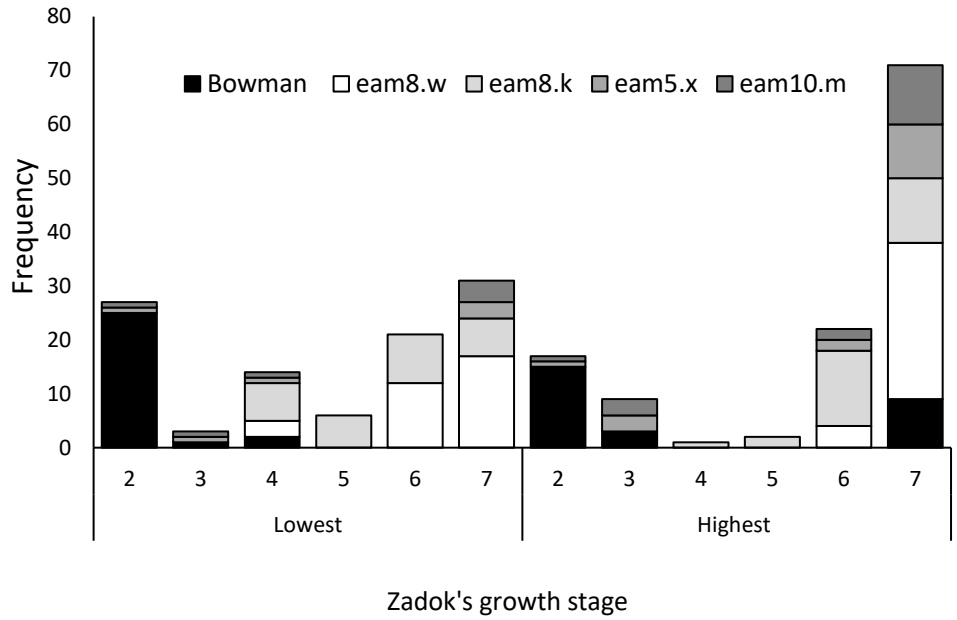


Figure 2.3.4.1 Frequency chart showing which development stage was most common for the developing tillers for each Bowman line at 60 days old. The lowest category (left) refers to the lowest zadok growth stage of any tiller on the plants, while highest refers to the highest zadok growth stage of any tiller.

Results and discussion

Early maturing lines had more plants with tillers at later development stages (seed development) than the parental line. Bowman is typical of most barley cultivars in that tillers began to develop around the time the third and fourth leaf were emerging on the main stem. It was observed that early maturing lines rapidly grow once they reach growth stage two (G2) (AHDB, 2018) on the Zadok scale when the first tillers starts to develop, they develop leaves at a faster rate and begin stem elongation earlier resulting in more tillers at later growth stages. Figure 2.3.4.1 shows that at 60 days old the majority of early maturing lines had more tillers at higher Zadok growth stages than the parental line. The majority of the parental line were still at the tillering stage when early maturing lines had tillers that were already developing seeds.

Tillers were counted and heights recorded for plants grown in green houses at 60 and 72 days old, as tiller number can be indicative of stress and growth strategy, were typically higher tiller numbers indicate less stress and tiller height relative to number indicate difference in biomass distribution. In crowded pots (any pot with more than

one plant per pot) and in pots (limited growing conditions for roots of mature plants) there is going to be some degree of stress even in well-watered conditions. For plants as at 60 days old the majority of Bowman plant in these recordings were less developed than the early maturing plants and this is reflected in their heights were the mean and inter quartile range was less than 30cm (figure 2.3.4.2, left top, left bottom) whereas at 72 days old when more Bowman plants had tillers at zadok growth stage 7 as well, the tallest Bowman tiller heights were the tallest of the barley lines (figure 2.3.4.2, right, top) although across the whole plant there were still young tillers and so a wider range of tiller heights which lowered the overall average. From observation, Bowman parent lines did the more typical behaviour of having one or two tillers, which would develop a full head of seed, and the other tillers would senesce. There was no explanation for the reduced average number of tillers between plants grown at 60 days old and 72 days old. This may be an artefact of growing in different locations in the green house or the batch of seeds used. The average number of tillers in the early maturing lines are more consistent in the two age groups with eam10.m and eam5.x plants having relatively around 3 to 4, while eam8 lines were closer to 2 and 3 (figure 2.3.4.2, bottom row). What this data does not show is beyond 72 days and main stem senescing early maturing plants would still produce tillers that would later develop a small number of seeds and this happened more when one to two seeds were planted per pot. Additionally from general husbandry Bowman parental lines were more often identifiable as producing the tallest and straightest stems. From the data I and presenting, my main conclusion is early maturing lines, especially those with mutations in HvELF3 gene produced less tillers than Bowman plants.

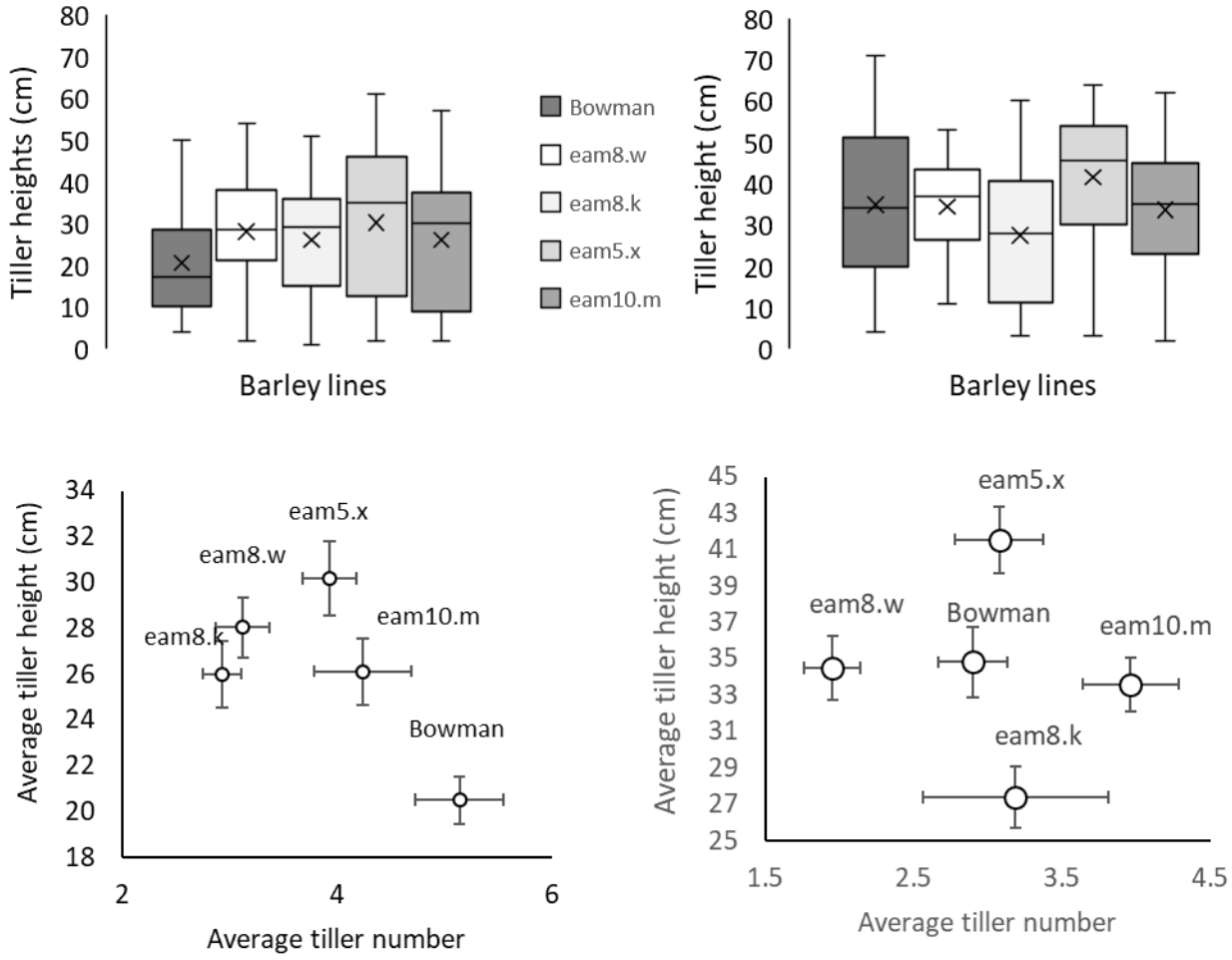


Figure 2.3.4.2 comparing tillering traits in bowman barley lines at 60 days old (left) and 72 days old (right). The range of heights of tillers is presented as bow and whisker plots (X= mean, quartile calculation is for the exclusive median) (top row) and the average tiller height relative to the average tiller number for each barley line (bottom row) are shown. For plants at 60 days old the replicate number Bowman (n= 28) eam8.w(n=32) eam8.k(n=29) eam5.x (n=29) eam10.m (n=29). 72 days replicate number Bowman (n=30) eam8.w(n=21) eam8.k (n=27) eam5.x (n=26) eam10.m (n=27) . Error bars are standard error.

Seed counts and seed weight data was collected as representing relative yield from Bowman plants. Collecting data on yield required all lines to have finished grain filling and seeds to be losing their green pigmentation. The first results shown are for seeds counted but not weighed as grain filling had not been completed from the plants measured at 72 days old with 3 plants per pot (the same group of plants tiller data had been collected from). From seeds counts alone at 72 days eam5.x plants produced more seed than the other lines (Figure 2.3.4.3). This result at 72 days old was not consistent with results from 90 days old and 130 days old plants (Figure 2.3.4.4, left side A and C) as total seed number was high from Bowman plants. The only consistent measurement was both eam8 lines producing the lightest and fewest seed.

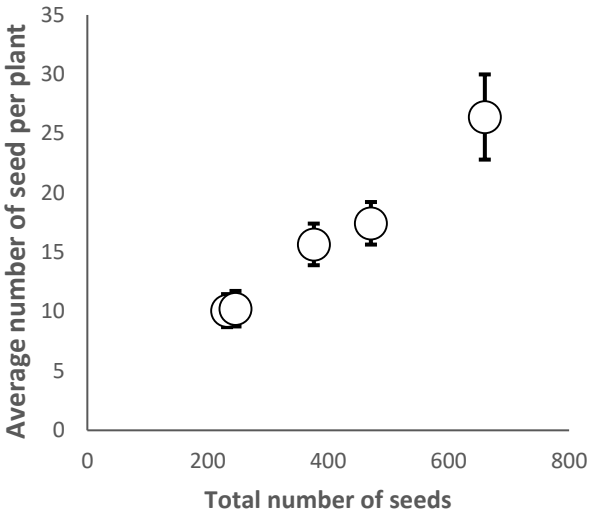


Figure 2.3.4.3 Average seed number per plant relative to total number of seeds from plants grown to 72 days of age with up to 3 plants grown per pot and 10 pots per barley line, Bowman (n=30) eam8.w (n=21) eam8.k (n=27) eam5.x (n=26) eam10.m (n=27, error bars are standard error of the mean.

The average weight per seed was taken as a measure to indicate some quality of the seed or at least indicate seed which had been packed with starch which would also indicate how successful the plant had transported it's nutrients up the plant into the seed. After 90 days was still too early to get collect this data, some heads had completed filling and drying and others had not, which is represented in the large error bars in figure Figure 2.3.4.4 B. After 130 days a much clearer picture could be attained as the majority of seeds were filled and dried, although the extra month may have been beyond the necessary time to wait. The end result after 130 days shows that eam10.m plants could collectively produce a similar number and weight of seed to Bowman lines, while other early maturing lines produced fewer and lighter seeds (Figure 2.3.4.4 C) which is consistent with trends even at 90 days (Figure 2.3.4.4 A). Although collectively eam10.m plants appear to have a high weight and high seed number on par with wild type when the average weight relative to seed number per plant was calculated it showed that eam10.m were more similar to other early maturing lines in having lighter seed weights.

An observation not represented in this data was for Bowman plants the number of seeds per head of a plant could be 16 to 24 seeds while for eam8 lines may be 6 to 12 seeds per head, eam10.m and eam5.x can be typically 10 to 18 seeds on the largest heads. In Bowman parental lines the number of tillers which development heads was

fewer than the number of tillers which developed heads and seed in early maturing lines, were almost every tiller developed heads and tillers kept being produced after the main stem senescence, - which may be due to a similar mechanism in Arabidopsis where changes in the evening complex inhibit senescence (Sakuraba et al., 2014).

It was difficult to choose a termination point that would be representative of all barley lines because when Bowman lines had reached their final development stages, the early maturing lines were continuing to produce new tillers and that changed some of the impression of how productive the plants were. Early maturing lines produced less seeds per head and divided their resources across more tillers, while also continued to produce tillers which may only produce one or two filled seed, or no developed seed. However the seeds which were collected, did germinate and grow into full plants.

In conclusion, collecting data on tillers and yield was more complicated than anticipated as factors such as location and conditions in the green house, number of plants per pot, size of pot, time of terminating and measuring plants all had an influence on tiller number and development. With more time and attention it would have been possible to make a standardised system with a set termination and more rigorous annotation of tiller, leaf and seed per head characteristics, which could then be used to apply drought treatments. Yield data and growth strategies involving tiller development do appear to be different in early maturing lines compared to parental lines in that early maturing lines, once they reach flowering and maturity do not senesce but continued to produce more while conditions are still suitable for growing.

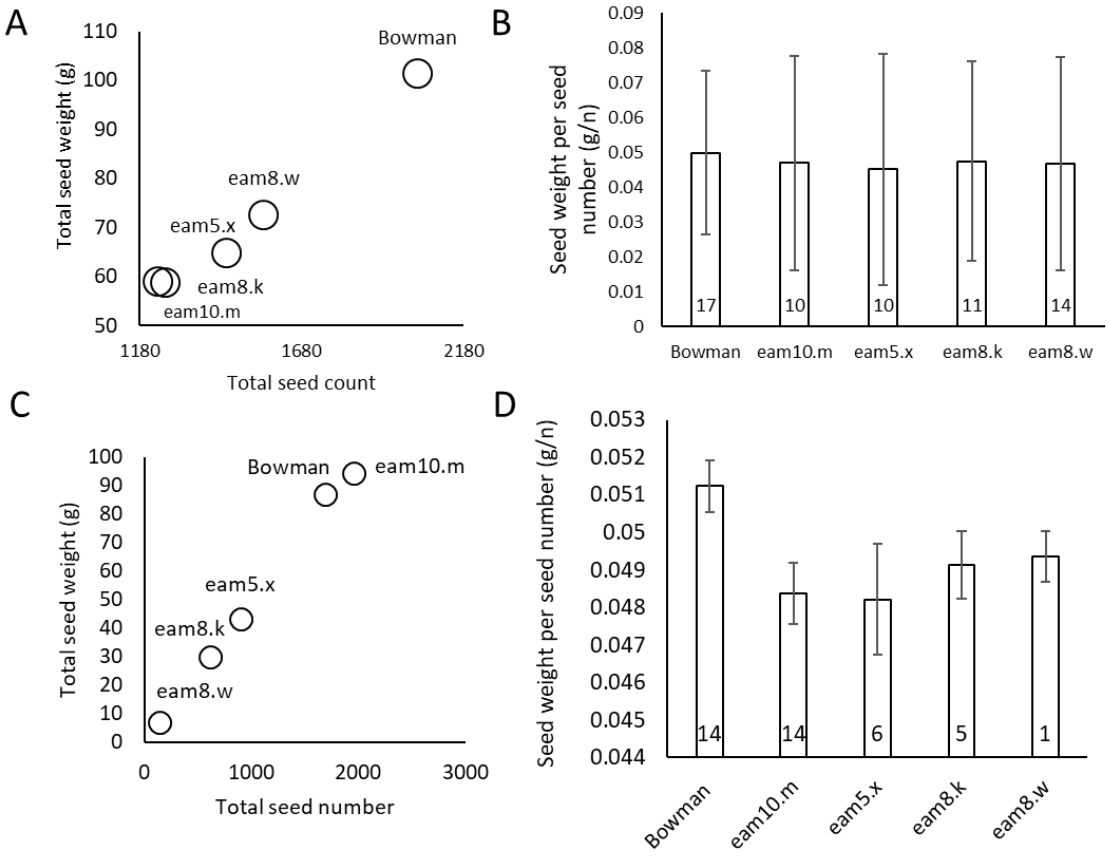


Figure 2.3.4.4 Seed weight and total seed counts for barley lines based from seeds collected from five plants grown per pot. For each plant total seed count and weight was recorded at 90 days old (A and B) and 130 days old (C and D). The data of average weight per seed as calculated by dividing the total weight of seed by total number of seeds per plant and averaging the value across barleys of the same barley line (B and D). Standard error pars in figures B and D are calculated from the total number of plants but the labels in bars indicate the number of pots that contained 5 plants.

2.3.5 Leaf and growth traits in response to osmotic treatments

Leaf organs are the primary location where transpiration and photosynthesis occurs and were therefore considered as important traits to compare between barely lines. Morphological features such as, number, size, shape, angle and movement of the leaves can all factor into water regulation while also indicating growth. Alongside leaves, Biomass is also indicative of plant performance. Differences in fresh weight and dry weight can indicate relative water content with separate measurements for root and shoot tissues allowing insight into allocation of biomass and water content in above and below ground biomass.

Methods

40 seeds per barley line (Antonella, Bowman, eam10.m, eam5.x, eam8.k,eam8.w) were cleaned using the method described in section 2.2.1, kept in 4°C for four days, moved to 20°C/18°C growth chambers (300 µmol m^{-2} s-1 fluorescent lights, 60-70% RH). When germinated seedlings were long enough (approximately 4 cm long roots and cotyledons) they were transferred as pairs to 10L boxes containing 10L low nutrient Hoagland solution to grow. Plants were grown for 4 weeks with Hoagland solution changed at after 2 weeks of transfer. At 4 weeks old, half the plants were measured for leaf width for each fully emerged leaf and fresh mass and dry mass weights, after the remaining seed husk was removed (Antonella n=17, Bowman n= 17, eam10.m n=8, eam5.x n=15, eam8.k n=7, eam8.w n=17). The remaining half of plants were transferred to 10L boxes containing fresh low nutrient solution with 0% PEG removed (Antonella n=8, Bowman n= 8, eam10.m n=4, eam5.x n=8, eam8.k n=4, eam8.w n=8) or 15% removed (Antonella n=8, Bowman n= 9, eam10.m n=4, eam5.x n=8, eam8.k n=3, eam8.w n=7). Plants were grown for 6 days and measured for leaf width for each leaf and fresh mass and dry mass weights. Water content was calculated as fresh mass take away dry mass, change in measured traits were calculated by subtracting values recorded from the plant harvested before treatment from their paired plant harvest after treatment. Root to shoot rations were calculated from dividing measurements for root tissues by the measurements for shoot tissues, thus smaller values indicates high values in shoot tissues, values at 1 or near indicate almost equal allocation of mass and water content, and values over one indicate more allocation to root tissues. Relative growth rate could have been. Relative growth rate calculation (mass day 1 – mass day 2/number of days) had the same pattern as change in fresh mass. Average and variance leaf widths for each plant was calculated from all the widths from one plant to associate with mass traits. Correlation coefficients were from linear trend lines calculated in Microsoft excel®.

Results and discussion

Average leaf widths and leaf number positively associated with total fresh weight when taking all the measurements across all the barley lines. Pre-treatment the association for both leaf number and average widths correlated with fresh weight (R^2 = 0.498 and R^2 = 0.491 respectively) shown as the white circles in figure 2.3.5aA and B. After six more days the association between fresh mass and leaf number grew stronger without stress treatment (R^2 = 0.617, black squares) and with stress treatment (R^2 = 0.603,grey diamonds) figure 2.3.5aA, whereas the association to leaf widths diverged depending on treatment. Plants grown in osmotic showed a positive association (R^2 = 0.583,grey diamonds) where heavier plants had wider leaves, but plants grown without osmotic stress the association diminished (R^2 = 0.0.089, black squares) as leaf widths tended to be around 0.5 to 0.8 cm range across plants weighing 1g up to 7g, indicating a limit to

how wide leaf blades could grow to, figure 2.3.5aB. Relating the change in fresh weight to change in average leaf widths showed similar trends (figure 2.3.5aC), where there was a positive association between the traits that was stronger for stressed plants (R^2 = 0.6379) more than unstressed plants (R^2 = 0.1815).

Examining how leaf number may have influence leaf variance for each barley line (figure 2.3.5aD) suggests that in eam5.x (3) and eam8.w lines (5) post treatment in both 0% PEG (black circles) and 15% PEG (grey squares) the average variance of leaf widths and leaf number across plants was much more variable than other lines. Looking at average leaf widths of leafs for each barley line (1 indicates the first leaf on the main stem and going up through main stem leafs from the first to last emerged leaves on other tillers.) A flaw in this presentation is not indicting which leaves were coming from which stem or tiller so the number assignment is more a reflection how many leaves present in each barley line in each condition and where there are error bars indicates that there was more than one plant that had that number of leaves. The information in figure 2.3.5a does not give insight into why variance was particularly distinct for eam8.w and eam5.x line. Looking at the bottom green bar there is some indication that early maturing lines show a response to osmotic stress in the width of their leaf but only eam18.m also shows a reduced width in the second leaf where other early maturing lines do not. Although it appears that post treatment plants grown in 15% PEG grew more leaves, the plants in this group were different plants to the plants pre-treatment and the leaf number may be more reflective of leaves the plants already had before treatment. Figure 2.3.5aE, mostly illustrates determining patterns and responses to osmotic conditions in leaf widths might not be consistent leaf to leaf.

An outcome of this preliminary work was measuring the leaf width of every leaf per plant was time consuming when scaled up to six barley lines and two treatments, alongside weight measurements, and since there was a positive association between leaf traits and fresh mass, I chose to only measure the weight of plants as an indicator of growth. As the overall weight would also factor in leaf lengths and stem mass and the overall size of the plant anyway which has the potential to be transpiring. Additionally leaf shape while a potentially informative factor does not capture the dynamic regulation of stomata and potential compensatory behaviour which may result in two morphologically different plants transpiring at the same rate.

Comparing change in fresh mass (figure 2.3.5b A) clearly shows the impact of osmotic stress (grey bars) and which barley lines had a greater relative response to the treatments (number labels, Average 15%- average 0%), notably Antonella being less responsive compared to the Spring lines. Plants which did not have a large change in 0% PEG conditions also tended to not have as strong a response to 15% PEG referring to eam5.x and eam8.w lines alongside Antonella. Bowman parental line, eam10.m and eam8.k lines appear to represent a “higher they could rise the further they would fall” comparing the change in fresh mass between non stressed and stressed plants.

To investigate how the total biomass measurement would relate to mass divided between above and below ground plant tissues, root:shoot ratio were plotted for dry biomass (g) and water content (g). The allocation of dry biomass is concentrated in the shoot material but in Antonella and eam5.x lines it is less biased compared to other Bowman lines but as the plants get older, regardless of osmotic treatment the ratio of dry biomass become very similar across all plant lines and remains larger in the shoot tissues. The allocation of water does change with age and osmotic treatment with post treatment plants having more water in the roots than pre-treatment indicated by higher values and the storage of water rises when plants have been osmotically stressed (figure 2.3.5b B).

To know if this distribution is due to more water being allocated to the root rather than the shoot or if it was more a reflection of greater water loss in shoot tissues, the average water content in root and shoot tissues was graphed (figure 2.3.5b C) showing clearly the change in ratio is due to water content being lower in bot root and shoot tissue but the reduction is much greater in shoot tissues.

In conclusion leaf measurements would not be collected in chapter 3, instead relative change in biomass as weights which would capture all the shoot tissue would be used to compare growth and stress response of plants. If differences are not found between water-uptake and total biomass that is a strong indicator that regardless of other physiological differences, water regulation and response to limited water availability across the barley lines is more similar than different.

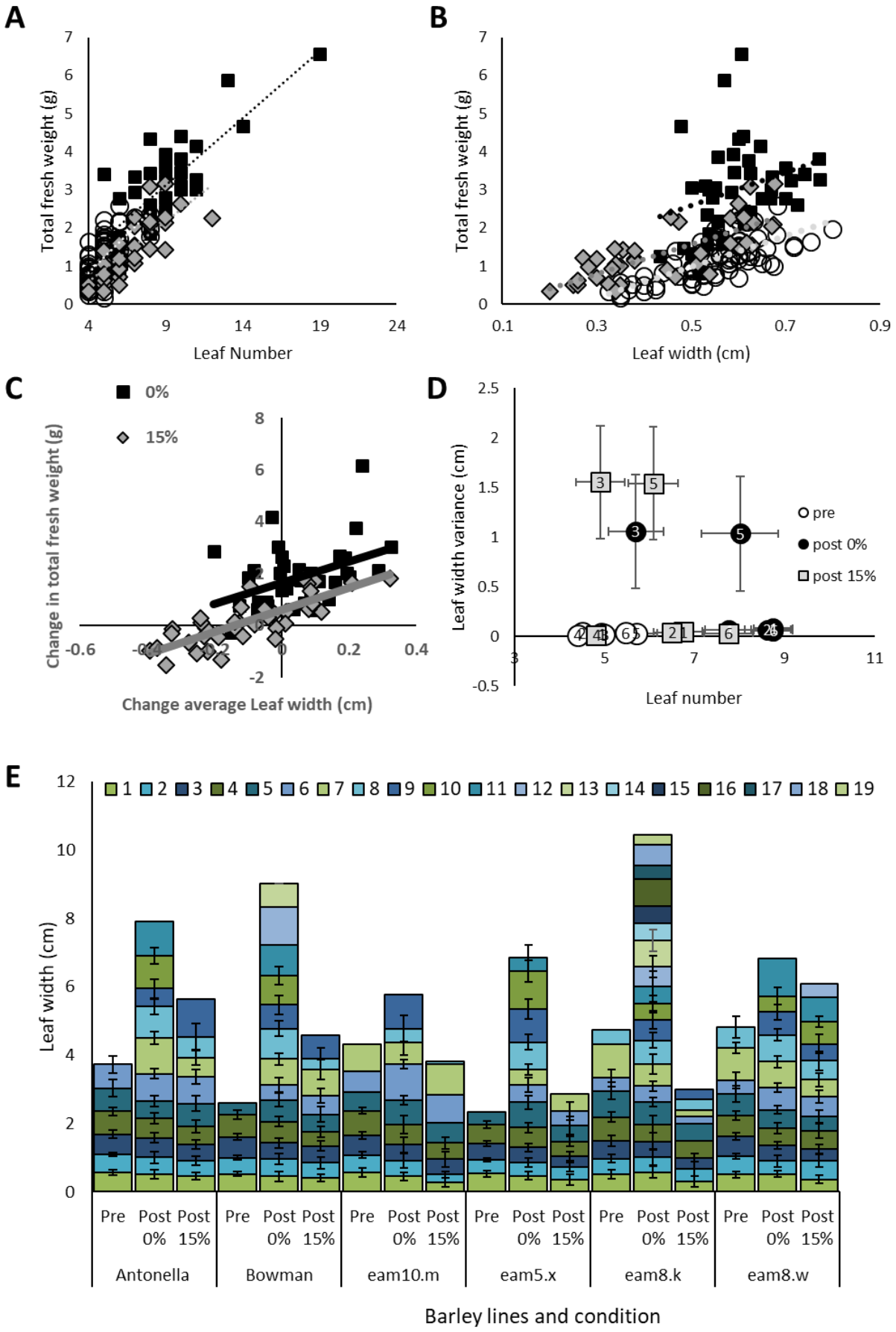


Figure 2.3.5a leaf width (cm) traits relative to leaf number and fresh weight (g). Total Leaf number positively correlated with fresh weight (A) pre-treatment (white circles, $R^2 = 0.498$),

post treatment 0% PEG (black squares, $R^2 = 0.617$), post treatment 15% PEG (grey diamonds, $R^2 = 0.603$). Average leaf width positively correlated with fresh weight (B) pre-treatment (white circles, $R^2 = 0.491$), post treatment 0% PEG (black squares, $R^2 = 0.089$), post treatment 15% PEG (grey diamonds$R^2 = 0.583$). Positive correlation between change in fresh weight and change in average leaf widths for plants in 0% PEG ($R^2 = 0.1815$, black line) and 15% PEG ($R^2 = 0.6379$, grey line) (C). Labels and Sample size for each barley line for pre-treatment (white circles), post treatment with 0% (black circles) and post treatment 15% (grey squares) is as follows: Antonella (1) n=16, 8, 8, eam10.m(2) n=8, 4, 4, eam5.x(3) n=15, 8, 7, eam8.k(4) n=7, 4, 3, eam8.w(5) n=15, 9, 8, Bowman(6) n=16 ,8, 9, error bars are standard error. Variance in leaf width appeared to be significantly affected in eam8.k and eam5.x lines in post stress (grey squares) and non-stress (black circles) conditions, error bars show standard error. Average leaf width and leaf number were highly variable across barely lines before and after treatment error bars are standard error with no error bar indicating the leaf width is representing only one plant that grew than many leaves.

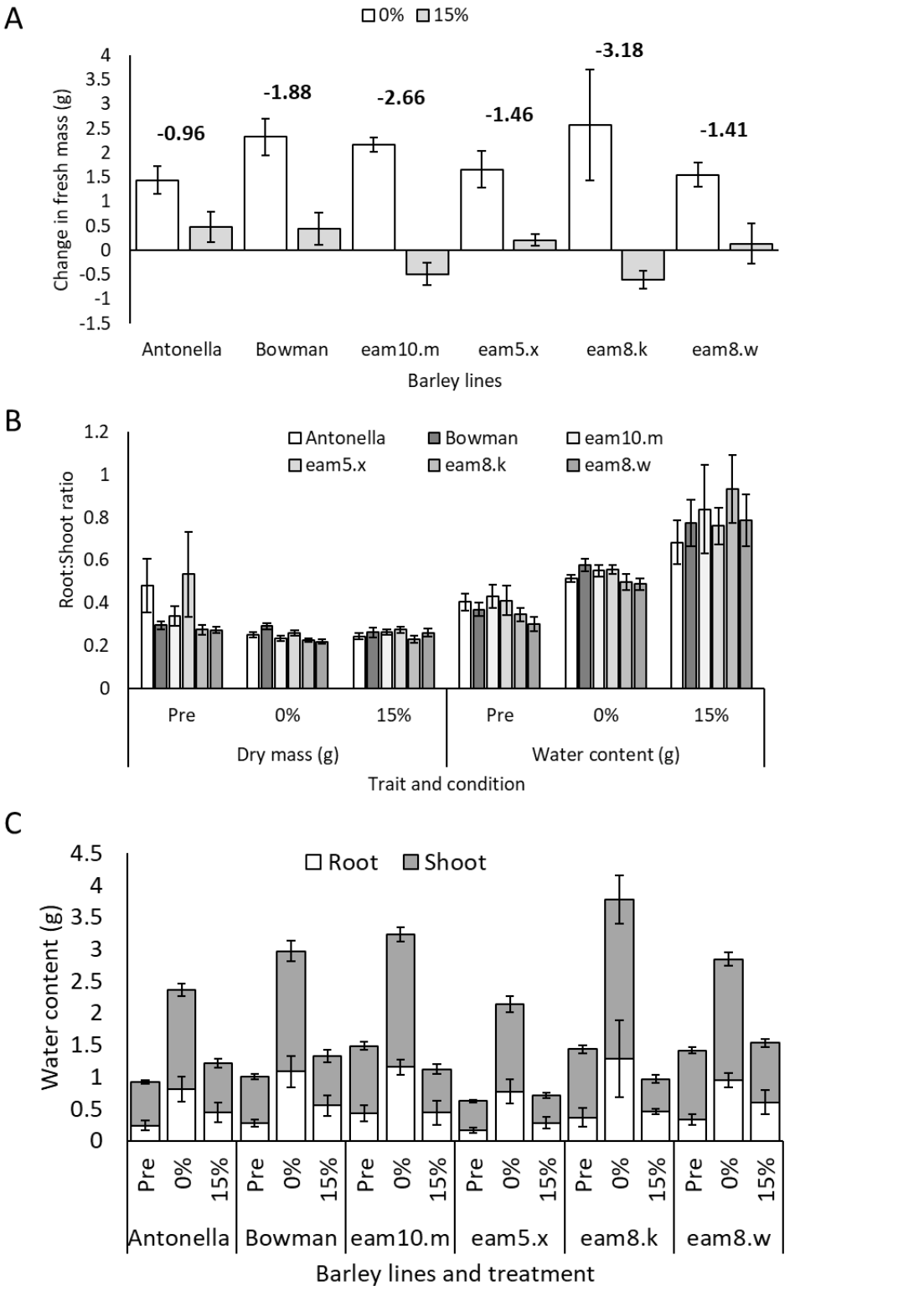


Figure 2.3.5b Biomass and water content responses to osmotic treatments. Change in fresh mass (g) in barley lines in response to osmotic treatment (A), the label above the bars calculated the difference between the average change in fresh mass of plants in 0% PEG to change in fresh mass of plants in 15% PEG calculated as (15% average -0% average) to show the largest average difference. Average change in Root to Shoot ratio of dry biomass and water content before (Pre) and after (0%, 15%) treatment (B).

Average water content (g) in roots (bottom grey bars) and shoots (top white bars) before (Pre) and after (0%, 15%) treatment (C). Errors bars are standard error (sd/√n).

2.3.6 Stomata density

Stomata are the "lungs" of plants. They are microscopic structures pores made from specialised guard cells on the aerial surfaces (Hetherington and Woodward, 2003; Zeiger et al., 1988). They operate by the turgor (cell pressure) of guard cells changing the stomata aperture (openness) (Condon et al., 2002; Hetherington and Woodward, 2003). Increasing turgor increases the opening of stomata, increasing stomatal conductance and gas exchange whereas reduction of cell turgor pressure reduces aperture, thus conductance and gas exchange, (Bartlett et al., 2016; McAdam et al., 2016; Mustilli et al., 2002; Schroeder et al., 2001; Tombesi et al., 2015). Passively, low water availability reduces turgor of guard cells because the hydraulic conductivity is reduced. Additionally lower water availability triggers an increase in the production of the hormone ABA, and this signalling increases stomatal closure. The hormone mechanic enables active adjustment (Kollist et al., 2014; Zeiger et al., 1988). Stomata aperture regulation allows dynamic and controlled regulation of water loss and CO2 uptake, to optimise the balance between water conservation and photosynthesis and content with environmental influences and threats such as external air humidity, wind speeds, temperatures, light intensity, soil moisture and biotic threats (Assmann and Jegla, 2016; Chaves et al., 2016; Farquhar and Sharkey, 1982; Mott Keith, 2009; Schroeder et al., 2001).

A conundrum of water regulation for plants is the need to lose water to gain water. Water can be moved through the plant by active transport, but this is energy expensive and more difficult the higher up the plant water needs to move to overcome gravity. Water loss through the stomata leaf epidermis is essential for proving the force to pulling water up the plant. Density (number of stomata per unit area), stomata index (ratio of stomata to epidermal cells) size, placement/patterning and aperture are all properties that can regulate water loss via the leaves to adapt. Stomata density are the simplest measurements to take and as such were used for the preliminary investigation into differences in stomata development between barely lines as stomata have been often proposed as a target characteristic to manipulate to improve crop water use efficiency and if early maturing mutant lines are already pre-disposed to have differing stomata traits that could be enlightening for considering their potential in industrial crop development.

To grow plants large numbers of seeds from the five Bowman lines were hydrated in distilled water, cleaned in three thirty minute washes of 33% thin bleach and triton solution, rinses with distilled water between and after washes.

Seeds were played on wet paper tower in large square petri dishes and stored in the dark at 4°C for up to 6 days, many germinating in the dark. When shoots started to appear, seeds were transferred to pots.

This was repeated five times with seeds being planted over one to three days depending on shoot emergence. The first three times was in October and second two times was in January. The main purpose was to bulk seeds. Germination of Bowman lines B285 was low at this time.

Depending on germination success up to five seedlings were planted in 10cm^3 square pots in a substrate mixture of Levingtons® F2+S compost, industrial terra green and industrial sand in a mixture ratio of 3:2:1. The lowest number of plants per pot was two. Five to six pots were placed into growing trays and water from the bottom once to twice a day, cared for by University of York Horticulture staff. Plants were grown in controlled glass house conditions with supplemented fluorescent lighting for 16 hours alongside natural day light over early October into mid-November When they reached their heading stage and vegetative growth was complete, around 39 to 45 days old for the main stem (around GS59 by the Tottman decimal code system) impressions of the abaxial side of the fourth leaf off the main stem were taken from three plants per pot. For the plants grown in January and sampled in February were sampled at 31 days old.

Impressions were taken by using clear nail polish and coat approximately 8-10cm of the leaf from the base to the centre. When the polish had dried, double sided tape was adhered to a glass microscope slide (25x75x1mm), the slide was placed against the abaxial base of the fourth leaf and the leaf was pressed along the exposed adhesive side of the tape on the slide. The slide was gently pulled away with the polish impression stuck to the slide.

Stomata were counted using a light microscope (model; Nikon Japn eclipse 50i, ocular lens; Nikon Japnan CFI 10X/22, graticule eye piece; Nikon uk NE81 Ø27mm cross lines graticules (10x10 square), scale bar; Nikon Japan 20x/0.5 plan flour DIC M/N2 ∞/0.17 WD2.1, magnification 200 and graticule eye piece area at x200 as 0.5mm^2. Points were measured all along width transect of the leaf at 2cm, 4cm and 6cm from the base of the impression to the tip (Figure 2.6.3a), with the average number of stomata measure across the transect being associated to that point. Average density of stomata per leaf was taken by averaging the average values for the three transect points.

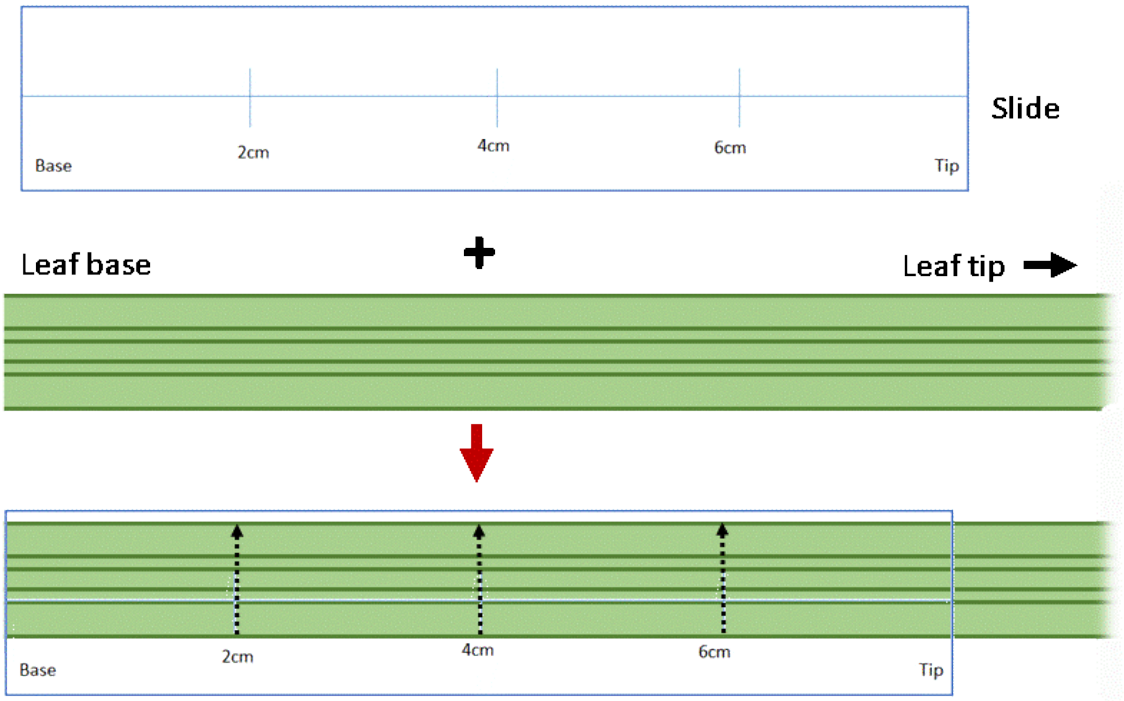


Figure 2.6.3a an illustration of the set up of the microscope slide to count Stomata against a "barley leaf". Base and Tip refer to the orientation the leaf impression was made. Base at the base of the leaf and Tip pointing towards the tip of the leaf. The slide's length created the sampling area and the lengths and dashed arrows indicate the point along the sampled leaf and direction of the three sampling transects.

In total 241, 129, 232,230, 248 leaves for Barley lines *eam10*, *eam5*, *eam8.k*, *eam8.w* and Bowman were measured respectively. Germination was poor for *eam5* for the first two groups and *eam8.w*for the third group therefore no plants of these lines were measured in these groups. Table2.3.6a summaries the representation of lines in each group and the date they were sampled.

Table 2.3.6a: **Total number of individual leaves sampled for each Bowman line.**

	16/11/2017	23/11/2017	26/11/2017	17/02/2018	25/02/2018	
Line	A	B	C	D	E	Total
lux-[eam10.m]	43	63	45	36	54	241
PHYC+ [eam5.x]			33	45	51	129
elf3-[eam8.k]	44	63	27	45	53	232
elf3-[eam8.w]	44	63		54	69	230
Bowman	45	63	42	45	53	248

To examine differences about stomata characteristics between the barley lines, stomata density was used as an indicator measure. Using nail polish, impressions were taken of the abaxial side of the fourth development barley leaf on the primary stem from the very base of the leaf towards the tip for 8cm. The impression was transferred to a microscope slide adhered with double sided tape. The number of stomata present in a 0.5mm^2 field of view were recorded multiple times along the width of the leaf impression at three 'points' from the base to the tip. The average stomata density of all the measurements at the three 'points' was calculated to give a single value for each making three values per leaf, per plant. This process was repeated five time and the results have been presented in figure 2.3.6b and summarised in table 2.3.6b.

The results from accumulating all the measurements across the experimental replications are shown in figure 2.3.6, f. Here significant difference between barley lines is detected in the nested ANOVA design ($P<0.001$, $F=45.255$, $Df=4$) which has nested factors of pots and points into the factor plants while the experimental group is independent because no interaction effect was detected when put into the model. This method also detected some pots were significantly different to each other ($P<0.001$, $F=3.048$, $Df=116$). Barley lines with similar stomata densities were *eam10* and *eam8* lines, whereas *eam5* and Bowman were significantly different from those lines and each other (figure 2.3.6b f). The point along the leaf where the measurements were taken did not show a statistically significantly different effect on the variances between lines but there is general trend in all lines for Stomata density to increase further from the base. The stomata densities of *eam10* and *eam8.k* had higher and similar stomata densities at all measured points, eam8.w was slightly lower densities then the former two. Meanwhile stomata densities in *eam5*were the lowest at all measured points than other lines while density in Bowman were between and much larger changes in stomata density from base towards the tip. This is highly variable within study groups and plants, but as a nested factor this trend is not significant. The above results are based on compiling data from five separate groups, annotated alphabetically from A to E. The groups themselves were significantly dissimilar ($P<0.001$, $F=155.958$, $Df=4$), Groups A and B were similar to each other as was C to D but they were different to each other and Group E was distinct from the rest. Some of these differences could be attributed to missing or poorly represented barley lines in group A to C.

Examining the groups separately shows the significant differences between barley lines are still present within each group ($P<0.001$) although the *eam8* lines vary in how consistently they differ against the averages of other lines, meanwhile lines *eam5* and *eam10* are more consistent relative to Bowman. Comparing graphs A to E in figure

2.3.6b shows when present *eam5* lines consistently had lower average stomatal densities than all other lines whereas *eam10* consistently had significantly higher densities than Bowman. *Eam8* lines in Groups A, B, D and E having higher stomata density averages than Bowman and lower or similar averages to *eam10* but vary by how much. Group C has the odd result of lines *eam5* and *eam8.k* having similar densities to Bowman and eam8.w lines having the highest stomata density averages.

Table2.3.6b: **summary of nested ANOVA results for stomata density measures for each group. P values of <0.001 denoted with *** and <0.01 denoted with **.**

Group	Factor	P-value	F Value	Degrees of freedom
A	Plant	$3.81 \times 10^{-7***}$	12.510	3
	Plant:Pot	0.148	1.412	16
	Plant:pot:point	0.969	0.595	40
B	Plant	$1.45 \times 10^{-8***}$	14.321	3
	Plant:Pot	$3.77 \times 10^{-5***}$	10.667	23
	Plant:pot:point	$2.27 \times 10^{-10***}$	4.751	54
C	Plant	$8.19 \times 10^{-7***}$	9.908	4
	Plant:Pot	$8.84 \times 10^{-6***}$	4.342	13
	Plant:pot:point	0.197	1.244	36
D	Plant	$6.79 \times 10^{-10***}$	14.368	4
	Plant:Pot	0.0084**	2.073	19
	Plant:pot:point	0.9397	0.678	48
E	Plant	$< 2 \times 10^{-16***}$	39.612	4
	Plant:Pot	$5.45 \times 10^{-13***}$	5.507	27
	Plant:pot:point	0.126	1.252	64
All	Group	$< 2 \times 10^{-16***}$	155.958	4
	Plant	$< 2 \times 10^{-16***}$	45.255	4
	Plant:pot	$< 2 \times 10^{-16***}$	3.048	112
	Plant:pot:point	0.987	0.787	242

The summary of the statistical tests are shown in table 2.2.6b. In all groups there is a highly significant difference between the barley lines but also detected differences between the pots. Examining interaction plots show the average stomata density for the pots of the same barley line could vary greatly, sometimes pots of one barley line could covering the range of stomata density in 0.5mm^2 area measured for the group, but in general reflected the trends when comparing the barley lines to each other, such as pots with Bowman and *eam5* lines tended to cluster at lower values and *eam10* lines tended to cluster at higher values. The odd averages in graph C can also be explained from examining the interaction plots of the average stomata density per pot. Plants from one pot in Group C appear to contribute to the higher overall average of *eam5* lines in that group, meanwhile the low *eam8.w* averages are due to the measurements for that line coming from plants from one pot.

Overall, there is clearer distinction between barley lines. Early maturing lines *eam10* and *eam8*, with loss of function mutations in clock genes *lux*- and *Hvelf3*- respectively have higher stomata densities than Bowman at the base of the 4th leaf and up to 6cm towards the tip. Early maturing lines *eam5*, with gain of function mutations in *PHYC*, have lower stomata densities than Bowman in this region.

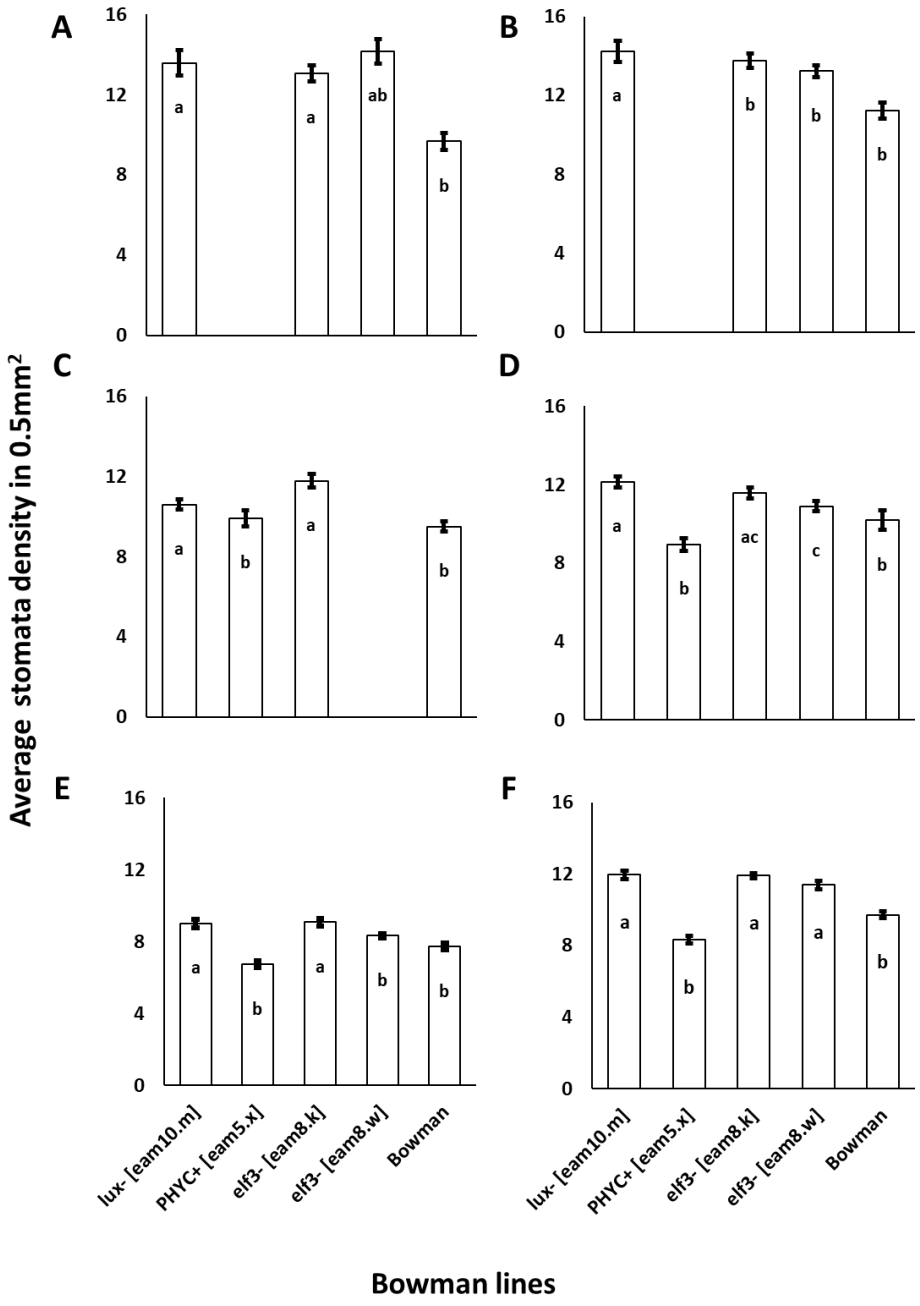


Figure 2.3.6b: Comparison of average stomata density over 0.5mm^2 surface areafrom2cm to 6cm from the base of the fourth fully grown leaf of Bowman barley (GS59[2]) in the Bowman parental line and the four early maturing mutants on the same background. Five separate cohorts (A to E) are depicted, statistical differences were detected between the groups and the results from each group are graphed and analysed separately. Graph A to E correspond to groups A to E respectively. Graph F average the measures across all the groups. Letters inside the bar graphs indicate significant differences with P value <0.01. Error bars show standard error of the means. Gaps indicate missing bowman lines where plants did not germinate or grow in that group.

Despite results here indicating that there is a replicable genotype difference when comparing just stomata at the base of the third leaf, other work carried out by Dr James Stevens in Larson lab in university of Essex taking images from the middle abaxial section of the third leaf found stomata density was complicated as results varied greatly depending on leaf and area of leaf measured, with inconclusive trade-off between size and density with the parental lines sometimes have lower or higher values than the mutant lines [comments from e-mail correspondence].

Difficultly with comparing plants is the influence of temperature, which speeds up barley development and Dr Stevens compared plants grown in warm conditions (20°C and 28°C). The combination of increased growth rate and comparing parental lines with the mutant lines with faster development than parental also a complicated stomata development discerning response as development stage was found to make a big difference to conclusions, the leaf chosen to sample and the trade-off between density and size were unclear with differences in early development between barley lines disappearing as time goes by.

It is not shown in the averages but images showed disparity in stomata characteristics across the width of the leaf e.g. size differences and density in stomata associating with placement on the leaf e.g. large stomata near the veins close to the centre of the leaf and smaller stomata at the edges. Although I randomised the order of slides, the eam5.x lines were more distinct with lower cell density and seemingly bigger stomata from observation. There were instances of what appeared as double stomata in mutant lines, which was consistent with observation in Dr Steven's study that used eam8.k and eam10.m lines. There weren't observational differences in subsidiary cells or their development which is expected to affect efficiency of stomata aperture in grasses (Raissig et al., 2017).

Finally, stomata size and conductance have effects that influence water regulation and this data does not reflect active regulation. Therefore the differences I have concluded from the measurements I did on just one area of the same leaf under unstressed conditions are unlikely to be universal or indicative of differences between these barley lines and a more intensive and controlled examination would be ideal. There are a lot of aspects to consider regarding stomata measurements (reviewed in (Bertolino et al., 2019) that stomata density alone is not necessarily indicative of water regulation whereas stomata size which can effect closure speed or stomatal conductance which gives and impression of water movement from the plant at a given time for a given area, that may or may not be representative of the plants overall water performance over a day or across the plant. Finally, over time stomata characteristics in younger leaves may change because of a systemic development response signalled from more mature leaves, because of the environment conditions they experienced (Casson and Gray, 2008; Casson and Hetherington, 2010; Chater et al., 2014; Pillitteri and Torii, 2012; Qi and Torii, 2018). Dr Stevens found differences between Bowman barley lines

diminished as the plants matured, this may indicate a greater environment adapting response in stomata characteristics than a genetically determined one.

2.3.7 Preliminary work Using lowering water levels as an indicator of water uptake to investigate differences between mutant and wild type of young plants

The volume of water a plant takes in from its environment can be indicative of its demand and suggestive of its use although efficiency may vary. Therefore, as a starting point this simple experiment aimed to test for differences in water uptake by measuring lowering water levels of a known volume, factoring in potential evaporation from the system. The outcomes would should whether or not young seedlings show differences in water uptake in a hydroponic system when they are and are not osmotically stressed. Other physiological measurements such as fresh weight and root characteristics were measured to look for differences in growth and correlate with total water uptake. Differences in water uptake can possibly indicate difference in rate of water consumption, water storage, overall water use and regulation.

Aims and Hypotheses

The aim of this experiment is to measure differences in water uptake as a basis for possible differences in water demand, ability to uptake water against a lower diffusion gradient when osmotically stressed and potentially adapt to the stress by calculating and comparing differences in water uptake over time.

Hypotheses:

H_1: Young winter cultivar barley will have more conservative water use relative to the spring cultivar, spring grow in a shorter time period during warmer times of the year whereas Antonella seedlings may have a slower rate of water use as their life cycle normally includes months of overwintering.

H_2: Mutant lines will use more water as circadian rhythms in TOC1 might be expected to be dampened and TOC1 has been to have a role in water regulation in regulating stomata response to stress.

Table 2.3.7: possible outcomes.

		Growth characteristics		
		Small weight gain	Median weight gain	large weight gain
Water-uptake	Low	Slow growth and water uptake may be causative	Fairly good water use efficiency	High water use efficiency
	Median	Not using much water for growth	Growing and likely using as much water as needed	Fairly good water use efficiency
	High	poor water use efficiency	Not very efficient water-use.	Growing a lot and possibly using necessary water as needed

Methods

Once roots showed in 4°C seeds were moved to a growth room, 17-21°C with 16 hours light (long day (LD)), with 30-40% humidity for 3 days to allow plants to grow shoots. When seedling stems were long enough plants were moved to 50ml macro centrifuge tubes containing ½ strength Hoagland's solution (appendix) secured using clean sponges. Controls to correct for evaporation were tubes with solution and sponges but no plants. Plants were moved to controlled growth cabinets with conditions set to 60-70% humidity, 12 hour Light:Dark cycles, light intensity ~300umol/m/s, 18-20°C Dark:Light) and placed in black boxes with tissue in gaps to reduce light exposure on the roots. Once plants were approximately 10cm with 1 to 2 leaves, 14 days old, they were ready to be placed to begin the experiment.
For each barley line tested half were exposed to stress by placing them in 15% PEG Hoaglands solution while the other half were in 0% PEG Hoaglands solution. There were 3 replicate controls for both the 15% and 0% solutions. The water level was recorded to the accuracy of 1ml using the volume measure on the side of the macro centrifuge tubes (Starlab®), starting at 47ml. Recording took place at approximately 24 hours intervals over seven days. After the seven days the plants were recorded for their total fresh mass, shoot fresh mass, root fresh mass, shoot dry weight, root dry weight using weighting scales, leaf number counted and the root structured scanned and analysed using WinRhizo® software.

All lines took up less water when osmotically stressed (fig 2.3.7a) and Bowman WT has the biggest difference in water uptake per day between stressed and unstressed plants. All plant and PEG combinations had significantly positive (results table 6 appendix)correlation between water uptake over time and time except Bowman WT ($P>0.1$) which suggests that on average Bowman WT plants did not take up more water over time. This correlation matches an observation in the data that at approximately 96 hours the uptake of the mutant diverges away from WT and by 168 hours the stressed mutant lines begin to distinguish from each other showing Bowman eam8.k, 284 and Antonella having greater water uptake than Bowman eam8.w under stress. Interestingly Bowman eam8.w also has the steepest correlation (indicating highest water uptake rate) when unstressed. Generally the most plants with a high increase in fresh weight (which did not correlate with total water uptake) were Bowman eam8.w (figure 2.3.7.b). According to correlation analysis only stressed Antonella ($P<0.01$), stressed ($P<0.05$) and unstressed ($P<0.02$) Bowman eam10.m, show significant correlations between water uptake and change in fresh weight.

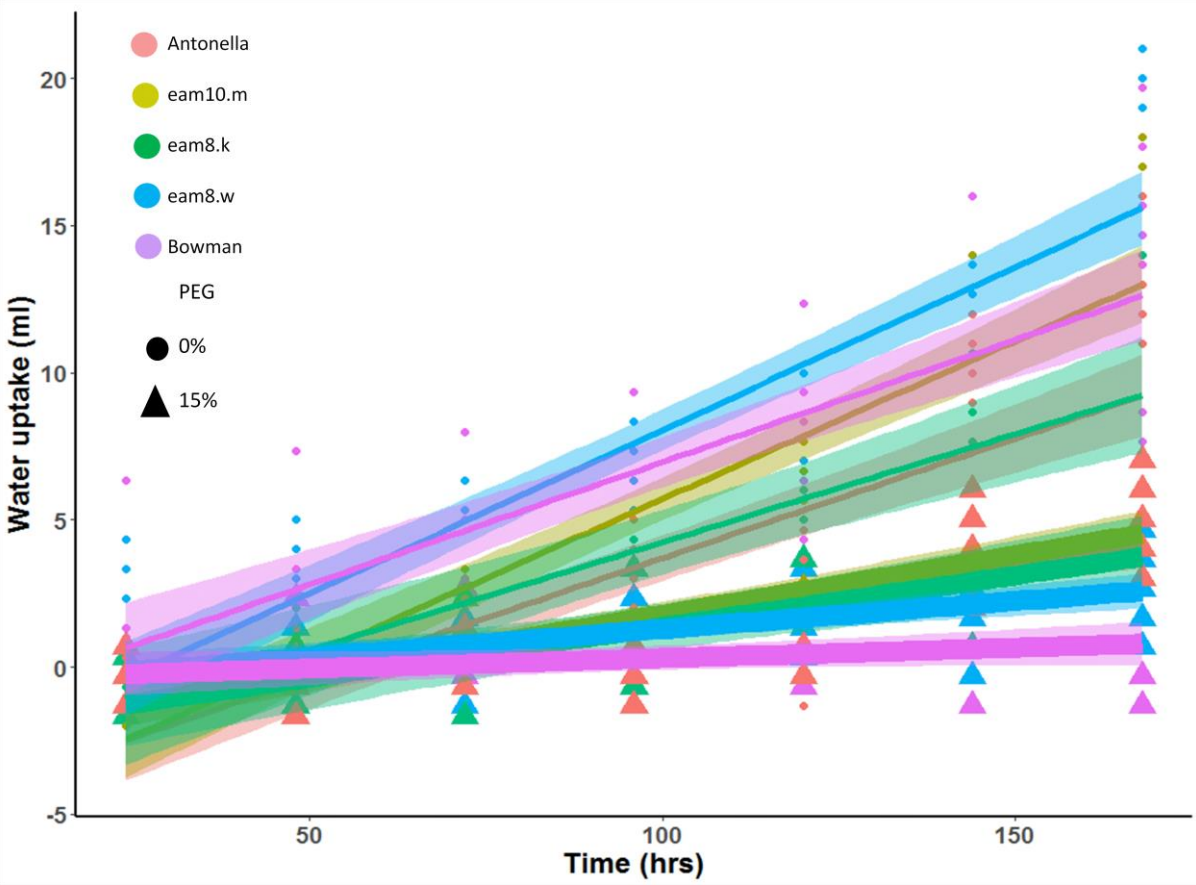


Figure 2.3.7a.: Osmotically stressed plants took up less water than non-osmotically stressed plants over time. Shaded areas show confidence intervals for each group. Groups were based on plant and treatment. Plant types are indicated by colour and include Bowman (n=7, fuchsia pink), Bowman 290 eam8.w (n=7, blue), Bowman 289 eam.k8 (n=7, green), Bowman 284 eam10.m (n=7, yellow-green), Antonella (n=7, orangey-red). PEG treatment is indicated by shape, 0% (small circles) and 15% (large triangles).

