The Causes and Consequences of Variation in Si Accumulation among Wheat Landraces

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Abstract

Silicon (Si) has long been regarded as a beneficial element for plants and is associated with improved stress tolerance. However, species vary in their ability to accumulate Si, which impacts on the benefits conferred from applying Si fertiliser in agriculture. Si accumulation likely also varies among genotypes within a species, but this possibility has not yet been extensively investigated. Wheat is an important staple food crop and known Si accumulator. In this study, significant differences in Si accumulation between wheat landraces were identified, allowing for the classification of high and low Si accumulating landraces. Whether the responses to varying levels of external Si, damage, osmotic stress, and drought varied between these two categories was then investigated.

Overall, this study highlights the importance of considering genotypic variation when examining the potential effects of applying Si fertiliser in agriculture. Significant differences in Si accumulation between wheat landraces were found at all levels of Si availability (Chapter 2). These differences were partially attributed to differences in transpiration rate and were not correlated with genetic differences or variation in putative Si transporter gene expression. Si did not affect spine density, but there was a negative correlation between Si accumulation and growth (Chapter 2). In Chapter 3, repeated damage caused a localised increase in Si concentration only in damaged leaves, although damage did not affect the density of silicified spines. The localised increase in Si was comparable among all landraces and required a minimum of two damage events. The expression of jasmonic acid-related genes was unaffected by Si. In Chapter 4, Si caused a small increase in osmotic stress tolerance for both high and low Si accumulating landraces. However, Si did not significantly improve growth or yield during drought for any landrace. Osmotic stress decreased Si accumulation for all landraces whereas drought increased it.

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Author's Declaration

I, Sarah Jane Thorne, 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.

Parts of Chapter 1 and 5 have been published as:

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However, instead of presenting the results of individual landraces, landraces were grouped together into high and low Si accumulator types. Only a subset of results from the thesis chapters were presented in this publication.

1 Chapter 1: General Introduction

1.1 The importance of wheat as a staple crop

Wheat (*Triticum aestivum*) is an important staple food crop worldwide, with around 734 million tonnes of wheat produced globally in 2018 (FAOSTAT). Wheat is the primary source of calories for 30 % of the global population, and the primary source of protein for 60 % of the global population (Chaves *et al*., 2013). However, the human population is expected to increase to 9.7 billion by 2050, increasing the pressure to produce more food (United Nations, 2019; Godfray *et al*., 2010). Presently, food insecurity affects one quarter of the world's population (FAO, 2020), but stagnating wheat yields are predicted to exacerbate food insecurity (Ray *et al*., 2012; Grassini *et al*., 2013). Between 1949 and 1978, global wheat production increased at a rate of 3.3 % per year, but between 1982 and 1991, the rate of increase was only 1.5 % per year (Mohammadi, 2018). Current increases in global wheat yield, which stand at 0.9 % per year, will be insufficient to meet the increased demand caused by a rising human population (Ray *et al*., 2013).

1.2 Factors that supress wheat yield

There are many abiotic and biotic stresses that supress wheat yields globally. At present, the global average wheat yield is approximately 3 t ha^{-1}, but there is much variation between countries, with the UK averaging over 7 t ha^{-1} compared to less than 2 t ha^{-1} in Australia (Hawkesford *et al*., 2013). This variation in yield is the result of different stresses that occur in different environments. Currently, drought stress is estimated to reduce wheat yields by approximately 20 % (Daryanto *et al*., 2016), with around 17 % of the global cultivated area affected by drought between 1980 and 2006 (Dai, 2013). Additionally, anthropogenic climate change is affecting global precipitation patterns and temperature (IPCC, 2014), which is predicted to decrease global wheat yields (Challinor *et al*., 2014; Asseng *et al*., 2015). In the future, global wheat production is predicted to decrease by 6 % for every 1 °C temperature increase (Asseng *et al*., 2015). Supporting this, in Europe, crop yield losses due to drought and heatwaves more than tripled in 1991-2015 compared to 1964-1990 (Brás *et al*., 2021).

Further yield losses are caused by soil salinisation, which affects significant amounts of agricultural land. It is estimated that a fifth of irrigated land is affected by soil salinity (FAO and ITPS, 2015), and current land clearing and irrigation practices are further increasing the problem of salinity (Munns and Gilliham, 2015). Although wheat is a moderately salt-

tolerant species (Maas and Hoffman, 1977), its yield can nevertheless be affected by saline conditions. In India, the yield of wheat grown in salt-affected land is 39 % lower compared to wheat grown in non-affected land (Qadir *et al*., 2014).

Between 2001 and 2003, 10.2 % of global wheat yield was lost due to pathogen infection (Oerke, 2006). In Australia, disease is estimated to cause annual wheat production losses of AUS$913 million (Murray and Brennan, 2009). Globally, viruses are estimated to cause plant yield losses of over $30 billion (Nicaise, 2014). Barley yellow dwarf is one of the main viruses affecting cereals, including wheat, and causes annual production losses of £10 million per year in the UK (Nicaise, 2014). In wheat, animal pests have been estimated to reduce yield by 8.7 % in the absence of pesticides (Oerke, 2006). Combined, pests and pathogens cause global wheat yield losses of 21.5 % (Savary *et al*., 2019).

1.3 Strategies to improve wheat yields

Several strategies are currently being used to improve wheat yields. Recent genomics advances have accelerated crop breeding programmes. For example, a recent genome wide association study (GWAS) in wheat identified 62 marker-trait associations for drought tolerance, which could be used in marker assisted selection to breed cultivars with improved drought tolerance (Mwadzingeni *et al*., 2017). However, breeding for improved stress tolerance is difficult due to the large number of genes involved, and the existence of complex genotype-environment interactions (Mohammadi, 2018; Araus *et al*., 2002; Sallam *et al*., 2019). Cultivars bred for improved disease resistance are often quickly overcome by the pathogen evolution (Chaves *et al*., 2013). Overall, breeding programmes are slow and expensive, and have yet to increase yields at the rate required to ensure future food security (Ahmar *et al*., 2020).

Pesticides are commonly employed to protect wheat against pests. Nevertheless, 7.9 % of wheat yield is lost every year due to herbivory, even after crop protection methods are applied (Oerke, 2006). Additionally, pesticides have a variety of negative environmental consequences including reducing insect diversity (Beketov *et al*., 2013) and negative effects on human health (Kim *et al*., 2017; Damalas and Eleftherohorinos, 2011). Insect pests rapidly develop resistance to novel pesticides (Bass *et al*., 2015). Thus, there is increasing interest in finding alternative methods to reduce herbivory.

Crop irrigation can be used to alleviate water limitations, but often creates new problems. Irrigation can increase competition for already scarce water resources and results in ground water being extracted at unsustainable rates (FAO and ITPS, 2015). In Bangladesh, using river water for irrigation has increased soil salinity in some downstream regions (FAO and ITPS, 2015). In some cases, wastewater is used for irrigation, but this is associated with risks both to the environment and human health (Khalid *et al*., 2018). It has been suggested that desalinised sea water be used for crop irrigation, but this has high energy requirements, can increase fertiliser requirements, and can result in soil salinisation (Martínez-Alvarez *et al*., 2016). Increasing crop tolerance to water stress and hence reducing the need for irrigation is important for ensuring sustainable agriculture in the future.

1.4 The potential of Si fertilisers

As discussed above, there are many problems associated with current strategies to limit stress-induced crop losses, notably the long time required for crop breeding, and the environmental costs associated with pesticide use and irrigation. A further alternative to increase crop yield is silicon (Si) fertilisation, which has been shown to improve plant tolerance to an array of biotic and abiotic stresses, including drought and herbivory (reviewed in Debona *et al*., 2017; Singh *et al*., 2020; Thorne *et al*., 2020). Si fertilisation could provide farmers with a quick and cheap method of improving crop yield, in contrast to the above methods which are often slow to take effect and too expensive to be implemented by small-holder farmers. Furthermore, the benefits of Si fertilisation have been found in a range of species including the major crops: rice, wheat, maize, and barley.

Si is the second most abundant element in the earth's crust after oxygen (Wedepohl, 1995) and is present in the soil in a variety of forms, with silicon dioxide (SiO_2) being the most prevalent (Sommer *et al*., 2006). However, plants only absorb Si from the soil in the form of silicic acid ($Si(OH)_4$), which is often scarce in the soil (Côté-Beaulieu *et al*., 2009). Typically, soils contain 100-500 µmol L^{-1} silicic acid, although the exact availability varies depending on soil type, temperature, and pH (Sommer *et al*., 2006). However, wheat accumulates around 1.75 % Si by dry weight (Deshmukh *et al*., 2020) and the potential exists for wheat to extract large amounts of bioavailable Si from the soil (reviewed in Guntzer *et al*., 2012; Ma and Yamaji, 2006). As wheat is harvested, plant matter, including Si, is removed from the field, which depletes soil Si levels over repeated cropping cycles (Vandevenne *et al.,* 2011). The rates of Si weathering are small compared to Si accumulation in crops, with the

result that many soils are, or risk being, Si deficient and require Si supplements (Savant *et al*., 1997; Schaller *et al*., 2021).

Si fertiliser could be used to improve crop yields and has fewer negative environmental impacts than other crop improvement strategies. Si fertiliser comes in several forms. Silicate slags are among the cheapest Si fertilisers and contain additional nutrients including potassium, nitrogen, and phosphorus, but typically contain only 10-15 % Si (Ito, 2015). Sodium- and potassium- silicate fertilisers and wollastonite are more expensive alternatives but contain 18-24 % Si. Cereal straw is rich in Si phytoliths and can be pyrolysed to produce cheap and readily available Si fertiliser (Li and Delvaux, 2019; de Tombeur *et al*., 2021). In some areas, Si fertiliser is already applied to crops, and this is expected to become increasingly common in the future (reviewed in Haynes, 2014). In other areas, as an alternative to Si fertiliser, straw is left on or returned to the field to reduce Si depletion (Schaller *et al.,* 2021).

In addition to its beneficial effect on crops, Si fertilisation could also be used to tackle current climate change. Through the process of enhanced weathering, silicate rocks react with atmospheric carbon dioxide to release silicic acid, which can be used by plants, and a bicarbonate leachate that stores carbon and reduces ocean acidification (Beerling *et al*., 2018). In sorghum, although no stress was experimentally imposed, basalt application resulted in a small improvement in seed yield as well as accelerating weathering in the field and thus capturing carbon (Kelland *et al*., 2020). A recent study has indicated that basalt application is likely to be an economically feasible method of carbon capture (Beerling *et al*., 2020).

Despite the potential benefits, there remain several issues associated with the use of Si fertiliser. Some cheap forms of Si fertiliser contain toxic amounts of aluminium and iron (Ito, 2015), which build-up in the soil over time, potentially leading to future yield losses and health issues. Si also negatively affects the value straw by reducing its digestibility (Zahoor *et al*., 2017), which inhibits the secondary use of crop residues as biofuels or as a feedstock for livestock (Gressel, 2008; Cougnon *et al*., 2020). Although smaller than the emissions associated with nitrogen-phosphorus-potassium (NPK) fertilisers, the mining and transport of Si fertilisers such as wollastonite create pollution which contributes to global climate change. Si in the soil can impact on rhizosphere microbial communities, affecting organic matter decomposition. For example, it was shown that Si delayed leaf litter

decomposition in the common reed, *Phragmites australis*, because it limited growth of fungal decomposers (Schaller *et al*., 2014). Further investigation into the long-term impact of Si fertiliser is required.

The economic feasibility of Si fertiliser depends on the crop, typical yield, current production costs, and the predicted yield increase with Si fertiliser. When production costs are low, larger yield gains are needed to offset the additional cost of Si fertiliser. Nevertheless, Si fertiliser can be used to improve abiotic and biotic stress tolerance, which may allow large yield increases when crops are produced in suboptimal environments (Thorne *et al*., 2020). While Si fertiliser is often used when growing rice in countries such as China and Japan, there is less evidence in the scientific literature that Si fertilisation is economically viable for other species such as wheat. Thus, further research is needed to evaluate the efficacy of Si fertilisers in a wider range of species and to establish the optimum level of Si fertilisation for a given soil-type for each species.

1.5 Si uptake and distribution in plants

Si transport has most extensively been studied in rice, where two Si transporters, Lsi1 and Lsi2, are used to transport silicic acid from the soil through the root (Ma *et al*., 2006, 2007a). Lsi1 homologs have now been identified in a range of plant species (Table 1.1), including wheat (Montpetit *et al*., 2012). Lsi1 is a plasma-membrane localised Nodulin-like 26 intrinsic protein (NIP) III aquaporin with six transmembrane domains and two Asn-Pro-Ala (NPA) motifs(Ma *et al*., 2006; Mitani *et al*., 2008). The NIP III aquaporin family is characterised by a unique aromatic arginine (ar/R) selectivity filter comprising of the amino acids Gly-Ser-Gly-Arg (GSGR). It has been suggested that this selectivity filter, combined with a precise 108 amino acid spacing between NPA domains, allows Si absorption (Mitani *et al*., 2008; Deshmukh *et al*., 2015, 2020). Supporting this, in tobacco, an amino acid substitution in Lsi1 has been proposed to explain its low Si accumulation (Coskun *et al*., 2019b).

In Si accumulators, Si uptake also involves a second transporter, Lsi2, which was first identified in rice (Ma *et al*., 2007a). Lsi2 is a member of a putative anion transporter family with eleven predicted transmembrane domains and is localised to the plasma membrane (Ma *et al*., 2007a). Lsi2 is hypothesised to function as an efflux transporter, such that Si efflux is an active process driven by the proton gradient across the plasma membrane (Ma *et al*., 2007a). Homologs of *OsLsi2* have been identified in several species (Table 1.1). In

tomato, a lack of functional Lsi2 transporters has been proposed to explain the low Si accumulation of this species (Sun *et al*., 2020). Nevertheless, many questions remain regarding the role of Lsi2 as a Si transporter and further investigation is needed to verify its putative role in Si transport (Coskun *et al*., 2021).

Table 1.1 List of identified Si transporters.

Plant species	Transporter	Cellular localisation	Effect of Si on gene expression	Reference
Barley	HvLsi1	Localised to distal side of exodermal and endodermal root cells; expression higher in basal region than tips	Unaffected	Chiba *et al*. (2009)
	HvLsi2	Roots: expression higher in basal region than tips	Decreased	Mitani *et al*. (2009a)
	HvLsi6	Root tips and mature region (epidermis and endodermis)	In roots: unaffected	
		Shoots: in leaf blades and sheaths; parenchyma cells of vascular bundle		Yamaji *et al*. (2012)
		Reproductive stage: nodes, awn, flag leaf blade and sheaf, peduncle		
Brachypodium	BdLsi1-1	Not examined	*Not measured*	Głazowska *et al*. (2018)
Cucumber	CSiT-1	Roots, mature leaves (less in young leaves, petals, stem)	3d: decreased	Wang *et al*. (2015b)
	CSiT-2		6 d: increased	
	CsLsi2	Highest in roots, also stem, laminae, petioles	Increased in roots only	Sun *et al*. (2018)
Date palm	PdNIP2-1	Roots	Unaffected	Bokor *et al*. (2019)
	PdNIP2-2			
Finger millet	EcLsi1	Roots and shoots	Increased	Jadhao *et al*. (2020)
	EcLsi2			
	EcLsi6			
Horsetail	EaLsi2-1	Roots and shoots	*Not measured*	Vivancos *et al*. (2016)
	EaLsi2-2			

	EaNIP3;1	Roots and shoots	Unaffected	
	EaNIP3;3	Roots and shoots	*Not measured*	Grégoire *et al*. (2012)
	EaNIP3;4	Roots	*Not measured*	
Grape vine	VvNIP2;1	Roots, green berries, flowers	Unaffected	Noronha *et al*. (2020)
Maize	ZmLsi1	Mainly in seminal roots; low in crown roots	Unaffected	Mitani *et al*. (2009b)
	ZmLsi6	Crown roots, leaf sheaf, blade		
	ZmLsi2	Roots- higher in basal region than in tips	Decreased	Mitani *et al*. (2009a)
Potato	StLsi1	Roots and leaves	Increased	Vulavala *et al*. (2016)
	StLsi2	Roots, leaves, tuber flesh, tuber skin, stolon, and stem	Unaffected	
Pumpkin	CmLsi1	Roots and shoots; in roots: distal side of root endodermis and exodermis	*Not measured*	Mitani *et al*. (2011)
	CmLsi2	Roots and shoots	*Not measured*	Mitani-Ueno *et al*. (2011)
Rice	OsLsi1	Distal side of root endodermis and exodermis	Decreased	Ma *et al*. (2006)
	OsLsi2	Proximal side of root endodermis and exodermis	Decreased	Ma *et al*. (2007a)
		Flowering stage, in nodes, unelongated stem		Yamaji *et al*. (2015)
	OsLsi3	During flowering stage, in nodes, peduncle, rachis, unelongated stem	*Not measured*	Yamaji *et al*. (2015)
	OsLsi6	Before heading, in xylem parenchyma cells of the leaf sheathes and blades	*Not measured*	Yamaji and Ma (2009)
		At reproductive stage, in node I (connected to flag leaf and panicle)		
Ryegrass	LpLsi1	Roots	Decreased	Pontigo *et al*. (2021)
Sorghum	SbLsi1	Not examined	*Not measured*	Markovich *et al*. (2015)

Soybean	GmNIP2-1 GmNIP2-2	Roots	Decreased	Deshmukh *et al*. (2013)
Tobacco	NtNIP2;1	Roots	Decreased	Zellner *et al*. (2019)
Tomato	SlLsi1 SlLsi2	Root tip and basal region, no polarity Roots	Unaffected Increased	Sun *et al*. (2020)
Wheat	TaLsi1	Roots	Unaffected	Montpetit *et al*. (2012)

The expression pattern of *OsLsi2* is similar to that of *OsLsi1* (Yamaji and Ma, 2011). In rice, Lsi1 and Lsi2 have different, polar, localisation patterns which are predicted to form an efficient directional transport system for Si uptake (reviewed in Ma and Yamaji, 2015). Lsi1 is capable of bidirectional transport (Mitani *et al*., 2008). However, active Si efflux by Lsi2 is hypothesised to create a concentration gradient promoting the uptake of Si from the soil (Ma *et al*., 2007a). In rice, both Lsi1 and Lsi2 are localised at the exodermis and endodermis in the mature region of main and lateral roots. However, Lsi1 is localised to the distal cell side, whereas Lsi2 is localised to the proximal side. Si is transported across the exodermis and endodermis symplastically, but apoplastically across the cortex (Figure 1.1; Ma *et al*., 2006; Yamaji and Ma, 2007).