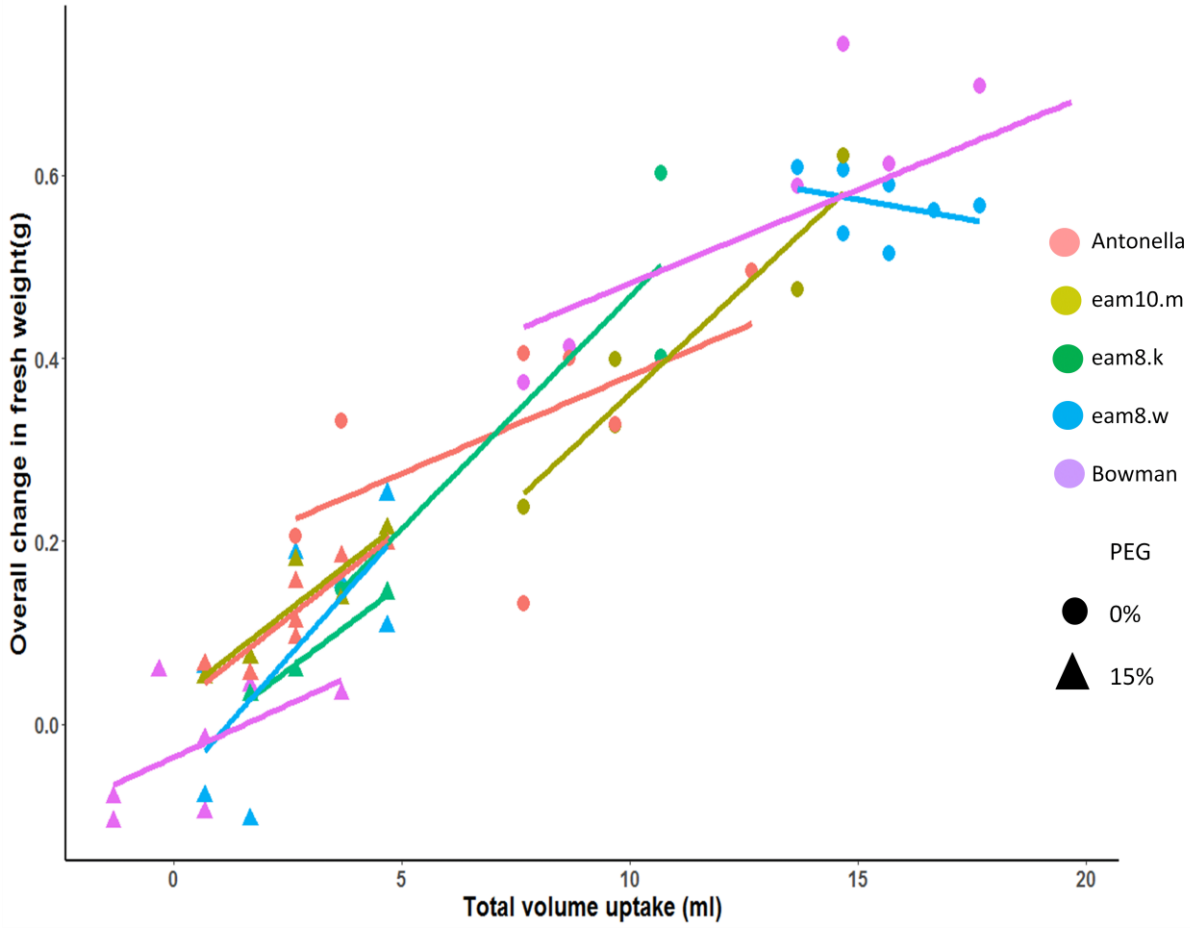


Figure 2.3.7b: Osmotically stressed plants took up less water and changed the least in total fresh weight. Groups were based on plant and treatment. Plant types are indicated by colour and include Bowman WT (n=14, fuchsia pink), Bowman 290 *eam8 (*n=14, blue), Bowman 289 *eam8* (n=6, green), Bowman 284 *eam10 (*n=10, yellow-green), Antonella WT *(*n=14, orangey-red). Peg treatment are indicated by shape, 0% (circles) and 15% (triangles).

Discussion

Although not statistically checked, it appears mutant lines and Antonella, at approximately 96 hours, increase water uptake. This may indicate the mutants adapting or 'growing out' by continuing to grow which perhaps results in more water diffusing into the plant. In terms of change in fresh weight some plants of Antonella and B289 grown without stress overlap with the stressed, this indicate might they have

lower water uptake and growth when undressed compared to the other plants therefore stress has less of an impact on their water uptake behaviour. This result and Antonella's relatively lower correlation value in unstressed conditions supports the first hypothesis. Antonella exposed to osmotic stress do generally take up less water and grow less but some unstressed plants are behaving in a similar range.

Based on the results of change in fresh weight and water uptake over time it seems Bowman WT was the most sensitive to being osmotically stressed, growing the least when stressed and water uptake not correlating with time. This supports the second hypothesis that Bowman mutants will use more water. A possible cause for this is Bowman WT has a more rhythmically expressed TOC1 gene, which is part of the ABA signalling pathway that induces stomatal closure in response to stress. Mutant lines with altered expression of TOC1 may have more stoma open and so more water is leaving the plant and because of hydraulic conductance this is helping some water enter the plant by slightly increasing the osmotic gradient between the external and internal environment of the roots. The steep correlation values (r^2 =0.99) for B289 are likely due to the lower sample size and conclusion will not be made on a small sample size for growth measurements.

Conclusions

Comparing daily change in water-uptake does not appear to reveal a distinct characteristic in the barley lines, although Antonella and B289 plants take up less water than other barley lines this is consistent in the daily measurements.

Overall change in fresh weight relative to total water uptake shows interesting patterns of variation in the test plants with B290 plants clustering, while other lines have much more variation between plants of the same genotype. Antonella plants show greater variation in change in fresh mass for a given amount of water taken up by the plant with some non-stressed plants having behaviour within close range to stressed plants. Stress homogenised plant fresh mass and water-uptake, severely limiting both at 15% PEG with the effects being the most pronounced in Bowman parental plants suggesting early maturing lines are slightly less susceptible to osmotic stress.

2.3.8 Effect of osmotic stress on growth performance and morphology in wild type and circadian mutants from a spring background.

Three different osmotic stress treatments were applied to plants grown in 20°C:18°C light/dark cycles using 5%, 10% and 15% 8000PEG (PEG).

The aim of this study was to determine if circadian mutants would responsed differently to Bowman under different severities of water-limitation and to compare the response of a winter cultivar to the spring cultivars. Previous studies have used 20% PEG over 4 to 5 days (Habte 2014) but with a similar osmolarity to the 15% PEG used in this research.

Methods

Plants were grown as described in section 2.2.1 to 2.2.7. Plants grown in 50ml centrifuge tubes using Barley lines described in 2.2.0. The data presented is collected from 3 experiment repeats of using 5 replicates for each Barley line and treatment combination totalling to 120 plants were experiment.

Analysis

Statistical analysis on the data was carried out in R studio™ using package 'lme4' (Bates et al., 2015) to make generalised mixed effect models to accommodate the right skew in the data with 'GAMMA' family when appropriate, (otherwise models were 'Guassian'). The 'emmeans' package (Lenth, 2018) was used for ANOVA and post-hoc analysis. Multiple models for each measured trait were created based on different combination of the factors being compared (e.g. including, barley lines, PEG treatment and the interaction effects of these two factors). Models were tested using Akaike's An Information Criterion (AIC) (Sakamoto et al., 1986) and based on the score factors were removed from the model because they were reducing the models predictive power. Factors dropped from the models were not showing a strong influence on the data compared to of the data was random, meaning there was no strong corrections due to the factor. This helped determine the strength of the genotype effects compared to the environment effects on growth traits.

Results

The focus here is on the sensitivity to osmotic stress between the barley lines to determine if alterations in the circadian regulation increased or decreased physiological water-uptake responses to different degrees of water stress. The work in section 1 compares a selection of physiological traits of young, 2 week old plants grown for seven days in increasing concentrations of PEG 0.05g/L (5%), 0.1g/L (10%), 0.15g/L (15%) (Figure 2.2.3.2) when grown in warm nights and low nutrient solution. In this section data was collected from three experimental repeats with five replicates for each genotype per osmotic treatment (n= 14 or 15 for all genotypes). Full data on the sample numbers and means are in supplementary table 3.3.1a and summary of statistical analysis results in table 2.2.8.3 and supplementary 3.3.1b.

It was expected that winter cultivars, due to having to overwinter would show evidence of a conservative growing and water-use strategy compared to the faster growing spring cultivars and the early maturing clock mutants to have different phenotypes to the parental line to demonstrate the influence of the mutant clock genes.

Table 2.2.8.3 **ANOVAs of Linear model results for all growth measurements in software 'R'. Significance levels set as Pr≤0.05. Plant refers to comparison across 5 barley lines: Bowman (n=15), Bowman eam8.w (n=15), Bowman *eam8.k* (n=15), Bowman *eam5.x* (n=14), Antonella (n=14). PEG refers to comparisons across the 2 osmotic stress treatments: 0% (n=64) and 15% (n=64). Interaction refers to analysis across the Barley line and PEG combination (n=7 per combination), non-significant interactions omitted from the table. Significance is indicated with *(P<0.1 = *, P<0.01=**, P<0.05 =***) P values rounded up, Df- degrees freedom within (W) and Between (B)**

	Barley lines				PEG				Interaction			
	Df		F	P	Df		F	P	Df		F	P
	W	B			W	B			W	B		
Total fresh weight (g) pre treatment	5	342	3.57	0.003 **								
Total fresh weight (g) post treatment	5	342	1.57	0.169	3	339	106.8	<0.000 ***	15	324	2.33	0.004 **
Shoot fresh weight (g)	5	342	1.87	0.098	3	339	134.1	<0.000 ***	15	324	2.54	0.001 **
Root fresh weight (g)	5	342	1.06	0.385	3	339	60.24	<0.000 ***	15	324	2.01	0.014*
Change in fresh weight (g)					3	344	64.87	<0.000 ***				
Total dry biomass (g)	5	342	1.91	0.092	3	339	58.70	<0.000 ***	15	324	1.83	0.03*
Shoot dry biomass (g)					3	344	51.15	<0.000 ***				
Root dry biomass (g)	5	342	4.46	0.000 ***	3	339	54.30	<0.000 ***	15	324	2.79	0.000 ***
Total water content (g)	5	342	1.35	0.243	3	339	107.5	<0.000 ***	15	324	2.43	0.002 **
Shoot water content (g)	5	342	1.74	0.128	3	339	133.6	<0.000 ***	15	324	2.76	0.000 ***
Root water content (g)					3	344	53.05	<0.000 ***				
Root:Shoot Dry Biomass ratio	5	342	11.9	<0.000 ***	3	339	4.41	0.005 ***				
Root:Shoot water content ratio	5	342	2.62	0.024 *	3	339	113.8	<0.000 ***				
Water uptake (ml)	5	342	2.03	0.074	3	339	322.7	<0.000 ***	15	324	2.05	0.012*
Accumulated root length (cm)	5	342	4.90	0.000 ***	3	339	15.35	<0.000 ***				
Total surface area (cm3)	5	341	3.25	0.007 **	3	338	42.59	<0.000				
Average diameter (mm)	5	340	1.26	0.281	3	337	0.31	0.82				
Average root volume (cm3)					3	342	7.68	<0.000 ***				
Total number of root tips	5	341	2.94	0.013 *	3	338	26.64	<0.000 ***	15	323	2.08	0.011*
Water uptake /total dry biomass (ml/g)					3	344	162.2	<0.000 ***				
Percentage of water as total biomass (%)					3	344	64.40	<0.000 ***				
Water uptake/ total water content stored (ml/g)	5	342	0.58	0.717	3	339	1.10	0.35				

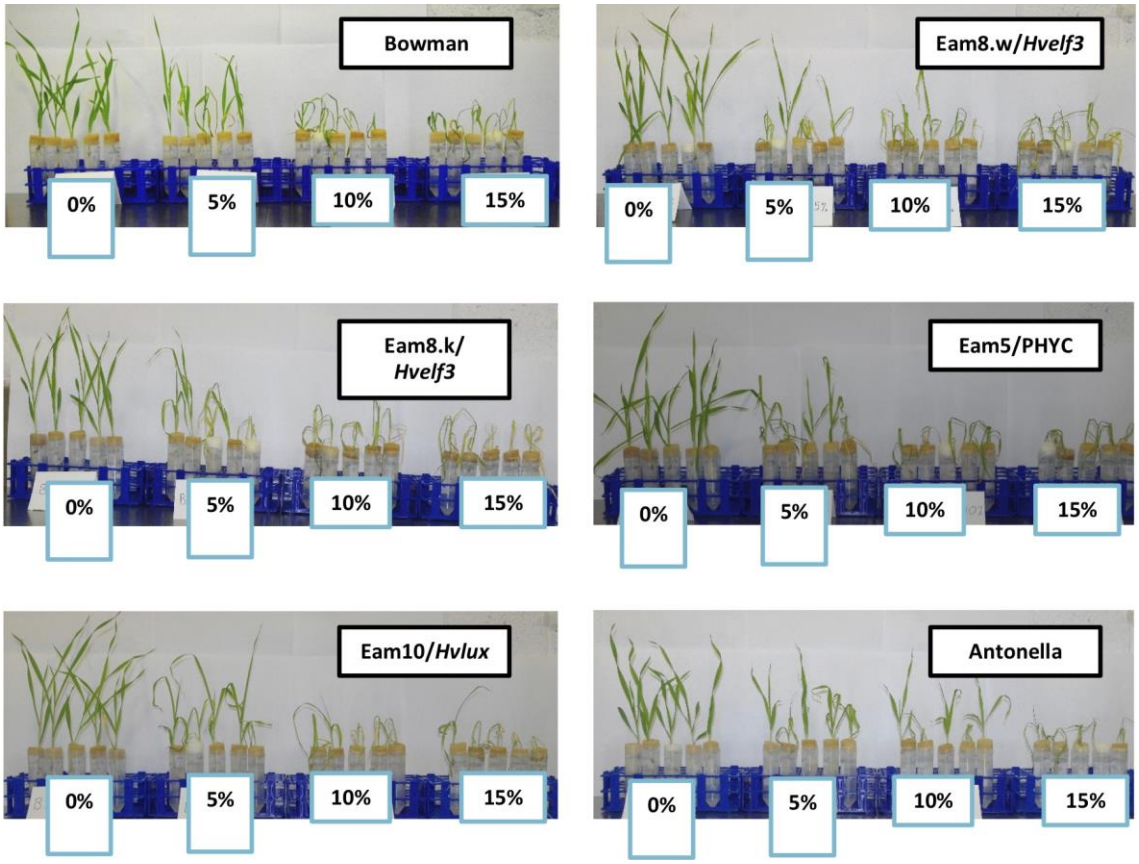


Figure 2.2.8.3.1: Photographs of one experimental group post treatment after being grown in warm, lon day light and dark condition with in Hoagland solution and 8000 PEG. In growth cabinets, plants were randomised to account for any variation in light or air quality in the cabinets, plants were grouped by genotype and PEG (%) for the photographs. Plants from left to right were grown in 0%, 5%, 10% and 15% PEG respectively and genotype is indicated by the black edged box in the top right of each photograph. The photographs show a strong visual impact of osmotic stress on young plants and different genotype responses.

The effect of the osmotic stress of plants was visibly apparent after the 7 days (figure 2.2.8.1) as well as difference between the winter cultivar Antonella and the Barley lines in the Spring background.

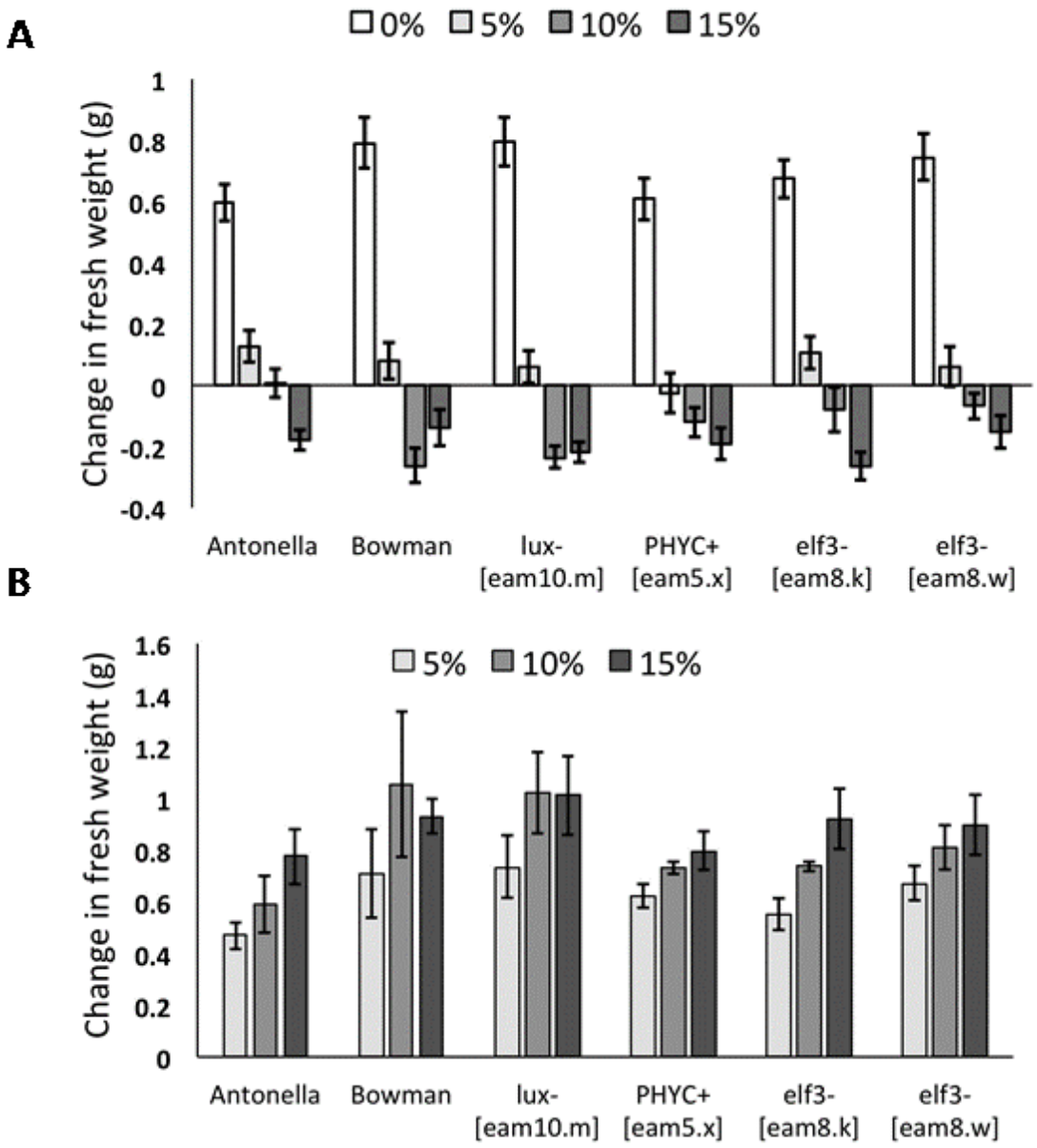


Figure 2.2.8.3.2 Change in fresh weight (A) and phenotypic change in fresh weight in response to osmotic stress (B). Bar's show the averages and error bars are the standard error of the means (s.e.).

Antonella were small plants and grew less over the treatment period relative to most Bowman lines except eam8.k whose average size before treatment and average growth was in between the Bowman parental line and a winter line (figure 2.2.8.3.2A). Growth was significantly and negatively impacted by chronic osmotic stress over 7 days, even in low osmotic stress conditions (5%). The phenotypic change (figure 2.2.8.3.2B) is representative of the trends across all traits, response to 10% and 15% PEG

Prior to treatment Antonella plants were significantly lighter (~0.57g) than Bowman plants (0.75g) (ANOVA $F_{(5,342)}$ = 3.5665, P=0.003) while the early maturing mutants were not statistically different to either of these lines with averages around (~0.6-0.7g).

The total fresh biomass post treatment was strong effected by the osmotic stress (ANOVA $F_{(3,339)}$ = 106.8234, P=<0.0001) as the unstressed plants total fresh mass was much greater than the stressed plants. There was a slight difference between some of the barely lines when stressed (ANOVA $F_{(15,324)}$ = 2.3325, P=0.003537), eam10.m lines were significantly more stressed from 0%, 5% and 10% PEG and Antonella was significantly more stressed from 0%, 5% and 15%. These lines were significantly affected by increasing stress, whereas the other barley lines there was a significant difference between unstressed (0% PEG) and stressed (5%, 10% and 15%), (ANOVA $F_{(15,324)}$ = 2.3325, P=0.003537). This is reflected in the total dry weight where there was a significant effect of PEG (ANOVA $F_{(3,339)}$ = 58.7047, P=<0.0001) as barley lines Bowman, eam10.m, eam5.x and eam8.w were significantly stressed between unstressed condition and to the stressed condition, while the stressed conditions had dry weights of a similar biomass. This was not true of all barley lines, and the different response to different levels of stress was statistically significant (ANOVA $F_{(15,324)}$= 1.8286, P=0.02996)The total dry weight of Antonella was significantly different between non stressed and the two most stressful conditions 10% and 15% PEG) while dry weights of this line overlapped with non-stressed plants and more severely stressed plants. The eam8.k lines the total dry weights were not significantly from 0% to 10% PEG or between the stressed measurements but dry weights between lines grow in 0% and those grown in 15% were significantly different to reach other.

The change in fresh mass partially accounted for differences between the initial fresh mass of plants before treatment. The total fresh mass at the end partially reflects initial fresh weight, correlating all fresh weight measurements before treatments with fresh weight measurements post treatment shows with none stressed plants (R^2 = 0.7572), and decreasingly with stressed plants 5% PEG (R^2 = 0.5704), 10% PEG (R^2 = 0.3411), 15% PEG (R^2 = 0.3373) reflecting an influence of initial fresh mass. The low initial mass of Antonella lines in particular may account for why the effect of osmotic stress between 0% PEG and 5% PEG treatments was not significant compared to other lines which had a greater fresh mass to lose from, however the difference between fresh weight before and after treatments (change in fresh mass) factored in initial difference between barley lines average weights. The response to osmotic conditions as change in fresh mass only detected the osmotic treatment as the influence, with no difference between barely lines within the stress treatment groups. The change in correlations reflect the similarity of the averages across each PEG treatment, 0% PEG averages were statistically higher than averages in 5% PEG which were in turn higher than averages in 10% and 15% PEG (ANOVA $F_{(3,344)}$ = 64.871, P=<0.0001) across all barley lines.

Of note is the effect of increasing PEG is not linear the difference in averages from 5% PEG to 0% PEG, is an exponential decline, whereas the difference from 5% to 10% and

15% PEG is much smaller with 10% and 15% averages starting to plateau out as the osmotic stress is approaching the limit of change for the plants. While looking at the change in MPa of the solutions (figure 3.3.1.1) the MPa of solutions gets exponentially more negative from 10-15% PEG.

Water traits

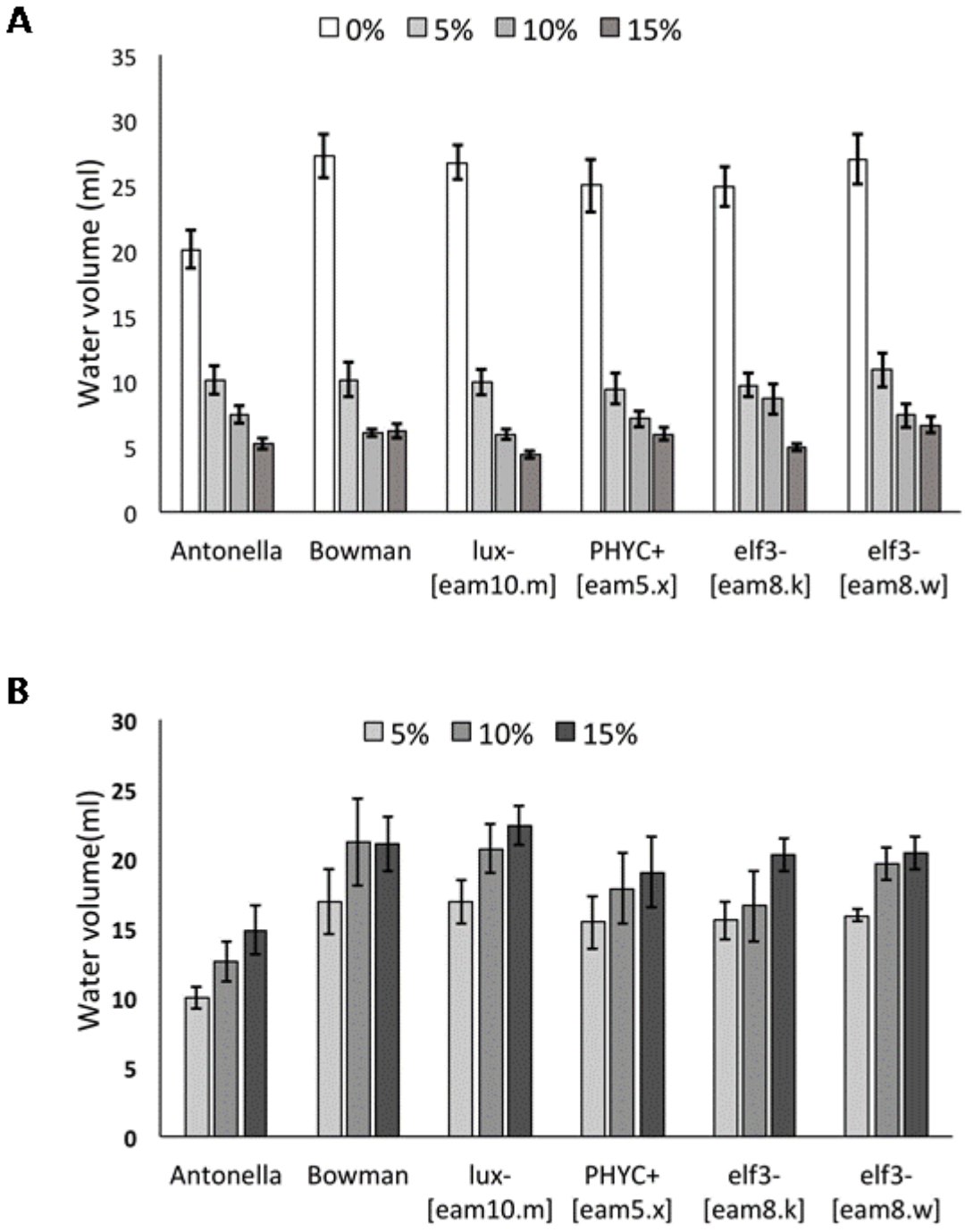


Figure 2.2.8.3 Water volume taken up by the plants over the course of the experiment in different osmotic stresses (A) and the phenotypic change in response to the osmotic stress (B). Bar's show the averages and error bars are the standard error of the means (s.e.).

Water traits measured included water-uptake, total water content and the percentage of total weight as water (Figure 2.2.8.2 A). The majority of the plants biomass was water, ~90-92% in plants grown without osmotic stress and ~80-88% in plants grown in stress (ANOVA $F_{(3,344)}$ = 64.395, P=<0.0001), and changes in fresh weight tended to correlate with total water content (0% R^2 = 0.841, 5% R^2 = 0.6676, 10% R^2 = 0.3554, R^2 = 0.4026) and a smaller change in fresh weight had a lower correlation. Water uptake was strongly affected by osmotic stress, as expected the higher the PEG treatment the less water was taken up, there was no statistical difference between the stressed conditions (5%-15% PEG) although averages were still declining (from 10%-15%) especially for Antonella, eam10.m and eam8.k lines (ANOVA $F_{(3,339)}$ = 322.7308, P=<0.0001).

In unstressed conditions there were some detectable differences between the barely lines (ANOVA $F_{(15,324)}$ =2.0484, P=0.01215), mainly between Antonella lines took up less water on average compared to all the Bowman lines except for eam8.k lines which were not statistically different to either Antonella or Bowman plants, meaning eam8,k lines took out on average less water than the other early maturing lines.

A similar pattern was reflected in the plants water content as there was a strong difference between plants grown with or without stress (ANOVA $F_{(3,339)}$ = 107.4681, P=<0.0001), as plants without stress had higher water content while plants grown in stress has lower and similar water content weights. A few lines had statistically detectable differences between osmotic stress treatments (ANOVA $F_{(15,324)}$ =2.4318, P=0.002265) where averages continued to decrease in Antonella the plants water content dropped significantly more when in 15% (0.27g) PEG relative to 5% and 10% (0.62g-0.48g) PEG treatments (and without PEG the average water content was 1.1g). Meanwhile eam10.m plants continued to decline significantly after 5% (0.65g) to 10% (0.28g) or 15% (0.25g) unlike other Bowman lines Figure 2.2.8.3 B).

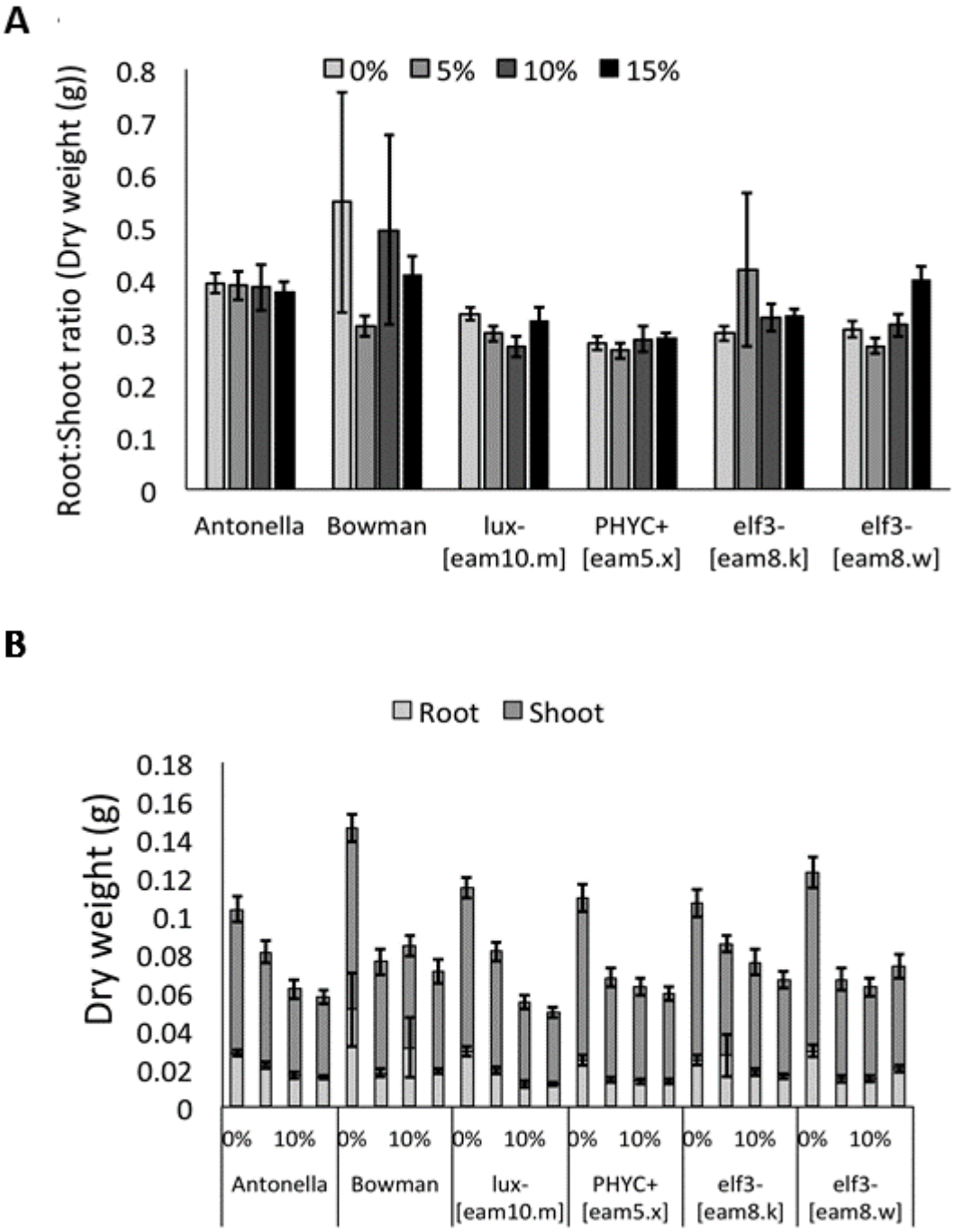


Figure 2.2.8.3 Averages of the Root:Shoot ratios in dry biomass (A) and total dry biomass of the root and shoots (B). In graph B the order of the bars goes from 0% to 15% left to right in each segment. Bar's show the averages and error bars are the standard error of the means (s.e.).

Resource allocation dry biomass patterns reflected how dry biomass distinguished between barley lines, particularly the Bowman parental line from the winter barely

cultivar, while water content allocation was less distinguishing between barley lines and more reflective of osmotic treatment (Figure 2.2.8.3)

Resource allocation was calculated from the dry biomass and water content of root organs relative to shoot organs. The allocation result shows that it is primarily the change in water content of tissues that are behind the response to osmotic stress in all barley lines as expected to correspond to water loss and water loss per organ type. The ratio of dry biomass in eam5.x across stress treatment was the lowest (0.26-0.29) (the majority of the biomass was in shoot tissues), the rest of the bowman lines were within a similar range (0.27-0.36) although eam8.k lines in 15% PEG had the highest ratio (0.39) relative to the other Barley lines, when in most treatments Antonella lines had higher ratios (0.38-0.39g) consistently. As all dry biomass ratios were skewed towards biomass being higher in shoot tissues but winter lines were slightly less bias, while early maturing lines with *HvPHYC* mutation had significantly more biomass accumulation in roots.

The ratios indicate that there were detectable statistical differences in cell biomass accumulation between barely lines (ANOVA $F_{(5,342)}$ = 11.8758, P=<0.0001) that was also independently influenced by osmotic treatment (ANOVA $F_{(3,339)}$ = 4.4106, P=0.00464) while the water content was more strongly influenced by osmotic treatment (ANOVA $F_{(3,339)}$ = 113.7948, P=<0.0001) and less influenced by barley lines (ANOVA $F_{(5,342)}$ = 2.6242, P=0.02404).

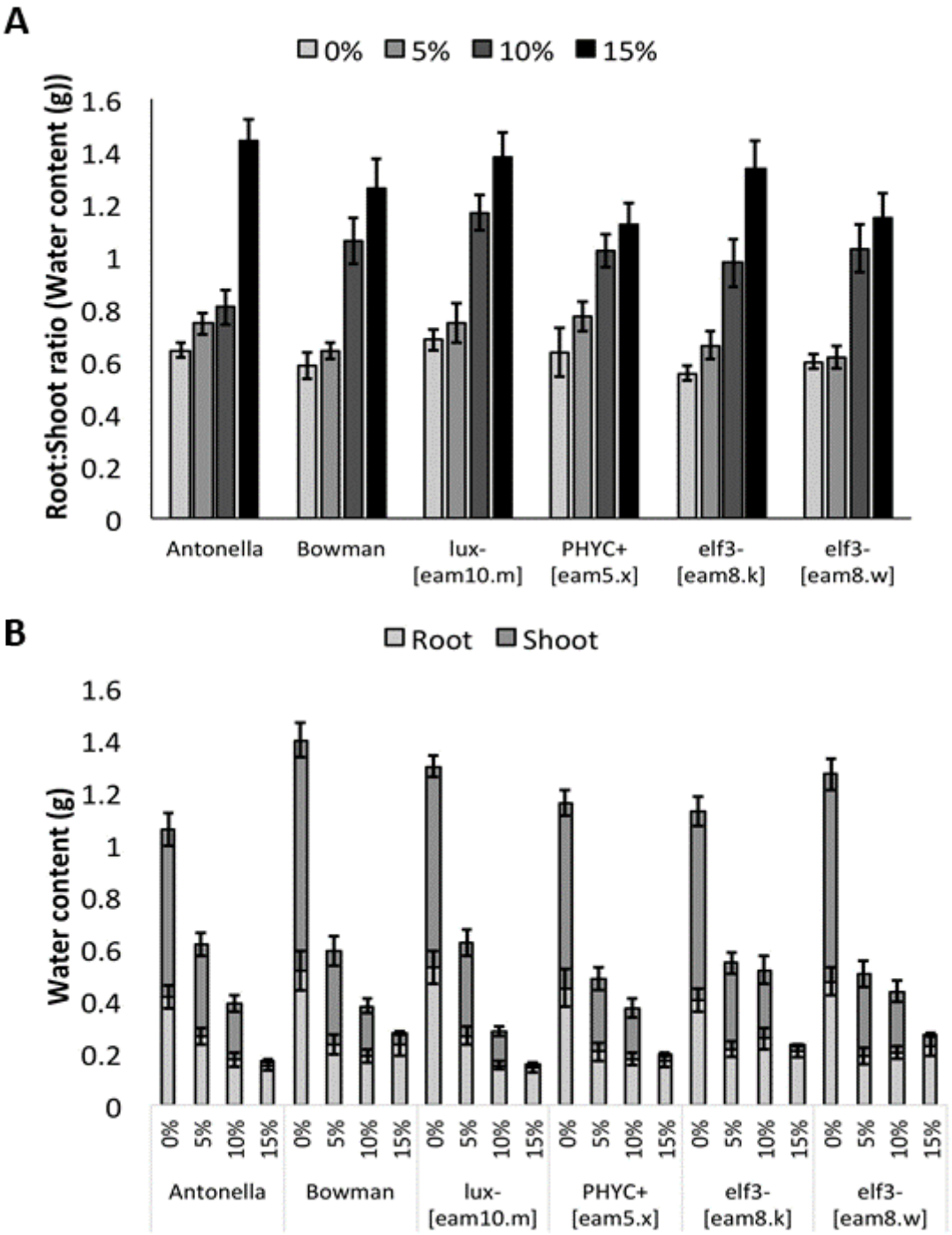


Figure 2.2.8.4 Averages of the Root:Shoot ratios in water content (A) and total water content of the root and shoots (B). In graph B the order of the bars goes from 0% to 15% left to right in each segment. Bar's show the averages and error bars are the standard error of the means (s.e.).

The ratio of water content in 0% and 5% followed a similar pattern to that seen in dry biomass were eam8 lines and Bowman had ratios around 0.55-0.57 in 0% and 0.61-0.68 in 5% that were relatively lower than Antonella, eam5.x and eam10.m in order of lowest ratio to highest ratio respectively in 0% and 5% PEG with a range 0.63-0.66 in 0% and 0.75-0.82 in 5% with eam10.m showing the largest shift in ratio between

treatments. As plants become more stressed the ratios shift more until there is a higher water content in the roots than shoots with 10% being very close to the point when there is approximately equal water in the shoot and root for eam5.x, eam8.w and Bowman lines, while eam8.k and eam10.m lines are more skewed to the roots (>1.1) and Antonella still have more water in the shoot (0.88). By 15% PEG, there is less water content in the shoot than root tissues of all barely lines and Antonella has the highest proportion of water in the roots relative to the shoot (1.44), eam10.m and eam8.k which had the highest ratios in 10% further skew from around 1.1-1.2 to rations of 1.33-1.38, whereas the other barley lines changed to a lesser extent from 1.02-1.05 in 10% PEG to 1.12-1.18 in 15% (Figure 2.2.8.4A).

The data in this section shows osmotic stress affected resource allocation in the same direction for all barley lines in both dry biomass and water content, water content noteably less distinguishing between barley lines compared to dry biomass was.

Root characteristics

Root traits, accumulated lengths, total surface area, average root volume and root analysed from WinRhizo® had detectable differences between barely lines and osmotic treatments.

Root lengths had significant differences between some barley lines, (ANOVA $F_{(5,342)}$ = 4.8994, P=0.000242) the bowman lines were significantly longer (>131cm) (or more variable eam10.m) than Antonella in non-stressed conditions and all plants grown in stressed conditions, while Antonella and eam10.m were significantly shorter in 5% (~47cm and ~70cm, respectively)and 10% (58cm and 48cm) osmotic stress treatment than the other bowman lines which were statistically undistinguished across the stress conditions with Antonella and eam10.m averages were statistically similar to the other lines in 15% and Antonella in 0% PEG(76cm to 123cm). For Bowman lines roots accumulated more length in unstressed conditions relative to stressed treatments (ANOVA $F_{(3,339)}$ = 15.3468, P=<0.0001)

Total surface area was also significantly bigger in non-stressed conditions than the stressed conditions (ANOVA $F_{(3,338)}$ = 42.5867, P=<0.0001). When stressed most plants had statistically similar surface areas, although Antonella and eam10.m in 5% and 10% have statistically different surface area to other lines but the averages are similar to the other lines in the same treatment, with Eam10.m have the highest surface area in 5% and lowest surface area in 10% (ANOVA $F_{(5,341)}$ = 3.2535, P=0.00697).

Average root volume was different between non stressed and stressed plants with volume being highest in Antonella (2.05 cm^3) while most Barley lines were around (1 cm^3 to 1.5 cm^3) and in general higher (>1cm^3) in non-stressed conditions than stressed (>1cm^3) (ANOVA $F_{(3,342)}$ = 7.6847, P=<0.0001). Although averages suggest more overlap

of eam10.m in 5% and 10% PEG (average 1-2.8cm^3), Bowman in 15% PEG (>1cm^3) and Antonella in 5% PEG (>1cm^3).

Finally root tips which indicated number of root hairs was similarly much higher in general in non- stressed conditions compared to all stressed conditions (ANOVA $F_{(3,338)}$ = 26.6371, P=<0.0001), except eam8.k plants which had lower averages within range of the number of root tips in stressed plants. ANOVA analysis detected influence of Barley lines (ANOVA $F_{(5,341)}$ = 2.9374, P=0.0131) and a interactions between Barley lines relative to PEG treatments (ANOVA $_{(15,323)}$ =2.0813, P=0.01058), as the number of root tips for Bowman and eam8.k plants grown in 15% PEG were higher than the average number of average total root tips grown compared to 5% and 10% PEG and thus more similar to plants grown in 0%.

Root tips are not a particularly distinguishing feature and despite statically differences being detected the overlap with means and difficult to understand patterns may come from artefacts.

Water-used relative to the dry biomass of the plant as an estimate of water-use efficiency.

Water –use efficiency is measured by calculating the change in biomass divided by the water consumed over the same periods of time. The measurements do not include pre-treatment dry biomass to calculate a change in biomass to divide against the water-uptake over the course of the experiment. As a replacement, water taken up for a given final biomass of the plant (Water uptake / total dry biomass) was calculated. The larger the value of this measurement the more indicative of inefficient water-use as plants could only use up to 50 ml.

Water-use efficiency as water-uptake per total dry biomass was strongly affected by osmotic stress treatment (ANOVA $F_{(3,344)}$ = 162.16, P=<0.0001) but not the barley lines, as every barley line became increasingly less efficient as osmotic stress became progressively more severe. Although not significant, Antonella water-use efficiency improved relative to Bowman and early maturing lines when plants were stressed, as Antonella efficiency had the lowest average in non-stressed conditions (197.8 ml g^{-1} compared to 209.5 ml g^{-1} -239.8 ml g^{-1}) and second lowest over eam10.m plants in 5% stress (126.8 ml g^{-1} compared to 134.3ml g^{-1} -165.3 ml g^{-1}) to having highest average in 10% PEG stressed conditions (125.5 ml g^{-1} compared to 113.9ml g^{-1} -95.3 ml g^{-1}) and second highest relative to eam5.x in 15% PEG stressed conditions (100.4 ml g^{-1} compared to 93.5 ml g^{-1} -82.2 ml g^{-1}).

2.3.9 Leaf mass area

Leaf shape (width) relative to fresh mass shows a positive association but another leaf trait is the mass of a leaf for a given area which can indicate density of cells, water and leaf thickness

Methods

In order to have information of the leaf mass per area for each genotype, to associate with patterns in other growth traits such as if higher weights could be due to denser leaf material.

Comparisons were only done on non-stressed plants but in both warm (18°C) and cold (4°C) night temperature conditions.

Barley seeds for Antonella, Bowman and fourth early maturing Bowman lines were cleaned in 33% bleach and triton as previously described then allowed to germinate on damp paper towel on petri dishes for one week in constant dark at 4°C. 100 plants were grown in 10L black boxes, capacity of 60 plants per box, with 10L in half strength Hoagland solutions for 3-4 weeks, no fresh water was added, plants were left to grow They were transferred to 10L black boxes filled with half strength hoagland solution (concentrations as previous described in table 2.2.2) and left to grow for 35 days without changing the solution. Boxes were placed in Weiss controlled growth cabinets set to, 60-70% humidity, 12 hour light (300 µmol m^{-2} s^{-1} flourescence) at 20 °C. Micro-centrifuge tubes were weighed and weights recorded before leaf discs were sampled. 3(technical replicates) x 0.6cm^2 leaf discs were from the 3rd leaf of plants into separate micro-centrifuge tubes and the tubes were reweighed. Technical replicates were averaged for each individual plant. These were then averaged to get a leaf mass per area. There was a total of up to 16 biological replicates.

Two to Three leaf discs of the third leaf per plant were sampled using a 0.6mm^2 hold punch (Rapesco PF25A001) near the middle section of the leaf and stored,1 leaf disc per micro- centrifuge. Leaf discs were only taken where the width of the leaf was ≥0.6mm^2. 16 to 17 biological replicates per barley line in each temperature were measured. Due to some errors and thin leafs not all plants were able to sample the two to three technical replicates. The sample size for each barley line and temperature condition are as follows, 43 discs for Antonella 18°C, 46 discs for Bowman 18°C , 40 discs for Bowman eam8.w 18°C , 45 discs for Bowman eam8.k 18°C, 48 discs for Bowman eam5.x 18°C, 47discs for Bowman eam10.m 18°C, 44 discs for Antonella 4°C, 48 discs for Bowman 4°C , 44 discs for Bowman eam8.w 4°C , 45 discs for Bowman eam8.k 4°C, 40 discs for Bowman eam5.x 4°C, 44discs for Bowman eam10.m 4°C.

Total fresh weights between the two night temperatures across the 6 genotypes were compared using two -way ANOVA in R.

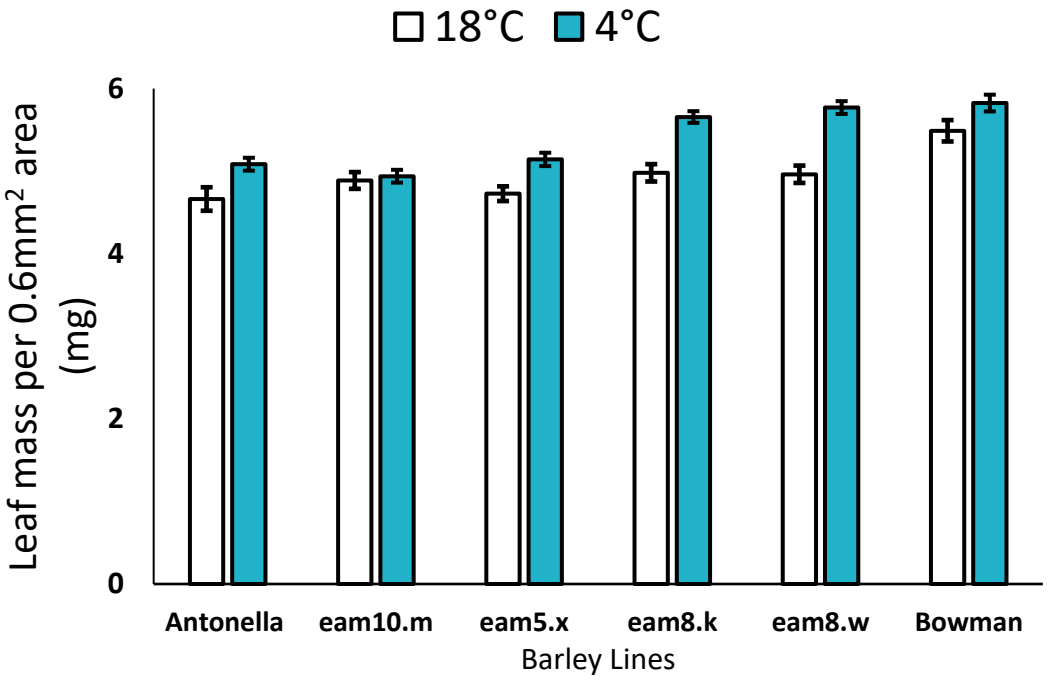


Figure 2.3.9: **Comparison of average leaf mass per 0.6mm^2 surface area of 3rd leaf for 40-45 days old plants in 6 barley lines, winter cultivar Antonella and five lines in spring cultivar Bowman including four early maturing lines, grown in warm night conditions (18 °C, white bars) and cold night conditions (4 °C, dark grey bars). Letters inside the bar graphs indicate significant differences with P value <0.01. Error bars show standard error of the means.**

Results and discussion

Comparing leaf mass per area to see if the barley lines differed in this aspect and if leaf mass per area was affected by temperature showed both factors to have statically significant influence on this trait. Plant genotype influenced leaf mass per area ($F_{5,522}$=21.341, P<0.001). Leaf mass per area at 18°C was significantly higher in Bowman plants compared to all other barley lines, but in 4°C, leaf mass per area was highest both eam8 lines, significantly higher relative to Antonlla, eam10.m and eam5.x but not Bowman, which was significantly higher than the average of eam10.m plants grown in 4°C. Temperature influenced leaf mass per area ($F_{1,522}$=59.28, P<0.001) as leaf mass was less per area when plants were grown in 18°C, this was statistically significant in all lines except Bowman. When grown in different night temperatures the relative average difference in leaf mass per area between barley lines changed patterns ($F_{5,522}$=3.486, P=0.00415), eam10.m and Bowman leaf mass per area was not affected by temperature but all other barley lines had higher average leaf masses per area in cold night temperatures with eam8 barley lines going from low averages in warm

nights to statistically higher averages, even higher than Bowman lines in cold nights (figure 2.3.9).

Cold nights slow plant growth, early maturing lines rapidly growth to flower and the cold nights slowly growth resulted in higher mass in the third leaf. Antonella and eam5.x lines were very similar to each other, and the two eam8 lines almost had similar averages and responses to different night temperatures. Bowman and eam10.m average leaf mass per area were almost unaffected by night temperatures, whereas Antonella and eam5.x lines average were also not statistically different between warm and cold nights but there is a higher average in cold nights.

2.3.10 Chlorophyll

Chlorophyll content has been shown to exhibit circadian rhythms (Pan et al., 2015; Sorek et al., 2014). Photosynthesis rate is proportional to chlorophyll content(Fleischer, 1935)and is influenced by the circadian clock (Dodd et al., 2015; Hennessey and Field, 1991). Water stress inhibits photosynthesis (Tezara et al., 1999). Regulation of chlorophyll content via the circadian clock may influence the effects of water stress in the plant. Therefore I will measure chlorophyll content and photosynthesis rates in barley plants.

The dramatic change in light and temperature accompanying the day and night transitions are powerful environmental signals and it is unsurprising the relationship between light and the circadian clock is often close. Light controls many aspects of plants physiology such as growth and responses to the environment. Unmasking light regulation is the usual way clock gene regulation can be validated. In addition to water being an essential component to photosynthesis and thus one of the main uses of water in plants chlorophylls and chlorophyll related molecules in leaves have been found to follow circadian rhythms in other photosynthesising species such as Arabidopsis, tobacco *(Nicotiana)* (Paulsen and Bogorad, 1988), red goosefoot *(Chenopodium rubrum*)(Chia-Looi and Gumming, 1972), mustard (*Brassicaceae)* (Gehring et al., 1977) and marine dinoflagellates (Prézelin and Sweeney, 1977). Therefore the amount of light pigments in the leaves and how they change over time could be indicative of photosynthetic potential and water use in the plant.

A small time series comparing the amount of leaf pigments in different barley lines grow in warm or cold night temperatures, the collected absorbance readings were used to calculate Chlorophyll a, Chlorophyll b, total chlorophyll, carotenoids concentrations as well as Phacophytinization quatientand chlorophyll a /chlorophyll b ratio. The plants were from the same experiment plants the leaf mass per area

measurements were collected from and both used a 0.6mm^2 hole punch to keep samples standardised.

Methods

Solutions: 80% buffered acetone

To make 80 mL of acetone made up to 100 mL with 20 mL of 2.5 mM sodium phosphate buffer, pH 7.8 dissolve 0.3091g Monosodium phosphate (FW 137.99) and 0.0698g Disodium phosphate (FW 268.1). Adjust with 1uL of 1M Mono and dibasic sodium phosphate (dilute 2mL into 18mL stock to make 20mL 2.5 mM sodium phosphate buffer) to pH the solution to 7.8 and store at 4°C.

In preparation of sampling chlorophyll, a single ethanol cleaned and dried, steel ball (3mm) was placed in each tube in a Quiagen 96 collection tubes (cat 19560) for four sets and stored in -80°C until sampling. Sampling took place at four time points 9AM-10AM, 2:30AM-3:30AM, 5:30PM-6:30PM, 9:30AM-10:30PM. At sampling one 96 collection microtubes was placed in a stereo foam holder with liquid nitrogen, making sure liquid nitrogen filled the collection tubes. Using a 0.6mm^2 hold punch, leaf disks from the youngest developed leaf (4th leaf) were sampled, two per leaf and each leaf disk went into a sperate tube. For each barley line at each temperature and time point 4 new barley plants were sampled.

	Ant		Bow		B290		B289		B285		B284	
18°C	1.1	1.2	1.1	1.2	1.1	1.2	1.1	1.2	1.1	1.2	1.1	1.2
	2.1	2.2	2.1	2.2	2.1	2.2	2.1	2.2	2.1	2.2	2.1	2.2
	3.1	3.2	3.1	3.2	3.1	3.2	3.1	3.2	3.1	3.2	3.1	3.2
	4.1	4.2	4.1	4.2	4.1	4.2	4.1	4.2	4.1	4.2	4.1	4.2
4°C	1.1	1.2	1.1	1.2	1.1	1.2	1.1	1.2	1.1	1.2	1.1	1.2
	2.1	2.2	2.1	2.2	2.1	2.2	2.1	2.2	2.1	2.2	2.1	2.2
	3.1	3.2	3.1	3.2	3.1	3.2	3.1	3.2	3.1	3.2	3.1	3.2
	4.1	4.2	4.1	4.2	4.1	4.2	4.1	4.2	4.1	4.2	4.1	4.2

Figure 2.3.10, illustrative example of 96 micro-tube collection rack layout, the first number indicates the replicate plant number (4 biological replicates per time point) and the second number indicates the technical replicate (2 technical replicates per plant), 18°C (top 4 rows, in darker shades) and 4°C (bottom 4 rows in lighter shades) refer to the temperature conditions at night. Plants sampled were Antonella (Ant, yellow Column 1, 2), Bowman (Bow, green Column 3, 4), eam8.w (B290, blue, Column 5, 6), eam8.k (B289, orange, column 7, 8), eam5.x (B285, red, column 9, 10), eam10.m (B284, purple, column 11, 12).

While the collection rack was still in liquid nitogen without the 96 well lid coverings on, the collection tubes were stored in -80°C until the liquid nitrogen evaporated off. Lids

were added to the microbe tubes in the freezer after 24 hours. After freezing the collection tubes were carried on ice to a mixer mill (Retsch, MM300), shaken for 15s, turned over, shaken again at the highest frequency. In a cold room 4°C, 1ml of ice cold 80% buffered acetone was added to each microtube. Lids were placed securely back on, the collection tubes were shaken vigorously by hand as they were taken to -20°C freezer where they stay for up to 30 hours with occasional hand shaking, until all the chlorophyll was in solution.

Once in solution collection tubes were Spun down for 5 minutes at 500xg (rcf) at 4°C then transported on ice in a covered Styrofoam box to the spectrophotometer (thermos scientific Evolution 60 V4). 80% Acetone buffer blanked the machine that was set to measure Absorbance at the following wavelengths 750 nm,415 nm,435 nm,470 nm,480 nm,663 nm,663.2 nm,663.6 nm,645 nm,646.6nm ,646.8 nm to calculate Chlorophyll, carotenoids and phaeophytins based on published work which had used 80% Acetone buffer. In each clean curette 500 µL of the sample was used for 1 reading.

Table 2.3.10 chlorophyll calculations and published references

Trait	Abbreviation	Unit	Calculation	Reference
Chlorophyll a	Chl a	µg/mL	12.25 ($A_{663.6}$) - 2.55 ($A_{646.6}$)	(Porra et al., 1989)
Chlorophyll b	Chl b	µg/mL	20.31 ($A_{646.6}$) – 4.91 ($A_{663.6}$)	
Total Chlorophyll	Chla+ b	µg/mL	17.76 ($A_{646.6}$) + 7.34 ($A_{663.6}$)	
Chlorophyll ratio	Chla/b		Chl a/Chl b	
Carotenoids		(µg/ml)	(1000 (A_{470}) – 1.82 (C_a) – 85.02(C_b))/ 198	(Lichtenthaler, 1987)
Phacophytinization quatient	pQa		A_{435}/A_{415}	(Sujetovienė, 2013)

Results and discussion

The relative concentration of chlorophyll pigments over 12 hours in barely lines grown in warm and cold night conditions was compared (Figure 2.3.10a). Results are presented in a box and whisker plot as the sample size was small. Results indicate higher amounts of chlorophyll pigments in Antonella lines compared to the spring lines at most points during the day except 5PM in both temperatures. The relatively higher concentrations of chlorophyll pigments in Antonella lines is most pronounced in the morning (9AM) to after noon (2PM) in plants grown in 4°C night temperatures. Additionally cold nights seems to increase Chlorophyll a concentrations and reduce variance in chlorophyll pigment concentrations and ratio compared to measurements taken from leaves grown in warm nights. There is an overlap in the range for most

barley lines to each other and across time. Only comparing averages (the x in the box) Antonella have higher concentrations of chlorophyll at 9AM (1 hour after lights have turned on) and concentration falls as the day progresses, whereas the average chlorophyll concentrations in spring barley lines rise slightly in the evening (5PM), this was most pronounced in eam8.w lines (Figure 2.3.10a E,F,G and H). In general cold nights slightly elevate chlorophyll concentrations and increase differences between time points. In Arabidopsis simulating temperature oscillations (using a gradual rise and falls in temperature over 12 hours with a temperature difference of ~7°C) for plants grown in laboratory conditions improves gene expression to me more like expression patterns of plants grown in field experiments (Song et al., 2018). The growth chamber plants were grown in for this project acclimates to the new temperature settings within an hour of switching to a new cycle, however the plants were grown in black 10L boxes with the 10L of water acting as a temperature buffer taking hours to change temperature resulting in roots being at a gradually warming temperature while shoots were in a relatively steady 20°C.

Alongside chlorophyll pigments, carentoid concentrations were calculated. Carentoids absorb wavelengths ranging from 400–550 nm and protect against photo-damage, particularly for chlorophyll. Results suggest concentrations of carentoids were highest from morning to afternoon, then started to decline by the early evening (5PM) before becoming almost completely absent after lights were turned off (9PM), figure Figure 2.3.10b A. Like chlorophyll pigments there is a tendency for average values to be higher and less variable when plants experience cold nights in the morning (9AM) although this distinction declines at the day goes on. Different to Chlorophyll it appear that plants grown in warm nights peak their carentoid concentrations in the afternoon (2PM) rather than 5PM. Light in the growth chambers is relatively constant throughout the light period of the diurnal cycle, so plants are not exposed to higher light intensities of midday sun nor the lower light intensities from suns lower in the sky, such as during long day photoperiods. The difference in diurnal light intensities is due to the angle of incidence affecting the spread of solar radiance over an area. Plants in growth chambers were growth in long photoperiod conditions. At 5PM light intensity is weaker, therefore this time of day in natural light would be an opportunity for plants to maximise light capture with increasing chlorophyll concentration per area of their leafs with less risk of photo-damage. Although the patterns are not strong in the collected data there is a suggestion of such an expected high concentration of carentoids and lower Chlorophyll after an approximate midday time point (2PM) to protect against high light intensity. Antonella having their highest concentrations of chlorophyll in the morning an hour after lights on, with Bowman cultivars tend to have their highest concentrations in the evening three hours before lights off could be a hint of a small circadian influence in barely plants and difference between winter and spring cultivars.

Phaeophytin is a product of chlorophyll breakdown, thus the Phacophytinization process can be indicative of environmental stress, or chlorophyll degradation during the extraction process. The Phacophytinization quotient refers to the ratio of

chlorophyll a to phaeophytin a, with lower values indicating more phaeophytin relative to chlorophyll. At every time point and in both temperature conditions the ratio was greater than 1.2, Figure 2.3.10b B. For plants grown in cold nights, the ratio stayed consistently close around 1.3 to 1.35 for the barley lines, whereas plants grown in warm nights showed more variation, most notably at 2PM when ratios increased indicating a greater reduction in phaeophytin a in samples that may coincide with the higher concentration of the protective carentoid pigments.