In other species, the absence of aerenchyma likely results in a different mechanism of Si uptake. In barley, silicic acid is taken up from the external solution by Lsi1 from the distal side of epidermal and cortical cells, as well as by hypodermal cells in the lateral roots (Chiba *et al*., 2009). In maize, Lsi1 is localised to the distal side of the epidermal and hypodermal cells in seminal and crown roots, as well as in the cortex cells of lateral roots (Mitani *et al*., 2009b). In both barley and maize, silicic acid is then transported to the endodermis by the symplastic pathway (Chiba *et al*., 2009; Mitani *et al*., 2009b). Finally, silicic acid is hypothesised to be released to the stele by Lsi2, which is localised at the endodermis without polar localisation (Mitani *et al*., 2009a). Although the *OsLsi2* homolog has not yet been identified in wheat, there is evidence that Si is absorbed by active transport in wheat (Jarvis, 1987; Rains *et al*., 2006; Casey *et al*., 2003; Rafi and Epstein, 1999).

The majority of absorbed silicic acid is transported from the roots to the shoots *via* the transpiration stream (reviewed in Ma and Yamaji, 2015). A homolog of Lsi1, Lsi6, is required to unload silicic acid from the xylem and into the shoot (Mitani *et al*., 2009b; Yamaji *et al*., 2008, 2012). Lsi6 is localised at the adaxial side of the xylem parenchyma cells in the leaf sheaths and leaf blades (Yamaji *et al*., 2008). At the reproductive stage, silicic acid is deposited in the husk of rice and barley by the cooperative action of Lsi2, Lsi3, and Lsi6 (Yamaji *et al*., 2015).

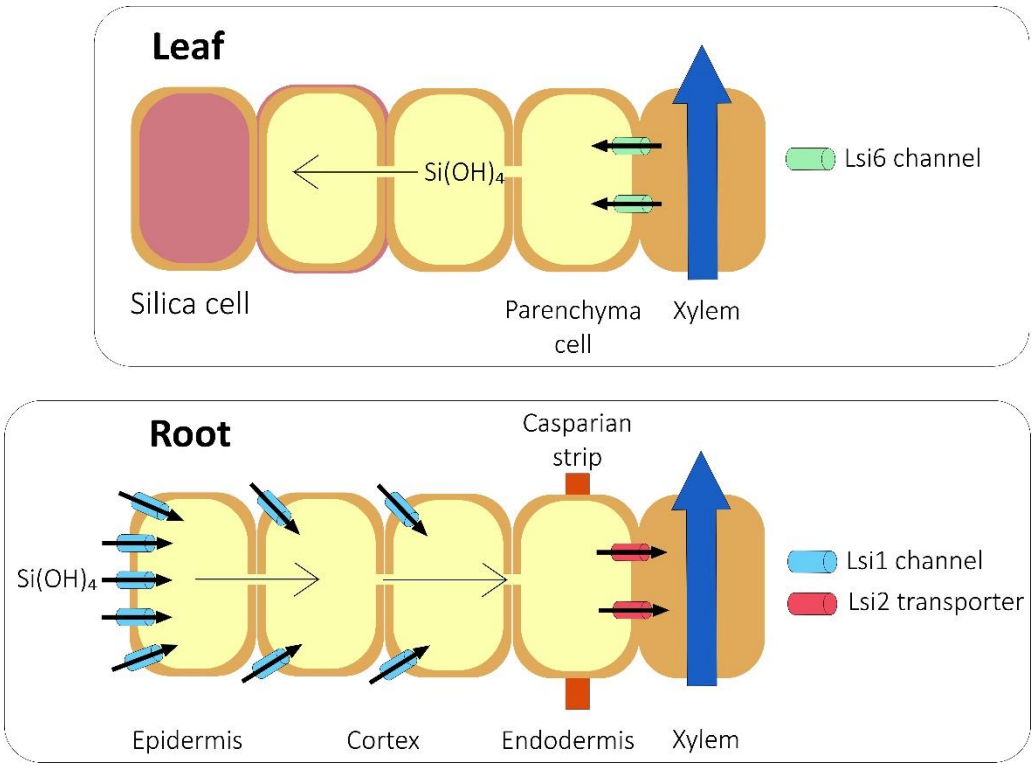

Figure 1.1 Si transport in a typical grass species. Silicic acid from the soil is transported into the root symplast by the action of aquaporins such as Lsi1 channels. The silicic acid then diffuses across the root into the endodermis. It is hypothesised that at the endodermis, Lsi2 transports silicic acid into the stelar apoplast from where it diffuses into the xylem and is transported to the shoot in the transpiration stream. In rice, the presence of aerenchyma means that Lsi2 is localised at both the exodermis and endodermis. In the shoot, silicic acid is unloaded from the xylem by further aquaporins such as Lsi6 and deposited in the cell walls and in specific silica cells, also known as phytoliths, which are silica-filled cells that can take a variety of different forms. *Based on Ma and Yamaji 2015.*

Supplying plants with Si affects the expression of Si transporter genes, although the response varies among species. In rice and soybean, the expression of Si transporters is usually decreased by Si supply (Ma *et al*., 2006; Deshmukh *et al*., 2013). It appears that high accumulation of silicic acid in the shoot results in a signal being produced that supresses Si transporter gene expression (Mitani-Ueno *et al*., 2016). However, some studies have found that *Lsi1* expression increases in rice in response to Si supply (Ye *et al*., 2013; Ma *et al*., 2015). Likewise, in cucumber, Si supply has been found to both decrease (Holz *et al*., 2019) and increase the expression of *Lsi1* (Wang *et al*., 2015a). In maize, barley, and wheat, *Lsi1*

expression appears to be unaffected by Si supply (Montpetit *et al*., 2012; Chiba *et al*., 2009; Mitani *et al*., 2009b).

In most species, the addition of Si decreases the expression of *Lsi2* (Ma *et al*., 2007a; Mitani-Ueno *et al*., 2011; Mitani *et al*., 2009a). However, in sorghum, both *Lsi1* and *Lsi2* have been shown to be upregulated in response to Si supply, although *Lsi6* is downregulated (Soukup *et al*., 2017). In cucumber, one *Lsi2* homolog was upregulated and one downregulated in response to increasing Si availability (Holz *et al*., 2019).

1.6 Si deposition

High levels of silicic acid result in its autopolymerisation into silica (Yoshida *et al*., 1962a). While most silicic acid is transported to the shoots, some is deposited in the roots, predominantly in the tangential and radial walls of endo- and exo-dermal tissues (Bennett, 1982; Lux *et al*., 2003). It appears that Si is integrated into the cell wall by cross-linking with other wall components, such as hemicelluloses, pectins, and phenolics (Sakai and Thom, 1979; Fleck *et al*., 2015; He *et al*., 2015). Recently, a mechanism of silica deposition has been proposed involving a unique lignin polymer, ASZ lignin, that catalyses the condensation of silicic acid into a silica aggregate (Zexer and Elbaum, 2020). The growth of the silica aggregate may then capture ferulic acid-bound hemicellulose and thus is incorporated into the cell wall (Soukup *et al*., 2017).

In the leaves, Si is deposited beneath the cuticle layer of the cell wall in epidermal cell layers (Yoshida *et al*., 1962b). Once Si is deposited, it is not remobilised (Samuels *et al*., 1991), and older leaves typically exhibit greater Si deposition (Sangster, 1970). Many plant structures also become silicified, including trichomes, leaf hairs, and spines (Hartley *et al*., 2015). Additionally, Si is deposited in the form of phytoliths, also known as silica cells, which can take a wide variety of different forms (reviewed in Shakoor *et al*., 2014). Although transpiration is needed to transport silicic acid into the leaves, depositing Si into phytoliths is an active process requiring biological factors such as proteins or sugars (Kumar *et al*., 2017). These biological factors are released into the apoplasm to induce silicification in the paramural space (Kumar and Elbaum, 2018). Recently, Siliplant1 (Slp1) has been identified as a protein involved in precipitating silica in sorghum silica cells (Kumar *et al*., 2020).

1.7 Si has a limited effect on unstressed plants

Si has been reported to induce a plethora of effects *in planta*, although the mechanisms underpinning these effects remain to be determined. Numerous studies have reported that Si regulates secondary metabolism to improve stress tolerance (reviewed in Ahanger *et al*., 2020). However, there is currently little evidence to support the idea that Si has a biochemical role, such that it interacts with intracellular processes such as gene expression. To the contrary, due to the lack of effect of Si on gene expression in a transcriptome analysis in Arabidopsis, Fauteux *et al.* (2006) concluded that Si does not affect plant metabolism. Instead, it is likely that the observed effects of Si are due to it acting as a mechanical barrier; the so-called "apoplastic obstruction hypothesis" (Coskun *et al*., 2019a).

The vast majority of studies report beneficial effects of Si only in the presence of some form of stress (e.g. Chen *et al*., 2016; Yan *et al*., 2020; Yeo *et al*., 1999). Nevertheless, a few studies have reported positive effects of Si in the absence of experimentally-imposed stress (Flores *et al*., 2019; Artyszak, 2018; Ligaba-Osena *et al*., 2020). In field-grown wheat, applying Si affected carbon turnover, phosphorous availability, and nitrogen use efficiency (Neu *et al*., 2017). In rice, Si accumulation correlated with lower levels of phenolic biosynthesis (Goto *et al*., 2002) as well as improving grain production and stimulating amino acid remobilisation (Detmann *et al*., 2012). It is unknown why some studies report effects of Si during unstressed conditions, but it is possible that these studies may have involved unintended, unreported, mild stress conditions that resulted in a Si effect being observed (Coskun *et al*., 2019a).

Supporting a limited role of Si in the absence of stress, several studies have reported that increasing Si availability results in very few changes in gene expression when plants are grown in optimal conditions (Watanabe *et al*., 2004; Chain *et al*., 2009; Rasoolizadeh *et al*., 2018). Using proteomics, Jang *et al*. (2018) identified only seven Si-regulated proteins in rice. Likewise, in unstressed conditions, Si had only a minimal effect on the metabolome of cowpea (Führs *et al*., 2012). However, other transcriptomic studies have reported numerous genes whose expression is affected by Si fertilisation in the absence of stress conditions (Holz *et al*., 2019; Brunings *et al*., 2009). Zhu *et al*. (2019) identified 1237 up- and 232 downregulated genes when Si was supplied to cucumber grown in the absence of experimentally imposed stress, which were mainly related to the plant stress response, metabolism, signalling, and ion homeostasis. In date palm, 263 metabolites were

differentially accumulated in either the leaves or roots in response to increased Si availability, including many antimicrobials, salicylic acid (SA)- and jasmonic acid (JA)-derivatives, and antioxidants (Jana *et al*., 2019). Differences in experimental design and growth conditions may explain these contrasting results.

1.8 Si increases tolerance to abiotic stress

Under stress conditions, Si has been reported to improve a range of physiological and biochemical parameters. Si is commonly found to reduce oxidative damage during abiotic stress (Gong *et al*., 2005; Pei *et al*., 2010; Liang *et al*., 2008; Cooke and Leishman, 2016). During drought and salinity stress, Si reduces oxidative damage by an average of 30 % (Thorne *et al*., 2020). This reduction in oxidative damage is achieved by increasing antioxidative enzyme activity (Figure 1.2). This has been reported to occur, for example, during alkaline (Abdel Latef and Tsran, 2016), freezing (Liang *et al*., 2008), drought (Tale Ahmad and Haddad, 2011), and salinity stress (Daoud *et al*., 2018). Additionally, Ma *et al*. (2016) linked Si treatment to increased expression of antioxidative enzyme genes. However, there are a few exceptions reporting contrasting effects of Si on antioxidative enzyme activity. Gong *et al*. (2008) reported decreased catalase (CAT) activity, and no difference in superoxide dismutase (SOD) or peroxidase (POX) activity in drought-stressed wheat. In drought-stressed sunflowers, the effect of Si on antioxidative enzyme activity varied among cultivars, and only 8 out of 12 cultivars tested had decreased H_2O_2 levels with Si (Gunes *et al*., 2008). Despite such exceptions, there is strong evidence for a link between Si accumulation and increased antioxidative enzyme activity.

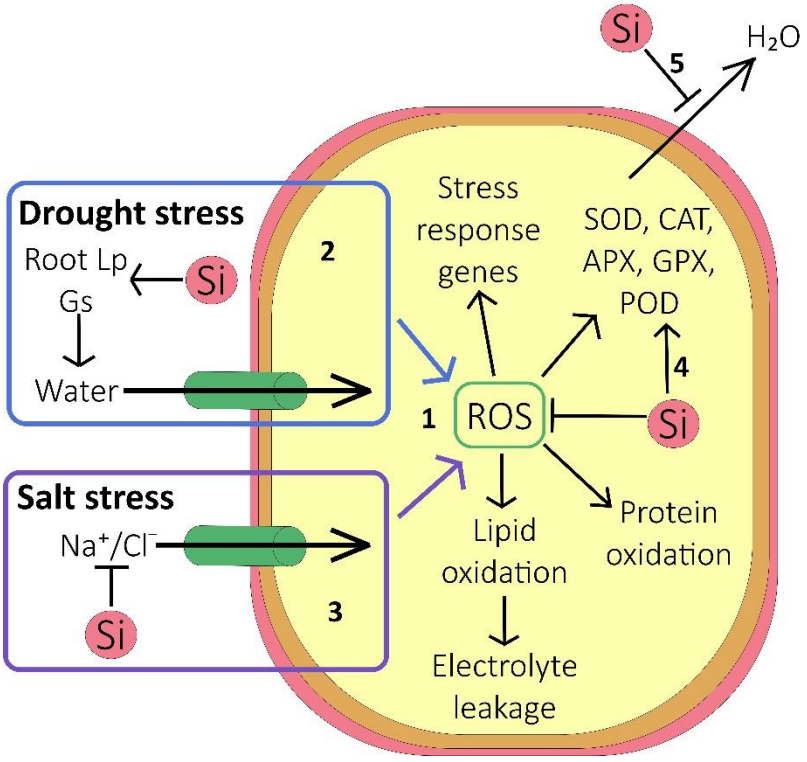

Figure 1.2: Effect of Si on oxidative damage. (1) During abiotic stress conditions, accumulation of reactive oxygen species (ROS) inside the cell causes protein oxidation, lipid oxidation (resulting in increased electrolyte leakage out of the cell), and activation of stress response genes. (2) During drought stress, Si increases the root hydraulic conductance (Lp) and stomatal conductance (G_s). This can allow more water to enter the cell and hence reduce the accumulation of ROS. (3) During salt stress, as well as improving the plant water status, Si reduces Na^+ and Cl^- accumulation in shoot by forming endodermal barriers in the root. This reduces the accumulation of ROS and limits ion toxicity. (4) Antioxidative enzymes are activated by increased cellular ROS, and their activity may be further increased by Si. These enzymes scavenge ROS within the cell, thus protecting it against oxidative damage. (5) Si deposited outside the cell reduces cuticular evapotranspiration, protecting the plant against water stress.

Plants use Si to improve photosynthetic parameters during drought (Gong *et al*., 2005; Sonobe *et al*., 2009), salinity (Daoud *et al*., 2018; Harizanova and Koleva-Valkova, 2019), zinc (Song *et al*., 2014) and cold stress (Joudmand and Hajiboland, 2019). During abiotic stress, Si accumulation is correlated with increases in the content of chlorophyll and other pigments in the leaf (Chen *et al*., 2011; Maghsoudi *et al*., 2016b; Hajiboland *et al*., 2017). Si-induced increases in stomatal conductance may also increase the photosynthetic rate

(Sonobe *et al*., 2009; Sattar *et al*., 2019; Gong and Chen, 2012; Amin *et al*., 2018). Si accumulation is also correlated with the upregulation of the activity of enzymes involved in photosynthetic pigment biosynthesis (Alamri *et al*., 2020). Phytoliths may protect cells from UV radiation, thus preventing photoinhibition and consequently improving photosynthetic capacity (Pierantoni *et al*., 2017).

Plant Si accumulation can improve plant water status during abiotic stress (Gong and Chen, 2012; de Camargo *et al*., 2019; Shi *et al*., 2016). This is partly a consequence of Si accumulation increasing water use efficiency (WUE; Chen *et al*., 2011; Hajiboland *et al*., 2017), as well as being correlated with increased levels of compatible solutes (Pei *et al*., 2010; Sayed and Gadallah, 2014; Hajiboland *et al*., 2017; Yang *et al*., 2019). Si modulates WUE *via* transpiration. In rice, Si deposited beneath the cuticle could reduce cuticular transpiration, and therefore limit water loss (Yoshida *et al*., 1962b). However, the majority of studies, in species including sorghum, cucumber, and tomato, report that Si accumulation increases transpiration during stress conditions (Liu *et al*., 2015; Wang *et al*., 2015b; Li *et al*., 2015; Shi *et al*., 2016). Alternatively, Si accumulation may affect transpiration through its effects on root hydraulic conductance (Chen *et al*., 2018). Si accumulation has been reported to improve root hydraulic conductance during both water stress (Sonobe *et al*., 2010; Shi *et al*., 2016), and salt stress (Wang *et al*., 2015b; Zhu *et al*., 2015) in several species.