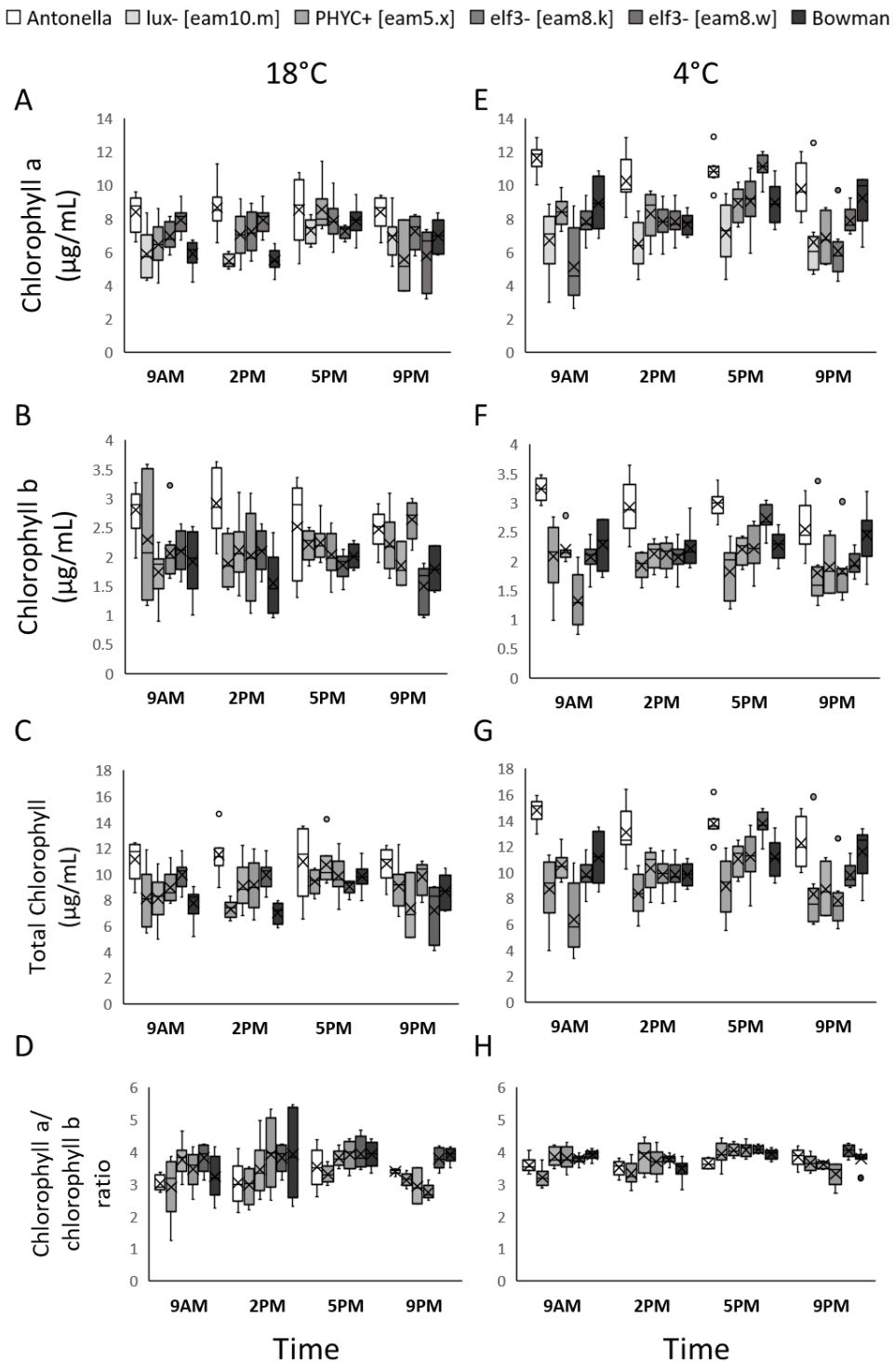


Figure 2.3.10a Comparison of Chlorophyll in the third leaf of barley plants grown in warm (18°C, left: A,B,C,D) or cold (4°C, right:E,F,G,H) night conditions over 12 hours in diurnal conditions. Pigments measurements were calculated from absorbance readings from 0.6mm^2 area in 1mL 80% buffered Acetone. The Chlorophyll measurements compared were chlorophyll a (A,E), chlorophyll b (B,F), total chlorophyll (C,G), and chlorophyll ratio a/b (D,H). Each bar indicates a different Barley line for each measured time point.

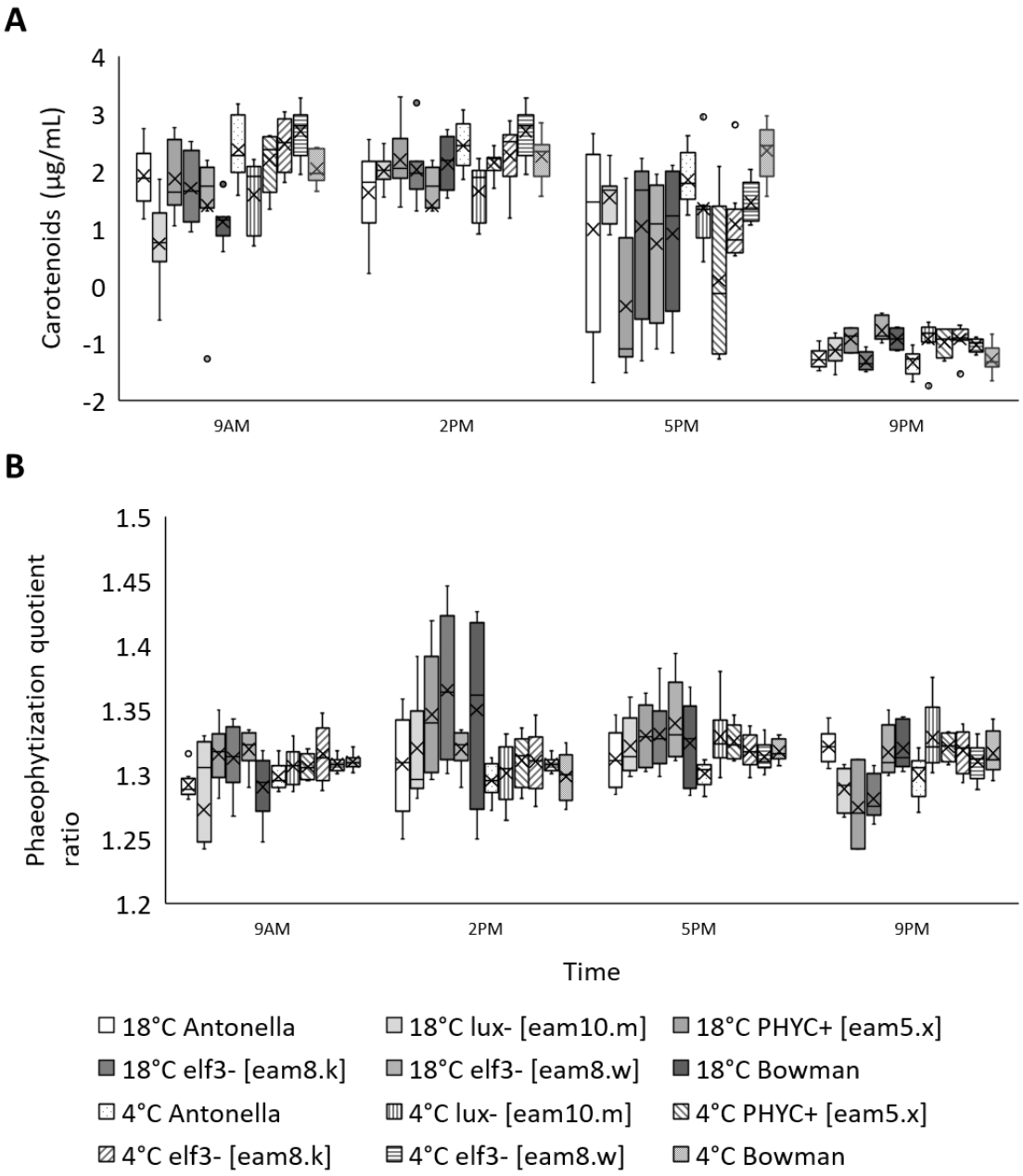


Figure 2.3.10b Comparison of leaf pigments carentoids and phaeophytization quotient ratio in the third leaf of barley plants grown in warm (18°C, left: A,B,C,D) or cold (4°C, right:E,F,G,H) night conditions over 12 hours in diurnal conditions. Pigments measurements were calculated from absorbance readings from 0.6mm^2 area in 1mL 80% buffered Acetone. The Chlorophyll measurements compared were chlorophyll a (A,E), chlorophyll b (B,F), total chlorophyll (C,G), and chlorophyll ratio a/b (D,H). Each bar indicates a different Barley line for each measured time point.

In conclusion, based on the data collected and small sample size, it is insufficient to conclude there were clear differences in leaf pigments between barley lines grown in cold or warm night over time. Carotenoids for all barley lines were almost completely absent in leaf discs sampled after light turned off. The ratio if Chlorophyll a and b was similar and consistent across barely lines and temperature conditions.

2.4 Discussion and conclusions

This chapter documented various preliminary work undertaken and general methods chosen as part of investigating the physiology of barely cultivar Bowman circadian mutant lines. Changing seed cleaning methods and new batches of seed improved germination success, and demonstrated 5 to 6 days at 4°C in the dark to stratify seeds improved germination success across barley lines.

The differences in Zadok growth stages across developing tillers of the barely lines at 60 days old was demonstrative of the speed of development of early maturing lines. Development rate has a potentially large effect on the plants water regulation, water demand increases as plant stems elongate and the plants canopy rises, yet speed of development could also impair max potential growth of leaf sizes or other rushed steps that may weaken a plants structural integrity. An observational note, when early maturing lines are grown in loam soil without additional clay or sand, or just grown in Levington F2 +sand soil without extra sand, the plants tended to have more flexible and leaning stems and lighter drooping leaves, whereas the slower developing bowman lines were firmer and straighter plants.

Root measurements in section 2.3.3 show eam8.w barley lines start with fewer and shorter primary roots compared to other bowman lines. In section 2.3.8, root characteristics of older plants were analysed and resulted suggested in most root morphology traits including root lengths and surface area there were not necessarily detectable differences between the matured barely lines. Root traits response to osmotic stress there could be potential interaction affects between barley bowman lines and osmotic stress. Based on this preliminary work more root analysis was done in chapter 3.

Leaf morphology can have an indirectly influence water transport by regulating the surface area from which plants interact with the air. Leaf number, widths and mass per area were measured for most bowman lines in sections 2.3.5 and 2.3.9. Leaf number and leaf widths were compared to the total fresh mass of the plant, to compare measurements which can give a more detailed image of a plants morphology to a measurement that encapsulates the plant in its entirety as either large or small. Leaf number while informative of growth stage if information on tiller number is also recorded, does not give information on the width or length of the leaf which is informative to know to compare surface area of the leaf canopy and potential area

from which water can be lost through the plant. An improved method would have been to scan leaves on a scanner to get an image and measurement of the total matured canopy at the time of measurement for each plant. In the interests of time constraints and sample dehydration, measurements were simplified to recording mass of the plants to compare. Furthermore, leaf mass per area was similar across all barley lines except Bowman in warm growing conditions (20°C/18°C) where Bowman lines have a heavier mass per leaf area. Growing in warm/ cold cycles (20°C/4°C) did not statistically significantly increased leaf mass per area in Bowman, but did in leaf mass per area in both eam8 lines. Cold night temperatures elevated the leaf mass per area in all barley lines except eam10.m. Leaf mass per area was not recorded for measurements in chapter 3.

Leaf morphology traits were highly variable as were leaf stomata density, leaf pigments, tiller number, plant heights and yield. Although a seemingly clear result of eam10.m and both eam8 lines having a higher stomata density at the base of the third leaf, unpublished work by Dr Stevens (2019) in the same Bowman lines found density alongside other stomata traits to be variable from leaf to leaf, leaf section to leaf section and changing with plant age (section 2.3.6). Plant age was similarly an important influence on average tiller number, tiller heights and seed yield when comparing bowman lines (section 2.3.4) and development rate could be influenced by seasonal influences in the glass houses. Measuring plants later tended to shown Bowman parental lines would grow stems longer than the early maturing lines and Bowman lines tended to produce high seeds per head, high total number of seeds and high total seed weight, only eam10.m plants appeared to produce as much yield. Data presented at the Monogram conference 2018 by Monika Spiller of Syngenta also suggested in field studies that eam10.m plants could compete with Elite barley cultivars. Although their work also suggested in different geographic conditions, (changes in soil and climate) that eam8 lines could produce more seed by weight than Elite barley cultivars on the market or severely underperform. This data is interesting as it suggests a change in adaptability in the mutants with genetic defects in their circadian clock systems as well as relating to my observations of how differently the early maturing plants grow depending on soil. An interesting analysis could be to examine the lignin and silicon composition of bowman line stems when grown in different soils. As tillers complicate measurements, to compare water regulation I focused on establishing plants around 2 to 3 weeks old, at the age plants could typically depend on their seed stores for nutrients.

Alongside simplification, smaller plants fit into 50ml centrifuge tubes from which plants could be grown in nutrient solution and water uptake by the plant could be measured by the drop in water volume inside the centrifuge tube (section 2.3.7). Using this method water uptake for some Bowman lines was compared with different osmotic stresses. 15% PEG had a big effect on water uptake for all lines and in non-stressed plants there appeared to differences in water-uptake between lines. In order of plants with the highest water uptake to lower water uptake in 0% PEG they were as follows,

eam8.w, Bowman and eam18.m and then Antonella and eam8.k. The difference between the eam8 lines is interesting as both have mutations in *HvELF*but eam8.k is a single point mutation and eam8.w has large deletions, insertions and deletions. *ELF3*among other circadian clock genes have temperature –dependent splice form, warm temperature dependent splice forms in Arabidopsis (Gil and Park, 2019; Kwon et al., 2014). Circadian genes in barley have also been shown to have temperature sensitive splicing in HvCCA1 and HvPRR37 (Ppd-h1) to cold temperatures (Calixto et al., 2016). This experiment was grown in warm cycle conditions, and although eam8 plants show dampened rhythmicity (Dakhiya et al., 2017; Faure et al., 2007) that does not mean all forms of the gene have lost function. This method will be used more in chapter 3 with a similar analysis to section 2.3.8 leaving out leaf width measurements.

My preliminary results have not provided strong evidence of clear differences in physiology between genotypes that could not be potentially be explained as an indirect effect of development rate or too variable to make a judgment with the low sample sizes and experiment repeats in this chapter. Chapter 3 focuses on water-uptake, growth as changes in weight and biomass, resources allocation and root traits in young barley plants when challenged by different osmotic conditions and changes in night temperatures.

Chapter 3

3.1 Introduction

3.1.1 Circadian clock and water regulation

Water regulation is a critical aspect of a plant's survival and growth. This is a key subject of concern as climate forecasting predictions model drastic changes in water distribution and availability in the future, expected predictions suggest that wet regions will become wetter and dry regions become drier (Liu and Allan, 2013) both in frequency and duration. This is all alongside a rise in unpredictable extreme weather events with long dry spells followed by heavier rainfall and flash floods, with potential assoiations of changing fresh water reserves and viability of current agricultural land (IPCC, 2014). Circadian clock genes have been attributed to universally controlling key agricultural traits (Bendix et al., 2015) via transcriptional activity and protein function. The circadian clock has been postulated as a target for manipulating traits and productivity in food crops and overcoming environmental stress. A plant's growth is balanced by its capacity to maintain homeostasis against the negative roles of perturbations, stress, damage and disease. The role of circadian rhythms in water-regulation for dicotelydons has been described for water-use efficiency, stomata movement, aqua-porein recruitment and leaf canopy transpiration (Dios and Gessler, 2017; Gorton et al., 1989; Resco de Dios et al., 2016; Simon et al., 2019; Takase et al., 2011). Therefore this project hypothesised significant changes to barley's water-use (as uptake) and consequentially growth (as biomass) when the plants contained clock gene mutants.

3.1.2 Comparing Barley to the model system Arabidopsis

Mutations in circadian clock genes have been shown to influence water-use efficiency in Arabidopsius (Simon et al., 2019). There is conservation of clock genes and gene networks (Campoli et al., 2012a; Song et al., 2010). There are similarities in the *diel* regulation of the transcriptome between monocot and dicot species (Filichkin et al., 2011). There have been observations showing internal circadian clock regulated rhythms synchronising with external circadian signals associating with improved physiological performance and growth in numerous plants (Greenham and McClung, 2015; Harmer, 2009). There also appears to be some conserved functionality of circadian clocks between dicotelydons and monocotelydons, however this cannot be assumed. As demonstrated by comparing circadian regulation of carbon metabolism and growth in Arabipodisis and barley (Müller et al., 2014). Firstly, growth in Arabidopsis and other dicotelydons is rhythmic while in barley it is continuous (Poire et al., 2010; Walter et al., 2009). Secondly, there are two different pathways which regulate supply of carbohydrate substrates, one for starch and one for sucrose.

Storing sucrose or starch as a carbohydrate source for when plants are not photosynthesising can be species specific. Arabidopsis primarily uses the starch pathway, whereas barley primarily uses the sucrose pathway. Thirdly, the starch pathway is circadian clock dependent, while the sucrose pathway is not which underlines the difference between Arabidopsis rhythmic and Barley's continuous growth (Müller et al., 2014). Furthermore, studies have shown poor viability arrhythmic *Atelf3* mutants due to negative phenotypic responses to light, and additional sensitivity to other stresses (Green et al., 2002) and there is a visible difference to the severity of the phenotypic change comparing *Atelf3* to *AtELF3* and *Hveam8.w* to Bowman parental background *(Hv.EAM8).* In Arabidopsis the evening complex, which is a complex of proteins formed from the combination of the clock genes *LUX, ELF3* and *ELF4,* is an integral part of the clock with direct regulation of plant growth processes including water-use efficiency (Dixon et al., 2016; Helfer et al., 2011; Nusinow et al., 2011; Thines and Harmon, 2010). The evening complex is implicated to be connected to water-transport in the roots of Arabidopsis (Takase et al., 2011) and osmotic stress detection and response in barley via the *HvELF3* gene (Habte et al., 2014a). The circadian mutants in this project are orthologs to genes in Aradiposis that work in the evening complex (*ELF3* and *LUX*) or along the same pathways (*PHYC*).

The expectation of different water-use and growth outcomes can be understood by observing the morphological and development differences between *Atelf3* to *AtELF3* and Bowman *eam8* (*Hvelf3*) compared to Bowman parental line (*HvELF3*) as in figure 3.1.1. *Atelf3* mutant is smaller plant, has smaller and fewer leaves and already bolted compaed to *AtELF3. Hvelf3 plants are very similar to HvELF3 plants except they* have a more developed 4^{th} and emerging 5^{th} leaf compared to *HvELF3* where the 4^{th} leaf is emerging after growing over the same period of time. This could have some water regulatory impacts (e.g. greater water demand in the mutant) but the difference between plants is less drastic and can be mitigated by other mechanisms (e.g. stomata or uptake at the roots). To develop a picture of how clock mutations affect water-use the water-uptake of barley cultivar Bowman was compared alongside other growth traits.

Figure 3.1.1: Photographs of (TOP ROW) same age *Arabidopsis thaliana* (Wassilewskija (WS) background) grown in pot soil, in the same size pot (12cm diameter) in 16 hour long days for 4 weeks in 20°C, showing *elf3-4* (left) and Wild type ELF3 (WT) (right), photographs taken 2014, uncropped. (BOTTOM ROW) same age Barley (Bowman cultivar background) grown in hydroponics with half strength Hoagland solutions for 4 weeks in 12 hour long days in control growth cabinets, top row grown in 4°C night temperature and bottom row grown in 18°C night temperatures, showing Eam8.w (left) to parental line (right), photographs are cropped and the clear rule shows scale.

3.1.3 PHYC and temperature regulation

As well as water regulation there is evidence of thermoregulation and temperature-sensitive stimulation and repression of the evening complex (Mizuno et al., 2014). PHYC has been shown to contribute to clock period regulation in a temperature-

specific manner under white light (Edwards et al., 2015) as part of a upstream convergence of light and temperature signalling to the clock. Together the PHYC signalling pathway to the clock could be the mechanism of thermo-regulation on the evening complex. Thus over expression of *HvPHYC* may enhance temperature dependent regulation on the evening complex, impacting water regulation. Interestingly, *AtPHYC* and *HvPHYC* are another instance of differing functionality between dicotyledons and monocotyledons. Arabidopsis has five phytochromes (A,B,C,D,E) with PHYC having less of a role comparatively to the other 4 (Takano et al., 2005). In barley there are only three phytochromes (A,B,C) with PHYC having much more of a role to play in far-red light signalling, photo-period detection and seasonal flowering responses (Chen et al., 2014; Nishida et al., 2013; Woods et al., 2014).

3.1.4 The experiment system

3.1.4.1 Plants

Currently there is not a lot of physiological data on these early maturing barley mutants, particularly in regards to water-regulation and this chapter aims to characterise the similarities and differences of these mutants relative to the Spring parental line, where they all share *ppd-h1* Bowman backgrounds (Faure et al., 2012) making the early maturing mutants in clock and photo-period sensitive genes. The ppd-h1 allele is known to reduce photoperiod sensitivity, resulting in the barley flowering during long days, a huge development phenotype change. The early maturing barley mutants were identified by even faster maturity phenotypes, and are expected to have more phenotypic differences, which this study explores if those differences may relate to water regulation and temperature responses. The spring cultivars were statistically compared to a winter cultivar Antonella with Ppd-h1 alleles as a comparison of spring versus winter plants. Originally Antonella TILLING lines were being investigated in the Maria von Korff Lab at the Max Planck Institute at the time of this research looking for distinctive phenotypes and the parental line was added into these experiments to potentially be data that could be groundwork for any investigations involving the developing Antonella library by growing Antonella alongside Bowman a recognised mutant library cultivar. Despite work on the Antonella TILLING lines having setbacks, I kept the data for the winter cultivar in my analysis due to what I felt were interesting observations when clock mutants had similar growth and water regulation phenotypes to the winter cultivar rather than the parental line.

3.1.4.2 Hydroponics and osmosis

In order to reduce environmental variables – such as soil characteristics that can impact plant growth, hydroponics was used. Water is made up of mobile molecules. The tendency for water to move from one area to another is quantified as the ‘Water

Potential' with the unit Psi or Greek letter Ψ. Water potential is caused by gravity, mechanical pressure, matrix effects (surface tension, capillary action) and osmosis. Osmosis is the process of solvent molecules, passing through a semi- permeable membrane from an area of low solute concentration into a more concentrated area. Like diffusion the chemical composition of the environments drive the net flow of movement except osmosis is for solvent and requires a semi permeable barrier. The larger the difference between the concentrations of the area the higher the rate or net movement is. Typically for plants, water is the solvent and cell walls and membranes are the barriers. In ambient conditions, the environment within a plant cell has a higher concentration of solutes in their supernatant than the external environment that water can passively move into the cell. Plants avoid concentrations reaching equilibrium by regulating solute concentrations in cells and transporting water away from the roots.

External solutions may be described as hypertonic when the external environment has a high concentration of solutes than inside, resulting in higher osmotic pressure and movement of water from inside to outside.

When the concentration is equal on both sides of the membrane, the outside solution is described as isotonic and rate of molecule movement in both directions across the membrane is the same with little difference in osmotic pressure across solutions.

Hypotonic solutions have a low solute concentration and a lower osmotic pressure resulting in net movement of water into the hypertonic solution.

The osmotic potential is the potential of water molecules to move from a hypotonic solution to a hypertonic solution through the semi-permeable membrane, the greater the difference between the solutions the faster the rate of movement.

Osmotic pressure is a measure of the attraction of solutes for water. Osmotic pressure is thus defined by the hydrostatic pressure applied by a solution that would prevent inward flow of water across the semi-permeable membrane.

Osmotic stress occurs when a sudden or extreme change in solute concentration around a cell results in a rapid change in the movement of water across the membrane.

3.1.4.3 Plant cell adaptations to osmotic changes

Cells described to be in hypertonic condition have movement of water from the cells and "shock" is caused by the inhibition of transport into the cell. When there is too much movement of water into the cell, the cell can burst or undergo apoptosis. Plant cells are surrounded by cell walls, which protect against structure loss and cell damage. Excessive external movement of water from a cell causes it to become flaccid, continuous loss and eventually the cell membrane shrinks away from the cell wall

(plasmolysis) but the membranes adhesion to the more rigid cell wall prevents complete detachment. When more water is moving into the cells than out, the cell wall prevents bursting by limiting the swell of the cell and the cells become turgid. The majority of water inside the cell is stored in the vacuole. Water can be moved through cells via the cell-to-cell and and apoplastic pathways.

Water impermeable structures such as the Casparian bands and suberin lamellae in endodermal and exodermal cell walls of root tissues can block water movement and forcingredirection. The Casparian bands contributes to producing root pressure by forcing water from cell walls of the cortical layer of the roots into the cytoplasm of endodermal cells (inner cell layer that separates the vascular tissues from the cortex) that surround the pericycle. The pericycle is a layer of cells that encircle the vascular tissue in stems and roots. The pericycle provides support, structure and protection for the plants xylem and phloem in the stem and hold the plant upright. It is a primary tissue that maintains meristematic activity, is involved in lateral root initiation and development and secondary root growth contributing to the formation of the root system and giving rise to two secondary meristems in roots, the vascular cambium and cork cambium (Dubrovsky and Rost, 2012). In monocotyledons the pericycle is the generating layer of vascular tissues throughout the plants life and alongside the endodermis tissues have a meristematic function in roots, stem and leaves (Menezes et al., 2005). Alongside increasing the transverse osmotic pressure within cells before water enters the vascular tissues helping the sap rise through the stem, the forced movement of water into cells from the space between the cell wall and cell membrane helps the directional diffusion of water towards the stem in the apoplastic pathways and maintains the higher osmotic pressure (higher solute concentration) inside the cell wall, favouring osmosis into the cells. Furthermore moving water from the free flowing, passively, diffusing apoplastic pathway to the cell-to-cell pathway increases directional control over water flow as aquaporins can pump water molecules against osmotic gradients.

3.1.4.4 Creating controlled osmotic stress

Polyethylene glycol (PEG) can simulate limited water availability by manipulating the osmotic pressure outside the roots, changing the osmotic gradient and decreasing the ability of plants to passively intake water as solute concentration is increased in the external environment. Drought, freezing and salinity stress are also water limiting stresses that prevent or challenge water intake into the roots, or even cause passive diffusion from the roots into the soil but have additional stresses or effects such as temperature related damage or influx of salts, meaning the effects of these phenomenon are a combination of stresses that interact with water regulation. Large molecular weights of Polyethylene glycol such as 8000 PEG can increase the solute concentration but molecules are too large to be absorbed by the plant tissues. Over time even high molecular weight PEG can degrade and can eventually be absorbed by plants where the deposits may block vascular tissues and intracellular spaces changing the hydrology of the tissues and organs relative to other parts of the plant (Jacomini et al., 1988; Yaniv and Werker, 1983). PEG can also cause hypoxia (Verslues et al., 1998) due to decreasing oxygen movement by increasing solution viscosity. Compound effects of PEG and hypoxia can negatively affect root elongation as has been shown

with PEG induced osmotic pressures of -0.3MPa to -1.6MPa (Verslues et al., 1998). From observation, over time roots grown in PEG accumulate an off-white layer, with roots stiffer and shorter than plants grown without PEG (Figure 2.2.3). Regardless of the drawbacks PEG is still a frequently used method to impose uniform low water potentials on root systems and has been used to test the effects of osmotic stress on the barley circadian clock in the Bowman mutant lines previously with 20% PEG for up to 5 days (Habte et al., 2014a).

3.1.4.5 Importance of night temperatures in circadian rhythms and water regulation

In addition to water regulation and osmotic stress response, the effects on physiology of night temperatures, specifically warm nights (18°C) and cold nights (4°C) relative to warm (20°C) day temperatures were compared, mainly on the hypothesis that larger temperature oscillations co-ordinated with the light/dark cycles would create a larger signal to the barley clock and physiological differences between mutants and the parental line would be more clear. The interplay between photo period and temperature in barley has been noted to be complex, long days and warmer temperatures (25°C compared to 15°C) accelerated development, whereas the opposite was reported in short days (Hemming et al., 2012) and a difference in 2 °C in thermos-cycles compared to constant temperature could significantly delay flowering Barley (Karsai et al., 2008) as reviewed in (Capovilla et al., 2014) and 2 °C have been used to grow and entrain barley plants in other studies (Habte et al., 2014a). Barley (temperate cereal) preferentially prefers colder latitudes (Leff et al., 2004) and is typically sown in spring (March and April), in the fertile crescent for example March temperatures can range from 4-6°C minimum to 17-19°C maximum in Syria,(Climatestotravel.com, 2019) although more northern Latitudes regions such as Scotland spring temperatures are still low with maximum March temperatures around 10°C. This chapter will look at the effects of 2°C differences and 16°C differences in diurnal temperature cycles with and without osmotic stress.

3.1.4.6 Scope, expectations and aims of the experiment

Before examining more refined aspects of water regulation like stomata responses or Aquaporin recruitment or responses under constant light, the focus in the studies described in this chapter primarily focused on plant level differences in diurnal conditions between barley circadian mutants and their parental line in the spring cultivar Bowman, alongside a winter cultivar Antonella. The aim being to establish whether having a functional clock was essential for 'normal' water-uptake and growth phenotypes. Based on fluorescent measurements recorded by Dr. Rachel Green laboratory (Hebrew University, Jerusalem) and depicted in Figure 3.1.2, the barley circadian mutants have weaker (*Hvlux*) photosynthesis rhymes, arrhymic photosynthesis activity (*Hvelf3*) to almost non-existent (*HvPHYC*) endogenous photosynthetic activity. If strong differences were detectable at this level between the lines it could be indicative that process linked to water-use in barley does have a strong association circadian regulation. Water-stress inducing stomata closure limits gas exchange and thus photosynthesis is well studied, how the observed changes in the photosynthesis patterns in the circadian mutants by Dr. Green's group will relate to the

plants water-regulation is examined. Results in 2.2.8 stronly suggest barley plants in the same genetic background (Bowman) uptake water at a similar rate and consume relatively more water than Antonella. Perhaps the clock in Barley has influence on Photosynethsis, while water regulation is more environmentally regulated like carbohydrate metabolism.

Approaching the question of how circadian mutants in barley adapt their water-use was investigated by measuring water uptake and growth responses to varying degrees of induced and chronic limited water availability, simulated as increasing osmotic stress.

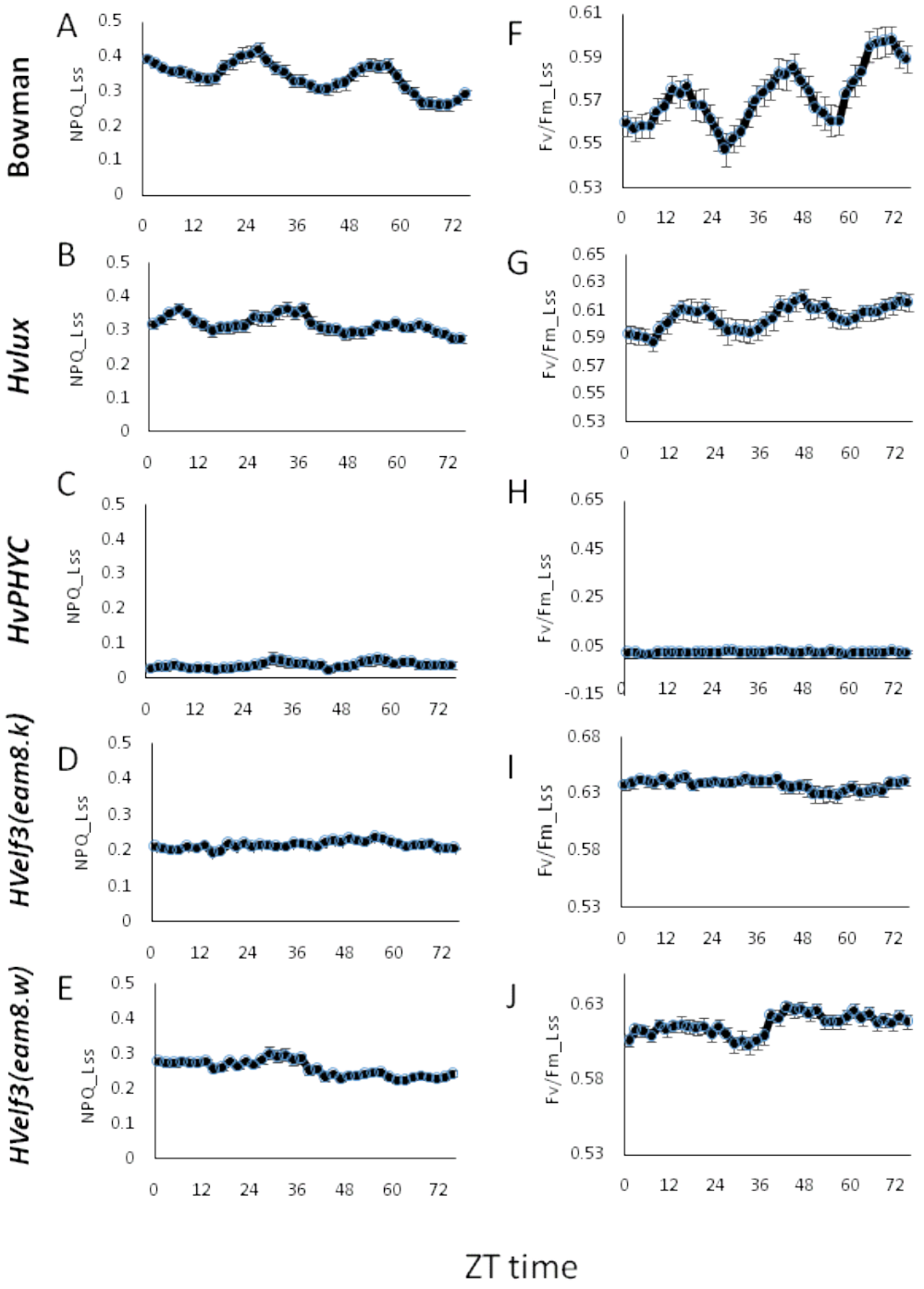


Figure 3.1.2 Arrhythmicy in early maturing mutants relative to the parental line represented by changes in **(left)** Non-photochemical quenching is measured by the quenching of chlorophyll fluorescence (Fm/Fm′) − 1 (NPQ_Lss) and **(right)** Maximum quantum yield of PSII photochemistry measured in the dark-adapted state (Fv/Fm_Lss) over time (ZT) of 75 hours of Bowman parental line **(A,F)** and early maturing mutants, *Hvlux*-**(B,G),** *HvPHYC+* **(C,H),** *Hvelf3*- (B289) **(D,I),***Hvelf3*- (B290) **(E,J),** sample number (n=18) and Error bars are the standard error. Seeds were given to Dr. Rachel Green (Hebrew University, Dept Plant and Environmental Sciences, Jerusalem, Israel) and plants were grown in their laboratory, were measurements were taken and data sent back.

The main physiology traits considered in this chapter are growth traits based on fresh mass and dry mass of the whole plant or shoot and root tissues, as well as change in fresh mass before and after treatment. The overall growth of the plant by change in weight is typically a good indicator of plant health with dry biomass indicating the number of cells plants have made and thus an indicator of converting energy into mass. Water measurements comparing total water taken up by the plant over seven days, the water content calculated from subtracting the dry mass measurements from the fresh mass measurements. Water-uptake provides an impression of water demand by the plant and transpiration. While water storage may show which plants on average retained more water.

Resource allocation based off of ratios calculated by dividing the root dry mass and water content measurements by the equivalent shoot measurements to provide an impression of where barley lines invested the majority of their energy into growth and if this differed between barley lines. As plants are grown in hydroponics it is expected investment into root mass will be lower as roots are not drying out or having to search for water in soil. Root morphology traits as analysed and calculated in WinRhizo® software. As roots were grown in hydroponics, it was expected to have influence on morphology. Water is absorbed through the roots via diffusion and active transport, requiring intimate contact between water molecule and root cells. To increase contact and thus absorption or to seek water, plants produce more root hairs. Plants can control direction of root growth towards water sources.

In hydroponic conditions plants are submerged in water and are in contact with water constantly (unless water levels decrease too far without being replenished), thus removing the need to "seek" water in their environment or the influence of soil texture to the roots ability to access oxygen or growth patterning. This could mean be the resulting root architecture is more genetically determined. The environmental factors that could affect root growth would be the osmotic potential of the water, the temperature and the light. The experiment design did not exclude light penetrating the tubes the plants grew in. Light (sunlight) can penetrate soil but intensity is greatly reduced, light is fragmented and scattered by soil particles and other factors such as moisture add to create complex light mosaics in the soil which can be detected by the phytochromes (root cap and meristem) and crytochromes (cortex cells beneath the epidermis in the shoot to root transition zone) in root tissues (Ciani, A. et al., 2005). The depth of light penetrated has reported to be in millimetres, with roots being very sensitive to low intensity light and affecting geo-trophic responses and architecture (Tester and Morris, 1987; Woolley and Stoller, 1978).

Handling of the tubes was observed to create some negative effects physiology (increased wilting compared to untouched plants of the same age), to minimise handling when recording daily drop in water-level methods of darkening the area by placing tube racks inside black plastic bags and in open 10L black plastic boxes was discontinued. Finally water-use efficiency can be estimated at different scales. Typical the plant physiology scale is usually calculated from the amount of CO2 taken in by the plant and fixed relative to water vapour leaving the stomata (Bacon, 2004; Condon et al., 2004; Willmer and Fricker, 1996). However for this research the plant level water use efficiency is based on the ratio of water taken up by the plant versus the total final dry mass of the plant at the end of the experiment, a variation of the agronomic level

that is calculated from the ratio of water used in crop production versus the biomass or yield (Condon et al., 2004; Medrano et al., 2015). Water-uptake in relation to water content can is an impression of the water turn over after seven takes relative to the water stored within the plant at time of measurement.

In order to move forward with the aim of the project, I wanted to be convinced that there were differences in the water management of the barley lines I am testing. Validating there are distinct differences between the physiology and water-management of the genotypes that impacted their growth and development would support deeper and more intensive examination of select genotypes.

Chapter 2 figure 2.3.7b indicates variation between barley lines in respect to their water-uptake and change in fresh weight, even with small and uneven sample sizes differences are detectable with simple measurements such as change in water-volume and plant weights.

In this chapter similar experiments were replicated to test potential variation in sensitivity and response to limited water availability and the effects of having larger changes in temperature between artificial night (the trough of the diurnal oscillation phase) and artificial day (peak of the diurnal oscillation phase) during long day 12 hour cycles. This was done by changing the temperatures during the artificial night phase, simulating a “warm” night with 18°C temperatures and “cold” nights with 4°C, creating a flux of 2°C or 16°C respectively from light to dark periods. In the growth cabinet’s temperatures would transition from one setting to the other within an hour twice each cycle, thus temperatures would be running at the set constant temperature (±1.0°C) for ten hours or more. In additional to introducing a new environmental variable to investigate, part of the motivation behind adding a larger temperature difference between the light and dark cycles was so add greater fluxes in environmental stimuli to encourage robust circadian rhythms.

Light and temperature are both important environmental inputs that circadian rhythmicity synchronises to when these inputs oscillate. Light is a well studied strong entraining signal on its own, however temperature has its own interactions with the circadian system, partly because of influence over mRNA and protein degradation rates which makes temperature influential in all biochemical and morphological properties in plants (Hatfield and Prueger, 2015; Sidaway-Lee et al., 2014) and because circadian genes have evolved to be temperature sensitive such as sensitivity to clock gene interacting with other genes and metabolites (Gil and Park, 2019). Additional there is evidence of “temperature-amplitude” coupling (Kurosawa et al., 2017) which seems to contribute to stabilising otherwise dynamic plasticity of circadian periods (Webb et al., 2019) as the amplitude of gene expression is temperature sensitive (higher temperatures tending to increase amplitude).

Light and temperature signals interact (Avello et al., 2019; Franklin et al., 2014), combinations of warm with light conditions transitioning to cold with dark condition in phase, phases tend to be stable with maximal amplitude of the measured circadian rhythms (Avello et al., 2019; Rensing and Ruoff, 2002b) (Avello et al., 2019; Rensing and Ruoff, 2002a). Immediate environmental temperatures can be volatile with extreme and frequent fluctuations, so the circadian system has evolved adaptability to diurnal and seasonal temperatures as well as a highly conserved mechanism to buffer clock output pathways to be insensitive to a physiological range of temperature fluctuations (Gould et al., 2006), the phenomenon is referred to as temperature compensation (Harmer et al., 2001), and helps plants maintain robust circadian rhythms. Plants have a optimal temperature range, when grown in temperature ranges that might induce stress, particularly heat stress maintaining circadian rhythms can weaken (Avello et al., 2019). Research has much to uncover regarding the extent of temperatures influence on the plant circadian clock.

In this study the 20°C standard temperature during the light cycles can be considered warm for barley plants, the 18°C night conditions would still be warm, with the difference in temperature for the light and dark cycles being 2°C which may not be enough to reset the clock (Somers et al., 1998) or enough of a temperature fluctuation for the plant to be responsive to. Instead of growing plants in a cooler 15°C, that would be a stronger temperature fluctuation without being stressful, I decided to grow plants in the more stressful 4°C which would be a bigger temperature fluctuation and take the opportunity to study the genotypes responses in their growth and water management to cold stress at night.
As with previous experiments, water-limiting conditions was simulated by inducing osmotic stress over the course of 1 week for young plants grown in hydroponics. In chapter 2 section 2.3.7 it shows high percentages of PEG solution (15%) homogenised plant growth response as water-uptake was severely reduced but there was indication of Bowman parental lines being more severely impacted by the stress whereas clock mutants had a physiological response more similar to Antonella lines. These results suggest a potential difference in water-uptake management by the clock mutants. Clock mutants were originally identified by their early flowering phenotypes and in these experiments it was observed spring cultivar plants at two to 4 weeks old developed faster than the winter type, growing new leaves earlier and taller stems, yet water-uptake was similar to the slower developing winter type.

This chapter sets out to examine these results in more depth by repeating them with a larger sample size while using the established experiment design from chapter two growing young plants in centrifuge tubes with standardised volume scales printed on the side. Previous work has established osmotic stress in the roots to cause changes in the circadian rhythms of clock genes, drought stress response genes and gas exchange measurements in Barley plant shoots and introgression lines for the *HvELF3* mutant in

cultivars Bowman and Scarlett (Habte et al., 2014a). The strong osmotic stress treatment should reduce water uptake and have negative impacts on barley plant growth, but the extent of the impacts are expected to be different across the genotypes.Additionally, the influence of colder temperatures during the dark period in the circadian cycle was also examined as another environmental influence on the relationship between plant genotype and water management. Temperature has a strong influence on water-management in plants. Temperature causes water molecules to move faster through the plant and leads to more transpiration and combined with evaporation from the soil, means warm temperatures are associated with water-loss and reducing water availability. Plants have evolved many mechanisms active and passive to regulate water transport. The circadian clock is understood to have a role in some of these mechanisms. In anticipation for the hottest period of the day, stomata have been recorded closing ahead of this period and opening later at predictable times demonstrating a circadian link (Legnaioli et al., 2009). Temperature is also speculated to be the main driver of carbon metabolism and growth in barley plants (Müller et al., 2014) and has been established as the main contributor to the process behind cyclic growth and growth rate in grasses (Matos et al., 2014; Poiré et al., 2010). Growth in all barley lines is expected to be highly responsive to temperature with low nightly temperatures expected to greatly reduced plant growth. The results are examined for significant difference between barley lines and any interaction between barely lines and other factors to indicate a potential role of the circadian clock in plant growth in response to water-limiting environments.

3.2 Methods

3.2.1 Plant material

The plant material in this experiment included the seeds as described in section 2.2.0 with the barley lines Antonella (winter cultivar), Bowman (parental line spring cultivar with *ppd-h1* alleles), Bowman *Hvlux*(B284, eam10.m), Bowman *HvPHYC* (B285, eam5.x), Bowman *Hvlelf3* (B289, eam8.k), Bowman *Hvelf3* (B2910, eam8.w), provided from stocks at the Max Planck Institute for plant breeding in Koln, Germany from Dr Maria von Korff's group and bulked in University of York Green Houses.

3.2.2 Growing conditions

Seeds were hydrated and then cleaned as described in section 2.2.1, submerging seeds in 33% bleach and 200µL/L triton for 30 minute, with 10 seconds of vortexed in centrifuge tubes, thrice with washes with ddH_2O in between and after, before being left to soak for another hour to ensure hydration. Seeds were placed in petri dishes with folded and soaked paper towel to maintain moisture. Lids were secured with tape.

Seeds were kept in a 4°C fridge for 6 days then moved to growth control cabinets (section 2.2.4) (Weiss Fitotron® SGC 120) in 12 hours 300 µmolm^{-2} s^{-1} light and dark cycles, with 60%-70% relative humidity. Plants were grown in either 20°C – light and 18 °C – dark diurnal cycles (warm night) (results in supplementary tables 3.3.1, 3.3.2, 3.3.3) or 20°C – light and 4 °C – dark diurnal cycles (cold night) (results in supplementary tables 3.3.3, 3.3.4). Plants had already developed roots by this time.

When plants cotyledons were long enough (~4 cm, after 5 days) seedlings were carefully removed from the paper towel were roots had usually grown into the paper. Seedlings were transfer to centrifuge tubes (50ml STARLAB™, E1450-0200, 34.9 mm × × 115.5 mm) of Hoagland solution (section 2.2.2). Plants in results section 1 of this chapter were grown in low nutrient Hoagland solution (1mM KNO_3,1mM $K_2HPO_4 \cdot 3H_2O$, 1mM $Ca(NO_3)_2 \cdot 4H_2O$, 1mM $MgSO_4.7H_2O$, 0.46mM H_3BO_3, 0.05M $MnCl_2 \cdot 4H_2O$, 0.2mM $ZnSO_4.7H_2O$, 0.1mM Na_2MoO_4, 0.2mM $CuSO_4$, 0.45mM$C_{10}H_{12}FeN_2NaO_8$) with their seeds. Plants in sections 2 to 4 were grown in half strength Hoagland solution (0.65M KNO_3, 0.2M $K_2HPO_4 \cdot 3H_2O$, 0.4M $Ca(NO_3)_2 \cdot 4H_2O$, 0.2M $MgSO_4.7H_2O$, 0.46mM H_3BO_3, 0.05M $MnCl_2 \cdot 4H_2O$, 0.2mM $ZnSO_4.7H_2O$, 0.1mM Na_2MoO_4, 0.2mM $CuSO_4$, 0.45mM$C_{10}H_{12}FeN_2NaO_8$, (table 2.2.2) with seeds removed as soon as they were transferred to hydroponics. Hoagland solution was filled to the 50ml mark on tubes. Seedling were secured with circular sponges (foam bungs, cut up into shorter cylinders) at the base of the cotyledon (shoot). Plants were left to grow until there were two weeks old from time of movement into the growth cabinets. Water level gradually declined drop in water level partially helped aerate the roots (figure 3.2.1). In order to compare plants of a similar development age grown in different night temperatures, half the plants were grown in cold night temperatures for one week longer and one group of plants for 4 weeks before being grown in treatment conditions.

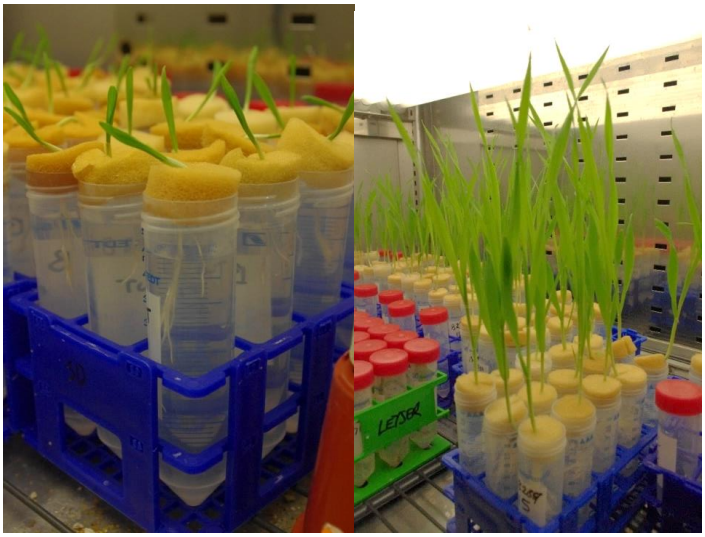


Figure 3.2.1 Photograph of seedlings in centrifuge tubes, held up by sponges (left) and plants after 14 days (right) in the growth chamber (Weiss Fitotron® SGC 120).

Before treatment, seed husks were removed, excess water was towel dried off the roots and the whole plant was weighed before being transferred into a new treatment solution with or without (0%) PEG. PEG treatments to induce osmotic stress was created by dissolving 5% (500g/10L), 10% (1KG/10L) and 15% (1.5KG/10L) PEG (8000 m.w.) into Hoagland solution and mixing until fully dissolved. In low concentration Hoagland solution pH was typically around 7.1-7.6, however in half strength Hoagland solution conditions were more acidic (pH 5.1-6.2). The starting volume was standardised across all tubes in each experiment (50mL). Control tubes with sponges and solutions for all treatments but no plants were included to record drop in volume of just evaporation to factor average evaporation values for each experiment into the final water uptake measurement of each plant grown in the same solution.

Plants were grown for seven days, each day the cabinet was opened for up to two hours as water volume of each tube was recorded without removing from the cabinet. On the seventh day plants were harvested to collect data as described in table 3.2.1. Root were dried on paper towel before plants were cut at the stem to root junction just above where roots join, the root and shoot organs were weighed separately, shoot tissue placed into paper bags, while roots were returned to the hydroponic solution to be scanned on a Epson (Epson Perfection V500 Photo flatbed scanner) through a custom-made, clear acrylic tray (A5 and A4 sizes, University of York, Biology Department Work shop). The images were processed in WinRhizo® software to collect data on global root morphology traits. After scanning roots were added into the same back as the matching shoot tissue before being dried for at least 72 hours at 60-70°C in drying ovens before being re-weighed. From the measured data additional measurements were calculated (summarized table 3.2.1). It was noted after analysis and data summarising that water uptake divided by total dry mass as a measure of water-use efficiency is incorrect and should be the dry mass divided by the water uptake to get a approximate measure of how much water is used to convert to plant matter instead calculations indicate how the amount of plant material affected water-uptake. It was intended to add the dry matter divided water-uptake to infer water-use efficiency like measurement (which would only be approximate as starting dry mass data would be missing. During the experimental period experiments were designed that had plants dried and weighed at the beginning to compare to dry weighs post treatment, to get a change in dry weight measurement to use to calculate water use efficiency more accurately however these experiments were incomplete and dropped from analysis.)

Table 3.2.1: Summary of abbreviations (Abbr.), units of measurement and method of measurement or calculation for each physiological trait examined in chapter 3.

Trait	Abbr.	Unit	Method of measurement
Total fresh weight pre treatment (Initial fresh weight)	IFW	g	Weighed before treatment
Total fresh weight pre treatment (Final fresh weight)	FFW	g	SFW+RFW
Change in fresh weight	CFW	g	FFW-IFW
Shoot fresh weight post treatment	SFW		Weighed after treatment
Root fresh weight post treatment	RFW	g	Weighed after treatment
Total dry biomass (Total dry weight)	TDW	g	SDW+RDW
Shoot dry biomass (Shoot dry weight)	SDW	g	Weighed after treatment and >72hours of drying at 70°C
Root dry biomass (Root dry weight)	RDW	g	Weighed after treatment and >72hours of drying at 70°C
Total water content	TWC	g	FFW-TDW
Shoot water content	SWC	g	SFW-SDW
Root water content	RWC	g	RFW-RDW
Root:Shoot Dry Biomass ratio	RSDW		RDW/SDW
Root:Shoot water content ratio	RDWC		RWC/SWC
Water uptake	WU	ml	Initial starting volume in 50ml centrifuge tube – (final volume after the end of treatment period+ the average of the final volumes from the control centrifuge tubes.)
Accumulated root lengths	RL	cm	Calculated using WinRhizo™ software. All root lengths detected added together.

Total root surface area	RSA	cm^2	Calculated using WinRhizo™ software based off the RL and RAD
Average root diameter	RAD	mm	Calculated using WinRhizo™ software. Averaging the calculated thickness of all the roots.
Total root volume	RV	cm^3	Calculated using WinRhizo™ software, calculated based off the RL, RSA and RAD
Total number of root tips	RT		Calculated using WinRhizo™ software, calculated as the number of detectible root ends
Water uptake per total dry biomass	WU/DW	ml/g	WU/TDW
Water uptake per total water content stored	WU/WC	ml/g	WU/TWC
Percentage of water as total biomass	W%	%	TWC/FFW

Statistical analysis on the data was carried out in R studio™ using package 'lme4' (Bates et al., 2015) to make generalised mixed effect models to accommodate the right skew in the data with 'GAMMA' family when appropriate and 'emmeans' package (Lenth, 2018) as post-hoc analysis.

In total, twenty two traits were compared, categorised as growth traits,(Total fresh weight (g) pre-treatment (FW), Total fresh weight (g) post treatment (FFW), Change in fresh weight (g) (CFW), Shoot fresh weight (g) post treatment (SFW), Root fresh weight (g) post treatment (RFW), Total dry biomass (g) (TDW), Shoot dry biomass (g) (SDW), and Root dry biomass (g) (RFW)), Water traits(Water uptake (ml) (WU), Total water content (g) (TDW), Shoot water content (g) (SDW), Root water content (g) (RDW), Percentage of water as total biomass (%) (W%)), resource allocation, (Root:Shoot Dry Biomass ratio (RSDW), Root:Shoot water content ratio (RDWC)), root morphology traits (Accumulated root length (cm) (RL), Total surface area (cm^3) (RSA), Average diameter (mm) (RAD), Average root volume (cm^3) (RV), Total number of root tips (RT)) and water-use efficiency (Water uptake per total dry biomass (ml/g), (WU/DW), Water uptake per total water content stored (ml/g) (WU/WC)).

The second section re-examines the responses when grown in high concentrated nutrient solution and only comparing the most severe induced water stress to non-stress conditions with a greater focus on other growth responses.

The third section compares the barley lines responses when grown in cold night temperatures relative to warm night temperatures.

Alongside light, temperature is a strong environmental signal to the clock. Temperature cycles in sections one and two had a difference of two degrees, making for warm nights. However, barely is often sown in early spring when differences between minimum and maximum temperatures can be over ten or fifteen degrees coinciding with day and night cycles, with temperatures getting below six degrees in some regions. Ford et al., 2016 found connections between HvELF3 (HvEAM8) and temperature sensitivity as well as circadian gene transcription being very responsive to warm temperatures while cold temperatures result in alternative splicing of circadian genes (Calixto et al., 2016). Therefore, potential differences in growth fitness were hypothesised to be possibly better detected when there is a greater temperature gradient, particularly with cold nights if the barley lines have different clocks. Against this hypothesis, Matos et al, (2014) with Brachypodium distachyon and Muller et al., (2018) with Barley have reported that temperature and not the circadian clock regulate growth and carbon availability respectively.

Section four will compare the water-uptake and growth responses of barley lines grown in cold nights but stressed at different early development stages specifically stressed after growing for 2 weeks, 3 weeks and 4 weeks. Barley circadian mutants do have faster developing phenotypes (Faure et al., 2012), their early maturing traits being their main identifiable feature and is detectable even in young plants by their leaf development. The number of leaves a plant has affects their size, water storage capacity but also number of stomata and hydraulic conductance. As such this accelerate growth when all environmental factors are the same, could indirectly be influencing differences in water-uptake and responses to water-availability between barely lines rather than direct circadian regulation.

The main physiology traits considered in this chapter are growth traits based on fresh mass and dry mass of the whole plant or shoot and root tissues, as well as change in fresh mass before and after treatment. The overall growth of the plant by change in weight is typically a good indicator of plant health with dry biomass indicating the number of cells plants have made and thus an indicator of converting energy into mass.

Water measurements comparing total water taken up by the plant over seven days, the water content calculated from subtracting the dry mass measurements from the fresh mass measurements,

Resource allocation based off of ratios calculated by dividing the root dry mass and water content measurements by the equivalent shoot measurements.

Root morphology traits as analysed and calculated in WinRhizo® software. As roots were grown in hydroponics, it was expected to have influence on morphology. Water is absorbed through the roots via diffusion and active transport, requiring intimate contact between water molecule and root cells. To increase contact and thus absorption or to seek water, plants produce more root hairs. Plants can control direction of root growth towards water sources. In hydroponic conditions plants are

submerged in water and are in contact with water constantly (unless water levels decrease too far without being replenished), thus removing the need to “seek” water in their environment or the influence of soil texture to the roots ability to access oxygen or growth patterning. This could mean be the resulting root architecture is more genetically determined. The environmental factors that could affect root growth would be the osmotic potential of the water, the temperature and the light. The experiment design did not exclude light penetrating the tubes the plants grew in. Meaning plants were exposed to more light that they would be in field conditions. Light (sunlight) can penetrate soil but intensity is greatly reduced, light is fragmented and scattered by soil particles and other factors such as moisture add to create complex light mosaics in the soil which can be detected by the phytochromes (root cap and meristem) and crytochromes (cortex cells beneath the epidermis in the shoot to root transition zone) in root tissues (Ciani, A et al., 2005). The depth of light penetrated has reported to be in millimetres, with roots being very sensitive to low intensity light and affecting geo-trophic responses and architecture (Tester and Morris, 1987). Handling of the tubes was observed to create some negative effects physiology (increased wilting compared to untouched plants of the same age), to minimise handling when recording daily drop in water-level methods of darkening the area by placing tube racks inside black plastic bags and in open 10L black plastic boxes was discontinued.

Finally water-use efficiency.

Water use efficiency can be estimated at different scales. Typical the plant physiology scale is usually calculated from the amount of CO2 taken in by the plant and fixed relative to water vapour leaving the stomata (Bacon, 2004; Condon et al., 2004; Willmer and Fricker, 1996) however for this research the plant level water use efficiency is based on the ratio of water taken up by the plant versus the total final dry mass of the plant at the end of the experiment, a variation of the agronomic level that is calculated from the ratio of water used in crop production versus the biomass or yield (Condon et al., 2004; Medrano et al., 2015).

3.3 Results

Comparison of barley lines grown in two different osmotic stress treatments in warm and cold nights.

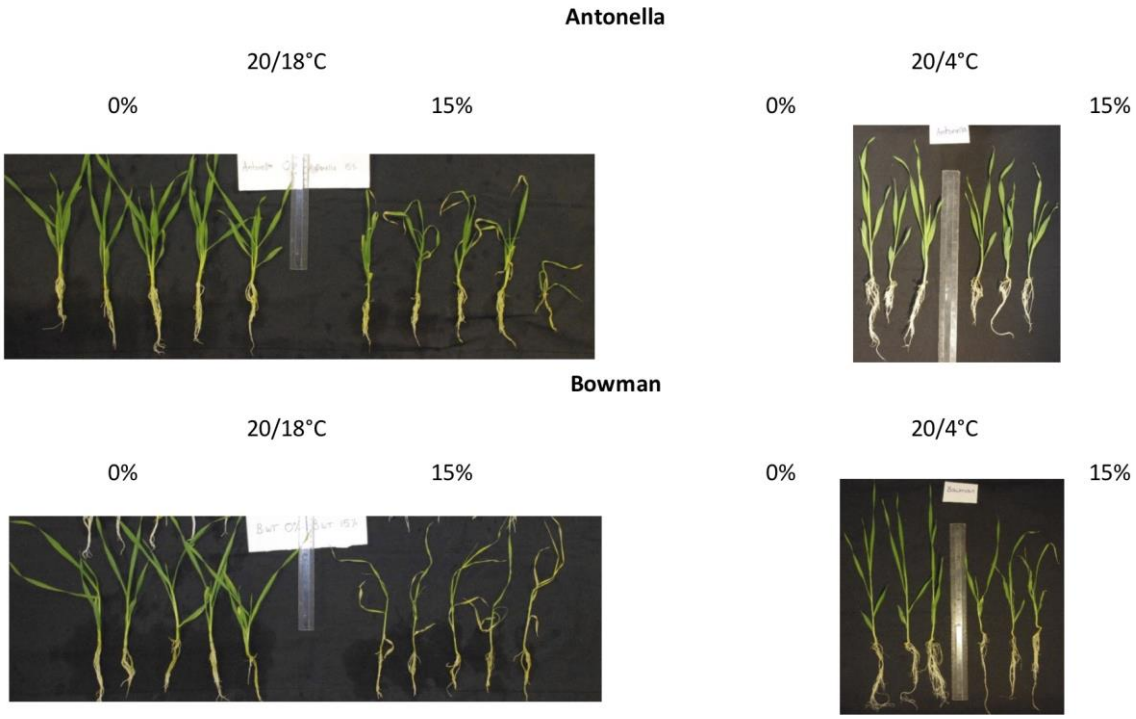


Figure 3.3.3.1 photograhs illustrating the the effects of warm or cold nights on young barley plant growth and response to osmotic stress. The photographs show that for plants germinated at the same time, when grown in cold nights (right) they develo slower, producing fewer and smaller leaves over the same growing period as plant grown in warm night (left), however while Antonella (top) plants show more resistance to osmotic stress than Bowman (bottom) after being grown in 15% PEG for a week, plants of both cultivars look healthier (less damages and wilted, more roots) than plants grown in warm nights and 15% PEG but not completely unaffected. This photo demonstrates cold night are benefiticial for plants during periods of water limitation.

This section will continue to examine similar traits as the previous two sections. Some data used in section 3 is re-used in this section to compare plants grown in warm night conditions to plants grown in cold night conditions. Data was selected by using the initial fresh weights pre treatment as as a proxy that within genotypes plants would be comparable prior to treatment. Instead of introduce cold nights as a shock and added stress, plants were grow from seedlings in either warm or cold night conditions to acclimate them to their diurnal environments.

3.4 Conclusions

There was evidence of Antonella having a more conservative growth pattern to spring barley types as fresh and dry biomass tended to be lower in non-stressed conditions but relatively higher or have a lower response when stressed. Winter barley tended to be relatively less skewed in biomass invested in shoot material, attributable to slower growth and overall lower water uptake and higher water use efficiency in non-stressed conditions.

Barley lines did not show large differences in growth or water uptake response to limited water availability and in this experimental system at the young growth stage before stem elongation did not show evidence of potential alternate strategies or physiology between winter lines, spring lines parental lines and mutant genotypes. At the same time, circadian clock mutants did not appear to be at a fitness disadvantage in either stressed or non-stressed osmotic conditions, further adding to the existing evidence that the functionality of the barely circadian clock is different to the circadian clock in Arabidopsis and changes in clock genes or even loss of function does not translate to dramatically detrimental phenotypes or fitness disadvantages, although the importance in development rate is evident even at a very early age.

Early maturing barley in spring cultivar Bowman were compared to their parental Bowman plants and a winter cultivar Antonella to investigate the responses for barley plants when they have changes in circadian clock genes with evidence of different clocks by way of changes in other clock gene expression levels and rhythms as well as changes in fluorescent rhythms.

The focus of the main part of the research was centred on establishing whether early maturing mutants had significantly different responses to limited water availability as induced by osmotic stress on the plants root in hydroponics.