Si accumulation is correlated with the accumulation of soluble sugars and amino acids, which increases the water potential gradient and subsequent water uptake (Zhu *et al*., 2015; Sonobe *et al*., 2010; Ming *et al*., 2012). However, the effect of Si accumulation on compatible osmolytes is not consistent, with Si being reported to increase (Tale Ahmad and Haddad, 2011; Alzahrani *et al*., 2018; Yang *et al*., 2019; Hajiboland *et al*., 2017) and decrease (Pei *et al*., 2010; Yin *et al*., 2014; Yang *et al*., 2019; Kang *et al*., 2016) the levels of proline and soluble sugars in drought-stressed plants. Generally, Si accumulation increases polyamine levels during drought and salt-stress (Wang *et al*., 2015b; Yin *et al*., 2016; Ali *et al*., 2018; Yin *et al*., 2019). Thorne *et al*. (2020) concluded that during drought or salinity stress, Si accumulation induces a small increase in compatible solutes.

Ionic toxicity due to salinity mainly stems from excess Na^+ and Cl^- ions and is significantly alleviated by Si fertilisation (Figure 1.3). Some studies have reported that Si increases potassium uptake, which could alleviate salinity stress by improving the tissue K^+:Na^+ ratio

(Tahir *et al*., 2010, 2006; Ali *et al*., 2012). Additionally, Si fertilisation consistently reduces accumulation of Na^+ (and Cl^-) in the shoot but not the root (Ahmad *et al*., 1992; Cooke and Leishman, 2016; Muneer *et al*., 2014). In salt-stressed rice, Si blocks apoplastic bypass flow in the root which occurs in regions where the Casparian strip is incomplete, and thus reduces ion transfer to the shoots (Gong *et al*., 2006; Yeo *et al*., 1999). Reduction of apoplastic bypass flow by Si also reduces nutrient accumulation and therefore improves tolerance to, for example, cadmium stress (Rizwan *et al*., 2012; Howladar *et al*., 2018).

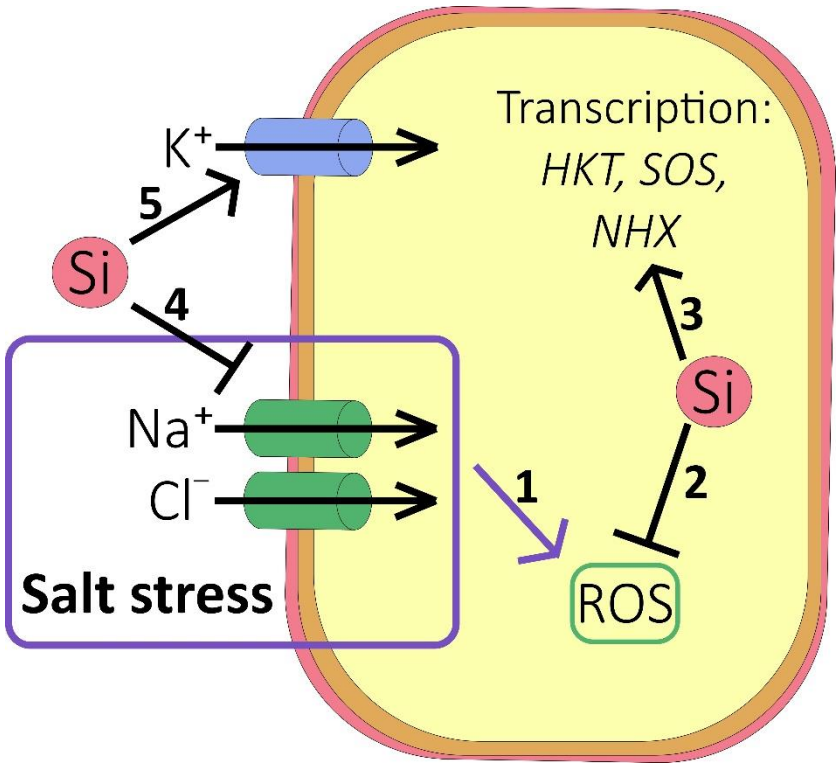

Figure 1.3: Effect of Si on salt accumulation. (1) During salt stress conditions, accumulation of Na^+ and Cl^- results in reactive oxygen species (ROS) accumulation and oxidative damage to the cell. (2) Si inhibits the production of ROS, protecting the cell against oxidative damage. (3) Si may increase the transcription of *HKT1, SOS*, and *NHX* transporters to relieve ion toxicity. (4) Si reduces root-to-shoot translocation of Na^+ and Cl^-. (5) Si may also stimulate accumulation of K^+ into the cell to improve the K^+:Na^+ ratio.

1.9 Si both prevents and reduces the negative effects of biotic stress

Si deposited in the cell wall can reduce plant susceptibility to pathogen stress. When infecting plants, pathogens release a range of molecules, known as effectors, into plant cells (reviewed in Jones and Dangl, 2006). According to the apoplastic obstruction

hypothesis, Si deposited in the apoplasm inhibits the release of pathogen effectors into the cytoplasm (Coskun *et al*., 2019a). Supporting this, Si was found to interfere with the signalling network between soybean and the hemibiotroph *Phytophthora sojae*, creating an incompatible reaction by preventing pathogen effectors from reaching plant receptors (Rasoolizadeh *et al*., 2018). Likewise, Si deposited in the cell wall can inhibit infection by rice blast (Kim *et al*., 2002) and brown spot (Ning *et al*., 2014).

In addition to acting as a mechanical barrier, Si supplementation is correlated with changes in defence-related enzyme activity that improve disease tolerance. In hydroponically-grown rice, plants that were switched from non-Si fertilised to Si fertilised growing conditions and simultaneously inoculated with bacterial blight showed the same high resistance as plants grown continuously with Si, with Si found to increase PPO and PAL activity, as well as increase the expression of several defence genes (Song *et al*., 2016). Si reduced tan spot disease severity in wheat, with enzymes involved in plant defence showing higher activity in plants supplied with Si (Dorneles *et al*., 2017). Likewise, in response to blast infection in wheat, Si increased the expression of defence-related genes, which was correlated with reduced disease severity (Cruz *et al*., 2015). Other studies have also correlated Si with increased defence-related enzyme activity (Gomes *et al*., 2005; Han *et al*., 2016). In sorghum, Si was found to enhance resistance to the fungus *Alternaria alternata* by stimulating biochemical defence reactions, rather than by acting as a mechanical barrier (Bathoova *et al*., 2021). However, using two near-isogenic rice lines with differing susceptibilities to rice blast, Cai *et al*. (2008) suggested that both Si-induced defence response and cell silicification contribute to Si-induced rice resistance.

However, in contrast to the commonly found increase during abiotic stress, during biotic stress conditions, Si accumulation is usually correlated with decreased antioxidative enzyme activity, despite also reducing oxidative damage (reviewed in Debona *et al*., 2017). Si deposition in the cell wall inhibits pathogen entry, thus reducing the need for antioxidative enzymes (Coskun *et al.,* 2019a). In wheat infected with powdery mildew, Si supplementation was correlated with decreased activity of antioxidant enzymes (Moldes *et al*., 2016). Likewise, Debona *et al*. (2014) found that while Si improves wheat resistance to leaf blast, plants treated with Si had lower levels of antioxidative enzyme activity. Other mechanisms appeared to be enhancing plant resistance, with Si-treated plants having higher GR enzyme activity, which may help them to maintain protein synthesis under infected conditions (Debona *et al*., 2014).

As well as reducing pathogen stress, Si fertilisation is also associated with beneficial effects during herbivory (Figure 1.4). Si deposited in the form of phytoliths, or as silicified structures including spines and trichomes on the leaf surface, reduces both insect and mammalian herbivory (reviewed in Hartley and DeGabriel, 2016). For example, Si reduced digestibility and caused mandible wear in African armyworm (Massey and Hartley, 2009). Silica phytoliths reduce plant digestibility to insect herbivory by inhibiting the crushing of plant cells, and thus the release of nutrients (Hunt *et al*., 2008). Furthermore, phytoliths can increase leaf abrasiveness, therefore deterring mammalian herbivores (Massey and Hartley, 2006; Massey *et al*., 2008). Si deposited in the root epidermis can also protect grasses from root herbivory (Moore and Johnson, 2017). However, the extent to which phytoliths, rather than grit on the plant surface, are responsible for mandible wear, is debated (Sanson *et al*., 2007), and the effectiveness of Si defences is likely herbivore-dependent (Hall *et al*., 2020a). Mir *et al*. (2019) found that, in rescuegrass, higher plant Si accumulation did not affect phytolith morphology, but it did reduce leaf tissue consumption by a grasshopper.

In response to herbivory, plants typically initiate jasmonic acid (JA) signalling to co-ordinate a defence response (reviewed in Howe and Jander, 2007). Several studies have examined the effect of Si on JA signalling, but with contrasting results. While there is general agreement that JA promotes Si accumulation, it is currently debated whether Si inhibits or promotes JA accumulation (Figure 1.4). Kim *et al*., (2011, 2014) and Hall *et al*. (2019) provide evidence that Si inhibits JA accumulation, while Ye *et al*. (2013) and Lin *et al*. (2019) argue that Si promotes JA accumulation. During unstressed conditions, Jang *et al*. (2018) found that Si increased JA levels at all time-points tested, although the extent of the increase varied depending on the concentration of Si used and the time-point.