Early work in warm cycling conditions did not produce convincing resulting in regards to water uptake, biomass accumulation and water content, with observed differences potentially being attributed to the physical changes in the mutant plants as they had accelerated development.

There is a sign of reduced water use efficiency as early maturing mutants used as much or slightly water than parental Bowman or winter Antonella but accumulated less biomass. This may be associated to less investment in vegetative growth and strengthen resources and more investment in growing fast, resulting in leaves with narrower leaf widths or deformed/irregular stomata (an observed double stomata phenomenon was noted about the early maturing plants, while the high variability in stomata sizes appeared to occur in parental barley lines.

Alongside altered fluorescent rhythms, which suggest chlorophyll accumulation and UV-protection pigmentrhythm irregularities, potential impaired stomata regulation

may mean photosynthesis is less efficient and plants have less energy for biomass. Results from my work from dry weights do not suggest that early maturing mutants are making less biomass relative to the parental line in the same conditions.

All barely lines were effected by increasing osmotic stress in the same way because decreasing water content and reduced biomass accumulation. As such it was difficult to conclude if there were any signs of different mechanism in water regulation or response to stress as overall plants were taken in similar amount of water and growth strongly correlated with water-uptake.

The genotypes with the greatest consistent statistical difference between them were the parental spring Bowman and the winter Antonella, while the early maturing lines having phenotypes “in between” a Spring and Winter type, or- more likely based on variance in a number of physiology measurements especially in non-stressed conditions, there is greater variability in physiology in plants with less functional circadian clock mechanisms and reduced light perception.

Water uptake correlated very strongly to water content, when plants were stressed the loss of water in the shoot tissue was the cause of biomass accumulations and water content ratios shifting towards root tissues rather than plants investing more in growing out more roots. It may have been an artefact of PEG and the limited growing space of the centrifuge tube but plants grown in PEG tended to have smaller root measurements that were similar in size in 5% or 15% PEG. Similarly, in non-stressed conditions spring cultivars appeared less efficient with water use, however after stress water taken up relative to biomass accumulated fell the most in Bowman. This was not due to Bowman plants becoming more efficient but rather they took up much less water when stressed but had grown faster prior to stress.

Although Osmotic stress was a useful means to examine water limitation, perhaps more interesting physiology can be explored in unstressed plants as the variability within a genotypes is more visible while stressing or repressing the plant diminish differences between types.

In warm conditions there are larger differences between barley lines. Heat accelerated Barley Bowman growth rates and breeding barely in high (20-28°C) temperatures has been used in research to speed up development and improve experiment turnover in long growing cereal crops, speed breeding also takes advantage of this mechanism.

Osmotic stress and cold nights result in growth and water-uptake being more homogenous and less variable but where osmotic stress harmed the plants survival with evidence of wilting, cold night temperatures tented to be associated with healthier, slower growing plants. Leaf mass per area in particular suggests that under cold nights biomass accumulation is greater and *Hvelf* early maturing mutants are very responsive to temperature changes. Water-use was greatly reduced in cold night conditions, which suggests in warm nights there is still a lot of transpiration. Furthermore plants grown in cold nights without osmotic stress used similar amounts

of water over the same period of time as plants that were grown in severe chronic osmotic stress but plants were healthy. The adverse effects of osmotic stress may be due to stress hormonal signalling. This research does not include application of hormones, however, using hormones such as ABA on plants grown in cold nights without PEG may determine how much the difference in plant health (wilting leaves, loss of water in shoot tissues, loss of chlorophyll) may be due to stress response and how much may be attributed to physically lacking water.

Hydroponic systems had additional advantages of water natural temperature buffering, especially when grown in black boxes which would take longer (up to 8 hours) to reach air temperatures resulting in plant roots grown in cold nights being in low temperatures even as the shoots were in warm temperatures and likewise cool down after the dark cycle began so roots would be warmer in the evenings, the thermodynamic effects may help physically modulate transpiration as low energy, cold water molecules surround the route and slow down rate of osmosis when the lights are one and plants are continuous under warm light conditions. In the evening after dark, plants may still transpire/respire and warmer water molecules can enter the plant at the root but are slower to leave from the cooler air temperatures around the leaves. Soils can have similar temperature insulating effects more so in the field than in pots.

It was expected that winter cultivars, due to having to overwinter would show evidence of a conservative growing and water-use strategy compared to the faster growing spring cultivars. Winter cultivars were expected to accumulate less biomass over the same period of time and take up less water. Results (appendices for 0% PEG treatments) indicate Spring barley did have larger increases in fresh biomass and take up more water than winter barley.

It was expected of early maturing plants, due to developing at a faster rate than Bowman would have larger increases in biomass and use water at a faster rate. However results (appendices, supplementary table 3.3.1.a) indicated that change in fresh biomass, total dry biomass and water uptake the early maturing lines accumulated less biomass and used less than the parential Bowman line on average. The *PHYC* mutant (eam5) in particular tended to be more similar to the winter cultivar in growth responses and water use than the other mutant lines. Based on raw data of experiments comparing different PEG percentages (supplementary 3.3.1a) early maturing lines develop faster using less water but developing less biomass. Analysing using generalised linear models showed all barley lines has statistically similar changes in fresh biomas and total dry biomass. With the latter eam8.k total dry biomass did not significantly differ between 0%, 5% and 10% PEG. Water uptake was more distinct between barley, Antonella were statistically less in 0% PEG to most Bowman lines except eam8.k (B289) but once in osmotic stress water uptake was similar across barley (supplementary 3.3.1b). However another batch of experiments only comparing 0% and 15% PEG in warm temperatures results were different. Bowman had significantly larger changes in fresh biomass, more total dry biomass, greater water uptake and higher water content (supplementary 3.3.2a). Early maturing lines eam10 (B284) and eam8 (B289, B290) lines were not statistically different to Bowman or Antonella in their

change in fresh biomass, eam5 (B285) had a similar change to Antonella and statistically less than Bowman. A similar pattern occurred for eam5 for total dry biomass being similar to Antonella but not statistically different to other Bowman. Meanwhile eam8 lines (B289, B290) unlike the previous set of experiements has a higher dry biomass than Bowman while eam10 (B284) was similar, and eam8.k (B289) was significantly greater than Antonella. For Water Uptake and water content, eam5 (B285) is similar to Antonella while other lines have a similar water content to Bowman and eam8 lines (B289, B290) used more water than Bowman (supplementary 3.3.2a and b). A similar pattern of Antonella and eam5 lines growing and using water at similar rates and slower than other lines persists in cold temperatures even when growth and water-use is overall reduced. Difference between eam10 (B284), eam8.w (B290) and eam5 (B285) in total dry biomass in cold termperatures without PEG, as eam5 lines have a statistically lower dry biomass than the other eam lines (supplementary 3.3.3c and d). Early maturing plants did not behave as expected, however the level of investigation of this research has not determined if this is directly circadian clock related.

Alongside data in chapter 2, the early maturing mutants potential strength in surviving drought conditions in field experiments may be connected to increased variability by decreased control over timing of development, in particular development of flowers and seed may occur faster, but are also more continuous as early maturing mutants take longer to senescence and will keep producing new tillers that produce some seed even after the parental lines have caught up in development and are beginning to senesce.

Additionally larger plants pre stress regardless of genotype tended to be less susceptible to osmotic stress, as heavier plants positively correlated with more water uptake even in stressed conditions and this could be due to a number of reasons. Larger plants have more canopy and thus more stomata to “pull” water through the plant, or more plant cells means more vacuoles to store water outside of the roots. Plants can be large by either faster development or by investing more per existing tissue. Typically faster growing plants tended to be bigger in general especially at early development stages that were the focus of this research when development is mostly in new leaves.

Root measurements, leaf widths and tiller number were less clear than other measurements as variance was high in these traits with reports from other laboratories that Stomata traits were also variable within genotypes and across development. In these traits there did not seem to be clear patterns in response to increasing osmotic stress despite showing a clear negative response to osmotic stress.

The spring barley types regardless of genotype leaned towards a “life in the fast lane” survival and development strategy, with early maturing mutants racing through development of new leaves and stem elongation to seed development quickly only to perpetuate at the seed development stage for a much longer time after the parental line has stopped seed development. Meanwhile the winter line was relatively slow and

steady type, although true to the cultivars designed, elite lineage, Antonella plants produced a lot of leaves while dormant before going onto stem elongation.

Chapter 4 discussion

4.1 Novel research of this thesis

My work has revealed that cool nights support growth of barley plants that are osmotically challenged. Whether the circadian clock modulates this process deserves further examinations.

4. 2 Expanding on the work in this project

Over the course of this research many methods were attempted or considered. The research results in chapter 2 covered a range of such work. Numerous large experiments generated extensive data that was collected to analyse. Were this work to continue a number of unrealised approaches could be used to explore the role of barley circadian clock genes in water regulation.

4.3 Molecular work examining the gene expression

As mentioned in section 2.8 a major and unfinished goal of the project was to analyse gene expression of circadian and water stress response genes in the Bowman mutants. This had not been done before in all of the Bowman mutant lines examined in this work. Alternatively comparing Antonella and Bowman as winter cultivar compared to a spring was also considered as a novel investigation with potential to get other winter and spring cultivars once the protocol was established. RNA extraction was optimised over the course of this project as one of the successes. The methods already work in other laboratories, but transferring them to York were a challenge. Additionally the ambition to sample every two hours to get a more detailed data on the gene expression in barley set compared to the more typical 4 hour period sampling was a large part of the planning and rigorous to carry out. Fortunately sampling and processing samples to extract RNA from this volume of samples, while intense work was achieved. Unfortunately the qPCR did not happen due to difficulties in getting housekeeping genes that would be stable in drought and cold or cloning them to be used to measure total RNA did not work by the time the project was finishing. Therefore if this work could be continued, building off the techniques learned and partial successes in making cDNA and clones I would analyse the gene expression of the hundreds of samples taken as part of this project.

4.4 Molecular work examining sugars

During the project there was the opportunity for sugars in the root and shoot tissue to be measured, Professor Ronan Sulpice (NUI Galway, Ireland) was a collaborator that would work together to measure samples taken in York in their laboratory using their established protocol. Measuring the effect of cold nights on barley complimented their research (Barros et al., 2020a). Antonella and Bowman cultivars, grown in cold and warm nights, before and after 0% or 15% PEG treatment was selected as the samples to examine, with 5 replicates for each cultivar, temperature, PEG treatment and tissue type sent. The results from this would have been very informative. Unfortunately plant material sent from York needed to be grinded to make finer powder and the results for a number of sugars measured (sucrose and starch) showed high standards of error and the material had degraded this did not produce reliable results.

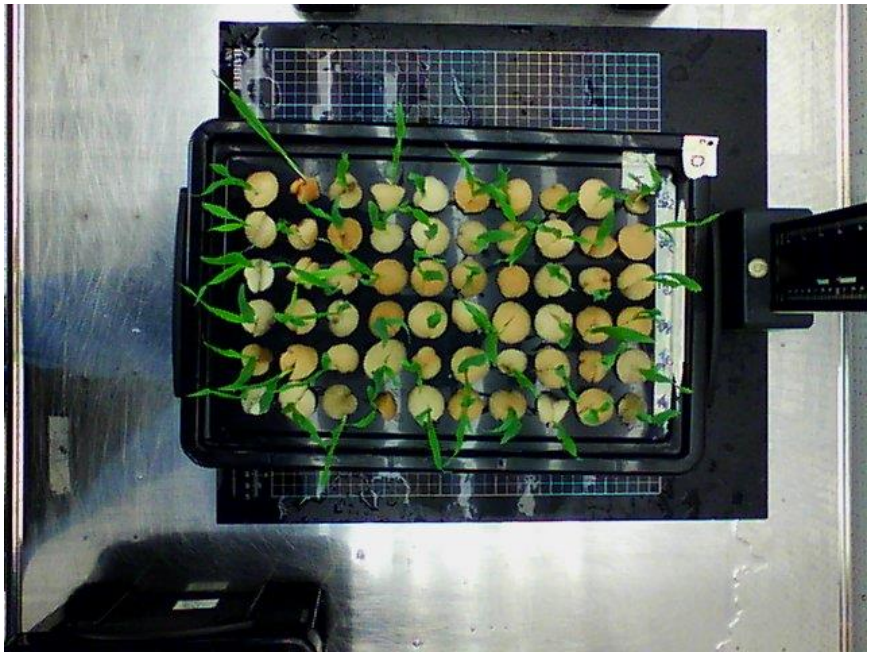
4.5 Physiological measurements in larger plants and longer term water-stress

Analysing growth performance was considered to be conducted in a large scale pot experiment. Here a single plant per pot and with drought conditions achieved by limiting water would have been implemented, to measure biomass, height and development in response to different watering conditions in the barley mutant lines. A limit of the hydroponic system was the size of the container. Plants could still reach develop maturity in hydroponics, growing to similar heights as those grown in soil in the 10L box system, but not in the 50ml centrifuge system. Drought is a different stress to osmotic stress, and roots would not be protected from drying out, making a pot experiment a good system to examine recovery from stress in the mutant lines.

Another investigation that could still use the developed 10L box hydroponic system with the addition of aerating the nutrient solutions would be to examine the effects on long-term mild osmotic stress (PEG8000 <5%). Here the focus would be on stronger osmotic stress over shorter time periods in this project did not find significant differences between the mutant lines and the parental lines.
During the project growth performance experiment, a comparison of plants at the same develop stage would be made. This would be when growing plants in different night temperatures, experimental repeats of cold night grown plants osmotically stressed at 2 weeks old was removed from analysis, alongside one experimental repeat of plants stressed at 4 weeks old. The effect of age on osmotic stress response has an Analysis that is already done [supplementary (tables 3.3.4a+b)].

4.6 Thermal imaging

Another area of investigation that was attempted but no completed was the use of thermal imaging to capture difference in water regulation between barley lines (Figure 4.6.1). The initial preliminary work revealed that thermal images can be captured. As water use results in a cooling process, the use of water by the plants can thus be measured as a change in temperature over time, alongside the physiological parameters explored just above. The trial of this process revealed distance between the camera and plants was very important as the humidity in the growth chamber could interfere and the black boxes filled with water could change temperature at a slower rate to the room.



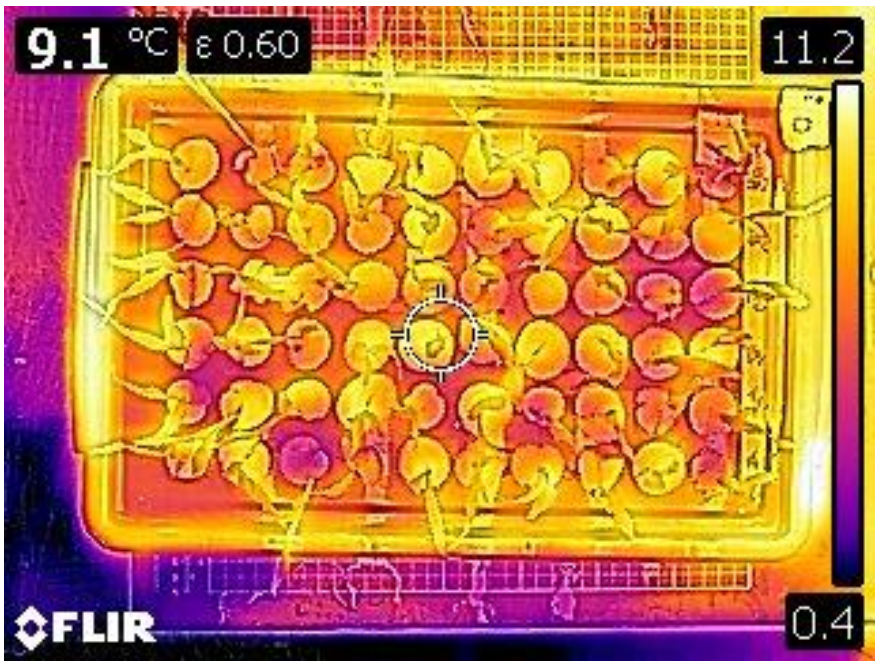


Figure 4.6.1 Thermal imaging of barley plants using FLIR™ C5 compact thermal imaging camera, **(top)** the position of the camera above the 10L box with randomised barley plants (order recorded on a spreadsheet), **(Bottom)** the view of the thermal imaging from the same height (the camera automatically zooms in closer), the bar on the right indicates the temperature range within the view e.g. 0.4°C is the lowest temperature recorded colourised in bue to black pixels while 11.2 °C is the highest and colourised in yellow to white pixels.

4.7 Stomata measurements

During the time on the project, taking leaf impressions without damaging the plants had been attempted. I was allowed to visit Professor Julie E. Gray's group in Sheffield to learn how they take leaf impressions and measurements from barley (Hughes et al., 2017). Protocols in this project developed to the stage of using dental paste to take an impression of the leaf surface. This mould could then be used repeatedly to look at leaf stomata of a single time point. Developing this further, multiple impressions from leaves could be taken to produce a time series data set showing for a given area and time how many stomata are present, open, semi-open or completely closed. Morphological measurements and development pattern of Stomata could also have been analysed with more time.

In addition to Stomata impressions, attempts were made to measure stomata activity using a LICOR 6000, a porometer and a portable infra red gas analyser to limited success. The porometer (delta-t AP4 porometer) worked the best with the experiment system in the growth chambers. However the time taken to collect data per sample was not practical in the limited sampling time and with the number of samples data was needed for as stomata activity data was intended to be collected as the physiological compliment to the gene expression analysis. With more time and under different conditions (e.g. working in a walk-in growth chamber or an experiment designed around the pace of the equipment with a smaller replicate number) information on stomata activity would have been interesting to collect as other lab groups have reported difference between circadian mutants based on time-series measuring gas exchanges.
Under ideal circumstances a Li-Cor portable photosynthesis system (e.g. Li6800) could be used to collect stomatal conductance, gas exchange and fluorescence data in real-time to construct comparative time series across the mutant lines and reveal active water regulation. Combined with Stomata impressions this could have given information on whether development and performance of stomata in circadian mutants underlines diurnal patterns in gas exchange activity and if they are different to the parental line particularly as *ArELF3* has been shown to have a influence via *ArFT1* on stomata performance (Hubbard and Webb, 2011), while interactions between the circadian Evening complex and *ArTOC1* could influence the ABA signalling pathway (Castells et al., 2010) with the potential for similar interactions to occur in barley.

4.8 Fluorescence

Photographs in this project suggest cold night plants were healthier than plants grown in warm night following osmotic stress treatment. As cold inhibits growth and water up-take the measurements taken to compare physiological response to stress it was difficult to examine deeper. Using Fluorescence. Delayed fluorescence was considered at the start of the project using the established “PIXIE” camera set in a constant dark growth cabinet. Initial testing used leaf cuttings suspended in water and left in the chamber for 4 days (A) and 6 days (B). This work was done very roughly at the beginning of the project, the output shown is the raw data of the program used to record the Fluorescence Emission after excitation (Figure 4.8.1A). Despite appearing that oscillations were appearing later in the time series (figure 4.8.1B), photographs taken at the time when the camera flashed were black images causing doubt that the system had worked.

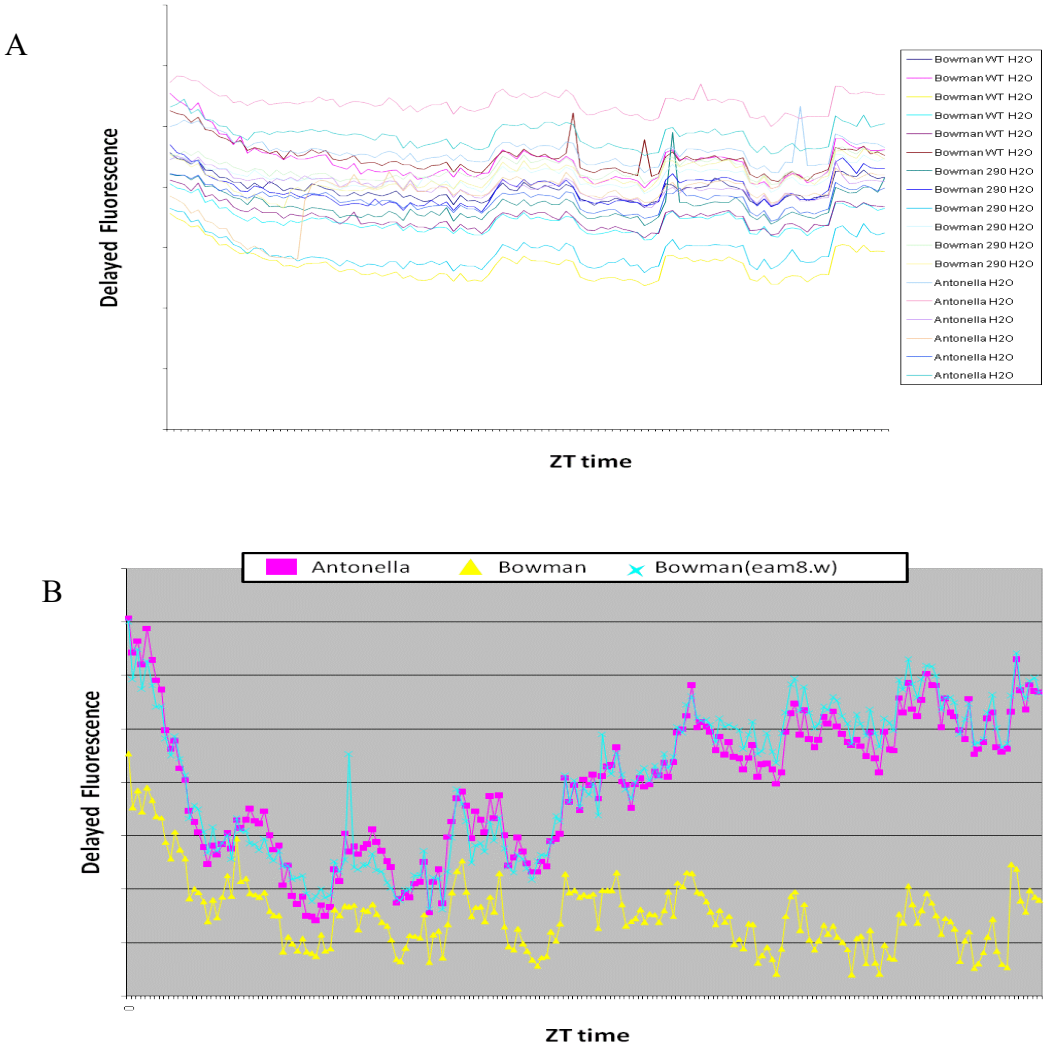


Figure 4.8.1 Illustrative graphs showing raw Fluorescence data taken using the Davis LAB "PIXIE" system in two separate trials, the excel output shown in (A) and the BRASS output shown in (B).

4.9 Final conclusion

This research project attempted to link the barley circadian clock with water-use, and the association between water stress response and cold stress response since it is well known to share pathways and be connected. It could be more successful to investigate water regulation associated with the barely circadian clock in association with the barley circadian clock associations to temperature response and regulation. Most research focuses on effects of warming, due to climate change, however to better understand the circadian clock in barley, studying the system in cool to cold conditions is recommended.

References

Abiko, M., Akibayashi, K., Sakata, T., Kimura, M., Kihara, M., Itoh, K., Asamizu, E., Sato, S., Takahashi, H., Higashitani, A., 2005. High-temperature induction of male sterility during barley (Hordeum vulgare L.) anther development is mediated by transcriptional inhibition. Sexual Plant Reproduction 18(2), 91-100.

ADM Germany, 2021. World Barley Production from 2008/2009 to 2020/2021 (in Million Metric Tons).

Agriculture, U.D.o., 2021. Worldwide Production of Grain in 2020/21, by Type (in Million Metric Tons).

AHDB, 2018. The growth stages of cereals. https://ahdb.org.uk/knowledge-library/the-growth-stages-of-cereals 2018).

Allaby, R.G., 2015. Barley domestication: the end of a central dogma? Genome Biology 16, 176.

Alqudah, A.M., Koppolu, R., Wolde, G.M., Graner, A., Schnurbusch, T., 2016. The Genetic Architecture of Barley Plant Stature. Frontiers in Genetics 7(117).

Anderson, P., Oelke, E., Simmons, S., 1985. Growth and development guide for spring barley.

Appels, R., Eversole, K., Stein, N., Feuillet, C., Keller, B., Rogers, J., Pozniak, C.J., Choulet, F., Distelfeld, A., Poland, J., Ronen, G., Sharpe, A.G., Barad, O., Baruch, K., Keeble-Gagnère, G., Mascher, M., Ben-Zvi, G., Josselin, A.-A., Himmelbach, A., Balfourier, F., Gutierrez-Gonzalez, J., Hayden, M., Koh, C., Muehlbauer, G., Pasam, R.K., Paux, E., Rigault, P., Tibbits, J., Tiwari, V., Spannagl, M., Lang, D., Gundlach, H., Haberer, G., Mayer, K.F.X., Ormanbekova, D., Prade, V., Šimková, H., Wicker, T., Swarbreck, D., Rimbert, H., Felder, M., Guilhot, N., Kaithakottil, G., Keilwagen, J., Leroy, P., Lux, T., Twardziok, S., Venturini, L., Juhász, A., Abrouk, M., Fischer, I., Uauy, C., Borrill, P., Ramirez-Gonzalez, R.H., Arnaud, D., Chalabi, S., Chalhoub, B., Cory, A., Datla, R., Davey, M.W., Jacobs, J., Robinson, S.J., Steuernagel, B., Ex, F.v., Wulff, B.B.H., Benhamed, M., Bendahmane, A., Concia, L., Latrasse, D., Bartoš, J., Bellec, A., Berges, H., Doležel, J., Frenkel, Z., Gill, B., Korol, A., Letellier, T., Olsen, O.-A., Singh, K., Valárik, M., Vossen, E.v.d., Vautrin, S., Weining, S., Fahima, T., Glikson, V., Raats, D., Číhalíková, J., Toegelová, H., Vrána, J., Sourdille, P., Darrier, B., Barabaschi, D., Cattivelli, L., Hernandez, P., Galvez, S., Budak, H., Jones, J.D.G., Witek, K., Yu, G., Small, I., Melonek, J., Zhou, R., Belova, T., Kanyuka, K., King, R., Nilsen, K., Walkowiak, S., Cuthbert, R., Knox, R., Wiebe, K., Xiang, D., Rohde, A., Golds, T., Čížková, J., Akpinar, B.A., Biyiklioglu, S., Gao, L., N'Daiye, A., Kubaláková, M., Šafář, J., Alfama, F., Adam-Blondon, A.-F., Flores, R., Guerche, C., Loaec, M., Quesneville, H., Condie, J., Ens, J., Maclachlan, R., Tan, Y., Alberti, A., Aury, J.-M., Barbe, V., Couloux, A., Cruaud, C., Labadie, K., Mangenot, S., Wincker, P., Kaur, G., Luo, M., Sehgal, S., Chhuneja, P., Gupta, O.P., Jindal, S., Kaur, P., Malik, P., Sharma, P., Yadav, B., Singh, N.K., Khurana, J.P., Chaudhary, C., Khurana, P., Kumar, V., Mahato, A., Mathur, S., Sevanthi, A., Sharma, N., Tomar, R.S., Holušová, K., Plíhal, O., Clark, M.D., Heavens, D., Kettleborough, G., Wright, J., Balcárková, B., Hu, Y., Salina, E., Ravin, N., Skryabin, K., Beletsky, A., Kadnikov, V., Mardanov, A., Nesterov, M., Rakitin, A., Sergeeva, E., Handa, H., Kanamori, H., Katagiri, S., Kobayashi, F., Nasuda, S., Tanaka, T., Wu, J., Cattonaro, F., Jiumeng, M., Kugler, K., Pfeifer, M., Sandve, S., Xun, X., Zhan, B., Batley, J., Bayer, P.E., Edwards, D., Hayashi, S., Tulpová, Z., Visendi, P., Cui, L., Du, X., Feng, K., Nie, X., Tong, W., Wang, L., 2018. Shifting the limits in wheat research and breeding using a fully annotated reference genome. Science 361(6403), eaar7191.

Arber, A., 2010. The Gramineae: a study of cereal, bamboo and grass. Cambridge University Press.

Assmann, S.M., Jegla, T., 2016. Guard cell sensory systems: recent insights on stomatal responses to light, abscisic acid, and CO2. Current Opinion in Plant Biology 33, 157-167.

Avello, P.A., Davis, S.J., Ronald, J., Pitchford, J.W., 2019. Heat the Clock: Entrainment and Compensation in Arabidopsis Circadian Rhythms. J Circadian Rhythms 17, 5-5.

Bacon, M.A., 2004. Water use efficiency in plant biology. Water use efficiency in plant biology.

Badr, A., Sch, R., El Rabey, H., Effgen, S., Ibrahim, H.H., Pozzi, C., Rohde, W., Salamini, F., 2000. On the origin and domestication history of barley (Hordeum vulgare). Molecular Biology and Evolution 17, 499-510.

Baldwin, I.T., Meldau, S., 2013. Just in time. Plant Signaling & Behavior 8, e24410.

Bargel, H., Barthlott, W., Koch, K., Schreiber, L., Neinhuis, C., 2004. Plant cuticles: Multifunctional interfaces between plant and environment, in: Hemsley, A.R., Poole, I.B.T.T.E.o.P.P. (Eds.), Academic Press, Oxford, pp. 171-III.

Barros, K.A., Esteves-Ferreira, A.A., Inaba, M., Meally, H., Finnan, J., Barth, S., Davis, S.J., Sulpice, R., 2020a. Diurnal patterns of growth and transient reserves of sink and source tissues are affected by cold nights in barley. Plant, Cell & Environment 43(6), 1404-1420.

Barros, K.A., Esteves-Ferreira, A.A., Inaba, M., Meally, H., Finnan, J., Barth, S., Sulpice, R., 2020b. Transient Carbon Reserves in Barley: Malate, Sucrose and Starch Are the Main Players, Their Quantitative Involvement Being Light Intensity Dependant. Frontiers in Plant Science 11(209).

Bartlett, M.K., Klein, T., Jansen, S., Choat, B., Sack, L., 2016. The correlations and sequence of plant stomatal, hydraulic, and wilting responses to drought. Proceedings of the National Academy of Sciences 113, 13098 LP - 13103.

Begović, L., Ravlić, J., Hrvoje, L., Leljak-Levanić, D., Cesar, V., 2015. The Pattern of Lignin Deposition in The Cell Walls of Internodes During Barley (Hordeum vulgare L.) Development. Acta Biologica Cracoviensia s. Botanica vol. 57(No 2).

Beier, S., Himmelbach, A., Colmsee, C., Zhang, X.-Q., Barrero, R.A., Zhang, Q., Li, L., Bayer, M., Bolser, D., Taudien, S., Groth, M., Felder, M., Hastie, A., Šimková, H., Staňková, H., Vrána, J., Chan, S., Muñoz-Amatriaín, M., Ounit, R., Wanamaker, S., Schmutzer, T., Aliyeva-Schnorr, L., Grasso, S., Tanskanen, J., Sampath, D., Heavens, D., Cao, S., Chapman, B., Dai, F., Han, Y., Li, H., Li, X., Lin, C., McCooke, J.K., Tan, C., Wang, S., Yin, S., Zhou, G., Poland, J.A., Bellgard, M.I., Houben, A., Doležel, J., Ayling, S., Lonardi, S., Langridge, P., Muehlbauer, G.J., Kersey, P., Clark, M.D., Caccamo, M., Schulman, A.H., Platzer, M., Close, T.J., Hansson, M., Zhang, G., Braumann, I., Li, C., Waugh, R., Scholz, U., Stein, N., Mascher, M., 2017. Construction of a map-based reference genome sequence for barley, Hordeum vulgare L. Scientific Data 4, 170044.

Bendix, C., Marshall, C.M., Harmon, F.G., 2015. Circadian clock genes universally control key agricultural traits. Molecular plant.

Bertolino, L.T., Caine, R.S., Gray, J.E., 2019. Impact of Stomatal Density and Morphology on Water-Use Efficiency in a Changing World. Frontiers in Plant Science 10, 225-225.

Boerjan, W., Ralph, J., Baucher, M., 2003. Lignin Biosynthesis. Annual Review of Plant Biology 54, 519-546.

Bonnett, G., Incoll, L., 1993. Effects on the stem of winter barley of manipulating the source and sink during grain-filling: I. Changes in accumulation and loss of mass from internodes. Journal of Experimental Botany 44(1), 75-82.

Bothmer, R.v., Sato, K., Komatsuda, T., Yasuda, S., Fischbeck, G., 2003. Chapter 2 The domestication of cultivated barley, in: Roland von Bothmer Helmut, K., Kazuhiro Sato, B.T.D.i.P.G., Breeding, T.v.H. (Eds.), Elsevier, pp. 9-27.

Boxall, S.F., Foster, J.M., Bohnert, H.J., Cushman, J.C., Nimmo, H.G., Hartwell, J., 2005. Conservation and Divergence of Circadian Clock Operation in a Stress-Inducible Crassulacean Acid Metabolism Species Reveals Clock Compensation against Stress. Plant Physiology 137(3), 969-982.

Bressan, R.A., Hasegawa, P.M., Handa, A.K., 1981. Resistance of cultured higher plant cells to polyethylene glycol-induced water stress. Plant Science Letters 21, 23-30.

Briggs, D., 1978. The morphology of barley; the vegetative phase, Barley. Springer, pp. 1-38.

Calixto, C.P.G., Simpson, C.G., Waugh, R., Brown, J.W.S., 2016. Alternative Splicing of Barley Clock Genes in Response to Low Temperature. PLOS ONE 11, e0168028.

Calixto, C.P.G., Waugh, R., Brown, J.W.S., 2015. Evolutionary Relationships Among Barley and Arabidopsis Core Circadian Clock and Clock-Associated Genes. Journal of Molecular Evolution 80, 108-119.

Campoli, C., Drosse, B., Searle, I., Coupland, G., von Korff, M., 2012a. Functional characterisation of HvCO1, the barley (Hordeum vulgare) flowering time ortholog of CONSTANS. The Plant Journal 69, 868-880.

Campoli, C., Drosse, B., Searle, I., Coupland, G., von Korff, M., 2012b. Functional characterisation of HvCO1, the barley (Hordeum vulgare) flowering time ortholog of CONSTANS. The Plant journal : for cell and molecular biology 69, 868-880.

Campoli, C., Pankin, A., Drosse, B., Casao, C.M., Davis, S.J., Korff, M., 2013. HvLUX1 is a candidate gene underlying the early maturity 10 locus in barley: phylogeny, diversity, and interactions with the circadian clock and photoperiodic pathways. New Phytologist 199, 1045-1059.

Campoli, C., Shtaya, M., Davis, S.J., von Korff, M., 2012c. Expression conservation within the circadian clock of a monocot: natural variation at barley Ppd-H1 affects circadian expression of flowering time genes, but not clock orthologs. BMC Plant Biology 12, 1-15.

Capovilla, G., Schmid, M., Posé, D., 2014. Control of flowering by ambient temperature. Journal of Experimental Botany 66, 59-69.

Casson, S., Gray, J.E., 2008. Influence of environmental factors on stomatal development. New Phytologist 178, 9-23.

Casson, S.A., Hetherington, A.M., 2010. Environmental regulation of stomatal development. Current Opinion in Plant Biology 13, 90-95.

Castells, E., Portolés, S., Huang, W., Mas, P., 2010. A functional connection between the clock component TOC1 and abscisic acid signaling pathways. Plant Signaling & Behavior 5(4), 409-411.

Chater, C.C.C., Oliver, J., Casson, S., Gray, J.E., 2014. Putting the brakes on: abscisic acid as a central environmental regulator of stomatal development. New Phytologist 202, 376-391.

Chaves, M.M., Costa, J.M., Zarrouk, O., Pinheiro, C., Lopes, C.M., Pereira, J.S., 2016. Controlling stomatal aperture in semi-arid regions—The dilemma of saving water or being cool? Plant Science 251, 54-64.

Chen, A., Li, C., Hu, W., Lau, M.Y., Lin, H., Rockwell, N.C., Martin, S.S., Jernstedt, J.A., Lagarias, J.C., Dubcovsky, J., 2014. PHYTOCHROME C plays a major role in the acceleration of wheat flowering under long-day photoperiod. Proceedings of the National Academy of Sciences 111 10037-10044.

Chia-Looi, A.-S., Gumming, B.G., 1972. Circadian rhythms of dark respiration, flowering, net photosynthesis, chlorophyll content, and dry weight changes in Chenopodium rubrum. Canadian Journal of Botany 50, 2219-2226.

Chutia, J., Borah, S.P., 2012. Water Stress Effects on Leaf Growth and Chlorophyll Content but Not the Grain Yield in Traditional Rice (Oryza sativa Linn.) Genotypes of Assam, India II. Protein and Proline Status in Seedlings under PEG Induced Water Stress. American Journal of Plant Sciences 3, 971-980.

Ciani, A., Goss, K.-U., Schwarzenbach, R.P., 2005. Light penetration in soil and particulate minerals. European Journal of Soil Science 56, 561-574.

Ciani, A., Goss, K.U., Schwarzenbach, R.P., 2005. Light penetration in soil and particulate minerals. European Journal of Soil Science 56(5), 561-574.

Clauw, P., Coppens, F., De Beuf, K., Dhondt, S., Van Daele, T., Maleux, K., Storme, V., Clement, L., Gonzalez, N., Inzé, D., 2015. Leaf Responses to Mild Drought Stress in Natural Variants of Arabidopsis. Plant Physiology 167 800-816.

Climatestotravel.com, 2019. https://www.climatestotravel.com/climate/syria. 2019).

Cockram, J., Jones, H., Leigh, F.J., O'Sullivan, D., Powell, W., Laurie, D.A., Greenland, A.J., 2007. Control of flowering time in temperate cereals: genes, domestication, and sustainable productivity. Journal of Experimental Botany 58 1231-1244.

Colt.55, 2006. This map shows the location and extent of the Fertile Crescent, a region in the Middle East incorporating Ancient Egypt; the Levant; and Mesopotamia, http://www.gnu.org/copyleft/fdl.html Microsoft Paint for Vista. wikipedia, wikimedia.org.

Condon, A.G., Richards, R.A., Rebetzke, G.J., Farquhar, G.D., 2002. Improving Intrinsic Water-Use Efficiency and Crop Yield. Crop science 42, 122-131.

Condon, A.G., Richards, R.A., Rebetzke, G.J., Farquhar, G.D., 2004. Breeding for high water-use efficiency. Journal of experimental botany 55, 2447-2460.

Consortium, I.W.G.S., Mayer, K.F., Rogers, J., Doležel, J., Pozniak, C., Eversole, K., Feuillet, C., Gill, B., Friebe, B., Lukaszewski, A.J., 2014. A chromosome-based draft sequence of the hexaploid bread wheat (Triticum aestivum) genome. Science 345(6194), 1251788.

Covington, M.F., Harmer, S.L., 2007. The Circadian Clock Regulates Auxin Signaling and Responses in Arabidopsis. PLoS Biol 5, e222.

Covington, M.F., Maloof, J.N., Straume, M., Kay, S.A., Harmer, S.L., 2008. Global transcriptome analysis reveals circadian regulation of key pathways in plant growth and development. Genome Biology 9, 1-18.

Dai, F., Nevo, E., Wu, D., Comadran, J., Zhou, M., Qiu, L., Chen, Z., Beiles, A., Chen, G., Zhang, G., 2012. Tibet is one of the centers of domestication of cultivated barley. Proceedings of the National Academy of Sciences 109, 16969-16973.

Dai, S., Wei, X., Pei, L., Thompson, R.L., Liu, Y., Heard, J.E., Ruff, T.G., Beachy, R.N., 2011. BROTHER OF LUX ARRHYTHMO is a component of the Arabidopsis circadian clock. The Plant Cell 23, 961-972.

Dakhiya, Y., Hussien, D., Fridman, E., Kiflawi, M., Green, R., 2017. Correlations between Circadian Rhythms and Growth in Challenging Environments. Plant Physiology 173, 1724 LP - 1734.

Daniels, R., Alcock, M., Scarisbrick, D., 1982. A reappraisal of stem reserve contribution to grain yield in spring barley (Hordeum vulgare L.). The Journal of Agricultural Science 98(2), 347-355.

Dawson, I.K., Russell, J., Powell, W., Steffenson, B., Thomas, W.T.B., Waugh, R., 2015. Barley: a translational model for adaptation to climate change. New Phytologist 206, 913-931.

del Moral, M.G., del Moral, L.G., 1995. Tiller production and survival in relation to grain yield in winter and spring barley. Field Crops Research 44(2-3), 85-93.

Deng, W., Clausen, J., Boden, S., Oliver, S.N., Casao, M.C., Ford, B., Anderssen, R.S., Trevaskis, B., 2015. Dawn and Dusk Set States of the Circadian Oscillator in Sprouting Barley Hordeum vulgareSeedlings. PLoS ONE 10, e0129781.

Digel, B., Tavakol, E., Verderio, G., Tondelli, A., Xu, X., Cattivelli, L., Rossini, L., von Korff, M., 2016. Photoperiod-H1 (Ppd-H1) controls leaf size in barley. Plant Physiology

Ding, Z., Doyle, M.R., Amasino, R.M., Davis, S.J., 2007. A complex genetic interaction between Arabidopsis thaliana TOC1 and CCA1/LHY in driving the circadian clock and in output regulation. Genetics 176.

Dios, V., Gessler, A., 2017. Circadian regulation of photosynthesis and transpiration from genes to ecosystems. Environmental and Experimental Botany.

Dixon, L.E., Knox, K., Kozma-Bognar, L., Southern, M.M., Pokhilko, A., Millar, A.J., 2016. Temporal Repression of Core Circadian Genes Is Mediated through EARLY FLOWERING 3 in Arabidopsis. Current Biology 21, 120-125.

Dodd, A.N., Belbin, F.E., Frank, A., Webb, A.A.R., 2015. Interactions between circadian clocks and photosynthesis for the temporal and spatial coordination of metabolism. Frontiers in Plant Science 6, 245.

Dodd, A.N., Parkinson, K., Webb, A.A.R., 2004. Independent circadian regulation of assimilation and stomatal conductance in the ztl-1 mutant of Arabidopsis. New Phytologist 162, 63-70.

Dodd, A.N., Salathia, N., Hall, A., Kévei, E., Tóth, R., Nagy, F., Hibberd, J.M., Millar, A.J., Webb, A.A.R., 2005. Plant Circadian Clocks Increase Photosynthesis, Growth, Survival, and Competitive Advantage. Science 309 630-633.

Dodig, D., Kandić, V., Zorić, M., Nikolić-Đorić, E., Nikolić, A., Mutavdžić, B., Perović, D., Šurlan-Momirović, G., 2018. Comparative kernel growth and yield components of two-and six-row barley (Hordeum vulgare) under terminal drought simulated by defoliation. Crop and Pasture Science 69(12), 1215-1224.

Dolgin, E., 2009. Maize genome mapped. Nature.

Druka, A., Franckowiak, J., Lundqvist, U., Bonar, N., Alexander, J., Houston, K., Radovic, S., Shahinnia, F., Vendramin, V., Morgante, M., 2010. Genetic dissection of barley morphology and development. Plant physiology, pp-110.

Druka, A., Muehlbauer, G., Druka, I., Caldo, R., Baumann, U., Rostoks, N., Schreiber, A., Wise, R., Close, T., Kleinhofs, A., 2006. An atlas of gene expression from seed to seed through barley development. Functional & integrative genomics 6(3), 202-211.

Dubrovsky, J.G., Rost, T.L., 2012. Pericycle.

Dunford, R.P., Yano, M., Kurata, N., Sasaki, T., Huestis, G., Rocheford, T., Laurie, D.A., 2002. Comparative mapping of the barley Ppd-H1 photoperiod response gene region, which lies close to a junction between two rice linkage segments. Genetics 161(2), 825-834.

Eckardt, N.A., 2000. Sequencing the Rice Genome. The Plant Cell 12(11), 2011-2017.

Edwards, K.D., Guerineau, F., Devlin, P.F., Millar, A.J., 2015. Low-temperature-specific effects of PHYTOCHROME C on the circadian clock in Arabidopsis suggest that PHYC underlies natural variation in biological timing. bioRxiv.

Ellis, R., Pieta Filho, C., 1992. The development of seed quality in spring and winter cultivars of barley and wheat. Seed Science Research 2(1), 9-15.

European Commision, 2013. EU Cereal farms report 2013 based on FADN data. pp. 3-3.

FAO, 2021. Global Grain Harvested Area in 2019, by Type (in Million Hectares).

FAO and USDA, U.S.D.o.A., 2021. Major Barley Producers Worldwide in 2020/2021, by Country (in Million Metric Tons).

Farquhar, G.D., Sharkey, T.D., 1982. Stomatal Conductance and Photosynthesis. Annual Review of Plant Physiology 33, 317-345.

Faure, S., Higgins, J., Turner, A., Laurie, D.A., 2007. The flowering locus FT-like gene family in barley (Hordeum vulgare). Genetics 176.

Faure, S., Turner, A.S., Gruszka, D., Christodoulou, V., Davis, S.J., von Korff, M., Laurie, D.A., 2012. Mutation at the circadian clock gene EARLY MATURITY 8 adapts domesticated barley (Hordeum vulgare) to short growing seasons. Proceedings of the National Academy of Sciences 109, 8328-8333.

Filichkin, S.A., Breton, G., Priest, H.D., Dharmawardhana, P., Jaiswal, P., Fox, S.E., Michael, T.P., Chory, J., Kay, S.A., Mockler, T.C., 2011. Global Profiling of Rice and Poplar Transcriptomes

Highlights Key Conserved Circadian-Controlled Pathways and cis-Regulatory Modules. PLoS ONE 6, e16907.

Fincher, G.B., 1976. Ferulic acid in barley cell walls: a fluorescence study. Journal of the Institute of Brewing 82, 347-349.

Ford, B., Deng, W., Clausen, J., Oliver, S., Boden, S., Hemming, M., Trevaskis, B., 2016. Barley (Hordeum vulgare) circadian clock genes can respond rapidly to temperature in an EARLY FLOWERING 3-dependent manner. Journal of Experimental Botany

Franckowiak, J., Foster, A., Pederson, V., Pyler, R., 1985. Registration of 'Bowman'barley. Crop Science 25(5), 883-883.

Franklin, K.A., Toledo-Ortiz, G., Pyott, D.E., Halliday, K.J., 2014. Interaction of light and temperature signalling. Journal of Experimental Botany 65(11), 2859-2871.

Franks, P.J., Farquhar, G.D., 2007. The mechanical diversity of stomata and its significance in gas-exchange control. Plant physiology 143, 78-87.

Friedt, W., Horsley, R.D., Harvey, B.L., Poulsen, D.M.E., Lance, R.C.M., Ceccarelli, S., Grando, S., Capettini, F., 2010. Barley Breeding History, Progress, Objectives, and Technology, Barley. pp. 160-220.

Gehring, H., Kasemir, H., Mohr, H., 1977. The capacity of chlorophyll-a biosynthesis in the mustard seedling cotyledons as modulated by phytochrome and circadian rhythmicity. Planta 133, 295-302.

Gil, K.-E., Park, C.-M., 2019. Thermal adaptation and plasticity of the plant circadian clock. New Phytologist 221(3), 1215-1229.

Goodspeed, D., Chehab, E.W., Min-Venditti, A., Braam, J., Covington, M.F., 2012. Arabidopsis synchronizes jasmonate-mediated defense with insect circadian behavior. Proceedings of the National Academy of Sciences 109 4674-4677.

Gorton, H.L., Williams, W.E., Binns, M.E., Gemmell, C.N., Leheny, E.A., Shepherd, A.C., 1989. Circadian Stomatal Rhythms in Epidermal Peels from Vicia faba. Plant Physiology 90 1329-1334.

Gould, P.D., Locke, J.C., Larue, C., Southern, M.M., Davis, S.J., Hanano, S., Moyle, R., Milich, R., Putterill, J., Millar, A.J., 2006. The molecular basis of temperature compensation in the Arabidopsis circadian clock. The Plant Cell 18(5), 1177-1187.

Graf, A., Schlereth, A., Stitt, M., Smith, A.M., 2010. Circadian control of carbohydrate availability for growth in Arabidopsis plants at night. Proceedings of the National Academy of Sciences 107, 9458-9463.

Grando, S., Macpherson, H.G., 2005. Food barley: importance, uses and local knowledge. Proceedings of the International Workshop on Food Barley Improvement, Hammamet, Tunisia, 14-17 January, 2002., x + 156 pp.

Grantz, D.A., Zeiger, E., 1986. Stomatal responses to light and leaf-air water vapor pressure difference show similar kinetics in sugarcane and soybean. Plant Physiology 81, 865-868.

Green, R.M., Tingay, S., Wang, Z.-Y., Tobin, E.M., 2002. Circadian Rhythms Confer a Higher Level of Fitness to Arabidopsis Plants. Plant Physiology 129 576-584.

Green, R.M., Tobin, E.M., 1999. Loss of the circadian clock-associated protein 1 in Arabidopsis results in altered clock-regulated gene expression. Proceedings of the National Academy of Sciences of the United States of America 96 7, 4176-4179.

Greenham, K., McClung, C.R., 2015. Integrating circadian dynamics with physiological processes in plants. Nat Rev Genet 16, 598-610.

Guan, J., Garcia, D.F., Zhou, Y., Appels, R., Li, A., Mao, L., 2020. The Battle to Sequence the Bread Wheat Genome: A Tale of the Three Kingdoms. Genomics, Proteomics & Bioinformatics 18(3), 221-229.

Haas, M., Schreiber, M., Mascher, M., 2019. Domestication and crop evolution of wheat and barley: Genes, genomics, and future directions. Journal of Integrative Plant Biology 61, 204-225.

Habte, E., Müller, L.M., Shtaya, M., Davis, S.J., Von Korff, M., 2014a. Osmotic stress at the barley root affects expression of circadian clock genes in the shoot. Plant, Cell & Environment 37, 1321-1337.

Habte, E., MÜLler, L.M., Shtaya, M., Davis, S.J., Von Korff, M., 2014b. Osmotic stress at the barley root affects expression of circadian clock genes in the shoot. Plant, Cell & Environment 37(6), 1321-1337.

Hanano, S., Domagalska, M.A., Nagy, F., Davis, S.J., 2006. Multiple phytohormones influence distinct parameters of the plant circadian clock. Genes to Cells 11, 1381-1392.

Harlan, J.R., Zohary, D., 1966. Distribution of wild wheats and barley. Science. 153.

Harmer, S.L., 2009. The Circadian System in Higher Plants. Annual Review of Plant Biology 60, 357-377.

Harmer, S.L., Hogenesch, J.B., Straume, M., Chang, H.-S., Han, B., Zhu, T., Wang, X., Kreps, J.A., Kay, S.A., 2000. Orchestrated Transcription of Key Pathways in Arabidopsis by the Circadian Clock. Science 290, 2110-2113.

Harmer, S.L., Panda, S., Kay, S.A., 2001. Molecular bases of circadian rhythms. Annual review of cell and developmental biology 17(1), 215-253.

Hassidim, M., Dakhiya, Y., Turjeman, A., Hussien, D., Shor, E., Anidjar, A., Goldberg, K., Green, R.M., 2017. CIRCADIAN CLOCK ASSOCIATED1 (CCA1) and the Circadian Control of Stomatal Aperture. Plant Physiology 175(4), 1864-1877.

Hatfield, J.L., Prueger, J.H., 2015. Temperature extremes: Effect on plant growth and development. Weather and climate extremes 10, 4-10.

Haworth, M., Scutt, C.P., Douthe, C., Marino, G., Gomes, M.T.G., Loreto, F., Flexas, J., Centritto, M., 2018. Allocation of the epidermis to stomata relates to stomatal physiological control: stomatal factors involved in the evolutionary diversification of the angiosperms and development of amphistomaty. Environmental and experimental botany 151, 55-63.

Hayes, K.R., Beatty, M., Meng, X., Simmons, C.R., Habben, J.E., Danilevskaya, O.N., 2010. Maize global transcriptomics reveals pervasive leaf diurnal rhythms but rhythms in developing ears are largely limited to the core oscillator. PLoS One 5.

Helfer, A., Nusinow, D.A., Chow, B.Y., Gehrke, A.R., Bulyk, M.L., Kay, S.A., 2011. LUX ARRHYTHMO encodes a nighttime repressor of circadian gene expression in the Arabidopsis core clock. Current biology : CB 21, 126-133.

Hemming, M.N., Walford, S.A., Fieg, S., Dennis, E.S., Trevaskis, B., 2012. Identification of high-temperature-responsive genes in cereals. Plant Physiology 158, 1439-1450.

Hennessey, T.L., Field, C.B., 1991. Circadian Rhythms in Photosynthesis : Oscillations in Carbon Assimilation and Stomatal Conductance under Constant Conditions. Plant Physiology 96, 831-836.

Hepworth, C., Caine, R.S., Harrison, E.L., Sloan, J., Gray, J.E., 2018. Stomatal development: focusing on the grasses. Current opinion in plant biology 41, 1-7.

Herrero, E., Kolmos, E., Bujdoso, N., Yuan, Y., Wang, M., Berns, M.C., Uhlworm, H., Coupland, G., Saini, R., Jaskolski, M., Webb, A., Gonçalves, J., Davis, S.J., 2012. EARLY FLOWERING4 recruitment of EARLY FLOWERING3 in the nucleus sustains the Arabidopsis circadian clock. The Plant Cell Online.

Hetherington, A.M., Woodward, F.I., 2003. The role of stomata in sensing and driving environmental change. Nature 424, 901.

Hill, K., Guerin, G., Hill, R., Watling, J., 2014. Temperature influences stomatal density and maximum potential water loss through stomata of Dodonaea viscosa subsp. angustissima along a latitude gradient in southern Australia. Australian Journal of Botany 62, 657-665.

Hoagland, D.R., Arnon, D.I., 1950. The water-culture method for growing plants without soil. Circular. California Agricultural Experiment Station 347.

Hohl, M., Schopfer, P., 1991. Water relations of growing maize coleoptiles: comparison between mannitol and polyethylene glycol 6000 as external osmotica for adjusting turgor pressure. Plant Physiology 95, 716-722.

Holmes, M.G., Klein, W.H., 1986. Photocontrol of Dark Circadian Rhythms in Stomata of Phaseolus vulgaris L. Plant Physiology 82 28-33.

Huang, C., Webb, M.J., Graham, R.D., 1996. Pot size affects expression of Mn efficiency in barley. Plant and Soil 178, 205-208.

Hubbard, Katharine E., Webb, Alex A.R., 2011. Circadian Rhythms: FLOWERING LOCUS T Extends Opening Hours. Current Biology 21, R636-R638.

Hubbard, Katharine E., Webb, Alex A.R., 2015. Circadian Rhythms in Stomata: Physiological and Molecular Aspects, in: Mancuso, S.S., S. (Ed.) Rhythms in Plants. Springer, Cham, pp. 231-255.

Hughes, J., Hepworth, C., Dutton, C., Dunn, J.A., Hunt, L., Stephens, J., Waugh, R., Cameron, D.D., Gray, J.E., 2017. Reducing Stomatal Density in Barley Improves Drought Tolerance without Impacting on Yield Plant Physiology 174(2), 776-787.

Jacomini, E., Bertani, A., Mapelli, S., 1988. Accumulation of polyethylene glycol 6000 and its effects on water content and carbohydrate level in water-stressed tomato plants. Canadian journal of botany 66, 970-973.

Janes, B.E., 1974. The effect of molecular size, concentration in nutrient solution, and exposure time on the amount and distribution of polyethylene glycol in pepper plants. Plant Physiology 54, 226-230.

Jetter, R., Schäffer, S., Riederer, M., 2000. Leaf cuticular waxes are arranged in chemically and mechanically distinct layers: evidence from Prunus laurocerasus L. Plant, Cell & Environment 23, 619-628.

Jung, H.-J.G., 2003. Maize stem tissues: ferulate deposition in developing internode cell walls. Phytochemistry 63, 543-549.

Karsai, I., Szűcs, P., Kőszegi, B., Hayes, P.M., Casas, A., Bedő, Z., Veisz, O., 2008. Effects of photo and thermo cycles on flowering time in barley: a genetical phenomics approach. Journal of Experimental Botany 59, 2707-2715.

Karsai, I., Szűcs, P., Mészáros, K., Filichkina, T., Hayes, P.M., Skinner, J.S., Láng, L., Bedő, Z., 2005. The Vrn-H2 locus is a major determinant of flowering time in a facultative × winter growth habit barley (Hordeum vulgare L.) mapping population. Theoretical and Applied Genetics 110, 1458-1466.

Kebede, A., Kang, M.S., Bekele, E., 2019. Chapter Five - Advances in mechanisms of drought tolerance in crops, with emphasis on barley, in: Sparks, D.L.B.T.A.i.A. (Ed.) Academic Press, pp. 265-314.

Khan, S., Rowe, S.C., Harmon, F.G., 2010. Coordination of the maize transcriptome by a conserved circadian clock. BMC Plant Biology 10, 1-15.

Kim, Y.X., Ranathunge, K., Lee, S., Lee, Y., Lee, D., Sung, J., 2018. Composite Transport Model and Water and Solute Transport across Plant Roots: An Update. Frontiers in Plant Science 9(193).

Kinmonth-Schultz, H.A., Golembeski, G.S., Imaizumi, T., 2013. Circadian clock-regulated physiological outputs: dynamic responses in nature, Seminars in cell & developmental biology. Elsevier, pp. 407-413.

Kirby, E., Faris, D., 1972. The effect of plant density on tiller growth and morphology in barley. The Journal of Agricultural Science 78(2), 281-288.

Kirby, E., Jones, H., 1977. The relations between the main shoot and tillers in barley plants. The Journal of Agricultural Science 88(2), 381-389.

Kollist, H., Nuhkat, M., Roelfsema, M.R.G., 2014. Closing gaps: linking elements that control stomatal movement. The New phytologist 203, 44-62.

Kreszies, T., Shellakkutti, N., Osthoff, A., Yu, P., Baldauf, J.A., Zeisler‐Diehl, V.V., Ranathunge, K., Hochholdinger, F., Schreiber, L., 2019. Osmotic stress enhances suberization of apoplastic barriers in barley seminal roots: analysis of chemical, transcriptomic and physiological responses. New Phytologist 221, 180-194.

Kubitzki, K., Huber, H., Rudall, P.J., Stevens, P.S., Stützel, T., 2010. Flowering Plants. Monocotyledons: Lilianae (except Orchidaceae). Springer Berlin Heidelberg.

Kunst, L., Samuels, A.L., 2003. Biosynthesis and secretion of plant cuticular wax. Progress in lipid research 42, 51-80.

Kurosawa, G., Fujioka, A., Koinuma, S., Mochizuki, A., Shigeyoshi, Y., 2017. Temperature–amplitude coupling for stable biological rhythms at different temperatures. PLOS Computational Biology 13(6), e1005501.

Kwon, Y.-J., Park, M.-J., Kim, S.-G., Baldwin, I.T., Park, C.-M., 2014. Alternative splicing and nonsense-mediated decay of circadian clock genes under environmental stress conditions in Arabidopsis. BMC plant biology 14, 136.

Leegood, R.C., 2008. Roles of the bundle sheath cells in leaves of C3 plants. Journal of Experimental Botany 59(7), 1663-1673.

Leff, B., Ramankutty, N., Foley, J.A., 2004. Geographic distribution of major crops across the world. Global Biogeochemical Cycles 18.

Legnaioli, T., Cuevas, J., Mas, P., 2009. TOC1 functions as a molecular switch connecting the circadian clock with plant responses to drought. The EMBO Journal 28, 3745-3757.

Leopold, A., 1949. The control of tillering in grasses by auxin. American Journal of Botany, 437-440.

Lichtenthaler, H.K., 1987. Chlorophylls and carotenoids: Pigments of photosynthetic biomembranes, in: Enzymology, B.T.M.i. (Ed.) Academic Press, pp. 350-382.

Liller, C.B., Neuhaus, R., von Korff, M., Koornneef, M., van Esse, W., 2015. Mutations in barley row type genes have pleiotropic effects on shoot branching. PLoS ONE 10, e0140246.

Liu, C., Allan, R.P., 2013. Observed and simulated precipitation responses in wet and dry regions 1850{\textendash}2100. Environmental Research Letters 8, 34002.

Lucas, W.J., Groover, A., Lichtenberger, R., Furuta, K., Yadav, S.-R., Helariutta, Y., He, X.-Q., Fukuda, H., Kang, J., Brady, S.M., Patrick, J.W., Sperry, J., Yoshida, A., López-Millán, A.-F., Grusak, M.A., Kachroo, P., 2013. The Plant Vascular System: Evolution, Development and FunctionsF. Journal of Integrative Plant Biology 55, 294-388.

Marcolino-Gomes, J., Rodrigues, F.A., Fuganti-Pagliarini, R., Bendix, C., Nakayama, T.J., Celaya, B., Molinari, H.B.C., de Oliveira, M.C.N., Harmon, F.G., Nepomuceno, A., 2014. Diurnal Oscillations of Soybean Circadian Clock and Drought Responsive Genes. PLoS ONE 9, e86402.

Mark, J.J., 2018. Fertile Crescent. Ancient History Encyclopedia.

Mascher, M., Gundlach, H., Himmelbach, A., Beier, S., Twardziok, S.O., Wicker, T., Radchuk, V., Dockter, C., Hedley, P.E., Russell, J., Bayer, M., Ramsay, L., Liu, H., Haberer, G., Zhang, X.-Q., Zhang, Q., Barrero, R.A., Li, L., Taudien, S., Groth, M., Felder, M., Hastie, A., Šimková, H., Staňková, H., Vrána, J., Chan, S., Muñoz-Amatriaín, M., Ounit, R., Wanamaker, S., Bolser, D., Colmsee, C., Schmutzer, T., Aliyeva-Schnorr, L., Grasso, S., Tanskanen, J., Chailyan, A., Sampath, D., Heavens, D., Clissold, L., Cao, S., Chapman, B., Dai, F., Han, Y., Li, H., Li, X., Lin, C., McCooke, J.K., Tan, C., Wang, P., Wang, S., Yin, S., Zhou, G., Poland, J.A., Bellgard, M.I., Borisjuk, L., Houben, A., Doležel, J., Ayling, S., Lonardi, S., Kersey, P., Langridge, P., Muehlbauer, G.J., Clark, M.D., Caccamo, M., Schulman, A.H., Mayer, K.F.X., Platzer, M., Close, T.J., Scholz, U., Hansson, M., Zhang, G., Braumann, I., Spannagl, M., Li, C., Waugh, R., Stein, N., 2017. A chromosome conformation capture ordered sequence of the barley genome. Nature 544, 427.