Transcriptomic studies provide further insight into the effect of Si on the plant pathogen response. In wheat, powdery mildew infection changed the expression of nearly 900 genes, but this response was not present in Si-treated plants, suggesting Si provides almost full protection against infection (Chain *et al*., 2009). A similar study in Arabidopsis found that while Si did not provide complete protection against powdery mildew infection, Si reduced the observed decrease in downregulated gene expression, causing the gene expression profile to be more similar to that observed in uninfected plants, and thus suggesting that Si had improved pathogen tolerance (Fauteux *et al*., 2006).

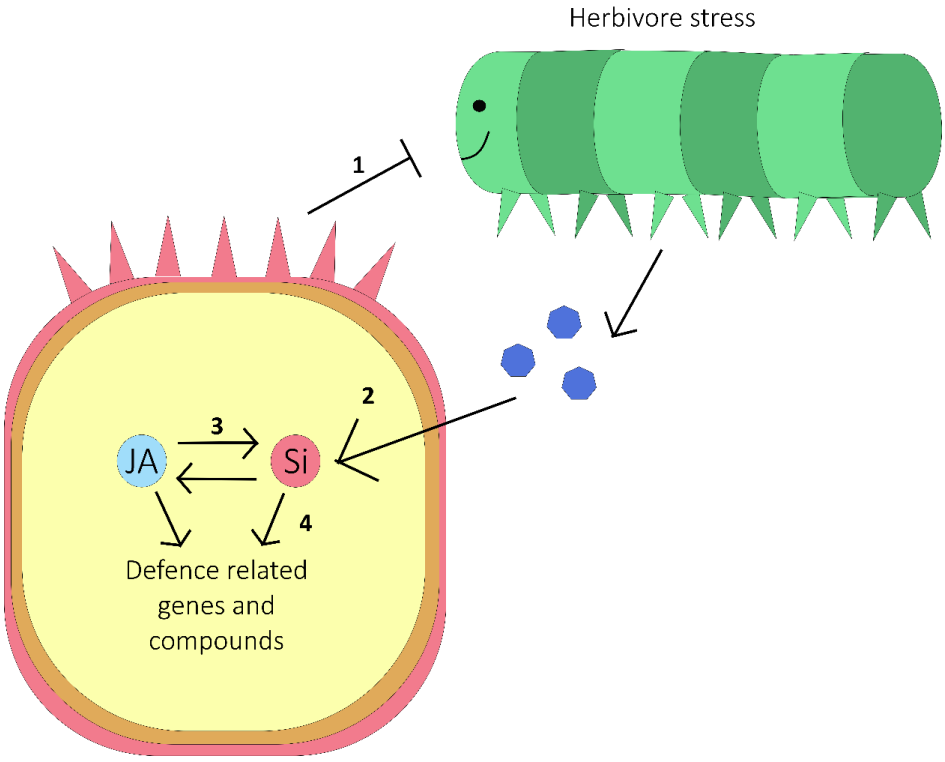

Figure 1.4 Effect of Si during herbivory. (1) Si phytoliths and other silicified structures increase insect mandible wear and reduce herbivory. (2) Herbivores release a variety of molecules, known as effectors, into the cell, activating the jasmonic acid (JA)-mediated defence response. Si deposited in the cell wall inhibits the entry of effectors. (3) Activation of the JA defence pathway increases Si accumulation. In turn, Si may promote JA biosynthesis, although Si-mediated inhibition of the JA pathway has also been reported. (4) Si increases the expression of defence-related genes and the accumulation of defence-related secondary metabolites.

1.10 Applying Si fertiliser in agriculture is not universally beneficial

Although the majority of studies report that Si supplementation improves stress tolerance, there are nevertheless studies that find no significant effect of growing plants with additional Si. In particular, although higher Si content can increase leaf abrasiveness and deter foliar herbivores, it has been suggested that plant Si content does not have a significant effect on phloem feeders (Massey *et al*., 2006; Rowe *et al*., 2020). In *Medicago sativa,* Si addition was positively correlated with an increase in aphid abundance on the plants, which was attributed to the increased plant biomass induced by Si (Johnson *et al*., 2017). A recent meta-analysis concluded that Si defences are more effective against chewing herbivores than fluid feeders (Johnson *et al*., 2020). Nevertheless, there are

examples of Si application correlating with a reduction in herbivory by phloem feeding herbivores (Yang *et al*., 2017) and the conclusion that Si accumulation has no effect on phloem feeders may be too generalised (Keeping and Kvedaras, 2008).

Few studies have examined whether plants can use Si to protect against viral infections, but it appears that a beneficial effect of Si accumulation during viral infection is not as universal as has been reported for other pathogens. In tobacco, Si-fertilised plants showed an enhanced defence response to tobacco ringspot virus (TRSV), but not to tobacco mosaic virus (TMV, Zellner *et al*., 2011). Similarly, Si supplementation improved tolerance to the viruses cowpea chlorotic mottle virus (CCMV) and cowpea mild mottle virus (CMMV) in mung bean, but not in yardlong bean or chickpea (Izaguirre-Mayoral *et al*., 2017).

In some studies, increased Si availability was correlated with beneficial effects on biochemical and physiological parameters but did not increase growth during stress (Rezende *et al*., 2017; Berni *et al*., 2020). In maize, increased Si accumulation decreased Zn accumulation, but overall, negatively impacted on growth during Zn stress (Bokor *et al*., 2014). Ruppenthal *et al*. (2016) found no effect of increasing soil Si availability through Si supplementation on growth in soybean during drought stress. While Si accumulation increased with increasing Si availability in non-stressed plants, there was no effect of Si supplementation on Si accumulation in drought-stressed plants, despite Si supplementation correlating with reduced membrane damage (Ruppenthal *et al.,* 2016). Likewise, in drought-stressed wheat, Sattar *et al*. (2019) reported Si supplementation significantly increased root, but not shoot, dry weight, whereas Xu *et al*. (2017) reported that Si supplementation increased wheat shoot fresh weight, but not dry weight, during osmotic stress. A lack of Si supplementation effect on growth during osmotic stress has also been reported in tall fescue (Vandegeer *et al*., 2021b) and barley (Hosseini *et al*., 2017; Maillard *et al*., 2018).

There is a plethora of reasons regarding why the literature is full of inconsistent results regarding the effect of Si in plants. Variation in experimental conditions likely explains much of the differences. Si takes time to accumulate (Deshmukh *et al*., 2020; Ma and Yamaji, 2006), and therefore, a more limited Si effect may be reported during short-term experiments. The failure of many studies to measure plant Si content prevents understanding of how plants use Si to improve stress tolerance and establishing the conditions in which Si supplementation is most likely to be beneficial. The use of foliar Si

fertiliser or seed priming compared to soil fertilisation is likely to further impact on the Si response, as will the use of different Si fertilisers such as silicate slag compared to Si-rich biochar. Some studies do not report balancing the ions when adding Si fertiliser (e.g. Ahmad *et al*., 2016; Joudmand and Hajiboland, 2019; Seleiman *et al*., 2019), and thus, their reported beneficial effect of Si may be the result of potassium or sodium fertilisation. Finally, the effect of Si appears to be species specific, with species able to accumulate higher levels of Si showing stronger responses when Si is readily available. Supporting this, transforming Arabidopsis with the wheat *Lsi1* gene increased Si accumulation and pathogen tolerance (Vivancos *et al*., 2015). Adding to this, genotypic variation is likely to further affect the Si response. Notably, studies using multiple genotypes often report different Si effects depending on the genotype (Farooq *et al*., 2015; Ali *et al*., 2018).

1.11 Variation in Si accumulation

Plants vary significantly in their ability to accumulate Si from the soil. A meta-analysis of over 700 plant species found that, in general, liverworts and horsetails accumulate more Si than angiosperms and gymnosperms (Hodson *et al*., 2005), although Si accumulation in liverworts and horsetails has been examined to a lesser extent compared to angiosperms. Several important monocot crop species, including wheat, barley, maize, and rice, accumulate large amounts of Si (Guntzer *et al*., 2012). Nevertheless, some dicots accumulate significant levels of Si, and there is significant variation in Si accumulation even within plant families (Katz, 2014; Deshmukh *et al*., 2020).

Additionally, there is also wide variation in Si uptake between cultivars of the same species (McLarnon *et al*., 2017; Mitani-Ueno *et al*., 2014, 2011; Chiba *et al*., 2009; Ma *et al*., 2007b; Hartley *et al*., 2015; Ma *et al*., 2003; Wu *et al*., 2006). However, in some cases, such genotype-specific variation is only apparent when plants are grown with an adequate supply of Si (Cotterill *et al*., 2007). In other cases, genotypes respond differently to increased Si availability, with four out of sixteen sorghum genotypes found not to accumulate significantly more Si in response to Si fertilisation (De Lima *et al*., 2019). Even with Si fertilisation, Deshmukh *et al*. (2020) did not find significant variation in Si accumulation between genotypes among eight species, including high and low Si-accumulating species.

Genotypic variation in Si accumulation capacity may influence the extent to which Si fertilisation can improve stress tolerance. However, to date, few studies have examined

whether there is a correlation between Si accumulation and the effect of Si. In chickpea, ten cultivars responded to drought stress by increasing Si accumulation to different extents, but this did not correlate with significant increases in growth (Gunes *et al*., 2007). Sapre and Vakharia (2017) found variation both in osmotic stress tolerance and Si accumulation among ten wheat cultivars, but the correlation between Si concentration and changes in oxidative damage was not significant. In sorghum, differences in aphid tolerance between three genotypes were not correlated to differences in Si accumulation (Sampaio *et al*., 2020). In sugarcane, the effect of Si under water deficit conditions varied among cultivars, with a significant positive effect on dry weight observed in only one out of four cultivars tested (de Camargo *et al*., 2019).

The causes of such genotypic variation in Si accumulation remain unknown but may relate to differences in Si transporters and/or their abundance. In pumpkin, the different ability of two pumpkin cultivars to take up Si has been attributed to a single amino acid change in the Lsi1 transporter (Mitani *et al*., 2011). However, in rice, the Lsi1 amino acid sequence appears to be strongly conserved among different cultivars (Mitani-Ueno *et al*., 2014). Instead, differences in rice Si accumulation may be the result of variation in Si transporter gene expression (Ma *et al*., 2007b; Wu *et al*., 2006). The high density of the Lsi1 transporter in rice compared to other species is hypothesised to explain high Si accumulation in this species (Mitani and Ma, 2005; Nikolic *et al*., 2006). Variation in other Si transporters may also be important. Talukdar *et al*. (2019) identified SNPs in *OsLsi2* and *OsLsi3* that were linked to differences in Si accumulation in a panel of 50 rice accessions, while Swain and Rout (2020) suggested that variation in *Lsi2* expression may explain variation in Si uptake among rice genotypes.

1.12 Aims and objectives

Wheat is an important crop species worldwide, but significant yield losses occur due to abiotic and biotic stresses. There is abundant evidence in the literature that Si can improve wheat tolerance to a variety of stresses. However, the magnitude of the Si effect is not consistent. One reason for this inconsistency could be that variation in genotype affects the response to Si. However, there is a lack of studies exploring the causes and consequences of genotype-specific Si responses. There is interest in using genetically diverse, traditional wheat landraces to breed new elite cultivars with enhanced stress tolerance (Lopes *et al*., 2015), yet whether such landraces vary in their response to Si fertilisation has not been investigated. Nevertheless, variation in the response to Si could have consequences for the

widespread use of Si fertilisation to improve plant stress tolerance. Notably, it remains to be determined whether there is a positive correlation between Si accumulation and a beneficial Si effect, or whether there are genotypes that do not receive benefits from Si fertilisation. To address this knowledge gap, this thesis uses genetically diverse wheat landraces to investigate the causes and consequences of variation in Si accumulation between genotypes. Overall, the main objectives of this thesis were:

1. To examine whether there is variation in Si accumulation among wheat landraces, and to determine the cause of any variation
2. To investigate whether mechanical damage induces landrace-specific responses in Si accumulation
3. To determine whether there is variation in the response of landraces to Si during osmotic and drought stress.

2 Chapter 2: Variation in Si accumulation among wheat landraces

2.1 Introduction

Wheat is an important food crop worldwide, and can accumulate significant levels of Si (Deshmukh *et al*., 2020; Hodson *et al*., 2005). For example, when grown for 30 days with 20 ppm Si, wheat accumulated 1.75 % Si in the leaves (Deshmukh *et al.,* 2020). Wheat uses Si to improve tolerance to a range of biotic and abiotic stresses in wheat. Si reduces the severity of pathogen infection, including powdery mildew (Moldes *et al*., 2016), spot blotch (Domiciano *et al*., 2010), and blast (Debona *et al*., 2014). Using Si fertilisation to increase the plant-available Si in the soil by 74 % improved wheat yield by around 15 % when plants were infected with tan spot and head blight (Pazdiora *et al*., 2021). Accumulation of Si in the shoots can also reduce herbivory in wheat (Cotterill *et al*., 2007; Griffin *et al*., 2015; Jeer *et al*., 2021). Furthermore, Si can improve wheat tolerance to drought (Ahmad *et al*., 2016; Gong *et al*., 2003), salinity (Ahmad *et al*., 1992; Saleh *et al*., 2017), and freezing stress (Liang *et al*., 2008). Grain yield increased by 70 % when wheat seeds were primed with 60 mM sodium silicate prior to drought-stress (Hameed *et al*., 2021).

Some of the mechanisms underpinning the beneficial effects of Si are currently debated. Si deposited in the cell wall can act as a physical barrier deterring herbivory (Massey and Hartley, 2009; Hartley *et al*., 2015) and preventing the entry of pathogens (Coskun *et al*., 2019a). Deposition on the plant surface, particularly near stomatal guard cells (Vandegeer *et al*., 2021b), suggests Si fertilisation could decrease transpiration, which has been reported in some wheat studies (Bukhari *et al*., 2020; Sattar *et al*., 2017). However, other studies in wheat have reported that Si increases transpiration, especially during drought stress (Gong and Chen, 2012; Javaid *et al*., 2019; Sattar *et al*., 2018). Another topic of debate is the location where Si manifests its activity; many studies focus only on Si accumulation in the shoot (e.g. Ahmad *et al*., 2007; Ali *et al*., 2012; Ma *et al*., 2016; Tahir *et al*., 2006), and the importance of root Si accumulation in improving stress tolerance remains unknown.

Si uptake in wheat roots is likely to involve at least two transporters: Lsi1 and Lsi2, analogous to what is found in rice (reviewed in Ma and Yamaji, 2015). Lsi1 is an aquaporin

involved in uptake of silicic acid from the soil (Ma *et al*., 2006) while Lsi2 likely functions as an active efflux transporter and has been suggested to be driven by the proton gradient across the plasma membrane (Ma *et al*., 2007a). Although *Lsi1* has been identified and characterised in wheat (Montpetit *et al*., 2012), the wheat *Lsi2* gene has not been formally characterised. Nevertheless, several studies have provided evidence that Si accumulation in wheat is an active process (Casey *et al*., 2003; Frick *et al*., 2020; Rains *et al*., 2006). When Si is readily available, wheat typically accumulates 1-2 % Si by dry weight (Ali *et al*., 2012; Deshmukh *et al*., 2020; Domiciano *et al*., 2010; Tahir *et al*., 2010). The majority of this Si is transported into the shoots (Jarvis, 1987), where it is deposited as special silica structures known as phytoliths in the epidermis cells, as well as in the cell wall (Mecfel *et al*., 2007; Ponzi and Pizzolongo, 2003). Structures including trichomes, macrohairs, and spines can also be silicified (Hartley *et al*., 2015). In roots, Si is deposited in the endodermis of seminal and adventitious roots, although the exact deposition pattern varies among cultivars (Bennett, 1982).

Si accumulation can vary between genotypes, but it remains to be determined whether this has any physiological relevance, for instance with respect to stress tolerance. Previous studies have found that significant variation in Si accumulation exists between cultivars in barley (Chiba *et al*., 2009; Ma *et al*., 2003), rice (Ma *et al*., 2007b), pumpkin (Mitani-Ueno *et al*., 2011), and tall fescue (McLarnon *et al*., 2017). Cotterill *et al*. (2007) reported significant variation among six wheat cultivars when Si fertiliser was used. The cause of this variation in Si accumulation is currently unknown. As silicic acid is transported to the shoot in the transpiration stream, it is possible that variation in Si accumulation between species and cultivars relates to differences in transpiration rate (Exley, 2015). However, recent evidence has indicated that differences in the expression levels of Si transporter genes may be the cause of differences in Si accumulation between cultivars (McLarnon *et al*., 2017). Differences in Si uptake between rice varieties have been linked to both differences in gene expression (Ma *et al*., 2007b; Wu *et al*., 2006) and sequence (Talukdar *et al*., 2019).

Landraces are traditional varieties that were bred by farmers to be locally-adapted to their environment (Zeven, 1998). Typically, landraces are highly tolerant to biotic and abiotic stresses, although produce lower yields compared to modern cultivars (Lopes *et al*., 2015; Zeven, 1998). Due to their high genetic diversity, landraces have been proposed as an

important source of novel alleles to increase crop adaptation to stressful environments (Dwivedi *et al*., 2016). Increasingly, landraces are being used in crop breeding programs to improve wheat stress tolerance (Lopes *et al*., 2015). It is hypothesised that there will be significant variation in Si accumulation among landraces, although the Si accumulation ability of landraces has not yet been investigated.