Matos, D.A., Cole, B.J., Whitney, I.P., MacKinnon, K.J.-M., Kay, S.A., Hazen, S.P., 2014. Daily Changes in Temperature, Not the Circadian Clock, Regulate Growth Rate in Brachypodium distachyon. PLOS ONE 9, e100072.

Matsushika, A., Makino, S., Kojima, M., Mizuno, T., 2000. Circadian waves of expression of the APRR1/TOC1 family of pseudo-responseregulators in Arabidopsis thaliana: Insight into the plant circadian clock. Plant Cell Physiol 41.

McAdam, S.A.M., Sussmilch, F.C., Brodribb, T.J., 2016. Stomatal responses to vapour pressure deficit are regulated by high speed gene expression in angiosperms. Plant, cell & environment 39, 485-491.

McAusland, L., Vialet‐Chabrand, S., Davey, P., Baker, N.R., Brendel, O., Lawson, T., 2016. Effects of kinetics of light‐induced stomatal responses on photosynthesis and water‐use efficiency. New Phytologist 211, 1209-1220.

McClung, C.R., 2011. The genetics of plant clocks, Advances in Genetics. pp. 105-139.

McClung, C.R., 2014. Wheels within wheels: new transcriptional feedback loops in the Arabidopsis circadian clock. F1000Prime Reports 6, 2.

McClung, C.R., 2019. The Plant Circadian Oscillator. Biology 8(1), 14.

McClung, R., 2010. A modern circadian clock in the common angiosperm ancestor of monocots and eudicots. BMC Evol Biol 8.

McClung, R.C., 2006. Plant Circadian Rhythms. The Plant Cell 18, 792-803.

McClung, R.C., 2009. Linking the loops. Science 13.

Medrano, H., Tomás, M., Martorell, S., Flexas, J., Hernández, E., Rosselló, J., Pou, A., Escalona, J.-M., Bota, J., 2015. From leaf to whole-plant water use efficiency (WUE) in complex canopies: limitations of leaf WUE as a selection target. The Crop Journal 3, 220-228.

Menezes, N.L.d., Silva, D.C., Arruda, R.C.O., Melo-de-Pinna, G.F., Cardoso, V.A., Castro, N.M., Scatena, V.L., Scremin-Dias, E., 2005. Meristematic activity of the Endodermis and the Pericycle in the primary thickening in monocotyledons: considerations on the "PTM". Anais da Academia Brasileira de Ciências 77, 259-274.

Merilo, E., Jõesaar, I., Brosché, M., Kollist, H., 2014. To open or to close: species‐specific stomatal responses to simultaneously applied opposing environmental factors. New Phytologist 202, 499-508.

Michael, T.P., Mockler, T.C., Breton, G., McEntee, C., Byer, A., Trout, J.D., Hazen, S.P., Shen, R., Priest, H.D., Sullivan, C.M., Givan, S.A., Yanovsky, M., Hong, F., Kay, S.A., Chory, J., 2008. Network Discovery Pipeline Elucidates Conserved Time-of-Day–Specific cis-Regulatory Modules. PLoS Genet 4, e14.

Michael, T.P., Salomé, P.A., Yu, H.J., Spencer, T.R., Sharp, E.L., McPeek, M.A., Alonso, J.M., Ecker, J.R., McClung, C.R., 2003. Enhanced fitness conferred by naturally occurring variation in the circadian clock. Science 302.

Minnesota, U.o., 2019a. Spring barley growth and development guide.

Minnesota, U.o., 2019b. Spring barley growth and development guide. University of Minnesota.

Mizuno, T., Nomoto, Y., Oka, H., Kitayama, M., Takeuchi, A., Tsubouchi, M., Yamashino, T., 2014. Ambient Temperature Signal Feeds into the Circadian Clock Transcriptional Circuitry Through the EC Night-Time Repressor in Arabidopsis thaliana. Plant and Cell Physiology 55, 958-976.

Molina-Cano, J.L., Conde, J., 1980. Hordeum spontaneum C. Koch em. Bacht, collected in southern Morocco. Barley Genetics Newsletter 10, 44-47.

Morrow, M.H.M., Algiers, K., Internal Anatomy of the Primary Stem. https://bio.libretexts.org/@go/page/37048. (Accessed 2021/11/12/.

Mott Keith, A., 2009. Opinion: Stomatal responses to light and CO2 depend on the mesophyll. Plant, Cell & Environment 32(11), 1479-1486.

Müller, L.M., Gol, L., Jeon, J.-S., Weber, A.P.M., Davis, S.J., von Korff, M., 2018. Temperature but not the circadian clock determines nocturnal carbohydrate availability for growth in cereals. bioRxiv, 363218.

Müller, L.M., von Korff, M., Davis, S.J., 2014. Connections between circadian clocks and carbon metabolism reveal species-specific effects on growth control. Journal of experimental botany 65, 2915-2923.

Muller, N.A., Wijnen, C.L., Srinivasan, A., Ryngajllo, M., Ofner, I., Lin, T., Ranjan, A., West, D., Maloof, J.N., Sinha, N.R., Huang, S., Zamir, D., Jimenez-Gomez, J.M., 2016. Domestication selected for deceleration of the circadian clock in cultivated tomato. Nat Genet 48, 89-93.

Muñoz-Amatriaín, M., Cuesta-Marcos, A., Hayes, P.M., Muehlbauer, G.J., 2014. Barley genetic variation: implications for crop improvement. Briefings in Functional Genomics 13, 341-350.

Murakami, M., Ashikari, M., Miura, K., Yamashino, T., Mizuno, T., 2003. The evolutionarily conserved OsPRR quintet: rice pseudo-response regulators implicated in circadian rhythm. Plant Cell Physiol 44.

Murakami, M., Tago, Y., Yamashino, T., Mizuno, T., 2007a. Characterization of the rice circadian clock-associated pseudo-response regulators in Arabidopsis thaliana. Biosci Biotechnol Biochem 71.

Murakami, M., Tago, Y., Yamashino, T., Mizuno, T., 2007b. Comparative overviews of clock-associated genes of Arabidopsis thaliana and Oryza sativa. Plant Cell Physiol 48.

Mustilli, A.-C., Merlot, S., Vavasseur, A., Fenzi, F., Giraudat, J., 2002. Arabidopsis OST1 Protein Kinase Mediates the Regulation of Stomatal Aperture by Abscisic Acid and Acts Upstream of Reactive Oxygen Species Production. The Plant Cell 14, 3089 LP - 3099.

Nagel, Dawn H.H., Kay, Steve A.A., 2012. Complexity in the Wiring and Regulation of Plant Circadian Networks. Current Biology 22, R648-R657.

Nakamichi, N., 2015. Adaptation to the Local Environment by Modifications of the Photoperiod Response in Crops. Plant and Cell Physiology 56 594-604.

Nevo, E., Fu, Y.-B., Pavlicek, T., Khalifa, S., Tavasi, M., Beiles, A., 2012. Evolution of wild cereals during 28 years of global warming in Israel. Proceedings of the National Academy of Sciences 109, 3412-3415.

Nevo, E., Shewry, P.R., 1992. Origin, evolution, population genetics and resources for breeding of wild barley, Hordeum spontaneum, in the Fertile Crescent. Barley: genetics, biochemistry, molecular biology and biotechnology., 19-43.

Newton, A.C., Flavell, A.J., George, T.S., Leat, P., Mullholland, B., Ramsay, L., Revoredo-Giha, C., Russell, J., Steffenson, B.J., Swanston, J.S., 2011. Crops that feed the world 4. Barley: a resilient crop? Strengths and weaknesses in the context of food security. Food Security 3, 141-178.

Nfus.org, 2018. What We Produce. NFU Scotland.

Ni, Z., Kim, E.D., Ha, M., Lackey, E., Liu, J., Zhang, Y., Sun, Q., Chen, Z.J., 2009. Altered circadian rhythms regulate growth vigour in hybrids and allopolyploids. Nature 457.

Nishida, H., Ishihara, D., Ishii, M., Kaneko, T., Kawahigashi, H., Akashi, Y., Saisho, D., Tanaka, K., Handa, H., Takeda, K., Kato, K., 2013. Phytochrome C is a key factor controlling long-day flowering in barley. Plant physiology 163, 804-814.

Nozue, K., Covington, M.F., Duek, P.D., Lorrain, S., Fankhauser, C., Harmer, S.L., Maloof, J.N., 2007. Rhythmic growth explained by coincidence between internal and external cues. Nature 448, 358-361.

Nusinow, D.A., Helfer, A., Hamilton, E.E., King, J.J., Imaizumi, T., Schultz, T.F., Farre, E.M., Kay, S.A., 2011. The ELF4-ELF3-LUX complex links the circadian clock to diurnal control of hypocotyl growth. Nature 475, 398-402.

Pan, W.-J., Wang, X., Deng, Y.-R., Li, J.-H., Chen, W., Chiang, J.Y., Yang, J.-B., Zheng, L., 2015. Nondestructive and intuitive determination of circadian chlorophyll rhythms in soybean leaves using multispectral imaging. Scientific Reports 5, 11108.

Pankin, A., Altmüller, J., Becker, C., von Korff, M., 2018. Targeted resequencing reveals genomic signatures of barley domestication. New Phytologist 218, 1247-1259.

Pankin, A., Campoli, C., Dong, X., Kilian, B., Sharma, R., Himmelbach, A., Saini, R., Davis, S.J., Stein, N., Schneeberger, K., 2014. Mapping-by-sequencing identifies HvPHYTOCHROME C as a candidate gene for the early maturity 5 locus modulating the circadian clock and photoperiodic flowering in barley. Genetics 198, 383-396.

Patil, V., McDermott, H.I., McAllister, T., Cummins, M., Silva, J.C., Mollison, E., Meikle, R., Morris, J., Hedley, P.E., Waugh, R., 2019. APETALA2 control of barley internode elongation. Development 146(11), dev170373.

Paulsen, H., Bogorad, L., 1988. Diurnal and Circadian Rhythms in the Accumulation and Synthesis of mRNA for the Light-Harvesting Chlorophyll a/b-Binding Protein in Tobacco. Plant Physiology 88, 1104 LP - 1109.

Peng, D., Chen, X., Yin, Y., Lu, K., Yang, W., Tang, Y., Wang, Z., 2014. Lodging resistance of winter wheat (Triticum aestivum L.): Lignin accumulation and its related enzymes activities due to the application of paclobutrazol or gibberellin acid. Field Crops Research 157, 1-7.

Peterson, C.A., Cholewa, E., 1998. Structural modifications of the apoplast and their potential impact on ion uptake. Zeitschrift für Pflanzenernährung und Bodenkunde 161, 521-531.

Pillitteri, L.J., Torii, K.U., 2012. Mechanisms of Stomatal Development. Annual Review of Plant Biology 63, 591-614.

Poets, A.M., Fang, Z., Clegg, M.T., Morrell, P.L., 2015. Barley landraces are characterized by geographically heterogeneous genomic origins. Genome biology 16, 1-11.

Poire, R., Wiese-Klinkenberg, A., Parent, B., Mielewczik, M., Schurr, U., Tardieu, F., Walter, A., 2010. Diel time-courses of leaf growth in monocot and dicot species: endogenous rhythms and temperature effects. Journal of experimental botany 61, 1751-1759.

Poiré, R., Wiese-Klinkenberg, A., Parent, B., Mielewczik, M., Schurr, U., Tardieu, F., Walter, A., 2010. Diel time-courses of leaf growth in monocot and dicot species: endogenous rhythms and temperature effects. Journal of Experimental Botany 61, 1751-1759.

Poorter, H., Bühler, J., van Dusschoten, D., Climent, J., Postma, J.A., 2012. Pot size matters: a meta-analysis of the effects of rooting volume on plant growth. Functional Plant Biology 39, 839-850.

Porra, R.J., Thompson, W.A., Kriedemann, P.E., 1989. Determination of accurate extinction coefficients and simultaneous equations for assaying chlorophylls a and b extracted with four different solvents: verification of the concentration of chlorophyll standards by atomic absorption spectroscopy. Biochimica et Biophysica Acta (BBA)-Bioenergetics 975, 384-394.

Post-Beittenmiller, D., 1996. Biochemistry and molecular biology of wax production in plants. Annual Review of Plant Physiology and Plant Molecular Biology 47, 405-430.

Pourkheirandish, M., Komatsuda, T., 2007. The importance of barley genetics and domestication in a global perspective. Annals of botany 100(5), 999-1008.

Prézelin, B.B., Sweeney, B.M., 1977. Characterization of Photosynthetic Rhythms in Marine Dinoflagellates. Plant Physiology 60, 388 LP - 392.

Qi, X., Torii, K.U., 2018. Hormonal and environmental signals guiding stomatal development. BMC Biology 16, 21.

Raissig, M.T., Matos, J.L., Anleu Gil, M.X., Kornfeld, A., Bettadapur, A., Abrash, E., Allison, H.R., Badgley, G., Vogel, J.P., Berry, J.A., Bergmann, D.C., 2017. Mobile MUTE specifies subsidiary cells to build physiologically improved grass stomata. Science 355, 1215 LP - 1218.

Rao, S., Jabeen, F.T.Z., 2013. In vitro selection and characterization of polyethylene glycol (PEG) tolerant callus lines and regeneration of plantlets from the selected callus lines in sugarcane (Saccharum officinarum L.). Physiology and Molecular Biology of Plants 19, 261-268.

Raschke, K., Fellows, M.P., 1971. Stomatal movement in Zea mays: shuttle of potassium and chloride between guard cells and subsidiary cells. Planta 101, 296-316.

Ray, J.D., Sinclair, T.R., 1998. The effect of pot size on growth and transpiration of maize and soybean during water deficit stress. Journal of Experimental Botany 49, 1381-1386.

Rehman, S., Kook, H.-S., Lim, J.-H., Park, M.-R., Ko, J.-C., Park, K.-G., Choi, J.-S., Park, T.-I., Kim, J.G., Lee, K.-S., 2004. Varietal responses of pollen development to salt stress in barley. Korean Journal of Crop Science 49(5), 407-409.

Rensing, L., Ruoff, P., 2002a. Temperature effect on entrainment, phase shifting, and amplitude of circadian clocks and its molecular bases. Chronobiology International 19(5), 807-864.

Rensing, L., Ruoff, P., 2002b. Temperature effect on entrainment, phase shifting, and amplitude of circadian clocks and its molecular bases. Chronobiology International 19(5), 807-864.

Resco de Dios, V., Gessler, A., Ferrio, J.P., Alday, J.G., Bahn, M., Del Castillo, J., Devidal, S., García-Muñoz, S., Kayler, Z., Landais, D., Martín-Gómez, P., Milcu, A., Piel, C., Pirhofer-Walzl, K., Ravel, O., Salekin, S., Tissue, D.T., Tjoelker, M.G., Voltas, J., Roy, J., 2016. Circadian rhythms have significant effects on leaf-to-canopy scale gas exchange under field conditions. GigaScience 5, 43.

Riaz, A., Kanwal, F., Börner, A., Pillen, K., Dai, F., Alqudah, A.M., 2021. Advances in Genomics-Based Breeding of Barley: Molecular Tools and Genomic Databases. Agronomy 11(5), 894.

Richardson, A., Franke, R., Kerstiens, G., Jarvis, M., Schreiber, L., Fricke, W., 2005. Cuticular wax deposition in growing barley (Hordeum vulgare) leaves commences in relation to the point of emergence of epidermal cells from the sheaths of older leaves. Planta 222(3), 472-483.

Rieu, I., Twell, D., Firon, N., 2017. Pollen development at high temperature: from acclimation to collapse. Plant physiology 173(4), 1967-1976.

Ruberti, I., Sessa, G., Ciolfi, A., Possenti, M., Carabelli, M., Morelli, G., 2012. Plant adaptation to dynamically changing environment: The shade avoidance response. Biotechnology Advances 30, 1047-1058.

Rudall, P.J., Chen, E.D., Cullen, E., 2017. Evolution and development of monocot stomata. American journal of botany 104, 1122-1141.

Rudall, P.J., Hilton, J., Bateman, R.M., 2013. Several developmental and morphogenetic factors govern the evolution of stomatal patterning in land plants. New Phytologist 200, 598-614.

Saisho, D., Takeda, K., 2011. Barley: Emergence as a New Research Material of Crop Science. Plant and Cell Physiology 52(5), 724-727.

Sakamoto, Y., Ishiguro, M., Kitagawa, G., 1986. Akaike information criterion statistics. Dordrecht, The Netherlands: D. Reidel 81(10.5555), 26853.

Sakuraba, Y., Jeong, J., Kang, M.-Y., Kim, J., Paek, N.-C., Choi, G., 2014. Phytochrome-interacting transcription factors PIF4 and PIF5 induce leaf senescence in Arabidopsis. Nature communications 5, 4636.

Salter, M.G., Franklin, K.A., Whitelam, G.C., 2003. Gating of the rapid shade-avoidance response by the circadian clock in plants. Nature 426, 680-683.

Samuels, L., Kunst, L., Jetter, R., 2008. Sealing plant surfaces: cuticular wax formation by epidermal cells. Annu. Rev. Plant Biol. 59, 683-707.

Sanchez, A., Shin, J., Davis, S.J., 2011. Abiotic stress and the plant circadian clock. Plant Signaling & Behavior 6, 223-231.

Sasani, S., Hemming, M.N., Oliver, S.N., Greenup, A., Tavakkol-Afshari, R., Mahfoozi, S., Poustini, K., Sharifi, H.-R., Dennis, E.S., Peacock, W.J., Trevaskis, B., 2009. The influence of vernalization and daylength on expression of flowering-time genes in the shoot apex and leaves of barley (Hordeum vulgare). Journal of experimental botany 60, 2169-2178.

Schäfer, N., Maierhofer, T., Herrmann, J., Jørgensen, M.E., Lind, C., von Meyer, K., Lautner, S., Fromm, J., Felder, M., Hetherington, A.M., 2018. A tandem amino acid residue motif in guard cell SLAC1 anion channel of grasses allows for the control of stomatal aperture by nitrate. Current Biology 28, 1370-1379.

Schreiber, M., Mascher, M., Wright, J., Padmarasu, S., Himmelbach, A., Heavens, D., Milne, L., Clavijo, B.J., Stein, N., Waugh, R., 2020. A Genome Assembly of the Barley 'Transformation Reference' Cultivar Golden Promise. G3 Genes|Genomes|Genetics 10(6), 1823-1827.

Schroeder, J.I., Allen, G.J., Hugouvieux, V., Kwak, J.M., Waner, D., 2001. Guard cell signal transduction. Annual review of plant physiology and plant molecular biology 52, 627-658.

Seung, D., Risopatron, J.P.M., Jones, B.J., Marc, J., 2012. Circadian clock-dependent gating in ABA signalling networks. Protoplasma 249, 445-457.

Shor, E., Green, R.M., 2016. The Impact of Domestication on the Circadian Clock. Trends in Plant Science 21, 281-283.

Sidaway-Lee, K., Costa, M.J., Rand, D.A., Finkenstadt, B., Penfield, S., 2014. Direct measurement of transcription rates reveals multiple mechanisms for configuration of the Arabidopsisambient temperature response. Genome Biology 15, R45.

Simmons, S.R., Rasmusson, D.C., Wiersma, J.V., 1982. Tillering in Barley: Genotype, Row Spacing, and Seeding Rate Effects 1. Crop Science 22(4), 801-805.

Simon, N.M.L., Comben, N.E., Hetherington, A.M., Dodd, A.N., 2019. A significant role for the circadian clock in the long-term water use efficiency of Arabidopsis. bioRxiv, 583526.

Somers, D.E., Webb, A.A., Pearson, M., Kay, S.A., 1998. The short-period mutant, toc1-1, alters circadian clock regulation of multiple outputs throughout development in Arabidopsis thaliana. Development 125(3), 485-494.

Song, Y.H., Ito, S., Imaizumi, T., 2010. Similarities in the circadian clock and photoperiodism in plants. Current opinion in plant biology 13, 594-603.

Song, Y.H., Ito, S., Imaizumi, T., 2013. Flowering time regulation: photoperiod- and temperature-sensing in leaves. Trends in Plant Science 18, 575-583.

Song, Y.H., Kubota, A., Kwon, M.S., Covington, M.F., Lee, N., Taagen, E.R., Laboy Cintrón, D., Hwang, D.Y., Akiyama, R., Hodge, S.K., Huang, H., Nguyen, N.H., Nusinow, D.A., Millar, A.J., Shimizu, K.K., Imaizumi, T., 2018. Molecular basis of flowering under natural long-day conditions in Arabidopsis. Nature Plants 4(10), 824-835.

Sorek, M., Díaz-Almeyda, E.M., Medina, M., Levy, O., 2014. Circadian clocks in symbiotic corals: The duet between Symbiodinium algae and their coral host. Marine Genomics 14, 47-57.

Soreng, R.J., Peterson, P.M., Romaschenko, K., Davidse, G., Teisher, J.K., Clark, L.G., Barberá, P., Gillespie, L.J., Zuloaga, F.O., 2017. A worldwide phylogenetic classification of the Poaceae (Gramineae) II: An update and a comparison of two 2015 classifications. Journal of Systematics and Evolution 55, 259-290.

Starling, T., 1980. Barley. Hybridization of Crop Plants, 189-202.

Stebbins, G.L., Shah, S.S., 1960. Developmental studies of cell differentiation in the epidermis of monocotyledons: II. Cytological features of stomatal development in the Gramineae. Developmental Biology 2, 477-500.

Steudle, E., 2000a. Water uptake by plant roots: an integration of views. Plant and soil 226, 45-56.

Steudle, E., 2000b. Water uptake by roots: effects of water deficit. Journal of experimental botany 51, 1531-1542.

Steudle, E., 2002. Transport of water in plants. Environment Control in Biology 40, 29-37.

Steudle, E., Jeschke, W.D., 1983. Water transport in barley roots. Planta 158, 237-248.

Sujetovienė, G., 2013. Biomonitoring of urban air quality in Kaunas City (Lithuania) using transplanted lichens. Biologija 59.

Taiz, L., Zeiger, E., 2003. Plant physiology. 3rd edn. Annals of Botany 91(6), 750-751.

Takano, M., Inagaki, N., Xie, X., Yuzurihara, N., Hihara, F., Ishizuka, T., Yano, M., Nishimura, M., Miyao, A., Hirochika, H., Shinomura, T., 2005. Distinct and cooperative functions of phytochromes A, B, and C in the control of deetiolation and flowering in rice. The Plant cell 17, 3311-3325.

Takase, T., Ishikawa, H., Murakami, H., Kikuchi, J., Sato-Nara, K., Suzuki, H., 2011. The circadian clock modulates water dynamics and aquaporin expression in Arabidopsis roots. Plant and Cell Physiology 52, 373-383.

Takata, N., Saito, S., Saito, C.T., Uemura, M., 2010. Phylogenetic footprint of the plant clock system in angiosperms: evolutionary processes of pseudo-response regulators. BMC Evol Biol 10.

Tavakoli, H., Mohtasebi, S., Jafari, A., 2009. Effects of moisture content, internode position and loading rate on the bending characteristics of barley straw. Research in Agricultural Engineering 55(2), 45-51.

Teagasc, 2017. Growth Stages. https://www.teagasc.ie/crops/crops/cereal-crops/spring-cereals/growth-stages/. 2017).

Tester, M., Morris, C., 1987. The penetration of light through soil. Plant, Cell & Environment 10, 281-286.

Tezara, W., Mitchell, V.J., Driscoll, S.D., Lawlor, D.W., 1999. Water stress inhibits plant photosynthesis by decreasing coupling factor and ATP. Nature 401, 914-917.

The International Barley Genome Sequencing Consortium, I., Mayer, K.F.X., Waugh, R., Langridge, P., Close, T.J., Wise, R.P., Graner, A., Matsumoto, T., Sato, K., Schulman, A., Muehlbauer, G.J., Stein, N., 2012. A physical, genetic and functional sequence assembly of the barley genome. Nature 491, 711-716.

Thines, B., Harmon, F.G., 2010. Ambient temperature response establishes ELF3 as a required component of the core Arabidopsis circadian clock. Proceedings of the National Academy of Sciences 107, 3257-3262.

Tombesi, S., Nardini, A., Frioni, T., Soccolini, M., Zadra, C., Farinelli, D., Poni, S., Palliotti, A., 2015. Stomatal closure is induced by hydraulic signals and maintained by ABA in drought-stressed grapevine. Scientific Reports 5, 12449.

Tottman, D.R., 1987. The decimal code for the growth stages of cereals, with illustrations. Annals of Applied Biology 110(2), 441-454.

Trivett, C.L., Evert, R.F., 1998. Ontogeny of the Vascular Bundles and Contiguous Tissues in the Barley Leaf Blade. International Journal of Plant Sciences 159, 716-723.

Turner, A., Beales, J., Faure, S., Dunford, R.P., Laurie, D.A., 2005. The pseudo-response regulator Ppd-H1 provides adaptation to photoperiod in barley. Science 310, 1031-1034.

Ullrich, S.E., 2010. Barley: Production, Improvement, and Uses. Wiley.

van den Berg, E., Labuschagne, M.T., 2012. The interaction of stem strength with plant density and nitrogen application in wheat progeny from parents with varying stem strength. Acta Agriculturae Scandinavica, Section B — Soil & Plant Science 62, 251-255.

Verslues, P.E., Agarwal, M., Katiyar‐Agarwal, S., Zhu, J., Zhu, J.K., 2006. Methods and concepts in quantifying resistance to drought, salt and freezing, abiotic stresses that affect plant water status. The Plant Journal 45, 523-539.

Verslues, P.E., Ober, E.S., Sharp, R.E., 1998. Root growth and oxygen relations at low water potentials. Impact of oxygen availability in polyethylene glycol solutions. Plant Physiology 116, 1403-1412.

Vico, G., Manzoni, S., Palmroth, S., Katul, G., 2011. Effects of stomatal delays on the economics of leaf gas exchange under intermittent light regimes. New Phytologist 192, 640-652.

Vogel, J., 2008. Unique aspects of the grass cell wall. Current Opinion in Plant Biology 11, 301-307.

Vogel, S., 1998. Floral biology, Springer, pp. 34-48.

Vosz, U., Wilson, M.H., Kenobi, K., Gould, P.D., Robertson, F.C., Peer, W.A., Lucas, M., Swarup, K., Casimiro, I., Holman, T.J., Wells, D.M., Peret, B., Goh, T., Fukaki, H., Hodgman, T.C., Laplaze, L., Halliday, K.J., Ljung, K., Murphy, A.S., Hall, A.J., Webb, A.A.R., Bennett, M.J., 2015. The circadian clock rephases during lateral root organ initiation in Arabidopsis thaliana. Nat Commun 6.

Walter, A., Silk, W.K., Schurr, U., 2009. Environmental Effects on Spatial and Temporal Patterns of Leaf and Root Growth. Annual Review of Plant Biology 60, 279-304.

Wang, G., Schmalenbach, I., von Korff, M., Léon, J., Kilian, B., Rode, J., Pillen, K., 2010. Association of barley photoperiod and vernalization genes with QTLs for flowering time and agronomic traits in a BC2DH population and a set of wild barley introgression lines. Theoretical and Applied Genetics 120, 1559-1574.

Wang, X., Wang, H., Wang, J., Sun, R., Wu, J., Liu, S., Bai, Y., Mun, J.-H., Bancroft, I., Cheng, F., Huang, S., Li, X., Hua, W., Wang, J., Wang, X., Freeling, M., Pires, J.C., Paterson, A.H., Chalhoub, B., Wang, B., Hayward, A., Sharpe, A.G., Park, B.-S., Weisshaar, B., Liu, B., Li, B., Liu, B., Tong, C., Song, C., Duran, C., Peng, C., Geng, C., Koh, C., Lin, C., Edwards, D., Mu, D., Shen, D., Soumpourou, E., Li, F., Fraser, F., Conant, G., Lassalle, G., King, G.J., Bonnema, G., Tang, H., Wang, H., Belcram, H., Zhou, H., Hirakawa, H., Abe, H., Guo, H., Wang, H., Jin, H., Parkin, I.A.P., Batley, J., Kim, J.-S., Just, J., Li, J., Xu, J., Deng, J., Kim, J.A., Li, J., Yu, J., Meng, J., Wang, J., Min, J., Poulain, J., Wang, J., Hatakeyama, K., Wu, K., Wang, L., Fang, L., Trick, M., Links, M.G., Zhao, M., Jin, M., Ramchiary, N., Drou, N., Berkman, P.J., Cai, Q., Huang, Q., Li, R., Tabata, S., Cheng, S., Zhang, S., Zhang, S., Huang, S., Sato, S., Sun, S., Kwon, S.-J., Choi, S.-R., Lee, T.-H., Fan, W., Zhao, X., Tan, X., Xu, X., Wang, Y., Qiu, Y., Yin, Y., Li, Y., Du, Y., Liao, Y., Lim, Y., Narusaka, Y., Wang, Y.,

Wang, Z., Li, Z., Wang, Z., Xiong, Z., Zhang, Z., 2011. The genome of the mesopolyploid crop species Brassica rapa. Nat Genet 43, 1035-1039.

Watts, G., Battarbee, R.W., Bloomfield, J.P., Crossman, J., Daccache, A., Durance, I., Elliott, J.A., Garner, G., Hannaford, J., Hannah, D.M., Hess, T., Jackson, C.R., Kay, A.L., Kernan, M., Knox, J., Mackay, J., Monteith, D.T., Ormerod, S.J., Rance, J., Stuart, M.E., Wade, A.J., Wade, S.D., Weatherhead, K., Whitehead, P.G., Wilby, R.L., 2015. Climate change and water in the UK – past changes and future prospects. Progress in Physical Geography 39 6-28.

Webb, A.A.R., Seki, M., Satake, A., Caldana, C., 2019. Continuous dynamic adjustment of the plant circadian oscillator. Nature Communications 10(1), 550.

Williams, M.L., Farrar, J.F., Pollock, C.J., 1989. Cell specialization within the parenchymatous bundle sheath of barley. Plant, Cell & Environment 12(9), 909-918.

Willmer, C.M., Fricker, M., 1996. Stomata. Chapman & Hall.

Wolfe, R.I., Franckowiak, J.D., 1990. Multiple dominant and recessive genetic marker stocks in spring barley. Barley Genetics Newsletter 20, 117-121.

Woods, D.P., Ream, T.S., Minevich, G., Hobert, O., Amasino, R.M., 2014. PHYTOCHROME C is an essential light receptor for photoperiodic flowering in the temperate grass, Brachypodium distachyon. Genetics 198, 397-408.

Woolley, J.T., Stoller, E.W., 1978. Light penetration and light-induced seed germination in soil. Plant Physiology 61, 597-600.

Yakir, E., Hilman, D., Harir, Y., Green, R.M., 2007. Regulation of output from the plant circadian clock. The FEBS journal 274, 335-345.

Yaniv, Z., Werker, E., 1983. Absorption and secretion of polyethylene glycol by Solanaceous plants. Journal of experimental botany 34, 1577-1584.

Yoshida, T., Anjos, L.d., Medeiros, D.B., Araújo, W.L., Fernie, A.R., Daloso, D.M., 2019. Insights into ABA-mediated regulation of guard cell primary metabolism revealed by systems biology approaches. Progress in Biophysics and Molecular Biology 146, 37-49.

Yoshida, T., Mogami, J., Yamaguchi-Shinozaki, K., 2014. ABA-dependent and ABA-independent signaling in response to osmotic stress in plants. Current opinion in plant biology 21, 133-139.

Yu, J.-W., Rubio, V., Lee, N.-Y., Bai, S., Lee, S.-Y., Kim, S.-S., Liu, L., Zhang, Y., Irigoyen, M.L., Sullivan, J.A., Zhang, Y., Lee, I., Xie, Q., Paek, N.-C., Deng, X.W., 2008. COP1 and ELF3 Control Circadian Function and Photoperiodic Flowering by Regulating GI Stability. Molecular Cell 32, 617-630.

Zadoks, J.C., Chang, T.T., Konzak, C.F., 1974. A decimal code for the growth stages of cereals. Weed Research 14, 415-421.

Zagotta, M.T., Hicks, K.A., Jacobs, C.I., Young, J.C., Hangarter, R.P., Meeks-Wagner, D.R., 1996. The Arabidopsis ELF3 gene regulates vegetative photomorphogenesis and the photoperiodic induction of flowering. The Plant Journal 10, 691-702.

Zakhrabekova, S., Gough, S.P., Braumann, I., Müller, A.H., Lundqvist, J., Ahmann, K., Dockter, C., Matyszczak, I., Kurowska, M., Druka, A., Waugh, R., Graner, A., Stein, N., Steuernagel, B., Lundqvist, U., Hansson, M., 2012. Induced mutations in circadian clock regulator Mat-a

facilitated short-season adaptation and range extension in cultivated barley. Proceedings of the National Academy of Sciences 109 4326-4331.

Zdepski, A., Wang, W., Priest, H.D., Ali, F., Alam, M., Mockler, T.C., Michael, T.P., 2008. Conserved Daily Transcriptional Programs in Carica papaya Tropical Plant Biology 1, 236-245.

Zeiger, E., Farquhar, G.D., Cowan, I.R., Goeschl, J.D., 1988. Stomatal Function. The Quarterly Review of Biology 63, 340-341.

Zhong, Y.-P., Li, Z., Bai, D.-F., Qi, X.-J., Chen, J.-Y., Wei, C.-G., Lin, M.-M., Fang, J.-B., 2018. In Vitro Variation of Drought Tolerance in Five Actinidia Species. Journal of the American Society for Horticultural Science 143, 226-234.

Zhou, M.X., 2009. Barley production and consumption, Genetics and improvement of barley malt quality. Springer, pp. 1-17.

Zohary, D., 1999. Monophyletic vs. polyphyletic origin of the crops on which agriculture was founded in the Near East. Genetic Resources and Crop Evolution 46, 133-142.

Zohary, D., 2017. The progenitors of wheat and barley in relation to domestication and agricultural dispersal in the Old World, The domestication and exploitation of plants and animals. Routledge, pp. 47-66.

Zohary, D., Hopf, M., 2000. Domestication of plants in the Old World: the origin and spread of cultivated plants in West Asia, Europe and the Nile Valley.

Zohary, D., Hopf, M., Weiss, E., 2012. Domestication of plants in the Old World: The origin and spread of domesticated plants in south-west Asia, Europe, and the Mediterranean Basin.

Appendices

Supplementary table 3.3.1a: Summary tables for the results in chapter 3.3.1 including all the 22 measured and calculated traits data minimum, maximum, means standard deviations to two decimal places and sample number for six barley lines grown in four different osmotic conditions induced with 0%, 5%, 10% and 15% PEG in 20°C/18°C temperature cycles of 12:12 hours.

		Total fresh weight (g) pre treatment											
PEG		Antonella		Bowman		eam10.m/Hvlux^{-}		eam5.x/HvPHYC^{+}		eam8.k/Hvelf3^{-}		eam8.w/Hvelf3^{-}	
0%	Min	0.32		0.40		0.42		0.31		0.36		0.33	
	Max	0.97		1.13		0.84		1.07		0.99		1.18	
	Mean (s.d.)	0.59	± 0.20	0.75	± 0.25	0.62	± 0.14	0.69	± 0.22	0.57	± 0.18	0.64	± 0.23
	n	14		15		15		14		15		15	
5%	Min	0.38		0.33		0.38		0.30		0.32		0.31	
	Max	0.90		1.20		0.93		0.83		0.79		0.86	
	Mean (s.d.)	0.57	± 0.16	0.63	± 0.26	0.66	± 0.18	0.58	± 0.19	0.51	± 0.16	0.51	± 0.16
	n	15		14		14		14		15		14	
10%	Min	0.35		0.25		0.31		0.40		0.36		0.25	
	Max	0.91		1.73		0.78		0.85		1.24		1.04	

	Mean (s.d.)	0.50	±	0.18	0.73	±	0.38	0.57	±	0.14	0.59	±	0.13	0.67	±	0.26	0.59	±	0.23
	n	14			15			15			14			15			14		
15 %	Min	0.27			0.26			0.28			0.33			0.31			0.26		
	Max	0.86			1.16			0.86			0.84			1.15			1.19		
	Mean (s.d.)	0.50	±	0.18	0.72	±	0.24	0.52	±	0.16	0.58	±	0.17	0.71	±	0.26	0.70	±	0.28
	n	15			13			14			15			15			14		
		Total fresh weight (g) post treatment																	
PEG		Antonella			Bowman			eam10.m/Hvlux^{-}			eam5.x/HvPHYC^{+}			eam8.k/Hvelf3^{-}			eam8.w/Hvelf3^{-}		
0%	Min	0.70			0.96			0.97			0.69			0.84			0.72		
	Max	2.05			2.64			2.42			2.09			2.17			2.36		
	Mean (s.d.)	1.20	±	0.41	1.54	±	0.52	1.41	±	0.41	1.32	±	0.39	1.19	±	0.36	1.39	±	0.47
	n	14			15			15			14			15			15		
5%	Min	0.26			0.28			0.22			0.17			0.24			0.26		
	Max	1.50			1.81			1.32			1.36			1.11			1.54		

	Mean (s.d.)	0.70	± 0.31	0.68	± 0.37	0.74	± 0.31	0.55	± 0.29	0.60	± 0.27	0.57	± 0.33
	n	15		14		14		14		15		14	
10 %	Min	0.23		0.12		0.14		0.20		0.13		0.17	
	Max	1.60		0.95		0.63		0.98		1.56		1.16	
	Mean (s.d.)	0.55	± 0.35	0.46	± 0.22	0.34	± 0.14	0.46	± 0.23	0.59	± 0.37	0.51	± 0.27
	n	14		15		15		14		15		14	
15 %	Min	0.18		0.24		0.19		0.16		0.14		0.14	
	Max	0.60		1.20		0.56		0.79		0.76		1.05	
	Mean (s.d.)	0.32	± 0.14	0.58	± 0.33	0.30	± 0.11	0.39	± 0.19	0.45	± 0.18	0.54	± 0.30
	n	15		13		14		15		15		14	
		Shoot fresh weight (g) post treatment											
PEG		Antonella		Bowman		eam10.m/Hvlux^{-}		eam5.x/HvPHYC^{+}		eam8.k/Hvelf3^{-}		eam8.w/Hvelf3^{-}	
0%	Min	0.46		0.64		0.61		0.51		0.56		0.49	

	Max	1.27			1.50			1.26			1.18			1.41			1.38	
	Mean (s.d.)	0.75	±	0.24	0.98	±	0.27	0.86	±	0.17	0.82	±	0.18	0.77	±	0.22	0.89	± 0.25
	n	14			15			15			14			15			15	
5%	Min	0.17			0.19			0.10			0.11			0.14			0.17	
	Max	0.92			1.14			0.80			0.72			0.64			0.96	
	Mean (s.d.)	0.41	±	0.19	0.42	±	0.23	0.44	±	0.19	0.33	±	0.17	0.37	±	0.17	0.37	± 0.21
	n	15			14			14			14			15			14	
10 %	Min	0.12			0.07			0.07			0.11			0.08			0.08	
	Max	0.78			0.49			0.36			0.64			0.88			0.74	
	Mean (s.d.)	0.30	±	0.18	0.24	±	0.11	0.18	±	0.08	0.26	±	0.15	0.32	±	0.23	0.29	± 0.18
	n	14			15			15			14			15			14	
15 %	Min	0.08			0.13			0.09			0.08			0.07			0.07	
	Max	0.36			0.71			0.27			0.49			0.44			0.67	

	Mean (s.d.)	0.16	± 0.08	0.30	± 0.19	0.14	± 0.05	0.21	± 0.12	0.22	± 0.10	0.29	± 0.18
	n	15		13		14		15		15		14	
		Root fresh weight (g) post treatment											
PEG		Antonella		Bowman		eam10.m/Hvlux^{-}		eam5.x/HvPHYC^{+}		eam8.k/Hvelf3^{-}		eam8.w/Hvelf3^{-}	
0%	Min	0.24		0.32		0.31		0.18		0.24		0.22	
	Max	0.85		1.19		1.16		1.27		0.77		1.05	
	Mean (s.d.)	0.45	± 0.17	0.57	± 0.27	0.55	± 0.24	0.50	± 0.27	0.41	± 0.15	0.50	± 0.22
	n	14		15		15		14		15		15	
5%	Min	0.10		0.09		0.10		0.07		0.08		0.09	
	Max	0.58		0.68		0.52		0.63		0.47		0.58	
	Mean (s.d.)	0.28	± 0.13	0.26	± 0.14	0.30	± 0.13	0.22	± 0.13	0.23	± 0.11	0.20	± 0.12
	n	15		14		14		14		15		14	
10%	Min	0.11		0.05		0.07		0.08		0.06		0.08	
	Max	0.82		0.52		0.31		0.42		0.68		0.41	

	Mean (s.d.)	0.24	± 0.18	0.22	± 0.12	0.16	± 0.06	0.20	± 0.09	0.27	± 0.16	0.22	± 0.09
	n	14		15		15		14		15		14	
15 %	Min	0.10		0.11		0.09		0.07		0.08		0.07	
	Max	0.30		0.51		0.30		0.40		0.36		0.48	
	Mean (s.d.)	0.17	± 0.07	0.28	± 0.14	0.16	± 0.06	0.18	± 0.09	0.22	± 0.09	0.26	± 0.14
	n	15		13		14		15		15		14	
		Change in fresh weight (g)											
PEG		Antonella		Bowman		eam10.m/Hvlux^{-}		eam5.x/HvPHYC^{+}		eam8.k/Hvelf3^{-}		eam8.w/Hvelf3^{-}	
0%	Min	0.33		0.40		0.42		0.37		0.18		0.19	
	Max	1.08		1.51		1.58		1.11		1.18		1.30	
	Mean (s.d.)	0.61	± 0.22	0.79	± 0.31	0.79	± 0.30	0.63	± 0.25	0.62	± 0.25	0.75	± 0.28
	n	14		15		15		14		15		15	
5%	Min	-0.21		-0.24		-0.27		-0.45		-0.18		-0.28	
	Max	0.60		0.61		0.42		0.53		0.49		0.69	

	Mean (s.d.)	0.13	±	0.19	0.05	±	0.21	0.07	±	0.20	-0.03	±	0.23	0.09	±	0.20	0.06	± 0.23
	n	15			14			14			14			15			14	
10 %	Min	-0.44			-0.78			-0.63			-0.26			-0.42			-0.35	
	Max	1.11			0.20			-0.09			0.35			0.53			0.22	
	Mean (s.d.)	0.05	±	0.35	-0.26	±	0.21	-0.23	±	0.12	-0.12	±	0.18	-0.08	±	0.27	-0.08	± 0.16
	n	14			15			15			14			15			14	
15 %	Min	-0.34			-0.49			-0.41			-0.55			-0.61			-0.61	
	Max	0.21			0.39			0.04			0.29			0.06			0.22	
	Mean (s.d.)	-0.18	±	0.12	-0.14	±	0.23	-0.22	±	0.11	-0.19	±	0.19	-0.26	±	0.17	-0.16	± 0.21
	n	15			13			14			15			15			14	

		Total dry biomass (g)					
PEG		Antonella	Bowman	eam10.m/Hvlux^{-}	eam5.x/HvPHYC^{+}	eam8.k/Hvelf3^{-}	eam8.w/Hvelf3^{-}
0%	Min	0.07	0.08	0.07	0.05	0.06	0.06

	Max	0.17			0.19			0.17			0.18			0.20			0.20	
	Mean (s.d.)	0.11	±	0.03	0.13	±	0.03	0.11	±	0.03	0.11	±	0.03	0.10	±	0.03	0.12	± 0.04
	n	14			15			15			14			15			15	
5%	Min	0.05			0.04			0.03			0.03			0.04			0.04	
	Max	0.15			0.16			0.12			0.11			0.10			0.14	
	Mean (s.d.)	0.08	±	0.03	0.08	±	0.03	0.08	±	0.02	0.07	±	0.02	0.07	±	0.02	0.07	± 0.03
	n	15			14			14			14			15			14	
10 %	Min	0.04			0.04			0.03			0.04			0.03			0.04	
	Max	0.12			0.12			0.10			0.10			0.14			0.13	
	Mean (s.d.)	0.07	±	0.02	0.07	±	0.02	0.06	±	0.02	0.07	±	0.02	0.08	±	0.03	0.07	± 0.02
	n	14			15			15			14			15			14	
15 %	Min	0.04			0.05			0.03			0.03			0.03			0.02	
	Max	0.09			0.12			0.07			0.09			0.10			0.12	

	Mean (s.d.)	0.06	± 0.02	0.08	± 0.02	0.05	± 0.01	0.06	± 0.02	0.07	± 0.02	0.08	± 0.03
	n	15		13		14		15		15		14	
		Shoot dry biomass (g)											
PEG		Antonella		Bowman		eam10.m/Hvlux^{-}		eam5.x/HvPHYC^{+}		eam8.k/Hvelf3^{-}		eam8.w/Hvelf3^{-}	
0%	Min	0.05		0.06		0.06		0.04		0.05		0.05	
	Max	0.13		0.15		0.13		0.14		0.15		0.15	
	Mean (s.d.)	0.08	± 0.02	0.09	± 0.03	0.09	± 0.02	0.09	± 0.02	0.08	± 0.02	0.09	± 0.03
	n	14		15		15		14		15		15	
5%	Min	0.03		0.03		0.03		0.03		0.03		0.03	
	Max	0.12		0.12		0.09		0.09		0.08		0.11	
	Mean (s.d.)	0.06	± 0.02	0.06	± 0.02	0.06	± 0.02	0.05	± 0.02	0.06	± 0.02	0.05	± 0.02
	n	15		14		14		14		15		14	
10%	Min	0.03		0.03		0.02		0.04		0.02		0.03	
	Max	0.11		0.10		0.07		0.08		0.12		0.10	

	Mean (s.d.)	0.05	± 0.02	0.05	± 0.02	0.04	± 0.01	0.05	± 0.01	0.06	± 0.02	0.05	± 0.02
	n	14		15		15		14		15		14	
15 %	Min	0.03		0.03		0.02		0.02		0.02		0.01	
	Max	0.07		0.09		0.06		0.07		0.08		0.09	
	Mean (s.d.)	0.04	± 0.01	0.06	± 0.02	0.04	± 0.01	0.05	± 0.01	0.05	± 0.02	0.06	± 0.02
	n	15		13		14		15		15		14	
		Root dry biomass (g)											
PEG		Antonella		Bowman		eam10.m/Hvlux^{-}		eam5.x/HvPHYC^{+}		eam8.k/Hvelf3^{-}		eam8.w/Hvelf3^{-}	
0%	Min	0.02		0.02		0.02		0.01		0.01		0.01	
	Max	0.04		0.05		0.04		0.05		0.05		0.05	
	Mean (s.d.)	0.03	± 0.01	0.03	± 0.01	0.03	± 0.01	0.03	± 0.01	0.02	± 0.01	0.03	± 0.01
	n	14		15		15		14		15		15	
5%	Min	0.01		0.01		0.01		0.01		0.01		0.01	
	Max	0.04		0.04		0.03		0.02		0.03		0.03	

	Mean (s.d.)	0.02	± 0.01	0.02	± 0.01	0.02	± 0.01	0.01	± 0.00	0.02	± 0.01	0.01	± 0.01
	n	15		14		14		14		15		14	
10 %	Min	0.01		0.01		0.01		0.01		0.01		0.01	
	Max	0.03		0.03		0.03		0.02		0.03		0.03	
	Mean (s.d.)	0.02	± 0.01	0.02	± 0.01	0.01	± 0.01	0.01	± 0.00	0.02	± 0.01	0.02	± 0.01
	n	14		15		15		14		15		14	
15 %	Min	0.01		0.01		0.01		0.01		0.01		0.01	
	Max	0.02		0.03		0.02		0.02		0.03		0.03	
	Mean (s.d.)	0.02	± 0.00	0.02	± 0.01	0.01	± 0.00	0.01	± 0.00	0.02	± 0.01	0.02	± 0.01
	n	15		13		14		15		15		14	
		Total water content (g)											
PEG		Antonella		Bowman		eam10.m/Hvlux^{-}		eam5.x/HvPHYC^{+}		eam8.k/Hvelf3^{-}		eam8.w/Hvelf3^{-}	
0%	Min	0.63		0.88		0.90		0.64		0.76		0.67	

	Max	1.88			2.44			2.27			1.90			1.97			2.16	
	Mean (s.d.)	1.09	±	0.38	1.40	±	0.50	1.30	±	0.39	1.20	±	0.36	1.08	±	0.33	1.27	± 0.43
	n	14			15			15			14			15			15	
5%	Min	0.20			0.23			0.18			0.14			0.20			0.22	
	Max	1.34			1.66			1.20			1.25			1.01			1.40	
	Mean (s.d.)	0.62	±	0.28	0.60	±	0.34	0.65	±	0.29	0.48	±	0.28	0.52	±	0.26	0.50	± 0.30
	n	15			14			14			14			15			14	
10 %	Min	0.18			0.08			0.11			0.16			0.10			0.12	
	Max	1.48			0.83			0.53			0.89			1.42			1.03	
	Mean (s.d.)	0.48	±	0.33	0.38	±	0.18	0.28	±	0.12	0.40	±	0.22	0.52	±	0.34	0.45	± 0.25
	n	14			15			15			14			15			14	
15 %	Min	0.13			0.20			0.15			0.13			0.11			0.12	
	Max	0.52			1.09			0.52			0.71			0.67			0.93	

	Mean (s.d.)	0.27	± 0.13	0.50	± 0.31	0.25	± 0.11	0.34	± 0.18	0.38	± 0.16	0.47	± 0.28
	n	15		13		14		15		15		14	
		Shoot water content (g)											
PEG		Antonella		Bowman		eam10.m/Hvlux^{-}		eam5.x/HvPHYC^{+}		eam8.k/Hvelf3^{-}		eam8.w/Hvelf3^{-}	
0%	Min	0.41		0.58		0.55		0.47		0.50		0.44	
	Max	1.15		1.35		1.15		1.06		1.25		1.23	
	Mean (s.d.)	0.67	± 0.22	0.88	± 0.24	0.77	± 0.16	0.73	± 0.16	0.70	± 0.19	0.79	± 0.22
	n	14		15		15		14		15		15	
5%	Min	0.13		0.15		0.07		0.08		0.11		0.13	
	Max	0.80		1.02		0.71		0.64		0.56		0.85	
	Mean (s.d.)	0.35	± 0.17	0.36	± 0.21	0.37	± 0.18	0.28	± 0.15	0.31	± 0.15	0.32	± 0.19
	n	15		14		14		14		15		14	
10%	Min	0.08		0.04		0.05		0.08		0.06		0.05	
	Max	0.68		0.40		0.28		0.56		0.76		0.65	

	Mean (s.d.)	0.25	± 0.16	0.19	± 0.10	0.13	± 0.06	0.21	± 0.14	0.26	± 0.20	0.24	± 0.17
	n	14		15		15		14		15		14	
15 %	Min	0.05		0.09		0.07		0.06		0.05		0.06	
	Max	0.31		0.63		0.22		0.43		0.37		0.58	
	Mean (s.d.)	0.11	± 0.07	0.25	± 0.17	0.11	± 0.05	0.17	± 0.11	0.17	± 0.09	0.23	± 0.16
	n	15		13		14		15		15		14	
		Root water content (g)											
PEG		Antonella		Bowman		eam10.m/Hvlux^{-}		eam5.x/HvPHYC^{+}		eam8.k/Hvelf3^{-}		eam8.w/Hvelf3^{-}	
0%	Min	0.23		0.07		0.29		0.17		0.22		0.21	
	Max	0.81		1.15		1.12		1.22		0.74		1.00	
	Mean (s.d.)	0.42	± 0.17	0.51	± 0.28	0.53	± 0.23	0.47	± 0.26	0.39	± 0.15	0.47	± 0.21
	n	14		15		15		14		15		15	
5%	Min	0.07		0.08		0.09		0.06		0.07		0.08	
	Max	0.54		0.64		0.50		0.61		0.45		0.54	

	Mean (s.d.)	0.26	±	0.12	0.24	±	0.13	0.28	±	0.12	0.21	±	0.13	0.21	±	0.11	0.19	±	0.12
	n	15			14			14			14			15			14		
10 %	Min	0.08			0.04			0.06			0.08			0.05			0.07		
	Max	0.80			0.50			0.29			0.41			0.66			0.40		
	Mean (s.d.)	0.23	±	0.18	0.19	±	0.10	0.15	±	0.06	0.19	±	0.09	0.26	±	0.15	0.21	±	0.09
	n	14			15			15			14			15			14		
15 %	Min	0.08			0.10			0.09			0.06			0.07			0.06		
	Max	0.28			0.48			0.29			0.38			0.34			0.46		
	Mean (s.d.)	0.15	±	0.06	0.26	±	0.14	0.14	±	0.06	0.17	±	0.08	0.21	±	0.09	0.24	±	0.13
	n	15			13			14			15			15			14		

		Root:Shoot Dry Biomass ratio					
PEG		Antonella	Bowman	eam10.m/Hvlux^{-}	eam5.x/HvPHYC^{+}	eam8.k/Hvelf3^{-}	eam8.w/Hvelf3^{-}
0%	Min	0.29	0.28	0.25	0.16	0.23	0.14

	Max	0.50		0.47		0.42		0.39		0.48		0.40		
	Mean (s.d.)	0.39	± 0.07	0.34	± 0.05	0.33	± 0.05	0.28	± 0.05	0.31	± 0.07	0.30	± 0.06	
	n	14		15		15		14		15		15		
5%	Min	0.27		0.17		0.17		0.17		0.16		0.19		
	Max	0.65		0.49		0.40		0.37		0.39		0.38		
	Mean (s.d.)	0.39	± 0.10	0.32	± 0.08	0.31	± 0.05	0.26	± 0.05	0.27	± 0.06	0.27	± 0.05	
	n	15		14		14		14		15		14		
10 %	Min	0.12		0.20		0.14		0.17		0.17		0.21		
	Max	0.86		0.45		0.38		0.38		0.53		0.57		
	Mean (s.d.)	0.39	± 0.18	0.31	± 0.08	0.27	± 0.07	0.27	± 0.07	0.33	± 0.10	0.31	± 0.09	
	n	14		15		15		14		15		14		
15 %	Min	0.24		0.22		0.18		0.21		0.24		0.29		
	Max	0.50		0.45		0.52		0.36		0.50		0.60		

	Mean (s.d.)	0.38	± 0.07	0.36	± 0.06	0.32	± 0.10	0.29	± 0.04	0.33	± 0.06	0.40	± 0.09
	n	15		13		14		15		15		14	
		Root:Shoot water content ratio											
PEG		Antonella		Bowman		eam10.m/Hvlux^{-}		eam5.x/HvPHYC^{+}		eam8.k/Hvelf3^{-}		eam8.w/Hvelf3^{-}	
0%	Min	0.50		0.09		0.44		0.37		0.36		0.42	
	Max	0.83		0.88		0.97		1.81		0.74		0.86	
	Mean (s.d.)	0.63	± 0.09	0.56	± 0.19	0.66	± 0.15	0.64	± 0.35	0.55	± 0.10	0.58	± 0.10
	n	14		15		15		14		15		15	
5%	Min	0.54		0.43		0.49		0.41		0.25		0.40	
	Max	1.26		0.92		1.68		1.11		0.93		1.06	
	Mean (s.d.)	0.75	± 0.16	0.67	± 0.13	0.82	± 0.29	0.77	± 0.21	0.68	± 0.19	0.61	± 0.15
	n	15		14		14		14		15		14	
10%	Min	0.57		0.47		0.68		0.59		0.56		0.52	
	Max	1.31		1.71		1.79		1.57		1.88		1.65	

	Mean (s.d.)	0.89	± 0.22	1.06	± 0.33	1.19	± 0.27	1.02	± 0.25	1.11	± 0.35	1.05	± 0.35
	n	14		15		15		14		15		14	
15 %	Min	0.68		0.71		0.87		0.60		0.54		0.60	
	Max	1.81		2.03		2.04		1.64		1.99		1.94	
	Mean (s.d.)	1.44	± 0.30	1.18	± 0.36	1.38	± 0.35	1.12	± 0.29	1.33	± 0.41	1.15	± 0.36
	n	15		13		14		15		15		14	
		Water uptake (ml)											
PEG		Antonella		Bowman		eam10.m/Hvlux^{-}		eam5.x/HvPHYC^{+}		eam8.k/Hvelf3^{-}		eam8.w/Hvelf3^{-}	
0%	Min	12.00		19.00		17.00		16.00		5.33		11.33	
	Max	31.00		43.00		34.00		42.33		36.00		38.00	
	Mean (s.d.)	20.83	± 5.12	27.24	± 6.32	26.78	± 4.89	26.05	± 6.62	23.58	± 6.93	27.04	± 7.24
	n	14		15		15		14		15		15	
5%	Min	2.33		4.33		5.33		4.33		5.33		6.33	
	Max	19.67		23.67		16.00		21.67		20.00		24.67	

	Mean (s.d.)	10.13	± 4.18	10.74	± 5.09	10.26	± 3.52	9.45	± 4.34	10.40	± 4.19	10.88	± 4.74
	n	15		14		14		14		15		14	
10 %	Min	4.00		3.67		3.00		4.67		3.33		3.67	
	Max	16.00		8.67		9.00		12.00		20.00		19.00	
	Mean (s.d.)	8.10	± 3.12	6.07	± 1.24	5.87	± 1.52	7.24	± 2.37	8.67	± 4.42	7.43	± 3.60
	n	14		15		15		14		15		14	
15 %	Min	2.00		2.67		2.67		2.67		3.00		2.67	
	Max	8.00		10.00		6.67		11.00		7.67		11.00	
	Mean (s.d.)	5.24	± 1.70	6.41	± 1.94	4.40	± 1.09	5.98	± 2.02	4.98	± 1.08	6.71	± 2.35
	n	15		13		14		15		15		14	
		Accumulated root length (cm)											
PEG		Antonella		Bowman		eam10.m/Hvlux^{-}		eam5.x/HvPHYC^{+}		eam8.k/Hvelf3^{-}		eam8.w/Hvelf3^{-}	
0%	Min	16.83		49.68		76.10		33.38		21.49		36.44	

	Max	177.09			364.48			230.70			301.82			186.53			220.93	
	Mean (s.d.)	105.57	±	47.01	157.90	±	74.87	136.07	±	46.20	139.70	±	80.56	105.34	±	47.30	131.40	±	60.44
	n	14			15			15			14			15			14		
5%	Min	13.03			12.70			5.52			10.02			41.66			31.19		
	Max	99.70			176.27			261.22			181.95			228.94			125.94		
	Mean (s.d.)	46.81	±	25.25	78.06	±	49.94	69.96	±	64.86	76.01	±	55.32	81.12	±	51.97	76.58	±	30.20
	n	15			14			14			14			15			14		
10 %	Min	16.05			16.41			9.18			26.57			17.85			29.41		
	Max	97.62			219.22			167.89			225.68			227.25			204.65		
	Mean (s.d.)	58.11	±	25.64	82.38	±	56.62	49.40	±	35.79	77.53	±	51.84	80.28	±	51.46	104.96	±	59.89
	n	14			15			15			14			15			14		
15 %	Min	16.15			17.02			23.94			7.88			9.87			11.52		
	Max	76.02			289.40			207.11			253.46			287.16			216.94		

	Mean (s.d.)	46.51	± 16.68	117.52	± 88.27	82.15	± 48.46	74.01	± 69.06	122.35	± 85.86	103.99	± 65.62
	n	15		13		14		15		15		14	
		Total surface area (cm^3)											
PEG		Antonella		Bowman		eam10.m/Hvlux$^-$		eam5.x/HvPHYC$^+$		eam8.k/Hvelf3$^-$		eam8.w/Hvelf3$^-$	
0%	Min	23.96		31.15		25.00		18.68		21.40		12.68	
	Max	55.80		78.86		72.61		88.52		59.36		70.50	
	Mean (s.d.)	39.71	± 10.19	48.60	± 15.13	41.15	± 12.22	40.57	± 17.99	34.29	± 11.51	40.69	± 14.05
	n	14		15		15		14		15		14	
5%	Min	12.66		11.09		8.28		7.10		9.93		15.40	
	Max	39.93		52.41		43.27		34.65		44.95		32.18	
	Mean (s.d.)	22.75	± 7.15	24.76	± 10.91	26.12	± 8.32	21.82	± 8.54	23.07	± 9.98	20.92	± 4.55
	n	15		14		14		14		15		14	
10%	Min	13.14		4.79		10.06		10.86		7.80		11.04	
	Max	38.37		50.54		36.38		38.90		47.02		39.84	

	Mean (s.d.)	20.08	± 6.83	25.46	± 12.07	17.24	± 7.04	21.91	± 8.46	25.13	± 9.71	26.18	± 9.55
	n	14		15		15		14		15		14	
15 %	Min	9.42		11.23		10.78		7.31		8.90		8.84	
	Max	40.06		51.03		50.24		48.78		50.78		60.52	
	Mean (s.d.)	17.69	± 7.44	31.59	± 13.03	20.55	± 9.93	21.50	± 11.14	28.01	± 13.04	27.81	± 12.89
	n	15		13		14		15		15		14	
		Average diameter (mm)											
PEG		Antonella		Bowman		eam10.m/Hvlux^{-}		eam5.x/HvPHYC^{+}		eam8.k/Hvelf3^{-}		eam8.w/Hvelf3^{-}	
0%	Min	0.52		0.62		0.48		0.61		0.58		0.52	
	Max	6.65		3.34		1.63		3.93		3.30		4.39	
	Mean (s.d.)	1.95	± 1.95	1.14	± 0.63	1.02	± 0.28	1.21	± 0.84	1.27	± 0.70	1.34	± 1.07
	n	14		15		15		14		15		14	
5%	Min	0.69		0.52		0.53		0.55		0.63		0.44	
	Max	4.93		5.57		24.52		7.82		2.20		1.99	

	Mean (s.d.)	2.01	± 1.22	1.43	± 1.22	3.27	± 5.98	1.52	± 1.82	1.04	± 0.45	1.05	± 0.52
	n	15		14		14		14		15		14	
10 %	Min	0.57		0.59		0.69		0.55		0.66		0.45	
	Max	3.38		3.76		9.75		2.02		4.24		3.34	
	Mean (s.d.)	1.30	± 0.66	1.27	± 0.78	2.02	± 2.62	1.11	± 0.47	1.32	± 0.95	1.02	± 0.70
	n	14		15		15		14		15		14	
15 %	Min	0.70		0.55		0.46		0.43		0.47		0.61	
	Max	4.11		4.93		1.43		4.60		6.10		6.35	
	Mean (s.d.)	1.41	± 0.92	1.40	± 1.25	0.92	± 0.33	1.50	± 1.21	1.18	± 1.36	1.26	± 1.43
	n	15		13		14		15		15		14	
		Total root volume (cm^3)											
PEG		Antonella		Bowman		eam10.m/Hvlux$^-$		eam5.x/HvPHYC$^+$		eam8.k/Hvelf3$^-$		eam8.w/Hvelf3$^-$	
0%	Min	0.32		0.60		0.42		0.43		0.35		0.23	

	Max	7.80		4.36		2.03		4.49		1.84		5.52	
	Mean (s.d.)	2.06	± 2.13	1.40	± 0.92	1.07	± 0.48	1.21	± 1.02	1.05	± 0.52	1.51	± 1.41
	n	14		15		15		14		15		14	
5%	Min	0.25		0.28		0.28		0.14		0.19		0.17	
	Max	2.85		3.10		26.06		4.81		1.66		1.60	
	Mean (s.d.)	1.19	± 0.81	0.86	± 0.74	2.80	± 6.49	0.87	± 1.17	0.62	± 0.41	0.58	± 0.40
	n	15		14		14		14		15		14	
10 %	Min	0.21		0.09		0.19		0.19		0.16		0.17	
	Max	1.44		2.46		6.86		1.49		2.52		2.58	
	Mean (s.d.)	0.64	± 0.33	0.79	± 0.61	1.09	± 1.81	0.59	± 0.31	0.82	± 0.63	0.67	± 0.57
	n	14		15		15		14		15		14	
15 %	Min	0.17		0.17		0.15		0.11		0.15		0.17	
	Max	2.14		3.25		1.46		2.20		2.88		3.64	

	Mean (s.d.)	0.70	±	0.61	1.05	±	0.90	0.47	±	0.31	0.75	±	0.55	0.72	±	0.64	0.82	±	0.84
	n	15			13			14			15			15			14		
		Total number of root tips																	
PEG		Antonella			Bowman			eam10.m/Hvlux^{-}			eam5.x/HvPHYC^{+}			eam8.k/Hvelf3^{-}			eam8.w/Hvelf3^{-}		
0%	Min	947.00			1268.00			851.00			370.00			359.00			292.00		
	Max	2464.00			2988.00			2397.00			5494.00			2202.00			2609.00		
	Mean (s.d.)	1667.50	±	525.62	1952.80	±	591.75	1518.87	±	384.37	1903.79	±	1319.60	1230.67	±	530.46	1575.43	±	619.38
	n	14			15			15			14			15			14		
5%	Min	252.00			368.00			336.00			311.00			360.00			431.00		
	Max	1733.00			1656.00			2601.00			1732.00			2432.00			1170.00		
	Mean (s.d.)	757.47	±	347.01	890.71	±	405.91	1008.21	±	543.91	901.36	±	475.53	803.53	±	468.86	784.71	±	246.50
	n	15			14			14			14			15			14		

10 %	Min	344.00			178.00			321.00			321.00			463.00			486.00		
	Max	1271.00			2170.00			2114.00			2309.00			2815.00			2036.00		
	Mean (s.d.)	736.14	±	294.23	1071.20	±	588.74	758.20	±	414.33	944.86	±	571.80	1106.67	±	581.38	1124.21	±	498.51
	n	14			15			15			14			15			14		
15 %	Min	225.00			196.00			325.00			346.00			280.00			407.00		
	Max	973.00			3212.00			1795.00			2098.00			3919.00			2709.00		
	Mean (s.d.)	626.40	±	196.92	1407.31	±	897.19	913.71	±	438.28	945.07	±	653.42	1476.73	±	1071.81	1091.50	±	611.49
	n	15			13			14			15			15			14		

Water uptake per total dry biomass (ml/g)

PEG		Antonella	Bowman	eam10.m/Hvlux^{-}	eam5.x/HvPHYC^{+}	eam8.k/Hvelf3^{-}	eam8.w/Hvelf3^{-}
0%	Min	160.71	63.92	186.21	170.87	62.75	136.55
	Max	248.37	266.06	321.14	313.73	295.45	333.33

	Mean (s.d.)	197.64	±	24.75	209.52	±	48.33	239.76	±	36.28	236.05	±	35.10	232.28	±	55.35	229.92	±	48.18
	n	14			15			15			14			15			15		
5%	Min	35.35			84.06			87.50			95.24			31.42			118.84		
	Max	215.96			223.88			162.88			222.22			465.12			219.70		
	Mean (s.d.)	126.77	±	41.66	134.30	±	37.12	121.96	±	26.70	141.04	±	37.89	151.17	±	91.51	165.30	±	35.85
	n	15			14			14			14			15			14		
10 %	Min	51.55			18.02			68.18			62.22			76.50			63.22		
	Max	213.33			175.00			229.89			145.83			147.06			160.00		
	Mean (s.d.)	125.50	±	47.49	95.31	±	39.26	113.92	±	41.09	110.82	±	23.41	113.66	±	24.64	112.99	±	28.98
	n	14			15			15			14			15			14		
15 %	Min	27.03			55.56			50.23			60.61			47.62			65.73		
	Max	170.94			145.83			130.95			163.12			172.84			126.98		
	Mean (s.d.)	100.39	±	41.40	85.63	±	21.49	93.54	±	25.55	105.91	±	32.82	84.23	±	35.09	91.04	±	18.10

	n	15		13		14		15		15		14	
		Percentage of water as total biomass (%)											
PEG		Antonella		Bowman		eam10.m/Hvlux^{-}		eam5.x/HvPHYC^{+}		eam8.k/Hvelf3^{-}		eam8.w/Hvelf3^{-}	
0%	Min	0.90		0.68		0.90		0.90		0.90		0.89	
	Max	0.92		0.93		0.94		0.93		0.93		0.93	
	Mean (s.d.)	0.91	± 0.01	0.90	± 0.06	0.92	± 0.01	0.91	± 0.01	0.91	± 0.01	0.91	± 0.01
	n	14		15		15		14		15		15	
5%	Min	0.75		0.83		0.80		0.80		0.61		0.81	
	Max	0.91		0.91		0.92		0.92		0.91		0.91	
	Mean (s.d.)	0.87	± 0.04	0.88	± 0.02	0.87	± 0.04	0.86	± 0.04	0.85	± 0.07	0.87	± 0.03
	n	15		14		14		14		15		14	
10%	Min	0.78		0.64		0.78		0.77		0.78		0.72	
	Max	0.93		0.89		0.89		0.91		0.91		0.91	
	Mean (s.d.)	0.86	± 0.04	0.82	± 0.07	0.83	± 0.03	0.84	± 0.04	0.85	± 0.04	0.85	± 0.05

	n	14		15		15		14		15		14	
15 %	Min	0.73		0.80		0.74		0.79		0.78		0.76	
	Max	0.87		0.91		0.92		0.90		0.89		0.89	
	Mean (s.d.)	0.81	± 0.04	0.85	± 0.04	0.82	± 0.05	0.84	± 0.03	0.84	± 0.03	0.84	± 0.04
	n	15		13		14		15		15		14	
		Water uptake per total water content stored (ml/g)											
PEG		Antonella		Bowman		eam10.m/Hvlux^{-}		eam5.x/HvPHYC^{+}		eam8.k/Hvelf3^{-}		eam8.w/Hvelf3^{-}	
0%	Min	14.37		13.50		14.71		16.77		7.03		16.24	
	Max	28.79		29.46		28.87		27.25		27.81		29.03	
	Mean (s.d.)	19.87	± 3.58	20.57	± 4.24	21.42	± 3.88	22.19	± 3.19	22.08	± 5.49	22.00	± 3.99
	n	14		15		15		14		15		15	
5%	Min	11.93		11.66		8.86		11.09		13.93		14.76	
	Max	27.07		26.67		30.13		37.80		96.15		30.65	
	Mean (s.d.)	17.20	± 4.48	18.57	± 4.50	17.81	± 5.86	22.73	± 8.42	24.48	± 19.66	23.31	± 4.51

	n	15		14		14		14		15		14	
10 %	Min	4.72		7.26		12.66		12.58		11.70		8.80	
	Max	46.88		83.33		54.64		37.27		35.60		50.85	
	Mean (s.d.)	21.54	± 10.94	21.25	± 17.43	24.06	± 12.52	21.41	± 8.22	19.40	± 6.84	19.46	± 9.80
	n	14		15		15		14		15		14	
15 %	Min	6.54		8.19		7.78		9.31		7.33		9.07	
	Max	50.51		21.30		33.67		43.93		40.94		23.81	
	Mean (s.d.)	24.40	± 12.69	15.10	± 4.86	20.05	± 7.81	21.31	± 9.45	16.35	± 8.99	16.89	± 5.10
	n	15		13		14		15		15		14	

Supplementary table 3.3.1b: Results of statistical analysis comparison of means from generalised linear models. P<0.01 (*), P<0.001(**), p<0.0001(***), with differences between bareley lines(Antonella (Ant), Bowman(Bow), Bowman eam10.m Hvlux- (eam10.m),Bowman eam5.x PHYC+

(eam5.x), Bowman eam8.k Hvelf- (eam8.k),Bowman eam8.w Hvelf- (eam8.w)) annotated with different letters based off of post hoc plots of confidence intervals calculates in the estimated means R package (emmeans).

Model F test results							**Post hoc**						
	Predictors	d.f.	d.f.	F	P		PEG	Ant	Bow	eam10.m	eam5.x	eam8.k	eam8.w
Total fresh weight (g) pre treatment	Barley lines						0%	a	b	ab	ab	ab	ab
Barley Lines		5	342	3.5665	0.003	**	5%	a	b	ab	ab	ab	ab
PEG							10%	a	b	ab	ab	ab	ab
Barley lines * PEG							15%	a	b	ab	ab	ab	ab
Total fresh weight (g) post treatment	Barley lines * PEG						0%	a	a	a	a	a	a
Barley Lines		5	342	1.5677	0.1687		5%	b	bc	b	bc	bc	bc
PEG		3	339	106.823	<0.0001	***	10%	bc	bc	c	bc	bc	bc

Barley lines * PEG		15	324	2.3325	0.00354	**	15%	c	bc	c	bc	bc	bc
Shoot fresh weight (g)	Barley lines						0%	a	b	ab	ab	a	ab
post treatment	* PEG												
Barley Lines		5	342	1.8734	0.098		5%	c	cd	c	cd	c	c
PEG		3	339	134.085	<0.0001	**	10%	cd	cd	d	cd	cd	cd
Barley lines * PEG		15	324	2.5411	0.0013	**	15%	d	cd	d	d	d	cd
Root fresh weight (g)	Barley lines						0%	a	ab	b	ab	abc	ab
post treatment	* PEG												
Barley Lines		5	342	1.0552	0.3852		5%	bc	bc	bc	c	c	c
PEG		3	339	60.2367	<0.0001	***	10%	c	c	c	c	bc	c
Barley lines * PEG		15	324	2.0097	0.0143	*	15%	c	c	c	c	bc	bc
Change in fresh weight (g)	PEG						0%	a	a	a	a	a	a
Barley Lines							5%	b	b	b	b	b	b

PEG		3	344	64.871	<0.0001	***	10%	c	c	c	c	c	c
Barley lines * PEG							15%	c	c	c	c	c	c
Total dry biomass (g)	Barley lines						0%	ab	a	a	a	ab	a
Barley Lines	* PEG	5	342	1.9132	0.09169		5%	bc	bc	bc	c	c	c
PEG		3	339	58.7047	<0.0001	***	10%	c	c	c	c	bc	c
Barley lines * PEG		15	324	1.8286	0.02996	*	15%	c	bc	c	c	c	bc
Shoot dry biomass (g)	PEG						0%	a	a	a	a	a	a
Barley Lines							5%	b	b	b	b	b	b
PEG		3	344	51.153	<0.0001	***	10%	bc	bc	bc	bc	bc	bc
Barley lines * PEG							15%	c	c	c	c	c	c
Root dry biomass (g)	Barley lines						0%	a	a	a	ab	ab	a
Barley Lines	* PEG	5	342	4.4638	0.0006	***	5%	b	bc	bc	c	bc	bc

PEG		3	339	54.2979	<0.0001	***	10%	c	c	c	c	c	c
Barley lines * PEG		15	324	2.7913	0.00043	***	15%	c	bc	c	c	c	bc
Total water content (g)	Barley lines						0%	a	a	a	a	a	a
Barley Lines	* PEG	5	342	1.3504	0.2428		5%	b	b	b	b	b	b
PEG		3	339	107.4681	<0.0001	***	10%	b	b	c	b	b	b
Barley lines * PEG		15	324	2.4318	0.002265	**	15%	c	b	c	b	b	b
Shoot water content (g)	Barley lines * PEG						0%	a	b	ab	ab	ab	ab
Barley Lines		5	342	1.7412	0.12474		5%	c	c	c	c	c	c
PEG		3	339	133.5978	<0.0001	***	10%	cd	cd	d	cd	cd	cd
Barley lines * PEG		15	324	2.7622	0.00049	***	15%	d	cd	d	d	d	cd
Root water content (g)	PEG						0%	a	a	a	a	a	a
Barley Lines							5%	b	b	b	b	b	b

PEG		3	344	53.045	<0.0001	***	10%	b	b	b	b	b	b
Barley lines * PEG							15%	b	b	b	b	b	b
Root:Shoot Dry Biomass ratio	Barley lines						0%	ab	c	cd	d	cd	c
Barley Lines	+ PEG	5	342	11.8758	<0.0001	***	5%	a	c	cd	d	cd	c
PEG		3	339	4.4106	0.00464	**	10%	ab	c	cd	d	cd	c
Barley lines * PEG							15%	b	c	cd	d	cd	c
Root:Shoot water content ratio	Barley lines + PEG						0%	a	a	e	a	a	e
Barley Lines		5	342	2.6242	0.02404	*	5%	b	b	f	b	b	f
PEG		3	339	113.7948	<0.0001	***	10%	c	c	g	c	c	g
Barley lines * PEG							15%	d	d	h	d	d	h
Water uptake (ml)	Barley lines						0%	a	b	b	b	ab	b

Barley Lines	* PEG	5	342	2.032	0.07382		5%	c	c	c	c	c	c
PEG		3	339	322.7308	<0.0001	***	10%	c	c	c	c	c	c
Barley lines * PEG		15	324	2.0484	0.01215	*	15%	c	c	c	c	c	c
Accumulated root length (cm)	Barley lines						0%	b	a	a	a	a	a
Barley Lines	+ PEG	5	342	4.8994	0.000242	***	5%	c	ab	c	bc	bc	b
PEG		3	339	15.3468	<0.0001	***	10%	c	ab	c	bc	bc	b
Barley lines * PEG							15%	bc	b	b	b	b	b
Total surface area (cm^3)	Barley lines						0%	a	a	a	a	a	a
Barley Lines	+ PEG	5	341	3.2535	0.00697	**	5%	c	b	c	bc	bc	bc
PEG		3	338	42.5867	<0.0001	***	10%	c	bc	c	bc	bc	bc
Barley lines * PEG							15%	bc	b	bc	bc	bc	bc

Average diameter (mm)	Barley lines						0%						
Barley Lines	+ PEG	5	340	1.2605	0.2806		5%						
PEG		3	337	0.3075	0.82		10%						
Barley lines * PEG							15%						
Total root volume (cm^3)	PEG						0%	a	a	a	a	a	a
Barley Lines							5%	b	b	b	b	b	b
PEG		3	342	7.6847	<0.0001	***	10%	b	b	b	b	b	b
Barley lines * PEG							15%	b	b	b	b	b	b
Total number of root tips	Barley lines						0%	ab	a	ab	a	b	ab
Barley Lines	* PEG	5	341	2.9374	0.0131	*	5%	b	b	b	b	b	b
PEG		3	338	26.6371	<0.0001	***	10%	b	b	b	b	b	b
Barley lines * PEG		15	323	2.0813	0.01058	*	15%	b	ab	b	b	ab	b

Water uptake per total dry biomass (ml/g)	PEG						0%	a	a	a	a	a	a
Barley Lines							5%	b	b	b	b	b	b
PEG		3	344	162.16	<0.0001	***	10%	c	c	c	c	c	c
Barley lines * PEG							15%	d	d	d	d	d	d
Percentage of water as total biomass (%)	PEG						0%	a	a	a	a	a	a
Barley Lines							5%	b	b	b	b	b	b
PEG		3	344	64.395	<0.0001	***	10%	c	c	c	c	c	c
Barley lines * PEG							15%	c	c	c	c	c	c
Water uptake per total water content stored (ml/g)	Barley lines + PEG						0%						
Barley Lines		5	342	0.5776	0.7171		5%						

PEG	3	339	1.0993	0.3495	10 %
Barley lines * PEG					15 %

Supplementary table 3.3.2a: Summary tables for the results in chapter 3.3.2 including all the 22 measured and calculated traits data minimum, maximum, means standard deviations to two decimal places and sample number for six barley lines grown in four different osmotic conditions induced with 0%, and 15% PEG in 20°C/18°C temperature cycles of 12:12 hours.

		Total fresh weight (g) pre treatment											
PEG		Antonella		Bowman		eam10.m/Hvlux^{-}		eam5.x/HvPHYC^{+}		eam8.k/Hvelf3^{-}		eam8.w/Hvelf3^{-}	
0%	Min	0.33		0.41		0.53		0.29		0.39		0.50	
	Max	1.46		1.34		1.47		1.31		1.50		1.59	
	Mean (s.d.)	0.65	± 0.23	0.83	± 0.23	0.84	± 0.23	0.70	± 0.21	0.90	± 0.25	0.91	± 0.26

	n	36			35			32			33			34			35		
15%	Min	0.32			0.45			0.40			0.33			0.32			0.35		
	Max	1.09			1.20			1.67			1.25			1.70			1.86		
	Mean (s.d.)	0.65	±	0.18	0.86	±	0.22	0.78	±	0.27	0.72	±	0.25	0.90	±	0.29	0.98	±	0.32
	n	37			36			37			36			36			37		

		Total fresh weight (g) post treatment																	
PEG		Antonella			Bowman			eam10.m/Hvlux^{-}			eam5.x/HvPHYC^{+}			eam8.k/Hvelf3^{-}			eam8.w/Hvelf3^{-}		
0%	Min	0.62			0.98			1.12			0.54			0.80			0.88		
	Max	2.50			3.10			2.81			2.37			2.48			2.71		
	Mean (s.d.)	1.36	±	0.42	1.76	±	0.41	1.65	±	0.40	1.45	±	0.40	1.72	±	0.38	1.74	±	0.42
	n	36			35			32			33			33			35		
15%	Min	0.24			0.32			0.27			0.25			0.22			0.42		
	Max	1.36			1.58			1.36			1.17			1.40			1.68		

	Mean (s.d.)	0.79	±	0.25	0.81	±	0.31	0.76	±	0.27	0.67	±	0.21	0.86	±	0.27	0.87	±	0.26
	n	37			36			37			36			36			37		
		Change in fresh weight (g)																	
PEG		Antonella			Bowman			eam10.m/Hvlux^{-}			eam5.x/HvPHYC^{+}			eam8.k/Hvelf3^{-}			eam8.w/Hvelf3^{-}		
0%	Min	0.30			0.49			0.38			0.25			0.41			0.00		
	Max	1.42			1.77			1.34			1.39			1.23			1.57		
	Mean (s.d.)	0.71	±	0.23	0.93	±	0.25	0.82	±	0.24	0.75	±	0.24	0.82	±	0.19	0.81	±	0.29
	n	36			35			32			33			33			36		
15%	Min	-0.30			-0.67			-0.90			-0.76			-0.73			-0.62		
	Max	0.49			0.40			0.43			0.33			0.39			0.37		
	Mean (s.d.)	0.14	±	0.16	-0.05	±	0.29	-0.02	±	0.27	-0.06	±	0.30	-0.04	±	0.30	-0.11	±	0.28
	n	37			36			37			36			36			37		
		Shoot fresh weight (g) post treatment																	
PEG		Antonella			Bowman			eam10.m/Hvlux^{-}			eam5.x/HvPHYC^{+}			eam8.k/Hvelf3^{-}			eam8.w/Hvelf3^{-}		

0%	Min	0.38			0.56			0.70			0.39			0.60			0.51		
	Max	1.65			2.02			1.91			1.62			1.48			1.77		
	Mean (s.d.)	0.88	±	0.29	1.12	±	0.28	1.06	±	0.27	0.93	±	0.26	1.12	±	0.22	1.11	±	0.29
	n	36			35			32			33			33			35		
15%	Min	0.13			0.17			0.15			0.13			0.16			0.15		
	Max	0.91			0.98			0.87			0.74			0.83			1.02		
	Mean (s.d.)	0.50	±	0.17	0.47	±	0.22	0.45	±	0.17	0.41	±	0.15	0.52	±	0.17	0.49	±	0.18
	n	37			36			37			36			36			37		

		Root fresh weight (g) post treatment																	
PEG		Antonella			Bowman			eam10.m/Hvlux^{-}			eam5.x/HvPHYC^{+}			eam8.k/Hvelf3^{-}			eam8.w/Hvelf3^{-}		
0%	Min	0.24			0.29			0.32			0.15			0.20			0.25		
	Max	0.85			1.08			0.94			1.08			1.48			1.13		
	Mean (s.d.)	0.48	±	0.14	0.64	±	0.19	0.59	±	0.17	0.52	±	0.19	0.60	±	0.24	0.63	±	0.19
	n	36			35			32			33			33			35		

15%	Min	0.12			0.12			0.08			0.09			0.04			0.16		
	Max	0.48			0.62			0.59			0.46			0.70			0.69		
	Mean (s.d.)	0.29	±	0.09	0.34	±	0.12	0.31	±	0.13	0.26	±	0.09	0.34	±	0.15	0.38	±	0.12
	n	37			36			37			36			36			37		

		Shoot dry biomass (g)																	
PEG		Antonella			Bowman			eam10.m/Hvlux^{-}			eam5.x/HvPHYC^{+}			eam8.k/Hvelf3^{-}			eam8.w/Hvelf3^{-}		
0%	Min	0.04			0.05			0.03			0.04			0.04			0.03		
	Max	0.19			0.19			0.24			0.16			0.20			0.21		
	Mean (s.d.)	0.09	±	0.03	0.11	±	0.03	0.11	±	0.04	0.10	±	0.03	0.12	±	0.04	0.11	±	0.04
	n	36			35			32			33			33			35		
15%	Min	0.04			0.04			0.04			0.02			0.02			0.01		
	Max	0.12			0.13			0.12			0.18			0.12			0.11		
	Mean (s.d.)	0.07	±	0.02	0.07	±	0.02	0.07	±	0.02	0.07	±	0.03	0.08	±	0.02	0.07	±	0.02
	n	37			36			37			36			36			37		

		Root dry biomass (g)																		
PEG		Antonella			Bowman			eam10.m/Hvlux^{-}			eam5.x/HvPHYC^{+}			eam8.k/Hvelf3^{-}			eam8.w/Hvelf3^{-}		
0%	Min	0.02			0.02			0.02			0.01			0.02			0.01		
	Max	0.07			0.07			0.08			0.05			0.07			0.07		
	Mean (s.d.)	0.04	±	0.01	0.04	±	0.01	0.04	±	0.01	0.03	±	0.01	0.04	±	0.01	0.04	±	0.01
	n	34			33			32			33			33			35		
15%	Min	0.01			0.01			0.01			0.01			0.01			0.02		
	Max	0.04			0.05			0.05			0.03			0.05			0.06		
	Mean (s.d.)	0.03	±	0.01	0.03	±	0.01	0.03	±	0.01	0.02	±	0.01	0.03	±	0.01	0.03	±	0.01
	n	34			36			37			36			36			37		

		Total dry biomass (g)																		
PEG		Antonella			Bowman			eam10.m/Hvlux^{-}			eam5.x/HvPHYC^{+}			eam8.k/Hvelf3^{-}			eam8.w/Hvelf3^{-}		
0%	Min	0.06			0.07			0.09			0.05			0.07			0.05		
	Max	0.26			0.26			0.33			0.21			0.26			0.29		

	Mean (s.d.)	0.13	±	0.05	0.14	±	0.04	0.16	±	0.06	0.12	±	0.04	0.16	±	0.05	0.15	± 0.06
	n	35			33			32			33			33			35	
15%	Min	0.05			0.05			0.05			0.03			0.03			0.03	
	Max	0.16			0.18			0.17			0.20			0.16			0.16	
	Mean (s.d.)	0.09	±	0.03	0.09	±	0.03	0.10	±	0.03	0.09	±	0.03	0.10	±	0.03	0.10	± 0.03
	n	34			36			37			36			36			37	

		Root:Shoot Dry Biomass ratio																
PEG		Antonella			Bowman			eam10.m/Hvlux^{-}			eam5.x/HvPHYC^{+}			eam8.k/Hvelf3^{-}			eam8.w/Hvelf3^{-}	
0%	Min	0.28			0.16			0.27			0.12			0.25			0.18	
	Max	0.85			0.61			1.55			0.48			0.83			0.50	
	Mean (s.d.)	0.42	±	0.11	0.37	±	0.09	0.44	±	0.22	0.31	±	0.08	0.39	±	0.12	0.36	± 0.07
	n	34			33			32			33			33			35	
15%	Min	0.25			0.27			0.28			0.13			0.25			0.25	
	Max	0.52			0.64			0.42			0.62			0.57			1.20	

	Mean (s.d.)	0.37	±	0.06	0.39	±	0.08	0.36	±	0.44	0.32	±	0.09	0.37	±	0.09	0.43	±	0.16
	n	34			36			37			36			36			37		
		Shoot water content (g)																	
PEG		Antonella			Bowman			eam10.m/Hvlux^{-}			eam5.x/HvPHYC^{+}			eam8.k/Hvelf3^{-}			eam8.w/Hvelf3^{-}		
0%	Min	0.01			0.51			0.64			0.35			0.55			0.45		
	Max	1.46			1.83			1.69			1.45			1.46			1.58		
	Mean (s.d.)	0.78	±	0.29	1.01	±	0.26	0.96	±	0.23	0.83	±	0.23	1.01	±	0.20	0.98	±	0.27
	n	35			35			32			33			33			35		
15%	Min	0.08			0.12			0.10			0.11			0.11			0.12		
	Max	0.79			0.88			0.77			0.68			0.71			0.91		
	Mean (s.d.)	0.43	±	0.15	0.40	±	0.20	0.38	±	0.15	0.34	±	0.14	0.45	±	0.17	0.42	±	0.17
	n	37			36			37			36			36			37		
		Root water content (g)																	

PEG		Antonella	Bowman	eam10.m/Hvlux^{-}	eam5.x/HvPHYC^{+}	eam8.k/Hvelf3^{-}	eam8.w/Hvelf3^{-}
0%	Min	0.23	0.26	0.30	0.14	0.18	0.24
	Max	0.78	1.02	0.87	1.04	1.41	1.09
	Mean (s.d.)	0.45 ± 0.13	0.60 ± 0.18	0.54 ± 0.17	0.49 ± 0.18	0.56 ± 0.23	0.59 ± 0.18
	n	33	33	32	33	33	35
15%	Min	0.10	0.11	0.07	0.08	0.03	0.15
	Max	0.44	0.57	0.54	0.44	0.65	0.63
	Mean (s.d.)	0.25 ± 0.08	0.31 ± 0.12	0.28 ± 0.12	0.23 ± 0.09	0.31 ± 0.14	0.35 ± 0.11
	n	34	36	37	36	36	37

Total water content (g)

PEG		Antonella	Bowman	eam10.m/Hvlux^{-}	eam5.x/HvPHYC^{+}	eam8.k/Hvelf3^{-}	eam8.w/Hvelf3^{-}
0%	Min	0.56	0.90	1.03	0.49	0.73	0.78
	Max	2.24	2.85	2.51	2.15	2.22	2.47
	Mean (s.d.)	1.24 ± 0.39	1.57 ± 0.36	1.50 ± 0.36	1.32 ± 0.37	1.57 ± 0.34	1.57 ± 0.39

	n	34			35			32			33			33			35		
15%	Min	0.17			0.26			0.17			0.22			0.17			0.36		
	Max	1.23			1.40			1.31			1.08			1.27			1.52		
	Mean (s.d.)	0.67	±	0.20	0.72	±	0.29	0.66	±	0.27	0.58	±	0.19	0.75	±	0.25	0.77	±	0.24
	n	37			36			37			36			36			37		

		Root:Shoot water content ratio																	
PEG		Antonella			Bowman			eam10.m/Hvlux^{-}			eam5.x/HvPHYC^{+}			eam8.k/Hvelf3^{-}			eam8.w/Hvelf3^{-}		
0%	Min	0.42			0.00			0.35			0.35			0.29			0.31		
	Max	0.84			0.96			0.96			1.32			1.75			1.78		
	Mean (s.d.)	0.58	±	0.10	0.58	±	0.22	0.57	±	0.15	0.60	±	0.18	0.56	±	0.27	0.63	±	0.25
	n	33			35			32			33			33			35		
15%	Min	0.34			0.34			0.70			0.28			0.04			0.35		
	Max	2.32			2.02			0.70			1.59			1.77			2.17		
	Mean (s.d.)	0.67	±	0.33	0.90	±	0.44	0.74	±	0.78	0.77	±	0.36	0.77	±	0.39	0.95	±	0.39

	n	34	36	37	36	36	37
		Water uptake (ml)					
PEG		Antonella	Bowman	eam10.m/Hvlux^{-}	eam5.x/HvPHYC^{+}	eam8.k/Hvelf3^{-}	eam8.w/Hvelf3^{-}
0%	Min	15.30	21.00	18.00	8.67	20.00	13.00
	Max	46.00	48.67	48.00	42.67	47.50	48.67
	Mean (s.d.)	28.30 ± 8.81	35.50 ± 6.44	34.85 ± 8.69	28.43 ± 7.51	38.12 ± 7.02	38.16 ± 9.51
	n	37	35	32	33	34	35
15%	Min	0.00	0.00	0.00	0.00	0.00	0.00
	Max	17.00	17.00	18.00	16.00	21.00	18.00
	Mean (s.d.)	10.35 ± 3.60	6.99 ± 3.98	7.41 ± 4.86	7.46 ± 4.58	9.14 ± 5.98	8.03 ± 4.51
	n	37	36	37	36	36	37
		Accumulated root length (cm)					
PEG		Antonella	Bowman	eam10.m/Hvlux^{-}	eam5.x/HvPHYC^{+}	eam8.k/Hvelf3^{-}	eam8.w/Hvelf3^{-}
0%	Min	31.67	58.71	64.15	31.81	36.28	79.42

	Max	247.17			419.17			273.22			357.96			435.33			390.44		
	Mean (s.d.)	101.01	±	61.56	178.12	±	104.80	137.95	±	58.47	108.58	±	77.87	162.61	±	93.18	218.78	±	101.09
	n	15			17			15			16			17			17		
15%	Min	16.96			29.02			7.08			25.37			44.56			34.57		
	Max	208.59			310.77			150.54			264.65			224.03			251.52		
	Mean (s.d.)	76.79	±	49.74	104.77	±	74.86	71.95	±	46.58	106.16	±	60.74	100.45	±	48.86	132.38	±	75.14
	n	15			19			20			19			19			21		

		Total surface area (cm^3)																	
PEG		Antonella			Bowman			eam10.m/Hvlux$^-$			eam5.x/HvPHYC$^+$			eam8.k/Hvelf3$^-$			eam8.w/Hvelf3$^-$		
0%	Min	33.05			29.57			35.78			7.14			22.68			36.41		
	Max	77.57			112.53			97.29			69.33			87.83			88.30		
	Mean (s.d.)	50.85	±	12.69	59.18	±	22.55	59.85	±	16.15	41.01	±	19.33	52.74	±	17.52	59.59	±	15.79

	n	15			17			15			16			17			17			
15%	Min	13.76			20.39			13.68			13.93			11.31			24.36			
	Max	44.90			57.55			74.89			72.37			71.69			85.06			
	Mean (s.d.)	29.98	±	9.73	39.01	±	10.03	35.05	±	15.51	38.30	±	14.22	35.79	±	16.86	49.93	±	14.49	
	n	15			19			20			19			19			21			

		Average diameter (mm)																		
PEG		Antonella			Bowman			eam10.m/Hvlux^{-}			eam5.x/HvPHYC^{+}			eam8.k/Hvelf3^{-}			eam8.w/Hvelf3^{-}			
0%	Min	0.99			0.64			0.65			0.62			0.59			0.48			
	Max	4.99			3.51			4.83			3.88			3.85			2.29			
	Mean (s.d.)	2.13	±	1.17	1.29	±	0.65	1.64	±	0.98	1.43	±	0.80	1.34	±	0.90	1.08	±	0.57	
	n	15			17			15			16			17			17			
15%	Min	0.62			0.50			0.54			0.67			0.73			0.65			
	Max	6.85			4.08			9.70			4.38			2.13			4.29			
	Mean (s.d.)	1.73	±	1.47	1.73	±	1.06	2.56	±	2.31	1.39	±	0.78	1.17	±	0.37	1.70	±	1.11	

	n	15			19			20			19			19			21						
		Total root volume (cm^3)																					
PEG		Antonella			Bowman			eam10.m/Hvlux$^-$			eam5.x/HvPHYC$^+$			eam8.k/Hvelf3$^-$			eam8.w/Hvelf3$^-$					
0%	Min	1.08			0.93			0.83			0.13			0.35			0.46					
	Max	6.19			5.67			11.74			5.06			5.77			4.22					
	Mean (s.d.)	2.67	±	1.58	1.86	±	1.15	2.72	±	2.62	1.59	±	1.32	1.81	±	1.37	1.62	±	0.95			
	n	15			17			15			16			17			17					
15%	Min	0.38			0.42			0.31			0.40			0.22			0.50					
	Max	6.25			3.79			10.45			3.83			3.13			5.09					
	Mean (s.d.)	1.38	±	1.43	1.72	±	1.15	2.29	±	2.45	1.31	±	0.80	1.11	±	0.75	2.10	±	1.43			
	n	15			19			20			19			19			21					
		Total number of root tips																					
PEG		Antonella			Bowman			eam10.m/Hvlux$^-$			eam5.x/HvPHYC$^+$			eam8.k/Hvelf3$^-$			eam8.w/Hvelf3$^-$					

0%	Min	1134.00			771.00			1246.00			224.00			345.00			1431.00		
	Max	3377.00			4783.00			3378.00			2876.00			4197.00			3269.00		
	Mean (s.d.)	1949.00	±	628.65	2172.06	±	1144.48	2282.93	±	546.25	1485.88	±	790.33	1968.24	±	1042.86	2184.00	±	560.33
	n	15			17			15			16			17			17		
15%	Min	541.00			462.00			372.00			427.00			351.00			832.00		
	Max	2323.00			2928.00			3008.00			3228.00			3819.00			3580.00		
	Mean (s.d.)	1171.40	±	524.15	1450.21	±	528.72	1127.60	±	618.02	1476.42	±	666.42	1413.74	±	935.97	1933.29	±	690.61
	n	15			19			20			19			19			21		

		Water uptake per total dry biomass (ml/g)					
PEG		Antonella	Bowman	eam10.m/Hvlux^{-}	eam5.x/HvPHYC^{+}	eam8.k/Hvelf3^{-}	eam8.w/Hvelf3^{-}
0%	Min	13.57	17.10	14.47	15.85	16.74	14.85
	Max	43.68	41.65	31.85	42.04	36.03	32.58

	Mean (s.d.)	24.38	±	6.91	23.00	±	5.68	23.44	±	4.36	21.88	±	4.62	24.63	±	3.96	24.36	±	4.08
	n	33			33			32			33			33			35		
15%	Min	0.00			0.00			0.00			0.00			0.00			0.00		
	Max	26.81			29.44			24.43			50.88			25.52			19.51		
	Mean (s.d.)	16.01	±	6.04	9.68	±	4.92	11.18	±	6.40	13.28	±	9.21	11.63	±	6.01	10.53	±	5.05
	n	34			36			37			36			36			37		

		Water uptake per total water content stored (ml/g)																	
PEG		Antonella			Bowman			eam10.m/Hvlux^{-}			eam5.x/HvPHYC^{+}			eam8.k/Hvelf3^{-}			eam8.w/Hvelf3^{-}		
0%	Min	13.57			17.10			14.47			15.85			16.74			14.85		
	Max	43.68			41.65			31.85			42.04			36.03			32.58		
	Mean (s.d.)	24.38	±	6.91	23.00	±	5.68	23.44	±	4.36	21.88	±	4.62	24.63	±	3.96	24.36	±	4.08
	n	33			33			32			33			33			35		
15%	Min	0.00			0.00			0.00			0.00			0.00			0.00		
	Max	26.81			80.60			24.43			50.88			25.52			19.51		

	Mean (s.d.)	16.01	±	6.04	22.25	±	19.83	11.18	±	6.40	13.28	±	9.21	11.63	±	6.01	10.53	±	5.05
	n	34			36			37			36			36			37		

		Percentage of water as total biomass (%)																	
PEG		Antonella			Bowman			eam10.m/Hvlux^{-}			eam5.x/HvPHYC^{+}			eam8.k/Hvelf3^{-}			eam8.w/Hvelf3^{-}		
0%	Min	0.89			0.89			0.88			0.90			0.88			0.56		
	Max	0.93			0.94			0.96			0.93			0.97			0.96		
	Mean (s.d.)	0.91	±	0.01	0.92	±	0.01	0.91	±	0.01	0.91	±	0.01	0.91	±	0.02	0.91	±	0.06
	n	33			33			32			33			33			35		
15%	Min	0.72			0.80			0.78			0.79			0.76			0.81		
	Max	0.90			0.91			0.93			0.92			0.95			0.94		
	Mean (s.d.)	0.87	±	0.03	0.88	±	0.03	0.87	±	0.03	0.86	±	0.04	0.87	±	0.03	0.88	±	0.02
	n	34			36			37			36			36			37		

Tables 3.3.2b **Results of statistical analysis comparison of means from generalised linear models for data used in chapter 3.3.2. P<0.01 (*), P<0.001(**), p<0.0001(***), with differences between bareley lines(Antonella (Ant), Bowman(Bow), Bowman eam10.m Hvlux- (eam10.m), Bowman eam5.x PHYC+ (eam5.x), Bowman eam8.k Hvelf- (eam8.k), Bowman eam8.w Hvelf- (eam8.w)) annotated with different letters based off of post hoc plots of confidence intervals calculates in the estimated means R package (emmeans).**

Model F test results							Post hoc (estimated means comparisons)						
	Model predictors	Within d.f.	Between d.f.	F	P	sig.	PEG	Antonella	Bowman	eam10.m	eam5.x	eam8.k	eam8.w
Total fresh weight (g) pre treatment	Barley Lines						0%	a	bc	b	ab	bc	c

Barley Lines	family: Gaussian	5	418	14.352	<0.0001	***							
PEG													
Barley lines * PEG													
Total fresh weight (g)	Barley Lines *PEG						0%	a	b	b	ab	b	b
post treatment													
Barley Lines	family: Gaussian	5	417	7.2571	<0.0001	***	15%	c	c	c	c	c	c
PEG		1	416	595.825	<0.0001	***							
Barley lines * PEG		5	411	2.7399	0.01893	*							
Shoot fresh weight (g)	Barley Lines +PEG						0%	ab	b	ab	a	b	b
post treatment													
Barley Lines	family: Gamma	5	417	4.4586	0.00057	***	15%	cd	d	cd	c	d	d
PEG		1	416	572.29	<0.0001	***							

Barley lines * PEG													
Root fresh weight (g)	Barley Lines +PEG						0%	a	b	ab	a	b	b
post treatment													
Barley Lines	family: Gaussian	5	417	7.437	<0.0001	***	15%	c	d	cd	c	d	d
PEG		1	416	283.803	<0.0001	***							
Barley lines * PEG													
Change in fresh weight (g)	Barley Lines *PEG						0%	a	b	ab	a	ab	ab
Barley Lines	family: Gaussian	5	418	1.8112	0.1094		15%	c	d	cd	d	d	d
PEG		1	417	1051.91	<0.0001	***							
Barley lines * PEG		5	412	5.2988	<0.0001	***							

Total dry biomass (g)	Barley Lines+PEG						0%	a	ab	ab	a	b	ab
Barley Lines	family: Gamma	5	411	3.9391	0.00169	**	15%	c	cd	cd	c	d	cd
PEG		1	410	160.1437	<0.0001	***							
Barley lines * PEG													
Shoot dry biomass (g)	Barley Lines +PEG						0%	a	ab	ab	ab	b	ab
Barley Lines	family: Gamma	5	417	2.5413	0.02785	*	15%	c	c	c	c	c	c
PEG		1	416	146.1337	<0.0001	***							
Barley lines * PEG													
Root dry biomass (g)	Barley Lines +PEG						0%	a	a	a	b	a	a
Barley Lines	family: Gamma	5	410	9.6752	<0.0001	***	15%	b	b	b	c	b	b

PEG		1	409	144.8941	<0.0001	***							
Barley lines * PEG													
Total water content (g)	Barley Lines +PEG						0%	a	b	ab	a	b	b
Barley Lines	family: Gamma	5	415	6.9658	<0.0001	***	15%	c	d	cd	c	d	d
PEG		1	414	601.056	<0.0001	***							
Barley lines * PEG													
Shoot water content (g)	Barley Lines *PEG						0%	a	b	ab	a	b	b
Barley Lines	family: Gaussian	5	416	5.0909	0.00015	***	15%	c	c	c	c	c	c
PEG		1	415	643.075	<0.0001	***							
Barley lines * PEG		5	410	3.578	0.00353	**							

Root water content (g)	Barley Lines +PEG						0%	a	b	ab	a	ab	b
Barley Lines	family: Gamma	5	409	7.0415	<0.0001	***	15%	c	d	cd	c	d	d
PEG		1	408	302.025	<0.0001	***							
Barley lines * PEG													
Root:Shoot Dry Biomass ratio	Barley Lines *PEG						0%	a	ab	a	b	a	ab
Barley Lines	family: Gamma	5	410	6.2767	<0.0001	***	15%	ab	b	ab	a	ab	b
PEG		1	409	0.2547	0.61406								
Barley lines * PEG		5	404	3.5137	0.004031	**							
Root:Shoot water content ratio	Barley Lines +PEG						0%	a	ab	ab	ab	ab	b
Barley Lines	family: Gaussian	5	411	2.3707	0.03871	*	15%	bc	c	bc	bc	bc	c

PEG		1	410	54.6717	<0.0001	***							
Barley lines * PEG													
Water uptake (ml)	Barley Lines *PEG						0%	a	b	b	a	b	b
Barley Lines	family: Gaussian	5	419	10.3644	<0.0001	***	15%	cd	c	c	c	c	d
PEG		1	418	1122.755	<0.0001	***							
Barley lines * PEG		5	413	8.6205	<0.0001	***							
Accumulated root	Barley Lines +PEG						0%	a	ab	a	a	a	b
length (cm)													
Barley Lines	family: Gamma	5	204	5.5668	<0.0001	***	15%	a	a	a	a	a	ab
PEG		1	203	25.4173	<0.0001	***							

Barley lines * PEG													
Total surface area (cm^3)	Barley Lines *PEG						0%	ab	a	a	b	ab	a
Barley Lines	family: Gamma	5	204	3.7651	0.0028	**	15%	b	b	b	b	b	ab
PEG		1	203	45.2466	<0.0001	***							
Barley lines * PEG		5	198	2.6179	0.02563	*							
Average diameter (mm)	Barley Lines +PEG						0%	ab	b	ab	b	b	b
Barley Lines	family: Gamma	5	204	3.5744	0.004042	**	15%	ab	ab	a	ab	b	ab
PEG		1	203	2.7253	0.10032								
Barley lines * PEG													
Total root volume (cm^3)	Barley Lines +PEG						0%	ab	ab	a	ab	ab	ab

Barley Lines	family: Gamma	5	204	2.4144	0.03744	*	15%	ab	ab	ab	b	b	ab
PEG		1	203	3.0162	0.08396								
Barley lines * PEG													
Total number of root tips	Barley Lines *PEG						0%	ab	ab	a	ab	ab	ab
Barley Lines	family: Gamma	5	204	2.1767	0.05822		15%	ab	ab	ab	b	b	ab
PEG		1	203	25.7061	<0.0001	***							
Barley lines * PEG		5	198	2.8139	0.01767	*							
Water uptake per total dry biomass (ml/g)	Barley Lines *PEG						0%	a	a	a	a	a	a
Barley Lines	family: Gaussian	5	410	4.1126	0.00118	**	15%	bc	b	c	c	c	c
PEG		1	409	342.7852	<0.0001	***							

Barley lines * PEG		5	404	3.3544	0.00557	**							
Percentage of water	Barley Lines +PEG						0%	a	a	a	a	a	a
as total biomass (%)													
Barley Lines	family: Gamma	5	409	0.9785	0.4306		15%	b	b	b	b	b	b
PEG		1	408	161.80 11	<0.0001								
Barley lines * PEG													
Water uptake per total	Barley Lines *PEG						0%	a	a	a	a	a	a
water content stored (ml/g)													
Barley Lines	family: Gaussian	5	409	5.1512	<0.0001	***	15%	b	b	b	b	b	a
PEG		1	408	138.27 76	<0.0001	***							

Barley lines * PEG	5	403	6.0161	<0.0001	***

Supplementary table 3.3.3a: Summary tables for the results in chapter 3.3.3 including summary statistics for all the 17 measured and calculated traits data minimum, maximum, means standard deviations to two decimal places and sample number for six barley lines grown in four different osmotic conditions induced with 0%, 5%, 10% and 15% PEG in 20°C/18°C and 20°C/4°C temperature cycles of 12:12 hours.

Total fresh weight (g) pre treatment

		Antonella			Bowman			eam10.m/Hvlux-		
PEG		18°C	4°C		18°C	4°C		18°C	4°C	
0%	Min	0.33	0.30		0.41	0.35		0.56	0.59	
	Max	0.87	0.71		1.06	0.95		1.23	0.98	
	Mean (s.d.)	0.58 ± 0.16	0.53 ± 0.13		0.70 ± 0.14	0.70 ± 0.16		0.79 ± 0.15	0.78 ± 0.11	
	n	16	13		16	12		17	13	
15 %	Min	0.34	0.3		0.51	0.4		0.58	0.6	
	Max	0.81	0.7		1.11	1.1		1.02	1.3	
	Mean (s.d.)	0.58 ± 0.11	0.5 ± 0.1		0.83 ± 0.18	0.7 ± 0.2		0.74 ± 0.12	0.9 ± 0.2	
	n	17	11		17	13		17	13	

		eam5.x/HvPHYC+			eam8.k/Hvelf3-			eam8.w/Hvelf3-		
		18°C	4°C		18°C	4°C		18°C	4°C	
0%	Min	0.31	0.37		0.69	0.44		0.62	0.50	
	Max	0.83	0.75		1.26	1.10		1.09	1.11	
	Mean (s.d.)	0.63 ± 0.13	0.53 ± 0.10		0.95 ± 0.16	0.72 ± 0.21		0.80 ± 0.10	0.73 ± 0.18	
	n	17	13		17	13		18	12	
15 %	Min	0.37	0.4		0.68	0.5		0.35	0.3	
	Max	0.91	0.8		1.13	1.2		1.10	1.2	
	Mean (s.d.)	0.63 ± 0.13	0.5 ± 0.1		0.90 ± 0.12	0.7 ± 0.2		0.79 ± 0.16	0.7 ± 0.2	
	n	17	12		17	13		16	12	

Total fresh weight (g) post treatment

		Antonella		Bowman		eam10.m/Hvlux-	
PEG		18°C	4°C	18°C	4°C	18°C	4°C
0%	Min	0.62	0.49	0.98	0.97	1.12	1.06
	Max	1.67	1.22	2.18	1.85	2.00	1.82

	Mean (s.d.)	1.17	±	0.29	0.89	±	0.20	1.59	±	0.32	1.33	±	0.28	1.54	±	0.26	1.46	± 0.23
	n	16			13			16			12			17			13	
15 %	Min	0.34			0.40			0.46			0.44			0.37			0.55	
	Max	0.99			0.88			1.29			1.09			1.10			1.53	
	Mean (s.d.)	0.72	±	0.16	0.63	±	0.15	0.84	±	0.27	0.69	±	0.19	0.73	±	0.22	1.04	± 0.27
	n	17			11			17			13			17			13	
		eam5.x/HvPHYC+						eam8.k/Hvelf3-						eam8.w/Hvelf3-				
		18°C			4°C			18°C			4°C			18°C			4°C	
0%	Min	0.60			0.68			1.27			0.83			0.88			0.89	
	Max	1.91			1.29			2.14			2.01			2.05			1.94	
	Mean (s.d.)	1.36	±	0.30	0.96	±	0.15	1.82	±	0.28	1.39	±	0.41	1.67	±	0.25	1.43	± 0.33
	n	17			13			16			13			18			12	
15 %	Min	0.25			0.46			0.63			0.57			0.42			0.46	
	Max	1.06			1.02			1.40			1.20			1.25			1.15	

	Mean (s.d.)	0.67	±	0.22	0.69	±	0.14	1.02	±	0.25	0.84	±	0.16	0.82	±	0.23	0.86	±	0.22
	n	17			12			17			13			16			12		
Change in fresh weight (g)																			
		Antonella						Bowman						eam10.m/Hvlux-					
PEG		18°C			4°C			18°C			4°C			18°C			4°C		
0%	Min	0.30			0.19			0.49			0.32			0.44			0.42		
	Max	0.84			0.51			1.15			0.94			1.25			0.98		
	Mean (s.d.)	0.58	±	0.14	0.36	±	0.09	0.88	±	0.22	0.63	±	0.22	0.75	±	0.20	0.67	±	0.17
	n	16			13			16			12			17			13		
15 %	Min	-0.20			0.05			-0.48			-0.67			-0.40			-0.19		
	Max	0.34			0.22			0.30			0.30			0.35			0.39		
	Mean (s.d.)	0.14	±	0.13	0.16	±	0.06	0.01	±	0.23	-0.03	±	0.26	-0.01	±	0.21	0.18	±	0.17
	n	17			11			17			13			17			13		
		eam5.x/HvPHYC+						eam8.k/Hvelf3-						eam8.w/Hvelf3-					
		18°C			4°C			18°C			4°C			18°C			4°C		

0%	Min	0.30			0.03			0.46			0.36			0.12			0.35		
	Max	1.08			0.65			1.09			1.12			1.13			0.91		
	Mean (s.d.)	0.73	±	0.20	0.43	±	0.16	0.85	±	0.18	0.68	±	0.22	0.87	±	0.22	0.70	±	0.18
	n	17			13			16			13			18			12		
15 %	Min	-0.43			0.07			-0.38			-0.30			-0.28			-0.09		
	Max	0.31			0.24			0.39			0.45			0.37			0.36		
	Mean (s.d.)	0.04	±	0.21	0.14	±	0.05	0.11	±	0.22	0.13	±	0.20	0.03	±	0.21	0.20	±	0.12
	n	17			12			17			13			16			12		

Shoot fresh weight (g) post treatment

		Antonella						Bowman						eam10.m/Hvlux-					
PEG		18°C			4°C			18°C			4°C			18°C			4°C		
0%	Min	0.38			0.33			0.56			0.59			0.70			0.57		
	Max	1.02			0.75			1.29			1.05			1.24			0.93		
	Mean (s.d.)	0.72	±	0.19	0.53	±	0.12	0.93	±	0.15	0.75	±	0.14	0.94	±	0.14	0.75	±	0.12
	n	16			13			16			12			17			13		

15 %	Min	0.13			0.26			0.20			0.26			0.15			0.26		
	Max	0.60			0.52			0.73			0.62			0.68			0.76		
	Mean (s.d.)	0.43	±	0.11	0.39	±	0.08	0.46	±	0.17	0.37	±	0.11	0.41	±	0.15	0.51	±	0.15
	n	17			11			17			13			17			13		
		eam5.x/HvPHYC+						eam8.k/Hvelf3-						eam8.w/Hvelf3-					
		18°C			4°C			18°C			4°C			18°C			4°C		
0%	Min	0.44			0.41			0.85			0.43			0.51			0.51		
	Max	1.16			0.63			1.41			1.03			1.27			1.01		
	Mean (s.d.)	0.84	±	0.18	0.50	±	0.06	1.16	±	0.20	0.75	±	0.21	0.98	±	0.16	0.78	±	0.15
	n	17			13			16			13			18			12		
15 %	Min	0.13			0.23			0.29			0.29			0.15			0.24		
	Max	0.62			0.54			0.83			0.58			0.72			0.68		
	Mean (s.d.)	0.40	±	0.15	0.38	±	0.08	0.59	±	0.17	0.43	±	0.09	0.44	±	0.16	0.47	±	0.12
	n	17			12			17			13			16			12		

Root fresh weight (g) post treatment																	
		Antonella						Bowman						eam10.m/Hvlux-					
PEG		18°C			4°C			18°C			4°C			18°C			4°C		
0%	Min	0.24			0.16			0.36			0.38			0.37			0.49		
	Max	0.66			0.47			0.97			0.82			0.94			0.93		
	Mean (s.d.)	0.45	±	0.10	0.36	±	0.09	0.66	±	0.18	0.58	±	0.15	0.60	±	0.16	0.71	±	0.12
	n	16			13			16			12			17			13		
15 %	Min	0.16			0.14			0.24			0.11			0.17			0.29		
	Max	0.41			0.38			0.58			0.47			0.46			0.77		
	Mean (s.d.)	0.28	±	0.06	0.24	±	0.07	0.38	±	0.12	0.31	±	0.10	0.32	±	0.08	0.53	±	0.13
	n	17			11			17			13			17			13		
		eam5.x/HvPHYC+						eam8.k/Hvelf3-						eam8.w/Hvelf3-					
		18°C			4°C			18°C			4°C			18°C			4°C		
0%	Min	0.17			0.26			0.38			0.37			0.36			0.33		
	Max	0.76			0.66			1.01			0.99			1.13			0.93		

	Mean (s.d.)	0.52	±	0.13	0.46	±	0.11	0.66	±	0.16	0.65	±	0.21	0.69	±	0.15	0.65	± 0.18
	n	17			13			16			13			18			12	
15 %	Min	0.12			0.23			0.31			0.26			0.16			0.20	
	Max	0.44			0.48			0.70			0.62			0.55			0.60	
	Mean (s.d.)	0.28	±	0.08	0.31	±	0.07	0.43	±	0.10	0.41	±	0.11	0.38	±	0.09	0.38	± 0.11
	n	17			12			17			13			16			12	

Shoot dry biomass (g)

		Antonella						Bowman						eam10.m/Hvlux-				
PEG		18°C			4°C			18°C			4°C			18°C			4°C	
0%	Min	0.04			0.04			0.05			0.07			0.03			0.08	
	Max	0.11			0.10			0.15			0.14			0.13			0.13	
	Mean (s.d.)	0.08	±	0.02	0.07	±	0.02	0.09	±	0.02	0.09	±	0.02	0.09	±	0.03	0.10	± 0.02
	n	16			13			16			12			17			13	
15 %	Min	0.04			0.04			0.04			0.05			0.04			0.05	

	Max	0.08			0.08			0.10			0.09			0.10			0.13		
	Mean (s.d.)	0.06	±	0.01	0.06	±	0.01	0.06	±	0.02	0.07	±	0.01	0.06	±	0.02	0.09	±	0.02
	n	17			11			17			13			17			13		
		eam5.x/HvPHYC+						eam8.k/Hvelf3-						eam8.w/Hvelf3-					
		18°C			4°C			18°C			4°C			18°C			4°C		
0%	Min	0.05			0.05			0.07			0.05			0.06			0.06		
	Max	0.12			0.09			0.17			0.14			0.14			0.14		
	Mean (s.d.)	0.08	±	0.02	0.07	±	0.01	0.13	±	0.03	0.10	±	0.03	0.10	±	0.02	0.10	±	0.02
	n	17			13			16			13			18			12		
15 %	Min	0.02			0.05			0.05			0.05			0.01			0.05		
	Max	0.10			0.09			0.12			0.09			0.10			0.11		
	Mean (s.d.)	0.06	±	0.02	0.06	±	0.01	0.08	±	0.02	0.07	±	0.01	0.06	±	0.02	0.08	±	0.02
	n	17			12			17			13			16			12		
Root dry biomass (g)																			
		Antonella						Bowman						eam10.m/Hvlux-					

PEG		18°C			4°C			18°C			4°C			18°C			4°C		
0%	Min	0.02			0.01			0.02			0.03			0.02			0.04		
	Max	0.04			0.04			0.05			0.05			0.08			0.06		
	Mean (s.d.)	0.03	±	0.01	0.03	±	0.01	0.03	±	0.01	0.04	±	0.01	0.05	±	0.01	0.05	±	0.01
	n	16			13			16			12			17			13		
15 %	Min	0.01			0.02			0.02			0.01			0.01			0.03		
	Max	0.04			0.03			0.04			0.04			0.04			0.05		
	Mean (s.d.)	0.02	±	0.01	0.02	±	0.01	0.03	±	0.01	0.03	±	0.01	0.02	±	0.01	0.04	±	0.01
	n	17			11			17			13			17			13		
		eam5.x/HvPHYC+						eam8.k/Hvelf3-						eam8.w/Hvelf3-					
		18°C			4°C			18°C			4°C			18°C			4°C		
0%	Min	0.01			0.02			0.03			0.03			0.01			0.03		
	Max	0.04			0.06			0.06			0.05			0.05			0.07		
	Mean (s.d.)	0.03	±	0.01	0.04	±	0.01	0.05	±	0.01	0.04	±	0.01	0.04	±	0.01	0.04	±	0.01
	n	17			13			16			13			18			12		

15 %	Min	0.01			0.02			0.02			0.02			0.02			0.02		
	Max	0.03			0.04			0.05			0.05			0.05			0.04		
	Mean (s.d.)	0.02	±	0.00	0.03	±	0.01	0.03	±	0.01	0.03	±	0.01	0.03	±	0.01	0.03	±	0.01
	n	17			12			17			13			16			12		
Total dry biomass (g)																			
		Antonella						Bowman						eam10.m/Hvlux-					
PEG		18°C			4°C			18°C			4°C			18°C			4°C		
0%	Min	0.06			0.05			0.07			0.10			0.09			0.11		
	Max	0.15			0.14			0.19			0.19			0.20			0.18		
	Mean (s.d.)	0.11	±	0.02	0.10	±	0.02	0.12	±	0.03	0.13	±	0.03	0.13	±	0.03	0.15	±	0.02
	n	16			13			16			12			17			13		
15 %	Min	0.05			0.05			0.06			0.07			0.05			0.08		
	Max	0.12			0.12			0.13			0.13			0.13			0.18		
	Mean (s.d.)	0.09	±	0.02	0.09	±	0.02	0.09	±	0.02	0.09	±	0.02	0.09	±	0.02	0.13	±	0.03

	n	17			11			17			13			17			13	
		eam5.x/HvPHYC+						eam8.k/Hvelf3-						eam8.w/Hvelf3-				
		18°C			4°C			18°C			4°C			18°C			4°C	
0%	Min	0.06			0.08			0.12			0.08			0.08			0.09	
	Max	0.16			0.14			0.23			0.19			0.19			0.21	
	Mean (s.d.)	0.11	±	0.02	0.11	±	0.02	0.17	±	0.03	0.13	±	0.03	0.14	±	0.03	0.14	± 0.03
	n	17			13			16			13			18			12	
15 %	Min	0.03			0.07			0.08			0.07			0.03			0.07	
	Max	0.13			0.13			0.16			0.13			0.15			0.14	
	Mean (s.d.)	0.09	±	0.02	0.09	±	0.02	0.11	±	0.02	0.11	±	0.02	0.09	±	0.03	0.11	± 0.02
	n	17			12			17			13			16			12	
Root:Shoot Dry Biomass ratio																		
		Antonella						Bowman						eam10.m/Hvlux-				
PEG		18°C			4°C			18°C			4°C			18°C			4°C	
0%	Min	0.31			0.28			0.29			0.34			0.31			0.39	
	Max	0.66			0.51			0.61			0.46			1.55			0.60	

	Mean (s.d.)	0.43	±	0.09	0.41	±	0.08	0.38	±	0.08	0.40	±	0.04	0.53	±	0.29	0.49	± 0.06
	n	16			13			16			12			16			13	
15 %	Min	0.30			0.33			0.27			0.14			0.14			0.35	
	Max	0.52			0.46			0.64			0.65			0.53			0.58	
	Mean (s.d.)	0.39	±	0.06	0.39	±	0.04	0.43	±	0.09	0.41	±	0.12	0.37	±	0.08	0.49	± 0.06
	n	17			11			17			13			17			13	
		eam5.x/HvPHYC+						eam8.k/Hvelf3-						eam8.w/Hvelf3-				
		18°C			4°C			18°C			4°C			18°C			4°C	
0%	Min	0.12			0.35			0.26			0.22			0.18			0.34	
	Max	0.48			0.79			0.67			0.61			0.50			0.50	
	Mean (s.d.)	0.33	±	0.08	0.55	±	0.14	0.39	±	0.10	0.39	±	0.11	0.39	±	0.07	0.43	± 0.05
	n	17			13			16			13			18			12	
15 %	Min	0.27			0.33			0.27			0.35			0.29			0.36	
	Max	0.62			0.52			0.57			0.67			1.20			0.49	

	Mean (s.d.)	0.37	±	0.09	0.43	±	0.07	0.40	±	0.09	0.48	±	0.09	0.48	±	0.21	0.43	± 0.05
	n	17			12			17			13			16			12	
Shoot water content (g)																		
		Antonella						Bowman						eam10.m/Hvlux-				
PEG		18°C			4°C			18°C			4°C			18°C			4°C	
0%	Min	0.34			0.29			0.51			0.51			0.64			0.50	
	Max	0.92			0.65			1.14			0.91			1.12			0.80	
	Mean (s.d.)	0.64	±	0.17	0.46	±	0.11	0.84	±	0.14	0.66	±	0.12	0.85	±	0.13	0.65	± 0.10
	n	16			13			16			12			17			13	
15 %	Min	0.08			0.22			0.14			0.19			0.10			0.21	
	Max	0.52			0.45			0.64			0.52			0.58			0.64	
	Mean (s.d.)	0.37	±	0.10	0.33	±	0.07	0.40	±	0.15	0.31	±	0.10	0.35	±	0.14	0.42	± 0.13
	n	17			11			17			13			17			13	
		eam5.x/HvPHYC+						eam8.k/Hvelf3-						eam8.w/Hvelf3-				
		18°C			4°C			18°C			4°C			18°C			4°C	

0%	Min	0.39			0.35			0.78			0.38			0.45			0.45		
	Max	1.03			0.55			1.24			0.91			1.13			0.89		
	Mean (s.d.)	0.76	±	0.16	0.43	±	0.05	1.03	±	0.17	0.65	±	0.18	0.88	±	0.15	0.68	±	0.14
	n	17			13			16			13			18			12		
15 %	Min	0.11			0.18			0.22			0.21			0.12			0.18		
	Max	0.53			0.45			0.71			0.49			0.62			0.57		
	Mean (s.d.)	0.33	±	0.14	0.31	±	0.08	0.51	±	0.16	0.36	±	0.08	0.38	±	0.14	0.39	±	0.11
	n	17			12			17			13			16			12		

Root water content (g)

		Antonella						Bowman						eam10.m/Hvlux-					
PEG		18°C			4°C			18°C			4°C			18°C			4°C		
0%	Min	0.23			0.14			0.34			0.35			0.34			0.45		
	Max	0.62			0.43			0.94			0.77			0.87			0.88		
	Mean (s.d.)	0.41	±	0.10	0.33	±	0.09	0.62	±	0.18	0.54	±	0.14	0.55	±	0.15	0.66	±	0.11
	n	16			13			16			12			17			13		

15 %	Min	0.15			0.12			0.22			0.10			0.15			0.26		
	Max	0.38			0.35			0.55			0.44			0.42			0.71		
	Mean (s.d.)	0.26	±	0.06	0.22	±	0.07	0.35	±	0.11	0.29	±	0.09	0.29	±	0.08	0.48	±	0.13
	n	17			11			17			13			17			13		
		eam5.x/HvPHYC+						eam8.k/Hvelf3-						eam8.w/Hvelf3-					
		18°C			4°C			18°C			4°C			18°C			4°C		
0%	Min	0.16			0.22			0.34			0.33			0.34			0.29		
	Max	0.72			0.60			0.95			0.94			1.09			0.87		
	Mean (s.d.)	0.49	±	0.12	0.42	±	0.11	0.61	±	0.16	0.61	±	0.21	0.65	±	0.15	0.61	±	0.17
	n	17			13			16			13			18			12		
15 %	Min	0.11			0.21			0.28			0.24			0.15			0.18		
	Max	0.41			0.44			0.65			0.58			0.49			0.55		
	Mean (s.d.)	0.25	±	0.08	0.28	±	0.06	0.40	±	0.09	0.38	±	0.10	0.35	±	0.08	0.35	±	0.11
	n	17			12			17			13			16			12		

Total water content (g)																	
		Antonella						Bowman						eam10.m/Hvlux-					
PEG		18°C			4°C			18°C			4°C			18°C			4°C		
0%	Min	0.56			0.43			0.90			0.87			1.03			0.95		
	Max	1.54			1.08			1.98			1.66			1.92			1.64		
	Mean (s.d.)	1.06	±	0.27	0.79	±	0.18	1.46	±	0.30	1.20	±	0.26	1.41	±	0.25	1.31	±	0.21
	n	16			13			16			12			17			13		
15 %	Min	0.28			0.35			0.38			0.37			0.30			0.47		
	Max	0.86			0.77			1.16			0.96			0.97			1.35		
	Mean (s.d.)	0.63	±	0.15	0.54	±	0.14	0.75	±	0.25	0.60	±	0.18	0.64	±	0.21	0.91	±	0.25
	n	17			11			17			13			17			13		
		eam5.x/HvPHYC+						eam8.k/Hvelf3-						eam8.w/Hvelf3-					
		18°C			4°C			18°C			4°C			18°C			4°C		
0%	Min	0.55			0.59			1.15			0.71			0.78			0.76		
	Max	1.75			1.16			1.94			1.83			1.86			1.77		

	Mean (s.d.)	1.25	±	0.28	0.85	±	0.14	1.65	±	0.26	1.26	±	0.38	1.53	±	0.23	1.29	± 0.30
	n	17			13			16			13			18			12	
15 %	Min	0.22			0.39			0.54			0.51			0.37			0.38	
	Max	0.94			0.89			1.27			1.07			1.11			1.01	
	Mean (s.d.)	0.58	±	0.20	0.59	±	0.13	0.91	±	0.23	0.74	±	0.14	0.73	±	0.20	0.74	± 0.20
	n	17			12			17			13			16			12	

Root:Shoot water content ratio

		Antonella			Bowman			eam10.m/Hvlux-		
PEG		18°C	4°C		18°C	4°C		18°C	4°C	
0%	Min	0.50	0.49		0.49	0.66		0.41	0.85	
	Max	0.84	0.92		0.96	1.00		0.96	1.21	
	Mean (s.d.)	0.66 ± 0.08	0.72 ± 0.13		0.73 ± 0.14	0.81 ± 0.12		0.65 ± 0.15	1.03 ± 0.11	
	n	16	13		16	12		17	13	
15 %	Min	0.51	0.47		0.52	0.38		0.57	0.93	

	Max	2.32			0.83			1.73			1.50			2.07			1.87		
	Mean (s.d.)	0.77	±	0.40	0.66	±	0.10	0.99	±	0.39	0.96	±	0.25	0.94	±	0.39	1.19	±	0.27
	n	17			11			17			13			17			13		
		eam5.x/HvPHYC+						eam8.k/Hvelf3-						eam8.w/Hvelf3-					
		18°C			4°C			18°C			4°C			18°C			4°C		
0%	Min	0.41			0.50			0.42			0.78			0.57			0.63		
	Max	0.82			1.33			1.03			1.11			1.78			1.05		
	Mean (s.d.)	0.65	±	0.10	0.98	±	0.22	0.61	±	0.19	0.93	±	0.12	0.76	±	0.25	0.88	±	0.11
	n	17			13			16			13			18			12		
15 %	Min	0.44			0.60			0.58			0.69			0.47			0.67		
	Max	1.59			1.16			1.44			2.65			2.17			1.24		
	Mean (s.d.)	0.85	±	0.29	0.93	±	0.15	0.84	±	0.25	1.12	±	0.49	1.06	±	0.45	0.89	±	0.17
	n	17			12			17			13			16			12		

Water uptake (ml)																			
		Antonella						Bowman						eam10.m/Hvlux-					

PEG		18°C			4°C			18°C			4°C			18°C			4°C		
0%	Min	15.30			8.00			24.30			11.00			19.00			13.00		
	Max	44.00			19.00			42.00			27.00			43.30			25.00		
	Mean (s.d.)	28.59	±	8.99	14.31	±	3.36	35.69	±	4.78	19.75	±	5.13	34.24	±	7.84	18.62	±	4.09
	n	17			13			16			12			17			13		
15 %	Min	2.00			3.00			2.00			0.00			1.00			0.00		
	Max	13.00			10.00			17.00			10.00			14.00			12.00		
	Mean (s.d.)	7.24	±	3.10	6.00	±	1.91	8.06	±	4.02	3.31	±	2.78	7.06	±	4.39	5.62	±	3.15
	n	17			11			17			13			17			13		
		eam5.x/HvPHYC+						eam8.k/Hvelf3-						eam8.w/Hvelf3-					
		18°C			4°C			18°C			4°C			18°C			4°C		
0%	Min	12.30			9.00			35.00			10.00			12.30			10.00		
	Max	38.30			16.00			44.00			33.00			43.30			26.00		
	Mean (s.d.)	26.48	±	6.07	12.77	±	2.19	39.92	±	3.04	20.31	±	6.89	37.61	±	7.02	19.75	±	5.00
	n	17			13			17			13			18			12		

15 %	Min	0.00			1.00			-1.00			1.00			2.00			1.00		
	Max	12.00			6.00			16.00			9.00			14.00			12.00		
	Mean (s.d.)	6.53	±	3.36	3.83	±	1.62	8.53	±	4.80	4.69	±	2.73	6.69	±	4.30	5.25	±	3.06
	n	17			12			17			13			16			12		

Water uptake per total dry biomass (ml/g)

		Antonella						Bowman						eam10.m/Hvlux-					
PEG		18°C			4°C			18°C			4°C			18°C			4°C		
0%	Min	210.73			115.38			203.97			85.47			208.79			97.50		
	Max	407.72			188.48			389.62			179.10			505.84			155.67		
	Mean (s.d.)	258.08	±	55.93	144.86	±	23.64	294.88	±	49.81	151.99	±	24.25	269.02	±	81.59	122.67	±	17.10
	n	16			13			16			12			17			13		
15 %	Min	32.84072			47.39336			28.73563			0			14.12429			0		
	Max	140.09			86.36			168.99			83.96			180.03			75.14		
	Mean (s.d.)	81.65	±	31.12	68.54	±	11.90	85.03	±	35.64	34.53	±	24.01	78.35	±	45.34	40.86	±	18.77

	n	17			11			17			13			17			13		
		eam5.x/HvPHYC+						eam8.k/Hvelf3-						eam8.w/Hvelf3-					
		18°C			4°C			18°C			4°C			18°C			4°C		
0%	Min	184.76			81.30			191.39			86.66			135.16			76.22		
	Max	517.57			161.66			300.16			208.20			490.93			175.91		
	Mean (s.d.)	243.51	±	72.00	119.90	±	20.18	237.09	±	39.41	150.71	±	27.09	288.53	±	86.47	137.61	±	26.69
	n	17			13			16			13			18			12		
15 %	Min	0			14.68429			-10.8108			7.942812			24.84472			11.58749		
	Max	201.15			71.09			131.04			75.31			136.67			105.17		
	Mean (s.d.)	77.04	±	42.55	42.19	±	18.41	72.87	±	35.33	43.06	±	22.24	71.33	±	35.57	46.33	±	25.31
	n	17			12			17			13			16			12		

Percentage of water as total biomass (%)

		Antonella						Bowman						eam10.m/Hvlux-					
PEG		18°C			4°C			18°C			4°C			18°C			4°C		
0%	Min	0.90			0.866			0.90			0.869			0.90			0.874		
	Max	0.93			0.907			0.94			0.922			0.96			0.910		

	Mean (s.d.)	0.91	±	0.01	0.887	±	0.010	0.92	±	0.01	0.901	±	0.014	0.91	±	0.02	0.896	±	0.010
	n	16			13			16			12			17			13		
15%	Min	0.82			0.843			0.83			0.808			0.80			0.843		
	Max	0.89			0.880			0.91			0.899			0.93			0.898		
	Mean (s.d.)	0.87	±	0.00	0.861	±	0.010	0.89	±	0.02	0.863	±	0.025	0.87	±	0.03	0.872	±	0.016
	n	17			11			17			13			17			13		
		eam5.x/HvPHYC+						eam8.k/Hvelf3-						eam8.w/Hvelf3-					
		18°C			4°C			18°C			4°C			18°C			4°C		
0%	Min	0.91			0.824			0.89			0.861			0.90			0.853		
	Max	0.93			0.912			0.93			0.919			0.95			0.915		
	Mean (s.d.)	0.92	±	0.01	0.886	±	0.021	0.91	±	0.01	0.902	±	0.015	0.92	±	0.01	0.897	±	0.016
	n	17			13			16			13			18			12		
15%	Min	0.79			0.835			0.85			0.859			0.87			0.814		
	Max	0.91			0.885			0.91			0.890			0.94			0.891		

	Mean (s.d.)	0.87	±	0.03	0.865	±	0.013	0.89	±	0.02	0.871	±	0.010	0.89	±	0.02	0.864	±	0.021
	n	17			12			17			13			16			12		
Water uptake per total water content stored (ml/g)																			
		Antonella						Bowman						eam10.m/Hvlux-					
PEG		18°C			4°C			18°C			4°C			18°C			4°C		
0%	Min	21.07			15.236			18.78			11.130			18.54			11.648		
	Max	31.45			22.602			38.33			20.674			29.00			16.219		
	Mean (s.d.)	25.96	±	2.96	18.295	±	2.434	25.11	±	4.86	16.464	±	2.531	24.19	±	3.15	14.143	±	1.182
	n	16			13			16			12			17			13		
15%	Min	7.09			7.45			4.19			0.00			2.45			0.00		
	Max	20.11			13.20			29.44			10.91			19.00			11.70		
	Mean (s.d.)	11.41	±	4.01	11.02	±	1.99	10.91	±	5.58	5.00	±	3.06	10.05	±	4.51	5.98	±	2.99
	n	17			11			17			13			17			13		
		eam5.x/HvPHYC+						eam8.k/Hvelf3-						eam8.w/Hvelf3-					
		18°C			4°C			18°C			4°C			18°C			4°C		

0%	Min	15.90			11.190			20.50			13.910			15.69			11.834		
	Max	38.61			24.795			33.09			18.462			29.62			18.729		
	Mean (s.d.)	21.64	±	4.80	15.461	±	4.065	24.65	±	3.67	15.966	±	1.603	24.48	±	3.37	15.382	±	1.938
	n	17			13			16			13			18			12		
15 %	Min	0.00			2.56			-1.85			1.31			3.53			2.65		
	Max	32.38			9.70			14.57			10.13			14.63			14.10		
	Mean (s.d.)	11.41	±	6.62	6.34	±	2.31	8.72	±	4.16	6.21	±	3.11	8.41	±	3.65	6.85	±	3.19
	n	17			12			17			13			16			12		

Tables 3.3.3b **Results of the ANOVA, F-test statistical analysis comparison of means from the generalised linear models on the same data set as data from table 3.3.3a, P<0.01 (*), P<0.001(**), p<0.0001(***), with differences between bareley lines(Antonella (Ant), Bowman(Bow), Bowman eam10.m Hvlux- (eam10.m),Bowman eam5.x PHYC+ (eam5.x), Bowman eam8.k Hvelf- (eam8.k),Bowman eam8.w Hvelf- (eam8.w)) annotated with different letters based off of post hoc plots of confidence intervals calculates in the estimated means R package (emmeans). The 'model' contains the family and predictor parameters that produced the 'glm' models which best fit the observations.**

	ANOVA						Post hoc							
	Model	Within d.f.	Between d.f.	F	P	sig.	°C	%	Ant	Bow	eam10.m-	eam5.x	eam8.k-	eam8.w-
Total fresh weight (g) pre treatment	Family: Gaussian						18°C	0%	c	b	b	c	a	b
Barley Lines	□	5	346	29.9468	<0.0001	***								
Temperature	□	1	345	23.9161	<0.0002	***	4°C	0%	c	b	ab	c	b	b
Plant*Temperature	□	5	340	4.4326	0.000633	***								
Total fresh weight (g) post treatment	family: Gamma													
Barley Lines	□	5	345	23.3148	<0.0001	***	18°C	0%	bc	a	ac	b	a	a
PEG	□	1	344	477.2836	<0.0001	***		15%	d	cd	d	d	c	cd
Temperature	□	1	343	28.0881	<0.0001	***	4°C	0%	cd	bc	ab	c	b	ab

Barley lines*PEG							15 %	d	d	bc	d	cd	cd
Barley lines*Temperature	□	5	338	4.377	0.000717	***							
PEG*Temperature	□	1	337	23.121	<0.0001	***							
Barley lines*PEG*Temp	□	10	327	2.4825	0.007043	**							
Shoot fresh weight (g) post treatment	family: Gamma					18°C	0%	bc	ab	ab	b	a	ab
Barley Lines	□	5	345	14.8847	<0.0001	***	15 %	d	d	d	d	c	d
PEG	□	1	344	457.3611	<0.0001	***	4°C	0%	cd	ab	ab	cd	ab	b
Temperature	□	1	343	58.8055	<0.0001	***	15 %	d	d	cd	d	d	cd
Barley lines*PEG													
Barley lines*Temperature	□	5	338	3.1895	0.007945	**							

PEG*Temperature	□	1	337	5.0012	0.026003	*							
Barley lines*PEG*Temp		10	327	1.9111	0.042897	*							
Root fresh weight (g)	family: Gaussian						18°C	0%	bc	a	a	ab	a	a
post treatment														
Barley Lines	□	5	345	23.8491	<0.0001	***		15 %	c	bc	b	b	b	bc
PEG	□	1	344	275.0287	<0.0001	***	4°C	0%	bc	ab	a	b	a	a
Temperature	□	5	339	2.4372	0.03449	*		15 %	c	c	ab	c	bc	bc
Barley lines*PEG	□	6	333	5.1874	<0.0001	***								
Barley lines*Temperature														
PEG*Temperature														
Barley lines*PEG*Temp														

Change in fresh weight (g)	family: Gaussian						18°C	0%	bc	a	ab	ab	a	a
Barley Lines	□	5	345	4.9373	0.000225	***		15%	d	d	d	d	d	d
PEG	□	1	344	846.9493	<0.0001	***	4°C	0%	c	ab	ab	bc	ab	ab
Temperature	□	1	343	9.236	0.002561	**		15%	cd	d	cd	cd	cd	c
Barley lines*PEG	□	5	338	9.0648	<0.0001	***								
Barley lines*Temperature	□	5	333	2.1282	0.061751									
PEG*Temperature	□	1	332	43.1109	<0.0001	***								
Barley lines*PEG*Temp														
Total dry biomass (g)	family: Gamma						18°C	0%	c	bc	bc	bc	a	b
Barley Lines	□	5	345	18.1055	<0.0001	***		15%	c	bc	c	c	bc	bc

PEG	□	1	344	134.3139	<0.0001	***	4°C	0%		bc	ab	bc	bc	ab
Temperature								15%	c	c	bc	c	bc	bc
Barley lines*PEG														
Barley lines*Temperature	□	6	338	5.1656	<0.0001	***								
PEG*Temperature	□	1	337	8.1909	0.004473	**								
Barley lines*PEG*Temp														
Shoot dry biomass (g)	family: Gaussian						18°C	0%	c	bc	bc	bc	a	b
Barley Lines	□	5	345	13.878	<0.0001	***		15%	c	c	c	c	bc	c
PEG	□	1	344	110.8949	<0.0001	***	4°C	0%	c	bc	ab	c	bc	ab
Temperature								15%	c	c	bc	c	c	bc

Barley lines*PEG	□	5	339	3.4181	0.005021	**							
Barley lines*Temperature	□	6	333	5.379	<0.0001	***							
PEG*Temperature	□	1	332	5.2859	0.022119	*							
Barley lines*PEG*Temp													
Root dry biomass (g)	family: Gamma						18°C	0%	bc	bc	ab	c	ab	b
Barley Lines		5	345	20.3439	<0.0001	***		15 %	c	c	c	c	bc	c
PEG		1	344	109.235	<0.0001	***	4°C	0%	c	b	a	ab	bc	ab
Temperature		1	343	15.5779	<0.0001	***		15 %	c	c	ab	c	bc	bc
Barley lines*PEG		5	338	0.5961	0.703009									
Barley lines*Temperature		5	333	5.6903	<0.0001	***								

PEG*Temperature		1	332	11.375	0.000834	***							
Barley lines*PEG*Temp	□	5	327	4.0445	0.001416	**							
Total water content (g)	family: Gaussian						18°C	0%	bc	ab	ab	b	a	ab
Barley Lines	□	5	345	23.1449	<0.0001	***		15 %	d	c	d	d	c	cd
PEG	□	1	344	506.4628	<0.0001	***	4°C	0%	ab	b	ab	c	b	ab
Temperature	□	1	343	34.932	<0.0001	***		15 %	d	d	c	d	cd	cd
Barley lines*PEG	□	5	338	4.2953	0.000845	***								
Barley lines*Temperature	□	5	333	3.9737	0.00163	**								
PEG*Temperature	□	1	332	24.9954	<0.0001	***								
Barley lines*PEG*Temp														

Shoot water content (g)	family: Gaussian						18°C	0%	c	b	b	bc	a	b
Barley Lines	□	5	345	16.7231	<0.0001	***		15%	d	de	de	de	d	de
PEG	□	1	344	572.181	<0.0001	***	4°C	0%	d	c	c	d	c	c
Temperature	□	1	343	94.234	<0.0001	***		15%	de	e	de	e	cd	de
Barley lines*PEG	□	5	338	5.0169	0.000191	***								
Barley lines*Temperature	□	5	333	3.7245	0.002706	**								
PEG*Temperature	□	1	332	50.2326	<0.0001	***								
Barley lines*PEG*Temp														
Root water content (g)	family: Gamma						18°C	0%	bc	ab	ab	b	ab	a
Barley Lines	□	5	345	27.4587	<0.0001	***		15%	c	c	c	bc	bd	c

PEG	□	1	344	294.7377	<0.0001	***	4°C	0%	c	ab	a	c	bc	bc
Temperature								15%	c	c	b	b	bc	c
Barley lines*PEG														
Barley lines*Temperature	□	6	338	4.9061	<0.0001	***								
PEG*Temperature														
Barley lines*PEG*Temp	□	11	327	2.3024	0.009995	**								
Root:Shoot Dry Biomass ratio	family: Gamma						18°C	0%	ab	a	b	a	a	a
Barley Lines	□	5	344	2.9758	0.012137	*		15%	a	ab	a	a	a	b
PEG							4°C	0%	a	ab	b	b	a	b
Temperature	□	1	343	9.9137	0.001792	**		15%	a	ab	b	ab	ab	ab
Barley lines*PEG	□	6	337	2.9742	0.007672	**								

Barley lines*Temperature	□	5	332	4.1643	0.001109	**								
PEG*Temperature														
Barley lines*PEG*Temp	□	6	326	3.0511	0.006436	**								
Root:Shoot water content ratio	family: Gamma						18°C	0%	a	ab	a	a	a	ab
Barley Lines	□	5	345	5.943	<0.0001	***		15 %	ab	bc	bc	bc	bc	bc
PEG	□	1	344	36.4439	<0.0001	***	4°C	0%	ab	ab	bc	bc	bc	bc
Temperature	□	1	343	25.6524	<0.0001	***		15 %	ab	bc	c	bc	bc	bc
Barley lines*PEG														
Barley lines*Temperature	□	5	338	4.7426	0.000335	***								
PEG*Temperature	□	1	337	15.7142	<0.0001	***								
Barley lines*PEG*Temp														

Water uptake (ml)	family: Gaussian						18°C	0%	c	ab	b	c	a	ab
Barley Lines	□	5	347	12.3966	<0.0001	***		15 %	f	fe	f	f	ef	f
PEG	□	1	346	1489.906	<0.0001	***	4°C	0%	de	d	e	d	d	d
Temperature	□	1	345	310.6912	<0.0001	***		15 %	f	f	f	f	f	f
Barley lines*PEG	□	5	340	9.5606	<0.0001	***								
Barley lines*Temperature														
PEG*Temperature	□	1	339	157.6949	<0.0001	***								
Barley lines*PEG*Temp														
Water uptake per total dry biomass (ml/g)	family: Gaussian						18°C	0%	ab	a	ab	b	b	a

Barley Lines	□	5	345	2.212	0.05273			15 %	de	e	de	de	de	de
PEG	□	1	344	895.838	<0.0001	***	4°C	0%	c	c	cd	cd	c	c
Temperature	□	1	343	263.757	<0.0001	***		15 %	d	de	d	de	de	de
Barley lines*PEG														
Barley lines*Temperature														
PEG*Temperature	□	1	342	92.62	<0.0001	***								
Barley lines*PEG*Temp														
Percentage of water as total biomass (%)	family: Gaussian						18°C	0%	ab	a	ab	a	ab	a
Barley Lines	□	5	345	3.7514	0.002959	**		15 %	c	cb	c	c	cb	cb
PEG	□	1	344	283.5209	<0.0001	***	4°C	0%	b	ab	b	bc	ab	b

Temperature	□	1	343	73.4659	<0.0001	***		15 %	c	c	bc	c	bc	c
Barley lines*PEG														
Barley lines*Temperature														
PEG*Temperature														
Barley lines*PEG*Temp	□	16	327	1.9613	0.015136	*								
Water uptake per total water content stored (ml/g)	family: Gaussian						18°C	0%	a	ab	ab	b	ab	ab
Barley Lines		5	345	4.7633	0.000324			15 %	cd	cd	d	cd	d	d
PEG		1	344	826.458	<0.0001	***	4°C	0%	bc	c	c	c	c	c
Temperature		1	343	195.1518	<0.0001	***		15 %	cd	d	d	d	d	d
Barley lines*PEG		5	338	1.6936	0.135597									

Barley lines*Temperature		5	333	1.2183	0.300135	
PEG*Temperature		1	332	37.0938	<0.0001	***
Barley lines*PEG*Temp	□	5	327	1.5957	0.160758	

Supplementary table 3.3.3c: Summary tables for the results in chapter 3.3.3 including summary statistics for all the 17 measured and calculated traits data minimum, maximum, means standard deviations to two decimal places and sample number for six barley lines grown in four different osmotic conditions induced with 0%, and 15% PEG in 20°C/18°C and 20°C/4°C temperature cycles of 12:12 hours using a smaller sample of 20°C/18°C grown plants that had similar starting fresh weights to plants grown in 20°C/4°C.

Total fresh weight (g) pre treatment

		Antonella			Bowman			eam10.m/Hvlux-		
PEG		18°C	4°C		18°C	4°C		18°C	4°C	
0%	Min	0.33	0.30		0.41	0.35		0.59	0.59	
	Max	0.69	0.71		0.97	0.95		0.97	0.98	
	Mean (s.d.)	0.55 ± 0.12	0.53 ± 0.13		0.70 ± 0.15	0.70 ± 0.16		0.77 ± 0.10	0.78 ± 0.11	
	n	13	13		13	12		12	13	
15%	Min	0.32	0.33		0.45	0.35		0.54	0.55	
	Max	0.73	0.69		1.11	1.11		1.60	1.35	

	Mean (s.d.)	0.52	±	0.12	0.47	±	0.11	0.77	±	0.21	0.71	±	0.20	0.87	±	0.28	0.86	±	0.22
	n	14			11			14			13			15			13		
		eam5.x/HvPHYC+						eam8.k/Hvelf3-						eam8.w/Hvelf3-					
		18°C			4°C			18°C			4°C			18°C			4°C		
0%	Min	0.40			0.37			0.39			0.44			0.50			0.50		
	Max	0.75			0.75			1.10			1.10			1.09			1.11		
	Mean (s.d.)	0.58	±	0.10	0.53	±	0.10	0.76	±	0.21	0.72	±	0.21	0.78	±	0.18	0.73	±	0.18
	n	13			13			13			13			12			12		
15%	Min	0.37			0.39			0.34			0.48			0.35			0.34		
	Max	0.84			0.83			1.26			1.19			1.18			1.22		
	Mean (s.d.)	0.55	±	0.11	0.54	±	0.11	0.74	±	0.22	0.71	±	0.20	0.74	±	0.19	0.65	±	0.24
	n	11			12			15			13			11			13		
Total fresh weight (g) post treatment																			
		Antonella						Bowman						eam10.m/Hvlux-					
PEG		18°C			4°C			18°C			4°C			18°C			4°C		
0%	Min	0.62			0.49			0.98			0.97			1.12			1.06		
	Max	1.51			1.22			2.04			1.85			2.30			1.82		
	Mean (s.d.)	1.16	±	0.26	0.89	±	0.20	1.56	±	0.33	1.33	±	0.28	1.60	±	0.27	1.46	±	0.23

	n	13			13			13			12			12			13		
15%	Min	0.24			0.40			0.32			0.44			0.36			0.55		
	Max	0.99			0.88			1.28			1.09			1.36			1.53		
	Mean (s.d.)	0.64	±	0.21	0.63	±	0.15	0.80	±	0.30	0.69	±	0.19	0.83	±	0.32	1.04	±	0.27
	n	14			11			14			13			15			13		
		eam5.x/HvPHYC+						eam8.k/Hvelf3-						eam8.w/Hvelf3-					
		18°C			4°C			18°C			4°C			18°C			4°C		
0%	Min	1.00			0.68			0.80			0.83			1.04			0.89		
	Max	1.64			1.29			2.14			2.01			2.46			1.94		
	Mean (s.d.)	1.29	±	0.19	0.96	±	0.15	1.56	±	0.41	1.39	±	0.41	1.68	±	0.36	1.43	±	0.33
	n	13			13			13			13			12			12		
15%	Min	0.25			0.46			0.22			0.57			0.42			0.46		
	Max	1.17			1.02			1.40			1.20			1.16			1.15		
	Mean (s.d.)	0.62	±	0.22	0.69	±	0.14	0.72	±	0.24	0.84	±	0.16	0.82	±	0.25	0.85	±	0.22
	n	11			12			15			13			11			13		
Change in fresh weight (g)																			
		Antonella						Bowman						eam10.m/Hvlux-					
PEG		18°C			4°C			18°C			4°C			18°C			4°C		

0%	Min	0.30			0.19			0.49			0.32			0.44			0.42		
	Max	0.84			0.51			1.15			0.94			1.33			0.98		
	Mean (s.d.)	0.62	±	0.16	0.36	±	0.09	0.85	±	0.22	0.63	±	0.22	0.83	±	0.22	0.67	±	0.17
	n	13			13			13			12			12			13		
15%	Min	-0.30			0.05			-0.40			-0.67			-0.90			-0.19		
	Max	0.34			0.22			0.30			0.30			0.43			0.39		
	Mean (s.d.)	0.12	±	0.16	0.16	±	0.06	0.04	±	0.19	-0.03	±	0.26	-0.03	±	0.35	0.18	±	0.17
	n	14			11			14			13			15			13		
		eam5.x/HvPHYC+ 18°C			4°C			eam8.k/Hvelf3- 18°C			4°C			eam8.w/Hvelf3- 18°C			4°C		
0%	Min	0.50			0.03			0.41			0.36			0.51			0.35		
	Max	0.94			0.65			1.09			1.12			1.57			0.91		
	Mean (s.d.)	0.71	±	0.13	0.43	±	0.16	0.80	±	0.23	0.68	±	0.22	0.90	±	0.28	0.70	±	0.18
	n	13			13			13			13			12			12		
15%	Min	-0.32			0.07			-0.52			-0.30			-0.39			-0.09		
	Max	0.33			0.24			0.39			0.45			0.32			0.36		
	Mean (s.d.)	0.06	±	0.18	0.14	±	0.05	-0.02	±	0.25	0.13	±	0.20	0.08	±	0.21	0.21	±	0.11
	n	11			12			15			13			11			13		

Shoot fresh weight (g) post treatment																	
		Antonella						Bowman						eam10.m/Hvlux-					
PEG		18°C			4°C			18°C			4°C			18°C			4°C		
0%	Min	0.38			0.33			0.56			0.59			0.71			0.57		
	Max	1.00			0.75			1.36			1.05			1.55			0.93		
	Mean (s.d.)	0.76	±	0.19	0.53	±	0.12	0.96	±	0.21	0.75	±	0.14	1.00	±	0.20	0.75	±	0.12
	n	13			13			13			12			12			13		
15%	Min	0.13			0.26			0.17			0.26			0.19			0.26		
	Max	0.57			0.52			0.83			0.62			0.87			0.76		
	Mean (s.d.)	0.40	±	0.14	0.39	±	0.08	0.47	±	0.18	0.37	±	0.11	0.48	±	0.21	0.51	±	0.15
	n	14			11			14			13			15			13		
		eam5.x/HvPHYC+						eam8.k/Hvelf3-						eam8.w/Hvelf3-					
		18°C			4°C			18°C			4°C			18°C			4°C		
0%	Min	0.62			0.41			0.60			0.43			0.73			0.51		
	Max	1.04			0.63			1.39			1.03			1.56			1.01		
	Mean (s.d.)	0.81	±	0.12	0.50	±	0.06	1.01	±	0.21	0.75	±	0.21	1.07	±	0.21	0.78	±	0.15
	n	13			13			13			13			12			12		
15%	Min	0.13			0.23			0.16			0.29			0.15			0.24		

	Max	0.74			0.54			0.71			0.58			0.82			0.68		
	Mean (s.d.)	0.39	±	0.16	0.38	±	0.08	0.45	±	0.15	0.43	±	0.09	0.49	±	0.20	0.47	±	0.12
	n	11			12			15			13			11			13		
Root fresh weight (g) post treatment																			
		Antonella						Bowman						eam10.m/Hvlux-					
PEG		18°C			4°C			18°C			4°C			18°C			4°C		
0%	Min	0.24			0.16			0.36			0.38			0.37			0.49		
	Max	0.51			0.47			0.97			0.82			0.87			0.93		
	Mean (s.d.)	0.40	±	0.07	0.36	±	0.09	0.60	±	0.20	0.58	±	0.15	0.60	±	0.13	0.71	±	0.12
	n	13			13			13			12			12			13		
15%	Min	0.12			0.14			0.12			0.11			0.11			0.29		
	Max	0.41			0.38			0.55			0.47			0.57			0.77		
	Mean (s.d.)	0.25	±	0.08	0.24	±	0.07	0.34	±	0.14	0.31	±	0.10	0.35	±	0.13	0.53	±	0.13
	n	14			11			14			13			15			13		
		eam5.x/HvPHYC+						eam8.k/Hvelf3-						eam8.w/Hvelf3-					
		18°C			4°C			18°C			4°C			18°C			4°C		
0%	Min	0.31			0.26			0.20			0.37			0.31			0.33		
	Max	0.67			0.66			1.01			0.99			0.90			0.93		

	Mean (s.d.)	0.48	±	0.09	0.46	±	0.11	0.55	±	0.24	0.65	±	0.21	0.60	±	0.19	0.65	± 0.18
	n	13			13			13			13			12			12	
15%	Min	0.12			0.23			0.04			0.26			0.16			0.20	
	Max	0.43			0.48			0.70			0.62			0.51			0.60	
	Mean (s.d.)	0.23	±	0.08	0.31	±	0.07	0.27	±	0.16	0.41	±	0.11	0.32	±	0.10	0.38	± 0.11
	n	11			12			15			13			11			13	
Shoot dry biomass (g)																		
		Antonella						Bowman						eam10.m/Hvlux-				
PEG		18°C			4°C			18°C			4°C			18°C			4°C	
0%	Min	0.04			0.04			0.05			0.07			0.06			0.08	
	Max	0.11			0.10			0.12			0.14			0.15			0.13	
	Mean (s.d.)	0.08	±	0.02	0.07	±	0.02	0.09	±	0.02	0.09	±	0.02	0.09	±	0.02	0.10	± 0.02
	n	13			13			13			12			12			13	
15%	Min	0.04			0.04			0.04			0.05			0.04			0.05	
	Max	0.08			0.08			0.10			0.09			0.12			0.13	
	Mean (s.d.)	0.06	±	0.01	0.06	±	0.01	0.06	±	0.02	0.07	±	0.01	0.08	±	0.02	0.09	± 0.02
	n	14			11			14			13			15			13	
		eam5.x/HvPHYC+						eam8.k/Hvelf3-						eam8.w/Hvelf3-				

		18°C			4°C			18°C			4°C			18°C			4°C		
0%	Min	0.06			0.05			0.04			0.05			0.03			0.06		
	Max	0.11			0.09			0.16			0.14			0.17			0.14		
	Mean (s.d.)	0.08	±	0.01	0.07	±	0.01	0.10	±	0.04	0.10	±	0.03	0.09	±	0.03	0.10	±	0.02
	n	13			13			13			13			12			12		
15%	Min	0.02			0.05			0.02			0.05			0.01			0.05		
	Max	0.06			0.09			0.10			0.09			0.10			0.11		
	Mean (s.d.)	0.05	±	0.01	0.06	±	0.01	0.06	±	0.02	0.07	±	0.01	0.06	±	0.02	0.08	±	0.02
	n	11			12			15			13			11			13		

Root dry biomass (g)

		Antonella						Bowman						eam10.m/Hvlux-					
PEG		18°C			4°C			18°C			4°C			18°C			4°C		
0%	Min	0.02			0.01			0.02			0.03			0.02			0.04		
	Max	0.04			0.04			0.04			0.05			0.05			0.06		
	Mean (s.d.)	0.03	±	0.01	0.03	±	0.01	0.03	±	0.01	0.04	±	0.01	0.04	±	0.01	0.05	±	0.01
	n	12			13			13			12			12			13		
15%	Min	0.01			0.02			0.01			0.01			0.01			0.03		
	Max	0.04			0.03			0.04			0.04			0.04			0.05		

	Mean (s.d.)	0.02	±	0.01	0.02	±	0.01	0.03	±	0.01	0.03	±	0.01	0.03	±	0.01	0.04	±	0.01
	n	14			11			14			13			15			13		
		eam5.x/HvPHYC+						eam8.k/Hvelf3-						eam8.w/Hvelf3-					
		18°C			4°C			18°C			4°C			18°C			4°C		
0%	Min	0.02			0.02			0.02			0.03			0.02			0.03		
	Max	0.04			0.06			0.06			0.05			0.05			0.07		
	Mean (s.d.)	0.03	±	0.00	0.04	±	0.01	0.04	±	0.01	0.04	±	0.01	0.03	±	0.01	0.04	±	0.01
	n	13			13			13			13			12			12		
15%	Min	0.01			0.02			0.01			0.02			0.02			0.02		
	Max	0.02			0.04			0.05			0.05			0.04			0.04		
	Mean (s.d.)	0.02	±	0.00	0.03	±	0.01	0.02	±	0.01	0.03	±	0.01	0.03	±	0.01	0.03	±	0.01
	n	11			12			15			13			11			13		

Total dry biomass (g)

		Antonella						Bowman						eam10.m/Hvlux-					
PEG		18°C			4°C			18°C			4°C			18°C			4°C		
0%	Min	0.06			0.05			0.07			0.10			0.09			0.11		
	Max	0.15			0.14			0.16			0.19			0.19			0.18		
	Mean (s.d.)	0.11	±	0.02	0.10	±	0.02	0.12	±	0.02	0.13	±	0.03	0.13	±	0.03	0.15	±	0.02

	n	13			13			13			12			12			13		
15%	Min	0.05			0.05			0.05			0.07			0.05			0.08		
	Max	0.12			0.12			0.14			0.13			0.16			0.18		
	Mean (s.d.)	0.08	±	0.02	0.09	±	0.02	0.09	±	0.03	0.09	±	0.02	0.11	±	0.03	0.13	±	0.03
	n	14			11			14			13			15			13		
		eam5.x/HvPHYC+						eam8.k/Hvelf3-						eam8.w/Hvelf3-					
		18°C			4°C			18°C			4°C			18°C			4°C		
0%	Min	0.08			0.08			0.07			0.08			0.05			0.09		
	Max	0.14			0.14			0.20			0.19			0.21			0.21		
	Mean (s.d.)	0.11	±	0.02	0.11	±	0.02	0.14	±	0.05	0.13	±	0.03	0.13	±	0.04	0.14	±	0.03
	n	13			13			13			13			12			12		
15%	Min	0.03			0.07			0.03			0.07			0.03			0.07		
	Max	0.09			0.13			0.14			0.13			0.13			0.14		
	Mean (s.d.)	0.07	±	0.01	0.09	±	0.02	0.09	±	0.03	0.11	±	0.02	0.09	±	0.03	0.11	±	0.02
	n	11			12			15			13			11			13		
Root:Shoot Dry Biomass ratio																			
		Antonella						Bowman						eam10.m/Hvlux-					
PEG		18°C			4°C			18°C			4°C			18°C			4°C		

0%	Min	0.31			0.28			0.16			0.34			0.27			0.39		
	Max	0.53			0.51			0.61			0.46			0.69			0.60		
	Mean (s.d.)	0.39	±	0.06	0.41	±	0.08	0.37	±	0.12	0.40	±	0.04	0.44	±	0.14	0.49	±	0.06
	n	12			13			13			12			12			13		
15%	Min	0.30			0.33			0.28			0.14			0.25			0.35		
	Max	0.52			0.46			0.64			0.65			0.48			0.58		
	Mean (s.d.)	0.39	±	0.06	0.39	±	0.04	0.41	±	0.09	0.41	±	0.12	0.36	±	0.05	0.49	±	0.06
	n	14			11			14			13			15			13		
		eam5.x/HvPHYC+ 18°C						eam8.k/Hvelf3- 18°C						eam8.w/Hvelf3- 18°C					
					4°C						4°C						4°C		
0%	Min	0.24			0.35			0.25			0.22			0.26			0.34		
	Max	0.40			0.79			0.69			0.61			0.50			0.50		
	Mean (s.d.)	0.35	±	0.05	0.55	±	0.14	0.41	±	0.13	0.39	±	0.11	0.38	±	0.08	0.43	±	0.05
	n	13			13			13			13			12			12		
15%	Min	0.21			0.33			0.25			0.35			0.33			0.36		
	Max	0.62			0.52			0.57			0.67			1.20			0.49		
	Mean (s.d.)	0.39	±	0.11	0.43	±	0.07	0.39	±	0.11	0.48	±	0.09	0.49	±	0.23	0.42	±	0.05
	n	11			12			15			13			11			13		

Shoot water content (g)																	
		Antonella						Bowman						eam10.m/Hvlux-			
PEG		18°C			4°C			18°C			4°C			18°C			4°C		
0%	Min	0.34			0.29			0.51			0.51			0.64			0.50		
	Max	0.89			0.65			1.23			0.91			1.40			0.80		
	Mean (s.d.)	0.68	±	0.18	0.46	±	0.11	0.87	±	0.19	0.66	±	0.12	0.91	±	0.18	0.65	±	0.10
	n	13			13			13			12			12			13		
15%	Min	0.08			0.22			0.12			0.19			0.14			0.21		
	Max	0.49			0.45			0.73			0.52			0.77			0.64		
	Mean (s.d.)	0.34	±	0.13	0.33	±	0.07	0.40	±	0.16	0.31	±	0.10	0.40	±	0.20	0.42	±	0.13
	n	14			11			14			13			15			13		
		eam5.x/HvPHYC+						eam8.k/Hvelf3-						eam8.w/Hvelf3-					
		18°C			4°C			18°C			4°C			18°C			4°C		
0%	Min	0.55			0.35			0.55			0.38			0.67			0.45		
	Max	0.94			0.55			1.22			0.91			1.39			0.89		
	Mean (s.d.)	0.73	±	0.11	0.43	±	0.05	0.91	±	0.18	0.65	±	0.18	0.98	±	0.18	0.68	±	0.14
	n	13			13			13			13			12			12		
15%	Min	0.11			0.18			0.11			0.21			0.12			0.18		

	Max	0.68			0.45			0.66			0.49			0.74			0.57		
	Mean (s.d.)	0.34	±	0.16	0.31	±	0.08	0.39	±	0.15	0.36	±	0.08	0.43	±	0.18	0.39	±	0.10
	n	11			12			15			13			11			13		
Root water content (g)																			
		Antonella						Bowman						eam10.m/Hvlux-					
PEG		18°C			4°C			18°C			4°C			18°C			4°C		
0%	Min	0.23			0.14			0.34			0.35			0.34			0.45		
	Max	0.47			0.43			0.94			0.77			0.82			0.88		
	Mean (s.d.)	0.38	±	0.07	0.33	±	0.09	0.57	±	0.20	0.54	±	0.14	0.56	±	0.13	0.66	±	0.11
	n	13			13			13			12			12			13		
15%	Min	0.10			0.12			0.11			0.10			0.09			0.26		
	Max	0.38			0.35			0.52			0.44			0.52			0.71		
	Mean (s.d.)	0.22	±	0.08	0.22	±	0.07	0.31	±	0.13	0.29	±	0.09	0.32	±	0.12	0.48	±	0.13
	n	14			11			14			13			15			13		
		eam5.x/HvPHYC+						eam8.k/Hvelf3-						eam8.w/Hvelf3-					
		18°C			4°C			18°C			4°C			18°C			4°C		
0%	Min	0.29			0.22			0.18			0.33			0.29			0.29		
	Max	0.63			0.60			0.95			0.94			0.85			0.87		

	Mean (s.d.)	0.45	±	0.09	0.42	±	0.11	0.51	±	0.23	0.61	±	0.21	0.57	±	0.18	0.61	± 0.17
	n	13			13			13			13			12			12	
15%	Min	0.11			0.21			0.03			0.24			0.15			0.18	
	Max	0.40			0.44			0.65			0.58			0.48			0.55	
	Mean (s.d.)	0.21	±	0.07	0.28	±	0.06	0.24	±	0.15	0.38	±	0.10	0.30	±	0.09	0.35	± 0.10
	n	11			12			15			13			11			13	

Total water content (g)

		Antonella						Bowman						eam10.m/Hvlux-				
PEG		18°C			4°C			18°C			4°C			18°C			4°C	
0%	Min	0.56			0.43			0.90			0.87			1.03			0.95	
	Max	1.36			1.08			1.91			1.66			2.11			1.64	
	Mean (s.d.)	1.06	±	0.24	0.79	±	0.18	1.44	±	0.31	1.20	±	0.26	1.47	±	0.25	1.31	± 0.21
	n	13			13			13			12			12			13	
15%	Min	0.17			0.35			0.26			0.37			0.30			0.47	
	Max	0.86			0.77			1.16			0.96			1.22			1.35	
	Mean (s.d.)	0.56	±	0.19	0.54	±	0.14	0.71	±	0.28	0.60	±	0.18	0.73	±	0.29	0.91	± 0.25
	n	14			11			14			13			15			13	
		eam5.x/HvPHYC+						eam8.k/Hvelf3-						eam8.w/Hvelf3-				

		18°C			4°C			18°C			4°C			18°C			4°C		
0%	Min	0.92			0.59			0.73			0.71			0.96			0.76		
	Max	1.51			1.16			1.94			1.83			2.25			1.77		
	Mean (s.d.)	1.18	±	0.17	0.85	±	0.14	1.42	±	0.37	1.26	±	0.38	1.55	±	0.32	1.29	±	0.30
	n	13			13			13			13			12			12		
15%	Min	0.22			0.39			0.17			0.51			0.36			0.38		
	Max	1.08			0.89			1.27			1.07			1.04			1.01		
	Mean (s.d.)	0.55	±	0.21	0.59	±	0.13	0.63	±	0.23	0.74	±	0.14	0.73	±	0.23	0.74	±	0.20
	n	11			12			15			13			11			13		

Root:Shoot water content ratio

		Antonella						Bowman						eam10.m/Hvlux-					
PEG		18°C			4°C			18°C			4°C			18°C			4°C		
0%	Min	0.43			0.49			0.31			0.66			0.35			0.85		
	Max	0.84			0.92			0.96			1.00			0.94			1.21		
	Mean (s.d.)	0.57	±	0.11	0.72	±	0.13	0.67	±	0.21	0.81	±	0.12	0.63	±	0.15	1.03	±	0.11
	n	13			13			13			12			12			13		
15%	Min	0.34			0.47			0.46			0.38			0.30			0.93		
	Max	2.32			0.83			1.43			1.50			1.63			1.87		

	Mean (s.d.)	0.78	±	0.47	0.66	±	0.10	0.81	±	0.27	0.96	±	0.25	0.90	±	0.40	1.19	±	0.27
	n	14			11			14			13			15			13		
		eam5.x/HvPHYC+						eam8.k/Hvelf3-						eam8.w/Hvelf3-					
		18°C			4°C			18°C			4°C			18°C			4°C		
0%	Min	0.41			0.50			0.29			0.78			0.31			0.63		
	Max	0.82			1.33			1.03			1.11			0.76			1.05		
	Mean (s.d.)	0.62	±	0.12	0.98	±	0.22	0.55	±	0.22	0.93	±	0.12	0.58	±	0.15	0.88	±	0.11
	n	13			13			13			13			12			12		
15%	Min	0.41			0.60			0.04			0.69			0.35			0.67		
	Max	1.32			1.16			1.44			2.65			2.17			1.24		
	Mean (s.d.)	0.72	±	0.31	0.93	±	0.15	0.71	±	0.42	1.12	±	0.49	0.87	±	0.52	0.89	±	0.16
	n	11			12			15			13			11			13		
Water uptake (ml)																			
		Antonella						Bowman						eam10.m/Hvlux-					
PEG		18°C			4°C			18°C			4°C			18°C			4°C		
0%	Min	18.70			8.00			22.33			11.00			23.00			13.00		
	Max	39.70			19.00			44.70			27.00			46.70			25.00		
	Mean (s.d.)	25.49	±	6.01	14.31	±	3.36	36.12	±	6.30	19.75	±	5.13	34.98	±	8.03	18.62	±	4.09

	n	13			13			13			12			12			13		
15%	Min	0.00			3.00			0.67			0.00			0.00			0.00		
	Max	17.00			10.00			17			10.00			17.00			12.00		
	Mean (s.d.)	10.79	±	4.37	6.00	±	1.91	8.86	±	4.65	3.31	±	2.78	6.38	±	4.74	5.62	±	3.15
	n	14			11			14			13			15			13		
		eam5.x/HvPHYC+						eam8.k/Hvelf3-						eam8.w/Hvelf3-					
		18°C			4°C			18°C			4°C			18°C			4°C		
0%	Min	17.33			9.00			20.00			10.00			21.67			10.00		
	Max	29.70			16.00			45.90			33.00			48.67			26.00		
	Mean (s.d.)	25.83	±	3.29	12.77	±	2.19	34.05	±	8.02	20.31	±	6.89	38.37	±	8.72	19.75	±	5.00
	n	13			13			13			13			12			12		
15%	Min	2.67			1.00			1.67			1.00			3.00			1.00		
	Max	12.00			6.00			19.00			9.00			16.00			23.00		
	Mean (s.d.)	7.79	±	2.63	3.83	±	1.62	8.51	±	4.81	4.69	±	2.73	9.33	±	4.02	6.62	±	5.57
	n	11			12			15			13			11			13		

Water uptake per total dry biomass (ml/g)

		Antonella						Bowman						eam10.m/Hvlux-					
PEG		18°C			4°C			18°C			4°C			18°C			4°C		

0%	Min	177.56			115.38			206.45			85.47			175.44			97.50		
	Max	318.65			188.48			424.21			179.10			438.24			155.67		
	Mean (s.d.)	245.91	±	43.51	144.86	±	23.64	310.85	±	66.69	151.99	±	24.25	270.13	±	80.40	122.67	±	17.10
	n	13			13			13			12			12			13		
15%	Min	0.00			47.39			10.67			0.00			0.00			0.00		
	Max	183.19			86.36			531.73			83.96			145.45			75.14		
	Mean (s.d.)	132.22	±	47.05	68.54	±	11.90	193.13	±	138.97	34.53	±	24.01	58.46	±	39.85	40.86	±	18.77
	n	14			11			14			13			15			13		
		eam5.x/HvPHYC+						eam8.k/Hvelf3-						eam8.w/Hvelf3-					
		18°C			4°C			18°C			4°C			18°C			4°C		
0%	Min	199.85			81.30			128.64			86.66			227.31			76.22		
	Max	282.86			161.66			396.48			208.20			525.25			175.91		
	Mean (s.d.)	236.01	±	29.77	119.90	±	20.18	269.54	±	70.92	150.71	±	27.09	316.06	±	77.74	137.61	±	26.69
	n	13			13			13			13			12			12		
15%	Min	43.79			14.68			14.14			7.94			36.01			11.59		
	Max	316.09			71.09			309.38			75.31			264.03			227.05		
	Mean (s.d.)	122.66	±	68.88	42.19	±	18.41	107.09	±	72.81	43.06	±	22.24	119.06	±	62.45	60.23	±	53.95
	n	11			12			15			13			11			13		

Percentage of water as total biomass (%)

		Antonella				Bowman				eam10.m/Hvlux-			
PEG		18°C		4°C		18°C		4°C		18°C		4°C	
0%	Min	0.90		0.87		0.90		0.87		0.90		0.87	
	Max	0.93		0.91		0.94		0.92		0.93		0.91	
	Mean (s.d.)	0.91	± 0.01	0.89	± 0.01	0.92	± 0.01	0.90	± 0.01	0.92	± 0.01	0.90	± 0.01
	n	13		13		13		12		12		13	
15%	Min	0.72		0.84		0.80		0.81		0.82		0.84	
	Max	0.89		0.88		0.91		0.90		0.90		0.90	
	Mean (s.d.)	0.86	± 0.04	0.86	± 0.01	0.88	± 0.03	0.86	± 0.02	0.86	± 0.03	0.87	± 0.02
	n	14		11		14		13		15		13	
		eam5.x/HvPHYC+				eam8.k/Hvelf3-				eam8.w/Hvelf3-			
		18°C		4°C		18°C		4°C		18°C		4°C	
0%	Min	0.90		0.82		0.88		0.86		0.91		0.85	
	Max	0.93		0.91		0.94		0.92		0.96		0.92	
	Mean (s.d.)	0.91	± 0.01	0.89	± 0.02	0.91	± 0.01	0.90	± 0.01	0.92	± 0.01	0.90	± 0.02
	n	13		13		13		13		12		12	
15%	Min	0.84		0.83		0.76		0.86		0.81		0.81	

	Max	0.92			0.88			0.95			0.89			0.94			0.89	
	Mean (s.d.)	0.88	±	0.03	0.87	±	0.01	0.87	±	0.04	0.87	±	0.01	0.89	±	0.03	0.86	± 0.02
	n	11			12			15			13			11			13	
Water uptake per total water content stored (ml/g)																		
		Antonella						Bowman						eam10.m/Hvlux-				
PEG		18°C			4°C			18°C			4°C			18°C			4°C	
0%	Min	17.55			15.24			18.42			11.13			17.54			11.65	
	Max	33.79			22.60			41.65			20.67			31.39			16.22	
	Mean (s.d.)	24.66	±	4.90	18.29	±	2.43	26.20	±	6.93	16.46	±	2.53	23.91	±	4.33	14.14	± 1.18
	n	13			13			13			12			12			13	
15%	Min	0.00			7.45			2.59			0.00			0.00			0.00	
	Max	25.04			13.20			53.66			10.91			18.91			11.70	
	Mean (s.d.)	18.45	±	6.13	11.02	±	1.99	22.86	±	14.16	5.00	±	3.06	8.60	±	5.56	5.98	± 2.99
	n	14			11			14			13			15			13	
		eam5.x/HvPHYC+						eam8.k/Hvelf3-						eam8.w/Hvelf3-				
		18°C			4°C			18°C			4°C			18°C			4°C	
0%	Min	17.23			11.19			16.74			13.91			19.24			11.83	
	Max	27.56			24.79			33.58			18.46			31.16			18.73	

	Mean (s.d.)	22.18	±	3.31	15.46	±	4.06	24.48	±	4.14	15.97	±	1.60	24.86	±	3.58	15.38	±	1.94
	n	13			13			13			13			12			12		
15%	Min	5.94			2.56			3.32			1.31			4.56			2.65		
	Max	50.88			9.70			25.52			10.13			19.51			32.41		
	Mean (s.d.)	17.16	±	12.14	6.34	±	2.31	13.06	±	5.53	6.21	±	3.11	13.09	±	4.46	8.82	±	7.47
	n	11			12			15			13			11			13		

*Tables 3.3.3d*Results of the ANOVA, F-test statistical analysis comparison of means from the generalised linear models on the same data set as data from table 3.3.3c, P<0.01 (*), P<0.001(**), p<0.0001(***), with differences between bareley lines(Antonella (Ant), Bowman(Bow), Bowman eam10.m Hvlux- (eam10.m),Bowman eam5.x PHYC+ (eam5.x), Bowman eam8.k Hvelf- (eam8.k),Bowman eam8.w Hvelf- (eam8.w)) annotated with different letters based off of post hoc plots of confidence intervals calculates in the estimated means R package (emmeans). The 'model' contains the family and predictor parameters that produced the 'glm' models which best fit the observations.

	ANOVA						**Post hoc**							
	Model	Within d.f.	Between d.f.	F	P	sig.	°C	%	Ant	Bow	eam10.m	eam5.x	eam8.k	eam8.w
Total fresh weight (g) pre treatment	family: Gamma	5	301	25.1442	<0.0001	***	18°C	0%	a	b	b	a	b	b
Barley Lines	□	1	300	2.8764	0.09092									
Temperature	□						4°C	0%	a	b	b	a	b	b
Plant*Temperature														
Total fresh weight (g)	family:													
post treatment	Gamma													
Barley Lines	□	5	301	15.4125	<0.0001	***	18°C	0%	ab	a	a	ab	a	a

PEG	□	1	300	347.1142	<0.0001	***		15%	c	bc	bc	c	bc	bc
Temperature	□	1	299	7.9779	0.00506	**	4°C	0%	bc	b	a	b	ab	ab
Barley lines*PEG								15%	bc	bc	ab	b	bc	bc
Barley lines*Temperature														
PEG*Temperature	□	1	298	19.3889	<0.0001	***								
Barley lines*PEG*Temp														
Shoot fresh weight (g) post treatment	family: Gaussian						18°C	0%	b	ab	a	b	a	a
Barley Lines	□	5	301	11.001	<0.0001	***		15%	c	c	c	c	c	c
PEG	□	1	300	394.6814	<0.0001	***	4°C	0%	bc	b	b	c	b	b
Temperature	□	1	299	56.5494	<0.0001	***		15%	c	c	c	c	c	c

Barley lines*PEG	□	5	294	3.4379	0.004918	**							
Barley lines*Temperature													
PEG*Temperature	□	1	293	40.6024	<0.0001	***							
Barley lines*PEG*Temp													
Root fresh weight (g) post treatment	family: Gamma						18°C	0%	bc	ab	a	b	ab	a
Barley Lines	□	5	301	16.9418	<0.0001	***		15 %	d	c	bc	cd	c	bc
PEG	□	1	300	181.4297	<0.0001	***	4°C	0%	b	a	a	ab	a	a
Temperature	□	1	299	7.9923	0.005015	**		15 %	d	bc	b	c	bc	bc
Barley lines*PEG														
Barley lines*Temperature														
PEG*Temperature														

Barley lines*PEG*Temp														
Change in fresh weight (g)	family: Gaussian						18°C	0%	ab	a	a	ab	a	a
Barley Lines	□	5	301	3.2115	0.007714			15%	c	c	c	c	c	c
PEG	□	1	300	633.1451	<0.0001	***	4°C	0%	bc	ab	ab	b	ab	ab
Temperature	□	1	299	5.6501	0.018097			15%	bc	c	bc	bc	c	bc
Barley lines*PEG	□	5	294	6.846	<0.0001	***								
Barley lines*Temperature														
PEG*Temperature	□	1	293	39.4135	<0.0001	***								
Barley lines*PEG*Temp														
Total dry biomass (g)	family: Gamma						18°C	0%	b	ab	ab	b	ab	ab

Barley Lines	□	5	301	13.938	<0.0001	***		15 %	bc	bc	b	c	bc	bc
PEG	□	1	300	93.172	<0.0001	***	4°C	0%	bc	ab	a	b	ab	a
Temperature	□	1	299	11.235	0.000906	***		15 %	bc	bc	ab	bc	bc	bc
Barley lines*PEG														
Barley lines*Temperature														
PEG*Temperature														
Barley lines*PEG*Temp														
Shoot dry biomass (g)	family: Gamma						18°C	0%	ab	ab	a	ab	a	a
Barley Lines	□	5	301	11.0514	<0.0001	***		15 %	b	b	ab	b	b	b
PEG	□	1	300	89.6724	<0.0001	***	4°C	0%	ab	a	a	ab	a	a
Temperature								15 %	b	b	ab	b	ab	ab
Barley lines*PEG														

Barley lines*Temperature													
PEG*Temperature	□	2	298	5.2905	0.005524	**							
Barley lines*PEG*Temp													
Root dry biomass (g)	family: Gaussian						18°C	0%	bc	bc	ab	bc	ab	abc
Barley Lines	□	5	300	16.2665	<0.0001	***		15 %	c	bc	bc	c	c	bc
PEG	□	1	299	72.0225	<0.0001	***	4°C	0%	bc	ab	a	ab	b	ab
Temperature	□	1	298	40.0724	<0.0001	***		15 %	bc	bc	ab	bc	bc	bc
Barley lines*PEG													
Barley lines*Temperature	□	1	293	3.2261	0.007494	**							
PEG*Temperature													
Barley lines*PEG*Temp													

Total water content (g)	family: Gaussian						18°C	0%	b	a	a	ab	ab	a
Barley Lines	□	5	301	15.7398	<0.0001	***		15 %	c	c	c	c	c	bc
PEG	□	1	300	383.6838	<0.0001	***	4°C	0%	bc	ab	ab	bc	ab	ab
Temperature	□	1	299	12.2274	0.0005439	***		15 %	c	c	bc	c	c	c
Barley lines*PEG	□	5	294	3.3386	0.0059942	**								
Barley lines*Temperature														
PEG*Temperature	□	1	293	22.2776	<0.0001	***								
Barley lines*PEG*Temp														
Shoot water content (g)	family: Gaussian						18°C	0%	b	ab	a	b	a	a
Barley Lines	□	5	301	10.5666	<0.0001	***		15 %	c	c	c	c	c	c

PEG	□	1	300	427.022	<0.0001	***	4°C	0%	bc	bc	bc	c	bc	b
Temperature	□	1	299	73.4152	<0.0001	***		15 %	c	c	bc	c	c	c
Barley lines*PEG	□	5	294	3.7037	0.002887	**								
Barley lines*Temperature														
PEG*Temperature	□	1	293	45.5346	<0.0001	***								
Barley lines*PEG*Temp														
Root water content (g)	family: Gamma						18°C	0%	b	ab	ab	b	ab	ab
Barley Lines	□	5	301	16.7415	<0.0001	***		15 %	c	bc	bc	c	c	bc
PEG	□	1	300	186.5422	<0.0001	***	4°C	0%	bc	ab	a	b	ab	ab
Temperature	□	1	299	6.6658	0.010305	*		15 %	c	bc	ab	bc	bc	bc
Barley lines*PEG														

Barley lines*Temperature													
PEG*Temperature	□	1	298	9.2447	0.002572	**							
Barley lines*PEG*Temp													
Root:Shoot Dry Biomass ratio	family: Gamma						18°C	0%	ab	ab	a	ab	ab	ab
Barley Lines								15 %	ab	ab	ab	ab	ab	ab
PEG							4°C	0%	ab	ab	b	b	ab	ab
Temperature	□	1	304	15.9238	<0.0001	***		15 %	ab	ab	ab	ab	ab	ab
Barley lines*PEG														
Barley lines*Temperature														
PEG*Temperature														
Barley lines*PEG*Temp	□	22	282	2.2941	0.001072	**								

Root:Shoot water content ratio	family: Gamma						18°C	0%	a	ab	ab	ab	a	a
Barley Lines	□	5	301	4.4604	0.000623	***		15 %	ab	ab	ab	ab	a	ab
PEG	□	1	300	16.2441	<0.0001	***	4°C	0%	a	ab	b	ab	b	ab
Temperature	□	1	299	49.4827	<0.0001	***		15 %	a	ab	ab	ab	ab	ab
Barley lines*PEG														
Barley lines*Temperature	□	5	294	2.337	0.0420519	*								
PEG*Temperature	□	1	293	10.1835	0.001571	**								
Barley lines*PEG*Temp														
Water uptake (ml)	family: Gaussian						18°C	0%	b	a	a	b	a	a
Barley Lines		5	301	8.3043	<0.0001	***		15 %	c	c	c	c	c	c
PEG		1	300	707.83	<0.0001	***	4°C	0%	c	bc	bc	c	bc	bc

Temperature		1	299	226.9501	<0.0001	***		15%	c	c	c	c	c	c
Barley lines*PEG		5	294	6.396	<0.0001	***								
Barley lines*Temperature		5	289	2.8589	0.01549	*								
PEG*Temperature		1	288	56.2363	<0.0001	***								
Barley lines*PEG*Temp	□	5	283	2.6071	0.02521	*								
Water uptake per total dry biomass (ml/g)	family: Gaussian						18°C	0%	ab	a	ab	ab	ab	a
Barley Lines		5	301	6.0994	<0.0001	***		15%	bc	b	c	bc	bc	bc
PEG		1	300	345.1707	<0.0001	***	4°C	0%	b	b	bc	bc	b	bc
Temperature		1	299	259.7503	<0.0001	***		15%	bc	c	c	c	c	bc
Barley lines*PEG		5	294	1.9458	0.086873									

Barley lines*Temperature		5	289	3.6049	0.003543	**							
PEG*Temperature		1	288	23.2646	<0.0001	***							
Barley lines*PEG*Temp	□	5	283	2.5822	0.026446	*							
Percentage of water as total biomass (%)	family: Gaussian						18°C	0%	ab	a	ab	ab	ab	a
Barley Lines								15%	b	b	b	b	b	b
PEG	□	1	305	192.0977	<0.0001	***	4°C	0%	b	ab	ab	b	ab	ab
Temperature	□	1	304	34.7022	<0.0001	***		15%	b	b	b	b	b	b
Barley lines*PEG														
Barley lines*Temperature	□	10	294	2.4509	0.007992	**							
PEG*Temperature	□	1	293	6.3857	0.012031	*							

Barley lines*PEG*Temp														
Water uptake per total water content stored (ml/g)	family: Gaussian						18°C	0%	a	a	a	a	a	a
Barley Lines	□	5	301	6.5105	<0.0001	***		15 %	ab	a	b	ab	b	b
PEG	□	1	300	173.9489	<0.0001	***	4°C	0%	ab	ab	b	b	b	b
Temperature	□	1	299	159.1544	<0.0001	***		15 %	b	b	b	b	b	b
Barley lines*PEG														
Barley lines*Temperature	□	5	294	3.2872	0.006673	**								
PEG*Temperature														
Barley lines*PEG*Temp	□	11	283	2.2124	0.013994	*								

Supplementary table 3.3.4a: Summary tables for the summary of descriptions for the data used in the analysis of physiological traits for data in section 3.4 for plants grown in cold nights (4°C) including all the 22 measured and calculated traits data minimum, maximum, means standard deviations to two decimal places and sample number for six barley lines grown in four different osmotic conditions induced with 0%, 5%, 10% and 15% PEG in 20°C/4°C temperature cycles of 12:12 hours of plants treated at 2 weeks, 3 weeks and 4 weeks4 week old plants .

Total fresh weight (g) pre treatment							
		Antonella			Bowman		
PEG		2 week	3 week	4 weeks	2 week	3 week	4 weeks
0%	Min	0.28	0.30	0.51	0.30	0.35	1.09
	Max	0.74	0.71	1.02	0.87	0.95	1.89

	Mean (s.d.)	0.41	±	0.11	0.53	±	0.13	0.73	±	0.16	0.56	±	0.15	0.70	±	0.16	1.46	±	0.25
	n	20			13			10			19			12			10		
15%	Min	0.26			0.33			0.16			0.27			0.35			1.05		
	Max	0.58			0.69			0.98			0.80			1.11			1.67		
	Mean (s.d.)	0.43	±	0.09	0.47	±	0.11	0.67	±	0.23	0.54	±	0.16	0.71	±	0.20	1.46	±	0.17
	n	18			11			10			19			13			10		
		eam10.m/Hvlux-									eam5.x/HvPHYC+								
		2 week			3 week			4 weeks			2 week			3 week			4 weeks		
0%	Min	0.28			0.59			0.79			0.25			0.37			0.89		
	Max	0.84			0.98			1.61			0.66			0.75			1.41		
	Mean (s.d.)	0.50	±	0.17	0.78	±	0.11	1.25	±	0.25	0.44	±	0.10	0.53	±	0.10	1.13	±	0.18
	n	14			13			10			19			13			10		
15%	Min	0.31			0.55			0.62			0.29			0.39			0.80		
	Max	0.87			1.35			1.58			0.68			0.83			1.36		
	Mean (s.d.)	0.53	±	0.16	0.86	±	0.22	1.17	±	0.30	0.44	±	0.10	0.54	±	0.11	1.14	±	0.16
	n	19			13			9			19			12			10		

		eam8.k/Hvelf3-						eam8.w/Hvelf3-					
		2 week		3 week		4 weeks		2 week		3 week		4 weeks	
0%	Min	0.32		0.44		1.28		0.29		0.50		1.08	
	Max	0.92		1.10		1.98		1.05		1.11		1.77	
	Mean (s.d.)	0.53	± 0.17	0.72	± 0.21	1.63	± 0.23	0.57	± 0.19	0.73	± 0.18	1.37	± 0.22
	n	19		13		10		19		12		10	
15%	Min	0.28		0.48		1.36		0.28		0.34		1.27	
	Max	0.88		1.19		2.02		0.88		1.22		1.71	
	Mean (s.d.)	0.53	± 0.18	0.71	± 0.20	1.64	± 0.20	0.55	± 0.17	0.65	± 0.24	1.46	± 0.15
	n	20		13		10		19		13		10	

Total fresh weight (g) post treatment

		Antonella						Bowman					
PEG		2 week		3 week		4 weeks		2 week		3 week		4 weeks	
0%	Min	0.50		0.49		0.79		0.68		0.97		1.80	
	Max	1.25		1.22		1.48		1.72		1.85		2.67	
	Mean (s.d.)	0.78	± 0.20	0.89	± 0.20	1.07	± 0.22	1.14	± 0.27	1.33	± 0.28	2.22	± 0.28

	n	20			13			10			19			12			10	
15%	Min	0.18			0.40			0.22			0.39			0.44			1.21	
	Max	0.77			0.88			1.02			1.12			1.09			1.81	
	Mean (s.d.)	0.54	±	0.14	0.63	±	0.15	0.73	±	0.26	0.68	±	0.21	0.69	±	0.19	1.52	± 0.22
	n	18			11			10			19			13			10	
		eam10.m/Hvlux-									eam5.x/HvPHYC+							
		2 week			3 week			4 weeks			2 week			3 week			4 weeks	
0%	Min	0.60			1.06			1.29			0.47			0.68			1.52	
	Max	1.81			1.82			2.47			1.24			1.29			2.21	
	Mean (s.d.)	1.04	±	0.31	1.46	±	0.23	1.96	±	0.35	0.92	±	0.51	0.96	±	0.15	1.85	± 0.23
	n	14			13			10			19			13			10	
15%	Min	0.29			0.55			0.65			0.25			0.46			0.68	
	Max	0.98			1.53			1.78			0.83			1.02			1.58	
	Mean (s.d.)	0.61	±	0.19	1.04	±	0.27	1.22	±	0.42	0.55	±	1.62	0.69	±	0.14	1.16	± 0.22
	n	19			13			9			19			12			10	
		eam8.k/Hvelf3-									eam8.w/Hvelf3-							

		2 week		3 week		4 weeks		2 week		3 week		4 weeks	
0%	Min	0.48		0.83		2.18		0.52		0.89		1.72	
	Max	1.87		2.01		3.07		1.74		1.94		2.70	
	Mean (s.d.)	1.06	± 0.41	1.39	± 0.41	2.55	± 0.29	1.14	± 0.33	1.43	± 0.33	2.17	± 0.25
	n	19		13		10		19		12		10	
15%	Min	0.31		0.57		1.43		0.36		0.46		1.08	
	Max	1.08		1.20		1.98		1.17		1.15		1.85	
	Mean (s.d.)	0.67	± 0.23	0.84	± 0.16	1.71	± 0.18	0.68	± 0.22	0.85	± 0.22	1.56	± 0.25
	n	20		13		10		19		13		10	

Change in fresh weight (g)

		Antonella						Bowman					
PEG		2 week		3 week		4 weeks		2 week		3 week		4 weeks	
0%	Min	0.19		0.19		0.26		0.34		0.32		0.64	
	Max	0.61		0.51		0.46		0.85		0.94		0.89	
	Mean (s.d.)	0.37	± 0.10	0.36	± 0.09	0.34	± 0.07	0.58	± 0.14	0.63	± 0.22	0.76	± 0.08
	n	20		13		10		19		12		10	

15%	Min	-0.08			0.05			-0.21			-0.06			-0.67			-0.17	
	Max	0.23			0.22			0.23			0.32			0.30			0.21	
	Mean (s.d.)	0.11	±	0.08	0.16	±	0.06	0.06	±	0.13	0.14	±	0.10	-0.03	±	0.26	0.06	± 0.14
	n	18			11			10			19			13			10	
		eam10.m/Hvlux-									eam5.x/HvPHYC+							
		2 week			3 week			4 weeks			2 week			3 week			4 weeks	
0%	Min	0.31			0.42			0.51			0.20			0.03			0.63	
	Max	1.01			0.98			0.91			0.65			0.65			0.90	
	Mean (s.d.)	0.54	±	0.17	0.67	±	0.17	0.71	±	0.12	0.48	±	0.03	0.43	±	0.16	0.72	± 0.08
	n	14			13			10			19			13			10	
15%	Min	-0.24			-0.19			-0.20			-0.05			0.07			-0.15	
	Max	0.27			0.39			0.29			0.34			0.24			0.35	
	Mean (s.d.)	0.09	±	0.15	0.18	±	0.17	0.05	±	0.17	0.11	±	0.06	0.14	±	0.05	0.03	± 0.18
	n	19			13			9			19			12			10	
		eam8.k/Hvelf3-									eam8.w/Hvelf3-							
		2 week			3 week			4 weeks			2 week			3 week			4 weeks	
0%	Min	0.14			0.36			0.42			0.23			0.35			0.55	

	Max	1.03		1.12		1.16		0.88		0.91		0.95		
	Mean (s.d.)	0.53	± 0.25	0.68	± 0.22	0.92	± 0.19	0.56	± 0.17	0.70	± 0.18	0.80	± 0.13	
	n	19		13		10		19		12		10		
15%	Min	-0.02		-0.30		-0.47		-0.17		-0.09		-0.36		
	Max	0.29		0.45		0.40		0.29		0.36		0.32		
	Mean (s.d.)	0.14	± 0.08	0.13	± 0.20	0.08	± 0.23	0.13	± 0.09	0.21	± 0.11	0.10	± 0.19	
	n	20		13		10		19		13		10		

Shoot fresh weight (g) post treatment

		Antonella			Bowman		
PEG		2 week	3 week	4 weeks	2 week	3 week	4 weeks
0%	Min	0.34	0.33	0.46	0.42	0.59	1.03
	Max	0.78	0.75	0.78	1.13	1.05	1.50
	Mean (s.d.)	0.48 ± 0.12	0.53 ± 0.12	0.61 ± 0.11	0.70 ± 0.17	0.75 ± 0.14	1.26 ± 0.15
	n	20	13	10	19	12	10
15%	Min	0.11	0.26	0.11	0.30	0.26	0.59
	Max	0.48	0.52	0.66	0.70	0.62	0.99

	Mean (s.d.)	0.34	±	0.09	0.39	±	0.08	0.43	±	0.16	0.44	±	0.11	0.37	±	0.11	0.80	±	0.13
	n	18			11			10			19			13			10		
		eam10.m/Hvlux-									eam5.x/HvPHYC+								
		2 week			3 week			4 weeks			2 week			3 week			4 weeks		
0%	Min	0.39			0.57			0.81			0.31			0.41			0.80		
	Max	1.04			0.93			1.39			0.77			0.63			1.30		
	Mean (s.d.)	0.67	±	0.18	0.75	±	0.12	1.15	±	0.19	0.58	±	0.00	0.50	±	0.06	1.05	±	0.15
	n	14			13			10			19			13			10		
15%	Min	0.15			0.26			0.27			0.18			0.23			0.30		
	Max	0.62			0.76			0.97			0.54			0.54			0.86		
	Mean (s.d.)	0.40	±	0.13	0.51	±	0.15	0.63	±	0.23	0.38	±	0.35	0.38	±	0.08	0.63	±	0.15
	n	19			13			9			19			12			10		
		eam8.k/Hvelf3-									eam8.w/Hvelf3-								
		2 week			3 week			4 weeks			2 week			3 week			4 weeks		
0%	Min	0.39			0.43			1.26			0.35			0.51			1.01		
	Max	1.20			1.03			1.71			1.08			1.01			1.45		
	Mean (s.d.)	0.70	±	0.24	0.75	±	0.21	1.46	±	0.16	0.72	±	0.21	0.78	±	0.15	1.21	±	0.14

	n	19			13			10			19			12			10	
15%	Min	0.22			0.29			0.66			0.18			0.24			0.36	
	Max	0.68			0.58			1.05			0.75			0.68			0.93	
	Mean (s.d.)	0.44	±	0.13	0.43	±	0.09	0.90	±	0.14	0.44	±	0.14	0.47	±	0.12	0.79	± 0.18
	n	20			13			10			19			13			10	

Root fresh weight (g) post treatment

		Antonella									Bowman							
PEG		2 week			3 week			4 weeks			2 week			3 week			4 weeks	
0%	Min	0.15			0.16			0.32			0.26			0.38			0.77	
	Max	0.48			0.47			0.70			0.66			0.82			1.20	
	Mean (s.d.)	0.30	±	0.09	0.36	±	0.09	0.46	±	0.11	0.43	±	0.12	0.58	±	0.15	0.96	± 0.14
	n	20			13			10			19			12			10	
15%	Min	0.07			0.14			0.11			0.09			0.11			0.54	
	Max	0.31			0.38			0.45			0.42			0.47			0.87	
	Mean (s.d.)	0.20	±	0.06	0.24	±	0.07	0.30	±	0.11	0.24	±	0.11	0.31	±	0.10	0.71	± 0.11
	n	18			11			10			19			13			10	

		eam10.m/Hvlux-			eam5.x/HvPHYC+		
		2 week	3 week	4 weeks	2 week	3 week	4 weeks
0%	Min	0.18	0.49	0.49	0.13	0.26	0.66
	Max	0.77	0.93	1.08	0.48	0.66	0.91
	Mean (s.d.)	0.37 ± 0.14	0.71 ± 0.12	0.81 ± 0.18	0.33 ± 0.67	0.46 ± 0.11	0.80 ± 0.09
	n	14	13	10	19	13	10
15%	Min	0.09	0.29	0.27	0.03	0.23	0.38
	Max	0.39	0.77	0.93	0.31	0.48	0.72
	Mean (s.d.)	0.21 ± 0.08	0.53 ± 0.13	0.59 ± 0.20	0.18 ± 0.40	0.31 ± 0.07	0.54 ± 0.08
	n	19	13	9	19	12	10
		eam8.k/Hvelf3-			eam8.w/Hvelf3-		
		2 week	3 week	4 weeks	2 week	3 week	4 weeks
0%	Min	0.09	0.37	0.71	0.17	0.33	0.71
	Max	0.67	0.99	1.36	0.66	0.93	1.25
	Mean (s.d.)	0.37 ± 0.18	0.65 ± 0.21	1.09 ± 0.19	0.42 ± 0.13	0.65 ± 0.18	0.96 ± 0.13
	n	19	13	10	19	12	10

15%	Min	0.08			0.26			0.67			0.12			0.20			0.63		
	Max	0.40			0.62			0.96			0.42			0.60			0.96		
	Mean (s.d.)	0.23	±	0.10	0.41	±	0.11	0.82	±	0.08	0.24	±	0.09	0.38	±	0.11	0.77	±	0.11
	n	20			13			10			19			13			10		

Shoot dry biomass (g)

		Antonella									Bowman								
PEG		2 week			3 week			4 weeks			2 week			3 week			4 weeks		
0%	Min	0.04			0.04			0.07			0.04			0.07			0.11		
	Max	0.11			0.10			0.11			0.14			0.14			0.16		
	Mean (s.d.)	0.06	±	0.02	0.07	±	0.02	0.08	±	0.01	0.08	±	0.02	0.09	±	0.02	0.14	±	0.01
	n	20			13			10			19			12			10		
15%	Min	0.02			0.04			0.01			0.04			0.05			0.06		
	Max	0.08			0.08			0.11			0.09			0.09			0.14		
	Mean (s.d.)	0.06	±	0.01	0.06	±	0.01	0.07	±	0.03	0.06	±	0.01	0.07	±	0.01	0.10	±	0.02
	n	18			11			10			19			13			10		
		eam10.m/Hvlux-									eam5.x/HvPHYC+								

		2 week	3 week	4 weeks	2 week	3 week	4 weeks
0%	Min	0.04	0.08	0.05	0.03	0.05	0.06
	Max	0.12	0.13	0.13	0.10	0.09	0.14
	Mean (s.d.)	0.08 ± 0.02	0.10 ± 0.02	0.08 ± 0.02	0.07 ± 0.05	0.07 ± 0.01	0.10 ± 0.03
	n	14	13	10	19	13	10
15%	Min	0.03	0.05	0.06	0.03	0.05	0.04
	Max	0.09	0.13	0.15	0.08	0.09	0.11
	Mean (s.d.)	0.06 ± 0.02	0.09 ± 0.02	0.08 ± 0.03	0.05 ± 0.06	0.06 ± 0.01	0.08 ± 0.02
	n	19	13	9	19	12	10

eam8.k/Hvelf3-

eam8.w/Hvelf3-

		2 week	3 week	4 weeks	2 week	3 week	4 weeks
0%	Min	0.04	0.05	0.07	0.04	0.06	0.09
	Max	0.14	0.14	0.18	0.13	0.14	0.21
	Mean (s.d.)	0.08 ± 0.03	0.10 ± 0.03	0.12 ± 0.04	0.08 ± 0.03	0.10 ± 0.02	0.11 ± 0.04
	n	19	13	10	19	12	10
15%	Min	0.04	0.05	0.05	0.03	0.05	0.08

	Max	0.14			0.09			0.14			0.11			0.11			0.15	
	Mean (s.d.)	0.07	±	0.02	0.07	±	0.01	0.10	±	0.03	0.06	±	0.02	0.08	±	0.02	0.11	± 0.02
	n	20			13			10			19			13			10	

		Root dry biomass (g)															
		Antonella									Bowman						
PEG		2 week			3 week			4 weeks			2 week			3 week			4 weeks	
0%	Min	0.01			0.01			0.03			0.01			0.03			0.04	
	Max	0.07			0.04			0.05			0.05			0.05			0.06	
	Mean (s.d.)	0.03	±	0.01	0.03	±	0.01	0.03	±	0.01	0.03	±	0.01	0.04	±	0.01	0.05	± 0.01
	n	16			13			10			14			12			10	
15%	Min	0.01			0.02			0.01			0.01			0.01			0.03	
	Max	0.03			0.03			0.04			0.04			0.04			0.06	
	Mean (s.d.)	0.02	±	0.01	0.02	±	0.01	0.02	±	0.01	0.02	±	0.01	0.03	±	0.01	0.05	± 0.01
	n	13			11			10			16			13			10	
		eam10.m/Hvlux-									eam5.x/HvPHYC+							
		2 week			3 week			4 weeks			2 week			3 week			4 weeks	

0%	Min	0.02			0.04			0.02			0.01			0.02			0.03	
	Max	0.05			0.06			0.06			0.04			0.06			0.05	
	Mean (s.d.)	0.03	±	0.01	0.05	±	0.01	0.04	±	0.01	0.02	±	0.02	0.04	±	0.01	0.04	± 0.01
	n	14			13			10			19			13			10	
15%	Min	0.01			0.03			0.02			0.01			0.02			0.02	
	Max	0.04			0.05			0.06			0.03			0.04			0.04	
	Mean (s.d.)	0.02	±	0.01	0.04	±	0.01	0.04	±	0.01	0.02	±	0.01	0.03	±	0.01	0.03	± 0.00
	n	19			13			9			19			12			10	
		eam8.k/Hvelf3-									eam8.w/Hvelf3-							
		2 week			3 week			4 weeks			2 week			3 week			4 weeks	
0%	Min	0.01			0.03			0.03			0.01			0.03			0.04	
	Max	0.06			0.05			0.07			0.05			0.07			0.07	
	Mean (s.d.)	0.03	±	0.01	0.04	±	0.01	0.06	±	0.01	0.03	±	0.01	0.04	±	0.01	0.05	± 0.01
	n	19			13			10			15			12			10	
15%	Min	0.01			0.02			0.03			0.01			0.02			0.05	
	Max	0.04			0.05			0.05			0.03			0.04			0.07	

	Mean (s.d.)	0.02	±	0.01	0.03	±	0.01	0.04	±	0.01	0.02	±	0.01	0.03	±	0.01	0.05	±	0.01
	n	20			13			10			14			13			10		

Total dry biomass (g)																			
		Antonella									Bowman								
PEG		2 week			3 week			4 weeks			2 week			3 week			4 weeks		
0%	Min	0.06			0.05			0.09			0.06			0.10			0.16		
	Max	0.16			0.14			0.16			0.15			0.19			0.23		
	Mean (s.d.)	0.09	±	0.02	0.10	±	0.02	0.12	±	0.02	0.10	±	0.03	0.13	±	0.03	0.20	±	0.02
	n	20			13			10			19			12			10		
15%	Min	0.03			0.05			0.02			0.06			0.07			0.09		
	Max	0.11			0.12			0.15			0.13			0.13			0.20		
	Mean (s.d.)	0.07	±	0.02	0.09	±	0.02	0.09	±	0.03	0.08	±	0.02	0.09	±	0.02	0.15	±	0.03
	n	18			11			10			19			13			10		
		eam10.m/Hvlux-									eam5.x/HvPHYC+								
		2 week			3 week			4 weeks			2 week			3 week			4 weeks		
0%	Min	0.06			0.11			0.08			0.04			0.08			0.09		

	Max	0.17			0.18			0.18			0.13			0.14			0.18	
	Mean (s.d.)	0.11	±	0.03	0.15	±	0.02	0.13	±	0.03	0.09	±	0.07	0.11	±	0.02	0.14	± 0.03
	n	14			13			10			19			13			10	
15%	Min	0.05			0.08			0.08			0.04			0.07			0.07	
	Max	0.12			0.18			0.21			0.11			0.13			0.14	
	Mean (s.d.)	0.08	±	0.02	0.13	±	0.03	0.12	±	0.04	0.07	±	0.04	0.09	±	0.02	0.11	± 0.02
	n	19			13			9			19			12			10	
		eam8.k/Hvelf3-									eam8.w/Hvelf3-							
		2 week			3 week			4 weeks			2 week			3 week			4 weeks	
0%	Min	0.06			0.08			0.11			0.05			0.09			0.14	
	Max	0.18			0.19			0.25			0.18			0.21			0.27	
	Mean (s.d.)	0.11	±	0.04	0.13	±	0.03	0.18	±	0.04	0.10	±	0.03	0.14	±	0.03	0.17	± 0.04
	n	19			13			10			19			12			10	
15%	Min	0.05			0.07			0.10			0.04			0.07			0.13	
	Max	0.16			0.13			0.18			0.11			0.14			0.19	
	Mean (s.d.)	0.10	±	0.03	0.11	±	0.02	0.14	±	0.03	0.08	±	0.02	0.11	±	0.02	0.16	± 0.02

	n	20	13	10	19	13	10

Root:Shoot Dry Biomass ratio

		Antonella			Bowman		
PEG		2 week	3 week	4 weeks	2 week	3 week	4 weeks
0%	Min	0.25	0.28	0.33	0.26	0.34	0.33
	Max	1.70	0.51	0.49	0.54	0.46	0.43
	Mean (s.d.)	0.49 ± 0.33	0.41 ± 0.08	0.39 ± 0.04	0.39 ± 0.08	0.40 ± 0.04	0.38 ± 0.03
	n	16	13	10	14	12	10
15%	Min	0.30	0.33	0.04	0.25	0.14	0.37
	Max	0.46	0.46	0.41	0.54	0.65	0.57
	Mean (s.d.)	0.39 ± 0.05	0.39 ± 0.04	0.32 ± 0.10	0.36 ± 0.08	0.41 ± 0.12	0.44 ± 0.06
	n	13	11	10	16	13	10

		eam10.m/Hvlux-			eam5.x/HvPHYC+		
		2 week	3 week	4 weeks	2 week	3 week	4 weeks
0%	Min	0.28	0.39	0.21	0.24	0.35	0.32
	Max	0.44	0.60	0.78	0.46	0.79	0.70

	Mean (s.d.)	0.35	±	0.04	0.49	±	0.06	0.54	±	0.18	0.33	±	0.43	0.55	±	0.14	0.46	±	0.13
	n	14			13			10			19			13			10		
15%	Min	0.31			0.35			0.33			0.21			0.33			0.33		
	Max	0.48			0.58			0.70			0.41			0.52			0.85		
	Mean (s.d.)	0.39	±	0.05	0.49	±	0.06	0.49	±	0.11	0.31	±	0.32	0.43	±	0.07	0.43	±	0.15
	n	19			13			9			19			12			10		
		eam8.k/Hvelf3-									eam8.w/Hvelf3-								
		2 week			3 week			4 weeks			2 week			3 week			4 weeks		
0%	Min	0.26			0.22			0.22			0.27			0.34			0.28		
	Max	0.48			0.61			0.89			0.46			0.50			0.65		
	Mean (s.d.)	0.35	±	0.06	0.39	±	0.11	0.51	±	0.18	0.35	±	0.06	0.43	±	0.05	0.49	±	0.12
	n	19			13			10			15			12			10		
15%	Min	0.11			0.35			0.29			0.29			0.36			0.32		
	Max	0.46			0.67			0.84			0.45			0.49			0.76		
	Mean (s.d.)	0.31	±	0.09	0.48	±	0.09	0.45	±	0.16	0.36	±	0.05	0.42	±	0.05	0.50	±	0.13
	n	20			13			10			14			13			10		

Shoot water content (g)

		Antonella			Bowman		
PEG		2 week	3 week	4 weeks	2 week	3 week	4 weeks
0%	Min	0.28	0.29	0.40	0.38	0.51	0.91
	Max	0.66	0.65	0.67	0.99	0.91	1.34
	Mean (s.d.)	0.42 ± 0.10	0.46 ± 0.11	0.53 ± 0.10	0.62 ± 0.15	0.66 ± 0.12	1.11 ± 0.14
	n	20	13	10	19	12	10
15%	Min	0.09	0.22	0.10	0.25	0.19	0.48
	Max	0.40	0.45	0.58	0.61	0.52	0.86
	Mean (s.d.)	0.28 ± 0.07	0.33 ± 0.07	0.36 ± 0.15	0.37 ± 0.10	0.31 ± 0.10	0.70 ± 0.12
	n	18	11	10	19	13	10
		eam10.m/Hvlux-			eam5.x/HvPHYC+		
		2 week	3 week	4 weeks	2 week	3 week	4 weeks
0%	Min	0.35	0.50	0.71	0.28	0.35	0.74
	Max	0.92	0.80	1.30	0.68	0.55	1.19
	Mean (s.d.)	0.59 ± 0.15	0.65 ± 0.10	1.07 ± 0.18	0.51 ± 0.45	0.43 ± 0.05	0.95 ± 0.14
	n	14	13	10	19	13	10

15%	Min	0.12			0.21			0.22			0.15			0.18			0.25		
	Max	0.53			0.64			0.82			0.46			0.45			0.76		
	Mean (s.d.)	0.34	±	0.12	0.42	±	0.13	0.55	±	0.21	0.32	±	0.34	0.31	±	0.08	0.55	±	0.13
	n	19			13			9			19			12			10		
		eam8.k/Hvelf3-									eam8.w/Hvelf3-								
		2 week			3 week			4 weeks			2 week			3 week			4 weeks		
0%	Min	0.34			0.38			1.16			0.30			0.45			0.91		
	Max	1.08			0.91			1.58			0.96			0.89			1.30		
	Mean (s.d.)	0.62	±	0.21	0.65	±	0.18	1.34	±	0.15	0.64	±	0.18	0.68	±	0.14	1.09	±	0.13
	n	19			13			10			19			12			10		
15%	Min	0.12			0.21			0.57			0.13			0.18			0.27		
	Max	0.59			0.49			1.00			0.65			0.57			0.81		
	Mean (s.d.)	0.36	±	0.12	0.36	±	0.08	0.80	±	0.13	0.38	±	0.12	0.39	±	0.10	0.68	±	0.16
	n	20			13			10			19			13			10		

Root water content (g)																			
		Antonella									Bowman								

PEG		2 week	3 week	4 weeks	2 week	3 week	4 weeks
0%	Min	0.08	0.14	0.29	0.24	0.35	0.72
	Max	0.47	0.43	0.65	0.61	0.77	1.13
	Mean (s.d.)	0.28 ± 0.10	0.33 ± 0.09	0.43 ± 0.10	0.41 ± 0.12	0.54 ± 0.14	0.91 ± 0.13
	n	20	13	10	19	12	10
15%	Min	0.06	0.12	0.10	0.07	0.10	0.49
	Max	0.31	0.35	0.42	0.41	0.44	0.81
	Mean (s.d.)	0.19 ± 0.07	0.22 ± 0.07	0.27 ± 0.11	0.23 ± 0.10	0.29 ± 0.09	0.67 ± 0.10
	n	18	11	10	19	13	10
		eam10.m/Hvlux-			eam5.x/HvPHYC+		
		2 week	3 week	4 weeks	2 week	3 week	4 weeks
0%	Min	0.17	0.45	0.46	0.12	0.22	0.62
	Max	0.73	0.88	1.02	0.44	0.60	0.86
	Mean (s.d.)	0.34 ± 0.14	0.66 ± 0.11	0.77 ± 0.17	0.31 ± 0.17	0.42 ± 0.11	0.76 ± 0.08
	n	14	13	10	19	13	10
15%	Min	0.07	0.26	0.25	0.02	0.21	0.36

	Max	0.35			0.71			0.88			0.28			0.44			0.68	
	Mean (s.d.)	0.19	±	0.08	0.48	±	0.13	0.55	±	0.19	0.16	±	0.43	0.28	±	0.06	0.51	± 0.08
	n	19			13			9			19			12			10	
		eam8.k/Hvelf3-									eam8.w/Hvelf3-							
		2 week			3 week			4 weeks			2 week			3 week			4 weeks	
0%	Min	0.07			0.33			0.65			0.15			0.29			0.67	
	Max	0.63			0.94			1.29			0.60			0.87			1.21	
	Mean (s.d.)	0.34	±	0.17	0.61	±	0.21	1.04	±	0.19	0.40	±	0.13	0.61	±	0.17	0.91	± 0.13
	n	19			13			10			19			12			10	
15%	Min	0.07			0.24			0.64			0.11			0.18			0.56	
	Max	0.38			0.58			0.92			0.42			0.55			0.91	
	Mean (s.d.)	0.21	±	0.10	0.38	±	0.10	0.78	±	0.07	0.23	±	0.09	0.35	±	0.10	0.72	± 0.11
	n	20			13			10			19			13			10	

Total water content (g)						
	Antonella			Bowman		
PEG	2 week	3 week	4 weeks	2 week	3 week	4 weeks

0%	Min	0.42			0.43			0.70			0.61			0.87			1.64		
	Max	1.09			1.08			1.31			1.58			1.66			2.44		
	Mean (s.d.)	0.69	±	0.19	0.79	±	0.18	0.95	±	0.20	1.04	±	0.25	1.20	±	0.26	2.02	±	0.26
	n	20			13			10			19			12			10		
15%	Min	0.15			0.35			0.20			0.33			0.37			1.05		
	Max	0.69			0.77			0.95			0.99			0.96			1.62		
	Mean (s.d.)	0.47	±	0.13	0.54	±	0.14	0.64	±	0.24	0.60	±	0.19	0.60	±	0.18	1.37	±	0.20
	n	18			11			10			19			13			10		
		eam10.m/Hvlux-									eam5.x/HvPHYC+								
		2 week			3 week			4 weeks			2 week			3 week			4 weeks		
0%	Min	0.54			0.95			1.17			0.41			0.59			1.43		
	Max	1.64			1.64			2.33			1.13			1.16			2.06		
	Mean (s.d.)	0.93	±	0.28	1.31	±	0.21	1.84	±	0.34	0.82	±	0.43	0.85	±	0.14	1.71	±	0.21
	n	14			13			10			19			13			10		
15%	Min	0.24			0.47			0.56			0.20			0.39			0.61		
	Max	0.86			1.35			1.67			0.72			0.89			1.44		

	Mean (s.d.)	0.53	±	0.17	0.91	±	0.25	1.10	±	0.39	0.48	±	0.66	0.59	±	0.13	1.06	±	0.20
	n	19			13			9			19			12			10		
		eam8.k/Hvelf3-									eam8.w/Hvelf3-								
		2 week			3 week			4 weeks			2 week			3 week			4 weeks		
0%	Min	0.41			0.71			2.03			0.45			0.76			1.58		
	Max	1.71			1.83			2.84			1.56			1.77			2.52		
	Mean (s.d.)	0.95	±	0.37	1.26	±	0.38	2.37	±	0.29	1.03	±	0.30	1.29	±	0.30	2.00	±	0.23
	n	19			13			10			19			12			10		
15%	Min	0.21			0.51			1.33			0.31			0.38			0.93		
	Max	0.96			1.07			1.80			1.07			1.01			1.68		
	Mean (s.d.)	0.57	±	0.22	0.74	±	0.14	1.57	±	0.16	0.60	±	0.21	0.74	±	0.20	1.39	±	0.23
	n	20			13			10			19			13			10		

Root:Shoot water content ratio													
		Antonella						Bowman					
PEG		2 week			3 week			4 weeks		2 week		3 week		4 weeks	
0%	Min	0.22			0.49			0.71		0.50		0.66		0.74	

	Max	0.99			0.92			0.97			0.87			1.00			0.90	
	Mean (s.d.)	0.66	±	0.17	0.72	±	0.13	0.80	±	0.07	0.66	±	0.10	0.81	±	0.12	0.81	± 0.05
	n	20			13			10			19			12			10	
15%	Min	0.44			0.47			0.07			0.30			0.38			0.78	
	Max	1.05			0.83			0.90			1.02			1.50			1.18	
	Mean (s.d.)	0.68	±	0.17	0.66	±	0.10	0.69	±	0.24	0.58	±	0.19	0.96	±	0.25	0.96	± 0.13
	n	18			11			10			19			13			10	
		eam10.m/Hvlux-									eam5.x/HvPHYC+							
		2 week			3 week			4 weeks			2 week			3 week			4 weeks	
0%	Min	0.39			0.85			0.58			0.40			0.50			0.70	
	Max	0.79			1.21			0.86			0.77			1.33			0.92	
	Mean (s.d.)	0.56	±	0.10	1.03	±	0.11	0.72	±	0.08	0.60	±	0.67	0.98	±	0.22	0.80	± 0.07
	n	14			13			10			19			13			10	
15%	Min	0.26			0.93			0.65			0.05			0.60			0.77	
	Max	1.24			1.87			1.60			0.78			1.16			1.45	
	Mean (s.d.)	0.61	±	0.27	1.19	±	0.27	1.05	±	0.25	0.50	±	0.77	0.93	±	0.15	0.96	± 0.19

	n	19			13			9			19			12			10	
		eam8.k/Hvelf3-									eam8.w/Hvelf3-						
		2 week			3 week			4 weeks			2 week			3 week			4 weeks	
0%	Min	0.21			0.78			0.45			0.35			0.63			0.67	
	Max	0.80			1.11			0.91			0.91			1.05			0.93	
	Mean (s.d.)	0.52	±	0.16	0.93	±	0.12	0.78	±	0.13	0.62	±	0.11	0.88	±	0.11	0.83	± 0.08
	n	19			13			10			19			12			10	
15%	Min	0.26			0.69			0.72			0.41			0.67			0.74	
	Max	0.75			2.65			1.44			1.30			1.24			2.49	
	Mean (s.d.)	0.57	±	0.16	1.12	±	0.49	1.00	±	0.19	0.60	±	0.19	0.89	±	0.16	1.16	± 0.47
	n	20			13			10			19			13			10	

Water uptake (ml)

		Antonella									Bowman						
PEG		2 week			3 week			4 weeks			2 week			3 week			4 weeks	
0%	Min	5.67			8.00			10.70			9.67			11.00			26.70	
	Max	19.67			19.00			20.70			28.67			27.00			34.70	

	Mean (s.d.)	11.03	±	4.02	14.31	±	3.36	15.90	±	2.96	17.16	±	5.95	19.75	±	5.13	30.60	±	2.66
	n	20			13			10			19			12			10		
15%	Min	2.67			3.00			2.70			3.00			0.00			1.70		
	Max	8.67			10.00			10.70			10.67			10.00			11.70		
	Mean (s.d.)	5.43	±	1.71	6.00	±	1.91	6.63	±	2.77	6.05	±	2.06	3.31	±	2.78	8.10	±	3.20
	n	18			11			10			19			13			10		
		eam10.m/Hvlux-									eam5.x/HvPHYC+								
		2 week			3 week			4 weeks			2 week			3 week			4 weeks		
0%	Min	5.33			13.00			21.70			6.67			9.00			23.70		
	Max	26.67			25.00			39.30			20.67			16.00			34.70		
	Mean (s.d.)	16.26	±	6.03	18.62	±	4.09	29.76	±	5.38	13.60	±	3.00	12.77	±	2.19	26.80	±	3.39
	n	14			13			10			19			13			10		
15%	Min	0.00			0.00			0.70			0.67			1.00			0.70		
	Max	8.67			12.00			11.70			7.67			6.00			11.70		
	Mean (s.d.)	4.56	±	2.49	5.62	±	3.15	6.92	±	4.05	3.68	±	11.00	3.83	±	1.62	5.90	±	3.60
	n	19			13			9			19			12			10		

		eam8.k/Hvelf3-			eam8.w/Hvelf3-		
		2 week	3 week	4 weeks	2 week	3 week	4 weeks
0%	Min	7.67	10.00	29.70	8.67	10.00	23.70
	Max	31.67	33.00	42.70	31.67	26.00	39.70
	Mean (s.d.)	17.91 ± 7.39	20.31 ± 6.89	36.80 ± 4.55	18.26 ± 6.77	19.75 ± 5.00	30.40 ± 4.45
	n	19	13	10	19	12	10
15%	Min	2.67	1.00	1.70	1.00	1.00	0.70
	Max	10.67	9.00	13.70	12.67	23.00	12.70
	Mean (s.d.)	6.11 ± 2.11	4.69 ± 2.73	8.50 ± 3.52	7.28 ± 3.04	6.62 ± 5.57	8.30 ± 3.98
	n	19	13	10	19	13	10

Percentage of water as total biomass (%)

		Antonella			Bowman		
PEG		2 week	3 week	4 weeks	2 week	3 week	4 weeks
0%	Min	0.79	0.87	0.88	0.88	0.87	0.90
	Max	0.93	0.91	0.90	0.93	0.92	0.92
	Mean (s.d.)	0.89 ± 0.03	0.89 ± 0.01	0.89 ± 0.00	0.91 ± 0.01	0.90 ± 0.01	0.91 ± 0.01

	n	20			13			10			19			12			10	
15%	Min	0.83			0.84			0.00			0.83			0.81			0.87	
	Max	0.92			0.88			0.93			0.91			0.90			0.94	
	Mean (s.d.)	0.87	±	0.02	0.86	±	0.01	0.78	±	0.26	0.88	±	0.02	0.86	±	0.02	0.90	± 0.02
	n	18			11			10			19			13			10	
		eam10.m/Hvlux-									eam5.x/HvPHYC+							
PEG		2 week			3 week			4 weeks			2 week			3 week			4 weeks	
0%	Min	0.88			0.87			0.91			0.87			0.82			0.91	
	Max	0.91			0.91			0.95			0.91			0.91			0.94	
	Mean (s.d.)	0.90	±	0.01	0.90	±	0.01	0.93	±	0.01	0.90	±	11.00	0.89	±	0.02	0.93	± 0.01
	n	14			13			10			19			13			10	
15%	Min	0.84			0.84			0.87			0.82			0.83			0.88	
	Max	0.88			0.90			0.94			0.90			0.88			0.94	
	Mean (s.d.)	0.86	±	0.01	0.87	±	0.02	0.90	±	0.02	0.87	±	0.83	0.87	±	0.01	0.91	± 0.01
	n	19			13			9			19			12			10	
		eam8.k/Hvelf3-									eam8.w/Hvelf3-							

		2 week	3 week	4 weeks	2 week	3 week	4 weeks
0%	Min	0.86	0.86	0.90	0.88	0.85	0.89
	Max	0.92	0.92	0.95	0.94	0.92	0.94
	Mean (s.d.)	0.90 ± 0.02	0.90 ± 0.01	0.93 ± 0.02	0.91 ± 0.02	0.90 ± 0.02	0.92 ± 0.01
	n	19	13	10	19	12	10
15%	Min	0.66	0.86	0.90	0.84	0.81	0.87
	Max	0.90	0.89	0.94	0.91	0.89	0.91
	Mean (s.d.)	0.85 ± 0.06	0.87 ± 0.01	0.92 ± 0.01	0.88 ± 0.02	0.86 ± 0.02	0.89 ± 0.01
	n	20	13	10	19	13	10

Water uptake per total dry biomass (ml/g)

		Antonella			Bowman		
PEG		2 week	3 week	4 weeks	2 week	3 week	4 weeks
0%	Min	50.03	115.38	117.07	102.51	85.47	139.80
	Max	211.23	188.48	156.05	282.85	179.10	173.78
	Mean (s.d.)	129.83 ± 42.97	144.86 ± 23.64	135.52 ± 10.07	168.54 ± 49.64	151.99 ± 24.25	155.70 ± 11.16
	n	20	13	10	19	12	10

15%	Min	51.49	47.39	10.07	41.15	0.00	13.79
	Max	133.10	86.36	98.62	131.28	83.96	97.74
	Mean (s.d.)	79.16 ± 24.41	68.54 ± 11.90	65.22 ± 29.14	77.16 ± 24.25	34.53 ± 24.01	54.97 ± 22.99
	n	18	11	10	19	13	10
		eam10.m/Hvlux-			eam5.x/HvPHYC+		
		2 week	3 week	4 weeks	2 week	3 week	4 weeks
0%	Min	79.13	97.50	177.29	103.72	81.30	157.06
	Max	186.02	155.67	302.67	186.75	161.66	254.84
	Mean (s.d.)	147.33 ± 28.39	122.67 ± 17.10	241.68 ± 43.06	144.06 ± 141.51	119.90 ± 20.18	197.97 ± 30.11
	n	14	13	10	19	13	10
15%	Min	0.00	0.00	8.12	10.12	14.68	9.60
	Max	72.70	75.14	87.35	89.66	71.09	92.78
	Mean (s.d.)	51.99 ± 22.87	40.86 ± 18.77	55.82 ± 28.15	50.91 ± 24.01	42.19 ± 18.41	50.84 ± 25.84
	n	19	13	9	19	12	10
		eam8.k/Hvelf3-			eam8.w/Hvelf3-		
		2 week	3 week	4 weeks	2 week	3 week	4 weeks
0%	Min	126.51	86.66	150.38	138.05	76.22	125.47

	Max	239.32			208.20			292.25			264.18			175.91			212.87	
	Mean (s.d.)	164.39	±	24.90	150.71	±	27.09	218.92	±	46.77	180.98	±	37.07	137.61	±	26.69	185.28	±	24.87
	n	19			13			10			19			12			10		
15%	Min	25.18			7.94			11.23			18.87			11.59			4.96		
	Max	105.33			75.31			107.00			142.58			227.05			74.57		
	Mean (s.d.)	65.44	±	20.96	43.06	±	22.24	61.53	±	26.19	91.75	±	28.26	60.23	±	53.95	49.41	±	22.26
	n	19			13			10			19			13			10		

Water uptake per total water content stored (ml/g)

		Antonella									Bowman								
PEG		2 week			3 week			4 weeks			2 week			3 week			4 weeks		
0%	Min	12.24			15.24			14.93			11.12			11.13			13.25		
	Max	21.71			22.60			21.07			22.73			20.67			17.32		
	Mean (s.d.)	15.62	±	2.26	18.29	±	2.43	16.78	±	1.66	16.31	±	2.99	16.46	±	2.53	15.27	±	1.38
	n	20			13			10			19			12			10		
15%	Min	5.93			7.45			1.66			5.53			0.00			1.51		
	Max	24.12			13.20			13.68			17.66			10.91			9.66		

	Mean (s.d.)	11.84	±	3.50	11.02	±	1.99	9.66	±	3.83	10.57	±	3.54	5.00	±	3.06	6.00	±	2.55
	n	18			11			10			19			13			10		
		eam10.m/Hvlux-									eam5.x/HvPHYC+								
		2 week			3 week			4 weeks			2 week			3 week			4 weeks		
0%	Min	9.92			11.65			15.02			12.33			11.19			13.85		
	Max	22.69			16.22			18.57			20.86			24.79			17.24		
	Mean (s.d.)	17.07	±	3.51	14.14	±	1.18	16.28	±	1.07	16.29	±	3.57	15.46	±	4.06	15.68	±	1.10
	n	14			13			10			19			13			10		
15%	Min	0.00			0.00			1.25			1.67			2.56			1.15		
	Max	13.27			11.70			9.11			18.54			9.70			9.61		
	Mean (s.d.)	8.09	±	3.63	5.98	±	2.99	5.88	±	2.83	7.76	±	15.70	6.34	±	2.31	5.24	±	2.78
	n	19			13			9			19			12			10		
		eam8.k/Hvelf3-									eam8.w/Hvelf3-								
		2 week			3 week			4 weeks			2 week			3 week			4 weeks		
0%	Min	13.78			13.91			14.28			14.34			11.83			14.03		
	Max	25.19			18.46			18.79			21.32			18.73			17.12		
	Mean (s.d.)	18.93	±	3.27	15.97	±	1.60	15.55	±	1.37	17.41	±	2.19	15.38	±	1.94	15.17	±	0.97

	n	19		13		10		19		12		10		
15%	Min	5.76		1.31		1.22		3.24		2.65		0.75		
	Max	17.16		10.13		8.38		17.18		32.41		8.49		
	Mean (s.d.)	11.02	± 2.53	6.21	± 3.11	5.29	± 2.02	11.83	± 2.92	8.82	± 7.47	5.67	± 2.44	
	n	19		13		10		19		13		10		

*Tables 3.3.4b*Results of the ANOVA, F-test statistical analysis comparison of means from the generalised linear models on the same data set as data from table 3.3.4a, P<0.01 (*), P<0.001(**), p<0.0001(***), with differences between bareley lines(Antonella (Ant), Bowman(Bow), Bowman eam10.m Hvlux- (eam10.m),Bowman eam5.x PHYC+ (eam5.x), Bowman eam8.k Hvelf- (eam8.k),Bowman eam8.w Hvelf- (eam8.w)) annotated with different letters based off of post hoc plots of confidence intervals calculates in the estimated means R package (emmeans). The 'model' contains the family and predictor parameters that produced the 'glm' models which best fit the observations.

	Anova						Post hoc							
	Model	Within d.f.	Between d.f.	F	P	sig.	Weeks	PEG	Ant	Bow	eam10.m	eam5.x	eam8.k	eam8.w
Total fresh weight (g) pre treatment	family: Gamma						2	0%	a	ab	ab	ab	ab	b
Barley Lines	➢	5	487	44.471	<0.0001	***	3	0%	ab	c	c	ab	c	bc
Age	➢	2	485	745.121	<0.0001	***	4	0%	c	d	e	e	d	d
Plant*Age	➢	10	475	17.879	<0.0001	***								
Total fresh weight (g) post treatment	family: Gamma													
Barley Lines	➢	5	487	39.7593	<0.0001	***	2	0%	b	c	bc	bc	c	c
PEG	➢	1	486	356.2769	<0.0001	***		15%	a	ab	ab	a	ab	ab

Age	➢	2	484	276.1796	<0.0001	***	3	0%	bc	c	c	bc	c	c
Barley lines*PEG								15%	ab	ab	bc	ab	bc	bc
Barley lines*Age	➢	10	474	3.0135	0.00105	**	4	0%	bc	d	d	d	d	d
PEG*Age	➢	2	472	24.9399	<0.0001	***		15%	abc	cd	c	c	d	cd
Barley lines*PEG*Age														
Shoot fresh weight (g) post treatment	family: Gamma						2	0%	b	c	c	bc	c	c
Barley Lines	➢	5	487	35.3452	<0.0001	***		15%	a	ab	ab	ab	ab	ab
PEG	➢	1	486	417.1861	<0.0001	***	3	0%	bc	c	c	bc	c	c
Age	➢	2	484	204.846	<0.0001	***		15%	ab	ab	bc	ab	ab	ab
Barley lines*PEG							4	0%	bc	d	d	d	d	d
Barley lines*Age	➢	10	474	2.5576	0.005128	**		15%	abc	cd	bc	bc	cd	cd

PEG*Age	➢	2	472	12.0542	<0.0001	***								
Barley lines*PEG*Age														
Root fresh weight (g) post treatment	family: Gaussian						2	0%	ab	bc	b	b	b	bc
Barley Lines		5	487	38.7318	<0.0001	***		15 %	a	ab	ab	a	ab	ab
PEG	➢	1	486	280.9109	<0.0001	***	3	0%	bc	c	cd	bc	cd	cd
Age	➢	2	484	477.6126	<0.0001	***		15 %	ab	ab	c	ab	bc	bc
Barley lines*PEG							4	0%	bc	de	d	d	e	de
Barley lines*Age	➢	10	474	16.3769	<0.0001	***		15 %	ab	cd	cd	c	d	d
PEG*Age	➢	2	472	3.7436	0.02437	*								
Barley lines*PEG*Age														

Change in fresh weight (g)	family: Gaussian						2	0%	bc	c	c	c	c	c
Barley Lines		5	487	10.8674	<0.0001	***		15%	ab	ab	ab	ab	ab	b
PEG		1	486	1096.380	<0.0001	***	3	0%	bc	cd	d	c	d	d
Age		2	484	8.4287	0.000254 3	***		15%	ab	a	ab	ab	ab	b
Barley lines*PEG		5	479	11.7385	<0.0001	***	4	0%	bc	d	d	d	d	d
Barley lines*Age		10	469	2.5093	0.006068 8	**		15%	ab	ab	ab	ab	ab	ab
PEG*Age		2	467	26.5504	<0.0001	***								
Barley lines*PEG*Age	➢	10	457	2.1631	0.018980 8	*								
Total dry biomass (g)	family: Gamma						2	0%	bc	bc	bc	bc	bc	bc
Barley Lines	➢	5	487	18.7727	<0.0001	***		15%	c	c	c	c	bc	c
PEG	➢	1	486	83.8038	<0.0001	***	3	0%	bc	ab	ab	bc	b	ab

Age	➢	2	484	136.7731	<0.0001	***		15%	bc	bc	b	bc	bc	bc
Barley lines*PEG							4	0%	bc	a	b	ab	ab	ab
Barley lines*Age	➢	10	474	4.0336	<0.0001	***		15%	bc	ab	bc	bc	ab	ab
PEG*Age	➢	2	472	3.883	0.02125	*								
Barley lines*PEG*Age														
Shoot dry biomass (g)	family: Gamma						2	0%	bc	b	b	bc	b	b
Barley Lines	➢	5	487	15.6027	<0.0001	***		15%	bc	bc	bc	c	bc	bc
PEG	➢	1	486	65.025	<0.0001	***	3	0%	bc	bc	ab	bc	b	ab
Age	➢	2	484	73.1628	<0.0001	***		15%	bc	bc	b	bc	bc	b
Barley lines*PEG							4	0%	b	a	b	ab	ab	ab
Barley lines*Age	➢	10	474	3.0412	0.0009507	***		15%	bc	ab	bc	bc	ab	ab
PEG*Age														

Barley lines*PEG*Age														
Root dry biomass (g)	family: Gamma						2	0%	b	b	ab	b	ab	ab
Barley Lines	➢	5	461	14.157	<0.0001	***		15 %	ab	ab	ab	a	ab	ab
PEG	➢	1	460	71.4731	<0.0001	***	3	0%	b	bc	c	bc	b	bc
Age	➢	2	458	155.035	<0.0001	***		15 %	ab	ab	bc	ab	b	b
Barley lines*PEG							4	0%	b	c	bc	bc	c	c
Barley lines*Age	➢	10	448	5.2365	<0.0001	***		15 %	ab	bc	bc	b	bc	c
PEG*Age	➢	2	446	7.2461	<0.0001	***								
Barley lines*PEG*Age														
Total water content (g)	family: Gaussian						2	0%	a	ab	ab	ab	ab	ab
Barley Lines	➢	5	487	42.0836	<0.0001	***		15 %	a	a	a	a	a	a

PEG	➢	1	486	446.8432	<0.0001	***	3	0%	ab	bc	b	ab	b	b
Age	➢	2	484	417.9659	<0.0001	***		15%	a	a	ab	a	a	a
Barley lines*PEG	➢	5	479	4.7216	0.0003224	***	4	0%	ab	cd	c	c	d	cd
Barley lines*Age	➢	10	469	13.6991	<0.0001	***		15%	a	bc	ab	ab	bc	bc
PEG*Age	➢	2	467	10.8603	<0.0001	***								
Barley lines*PEG*Age														
Shoot water content (g)	family: Gaussian						2	0%	a	b	ab	ab	b	b
Barley Lines	➢	5	487	36.3369	<0.0001	***		15%	a	a	a	a	a	a
PEG	➢	1	486	511.3772	<0.0001	***	3	0%	ab	b	b	ab	b	b
Age	➢	2	484	312.6111	<0.0001	***		15%	a	a	a	a	a	a

Barley lines*PEG	➢	5	479	6.8093	<0.0001	***	4	0%	ab	c	c	c	d	c
Barley lines*Age	➢	10	469	10.4614	<0.0001	***		15 %	a	b	ab	ab	bc	b
PEG*Age	➢	2	467	19.7891	<0.0001	***								
Barley lines*PEG*Age														
Root water content (g)	family: Gaussian						2	0%	ab	b	ab	ab	ab	b
Barley Lines	➢	5	487	38.2947	<0.0001	***		15 %	a	a	a	a	a	a
PEG	➢	1	486	82.0878	<0.0001	***	3	0%	ab	bc	c	b	bc	bc
Age	➢	2	484	467.5574	<0.0001	***		15 %	a	ab	bc	ab	ab	ab
Barley lines*PEG	➢	5	479	2.2414	0.04924	*	4	0%	b	d	cd	cd	d	d
Barley lines*Age	➢	10	469	16.2654	<0.0001	***		15 %	ab	c	bc	bc	cd	cd
PEG*Age	➢	2	467	3.8324	0.02234	*								
Barley lines*PEG*Age														

Root:Shoot Dry Biomass ratio	family: Gamma						2	0%	ab	ab	b	b	b	b
Barley Lines	➢	5	461	2.512	0.02937	*		15 %	ab	b	ab	b	b	b
PEG	➢	1	460	4.741	0.02997	*	3	0%	ab	ab	ab	a	ab	ab
Age	➢	2	458	36.9655	<0.0001	***		15 %	ab	ab	ab	ab	ab	ab
Barley lines*PEG							4	0%	ab	ab	a	ab	ab	ab
Barley lines*Age	➢	10	448	6.1786	<0.0001	***		15 %	ab	ab	ab	ab	ab	ab
PEG*Age														
Barley lines*PEG*Age														
Root:Shoot water content ratio	family: Gamma						2	0%	bc	bc	bc	bc	bc	bc
Barley Lines	➢	5	487	3.8899	0.001828	**		15 %	bc	bc	bc	bc	bc	bc
PEG	➢	1	486	9.8976	0.001761	**	3	0%	b	ab	ab	ab	ab	ab

Age	➢	2	484	164.5666	<0.0001	***		15%	bc	ab	b	ab	a	ab
Barley lines*PEG	➢	5	479	2.3797	0.037827	*	4	0%	ab	ab	b	ab	ab	ab
Barley lines*Age	➢	10	469	6.0216	<0.0001	***		15%	ab	ab	ab	ab	ab	a
PEG*Age	➢	2	467	6.6526	0.001416	**								
Barley lines*PEG*Age														
Water uptake (ml)	family: Gaussian						2	0%	b	c	bc	bc	c	c
Barley Lines	➢	5	486	17.8003	<0.0001	***		15%	ab	ab	a	a	ab	ab
PEG	➢	1	485	1182.4225	<0.0001	***	3	0%	bc	c	c	bc	c	c
Age	➢	2	483	118.8007	<0.0001	***		15%	ab	a	ab	a	ab	ab
Barley lines*PEG	➢	5	478	12.5968	<0.0001	***	4	0%	bc	de	de	d	e	de
Barley lines*Age	➢	10	468	2.9171	0.001481	**		15%	ab	ab	ab	ab	ab	ab

PEG*Age	➢	2	466	59.5422	<0.0001	***								
Barley lines*PEG*Age														
Water uptake per total dry biomass (ml/g)	family: Gaussian						2	0%	b	c	bc	bc	bc	ce
Barley Lines		5	486	5.2583	0.0001046	***		15%	ab	ab	a	a	a	ab
PEG		1	485	1341.1813	<0.0001	***	3	0%	bc	bc	b	b	bc	bc
Age		2	483	37.7978	<0.0001	***		15%	ab	a	a	a	a	ab
Barley lines*PEG		5	478	8.7411	<0.0001	***	4	0%	bc	bc	d	cd	d	cd
Barley lines*Age		10	468	7.2302	<0.0001	***		15%	a	a	a	a	ab	a
PEG*Age		2	466	24.4254	<0.0001	***								
Barley lines*PEG*Age	➢	10	456	3.6144	0.0001207	***								

Percentage of water as total biomass (%)	family: Gaussian						2	0%	b	bc	bc	bc	bc	bc
Barley Lines	➢	5	487	6.7324	<0.0001	***		15%	ab	b	ab	ab	a	b
PEG	➢	1	486	213.816	<0.0001	***	3	0%	bc	bc	bc	b	bc	bc
Age	➢	2	484	62.5698	<0.0001	***		15%	ab	ab	ab	ab	ab	ab
Barley lines*PEG							4	0%	bc	bc	c	c	c	c
Barley lines*Age	➢	10	474	6.5229	<0.0001	***		15%	ab	bc	bc	bc	c	bc
PEG*Age														
Barley lines*PEG*Age														
Water uptake per total water content stored (ml/g)	family: Gaussian						2	0%	c	c	c	c	c	c
Barley Lines		5	486	10.6757	<0.0001	***		15%	b	ab	ab	ab	b	b

PEG		1	485	822.8946	<0.0001	***	3	0%	c	c	bc	c	c	c
Age		2	483	31.263	<0.0001	***		15%	ab	a	a	a	a	ab
Barley lines*PEG		5	478	5.0761	0.0001536	***	4	0%	c	bc	c	bc	bc	bc
Barley lines*Age		10	468	3.5224	0.0001691	***		15%	ab	a	a	a	a	a
PEG*Age		2	466	8.6291	0.0002097	***								
Barley lines*PEG*Age		10	456	1.2146	0.2789903									