Crop genetic diversity panels are collections of genotypes, often including landraces, that aim to capture significant amounts of the genetic diversity present in crop species. The YoGI diversity panel is a collection of 350 genetically diverse wheat landraces taken from 65 countries and includes landraces adapted to a variety of different environmental conditions (Harper, unpublished). In this study, 98 landraces were selected from the YoGI panel. After determining the Si accumulation capacity of each these landraces, a selection that differed significantly in their Si accumulation was selected for more detailed experiments to investigate variation in Si accumulation and deposition when supplied with different amounts of Si, as well as potential mechanisms that could explain the observed variation in Si accumulation.

2.2 Methods

2.2.1 Experimental design and plant growth conditions

2.2.1.1 Experiment 1: Investigating diversity in wheat Si accumulation

To investigate whether there is significant variation in Si accumulation among genotypes, a diversity panel of 98 wheat (*Triticum aestivum*) landraces was taken from the YoGI biodiversity panel (Harper, unpublished). The panel was formed using material from the following collections: The International Maize and Wheat Improvement Center (CIMMYT), Mexico; Crop Research Institute, Prague; and John Innes Germplasm Resource Unit, the Biotechnology and Biological Sciences Research Council Designing Future Wheat programme. Due to space and cost limitations, it was not possible to use all 350 landraces that form the YoGI panel, and thus a subset of 98 landraces was selected, which was representative of the diversity found across the YoGI panel. A phylogenetic tree was constructed in TASSEL (Bradbury *et al*., 2007) and a minimum of one landrace was selected from each of 54 tree clusters, with two landraces selected from the largest clusters. Additionally, six more modern landraces were selected, as well as ten landraces that were known to be the fastest or slowest to reach the heading stage. A mixture of winter and

spring cultivars was selected. The landraces originate from countries across the globe, although the climate and soil properties of the regions of origin are unknown. A full list of landraces is available in Table 2.2.

Using the 98 landraces, a screen was conducted to investigate variation in Si accumulation among wheat landraces and to identify landraces that exhibited low and high Si accumulation. The screen was initially carried out between October 2017 and May 2018 with plants grown without Si fertilisation (–Si). However, due to the low plant Si accumulation observed, the screen was repeated between March and July 2019 with Si fertilisation (+Si). Four seeds of each landrace were planted in 0.5 L pots filled with F2+S compost (Levington) and treated with Calypso insecticide (Bayer). One week after germination, seedlings were thinned to two plants per pot. Plants were grown for seven weeks under controlled glasshouse conditions (16 h daylight; 20 °C /15 °C day/night). For +Si plants, starting one week after germination and continuing until harvest, plants received 100 mL 1.5 mM sodium metasilicate ($Na_2SiO_3.9H_2O$) twice weekly. As the +Si screen was conducted after the –Si screen, the sodium ions were not balanced between the two screens. Plants were watered as required with tap water to maintain the soil moisture content. For both screens, three temporally separate replicates were conducted, separated by at least two weeks. At harvest, shoot and root fresh weight were recorded. Roots were cleaned with tap water and excess water removed prior to weighing. Plants were then oven dried at 70 °C until constant mass was achieved and dry weight recorded.

2.2.1.2 Experiment 2: Effect of Si availability on Si accumulation

To further examine variation in Si accumulation between wheat landraces, as well as the potential causes of this variation, ten landraces that consistently accumulated high levels of Si and ten landraces that consistently accumulated low levels of Si in the –Si screen of landraces were grown hydroponically at three Si levels. Additional plants for the three highest and three lowest Si-accumulating landraces were grown and harvested weekly to investigate whether Si accumulation varies over time.

Seeds were germinated in sand for 10 d, then transferred to 9 L hydroponics boxes filled with ½-strength Hoagland's solution. Three different levels of Si were applied to the hydroponics boxes: no additional Si (–Si), 0.9, and 1.8 mM Si, to represent the range of Si

concentrations that are typically found in soils (Sommer *et al*., 2006) as well as the maximum solubility of silicic acid (Iler, 1979). Five temporally separate replicates were conducted, separated by at least two weeks. For each replicate, at each level of Si availability, all landraces were grown in one hydroponics box, such that three 9 L boxes were used for each replicate. Each hydroponics contained a total of 50 seedlings: one seedling of each of the twenty landraces, plus additional seedlings of the six landraces used for weekly harvests. Sodium metasilicate ($Na_2SiO_3.9H_2O$) was used for the Si treatments and sodium chloride was used to balance sodium levels across all levels of Si availability. The pH was adjusted to 5.6-6.0 using 1 M HCl or 0.1 M KOH. The nutrient solution was changed every 3-4 d and aerated throughout the experiment. Plants were grown in controlled glasshouse conditions (16 h daylight, 20 °C/15 °C day/night). To investigate whether Si accumulation varies over time, every week, from two to seven weeks after germination, one plant from each Si treatment for each of six landraces was harvested. The remaining plants were harvested six weeks after germination. At harvest, roots were washed in deionised water and then shoot and root fresh weights were recorded. Plants were oven-dried at 70 °C until constant mass was achieved and dry weight recorded.

2.2.2 Si measurements

For all experiments, shoot and root Si concentration was measured by portable X-ray fluorescence spectroscopy (P-XRF) using the method described in Reidinger *et al*. (2012). Dried leaf material was ball-milled (Retsch MM400 Mixer mill, Haan, Germany) and ground material was pressed at 10 tons into pellets using a manual hydraulic press with a 13 mm die (Specac, Orpington, UK). Si analysis (% Si dry weight) was performed using a P-XRF instrument (Nitron XL3t900 GOLDD analyser: Thermo Scientific Winchester, UK) held in a test stand (SmartStand, Thermo Scientific, Winchester, UK). The P-XRF machine was calibrated using Si-spiked synthetic methyl cellulose (Sigma-Aldrich, product no. 274429) and validated using Certified Reference Materials of NCS DC73349 'Bush branches and leaves' obtained from China National Analysis Center for Iron and Steel. To avoid signal loss by air absorption, the analyses were performed under a helium atmosphere (Reidinger *et al*., 2012). A reading of each side of the pellet was taken, approximately one hour apart, to account for *u*-drift in the instrument (i.e. variation in readings between consecutive runs using identical parameters; Johnson, 2014). The two readings were averaged to obtain the Si concentration (% dry weight).

2.2.3 Si transporter single nucleotide polymorphism identification

Using identified single nucleotide polymorphism (SNP) data provided by Dr Andrea Harper, SNPs in putative Si transporter genes were identified. Analysis of variance (ANOVA), conducted in R (version 3.6.1, R Core Team, 2020), was used to investigate whether any of the identified SNPs were associated with significantly different Si accumulation across the diversity panel of 98 landraces. Only SNPs with a minor allele frequency greater than 5 % were used.

2.2.4 Associative transcriptomics

Associative transcriptomics (AT) is a recently developed method that uses transcriptome data to test for associations between differences in gene sequence or expression and traits of interest (Harper *et al*., 2012). Polyploid species often display functional redundancy or differential expression of homoeologous genes, in addition to having large sequence repeat regions (Borrill *et al*., 2019). Such complex, polyploid genomes are not easily amenable to Genome Wide Association Study (GWAS) techniques, but by using transcriptome data instead of genomic data, AT aims to avoid some of the issues associated with applying GWAS to polyploid species such as wheat. For these analyses, Single Nucleotide Polymorphism (SNP) and transcript abundance data (reads per kb per million aligned reads; RPKM) from RNA sequencing of the leaves of 15-days-old wheat seedlings, grown in glasshouse conditions without additional Si, was provided by Dr Andrea Harper.

An association analysis was performed to look for SNPs correlating with differences in Si accumulation among the 98-landrace diversity panel when grown with Si. A mixed linear model (MLM) was fitted in R (version 3.6.1, R Core Team, 2020) using the GAPIT package (Lipka *et al*., 2012; Zhang *et al*., 2010). Population structure was incorporated using kernel-PCA and optimisation (PSIKO, Popescu *et al*., 2014) as a fixed effect. Relatedness was included as a random effect using a kinship matrix generated by GAPIT (Zhang *et al*., 2010). A Shapiro-Wilk test was used to evaluate whether Si values followed a normal distribution, and a logit transformation was applied to satisfy the assumption of normality. SNPs with a minor allele frequency of less than 5 % were excluded from the analysis to minimise the risk of spurious associations. A total of 179 254 SNP markers were used. Model fit was assessed using Q-Q plots between expected and observed log10(P) values, which indicated

that model over-fitting was a problem. A significance level of 0.05 was set after applying a false discovery rate (FDR) controlling procedure (Benjamini and Hochberg, 1995).

Gene expression marker (GEM) analysis was performed in R (version 3.6.1, R Core Team, 2020) using scripts provided by Dr Andrea Harper (Harper *et al*., 2012). A fixed effect linear model was used to test for associations between GEMs based on transcript abundance and shoot Si concentration. Population structure was incorporated using PSIKO as a fixed effect (Popescu *et al*., 2014). Transcripts with RPKM values less than 0.4 averaged across landraces were removed. A total of 46 248 GEMs was used for the analysis. Si values were logit transformed to satisfy the assumption of normality. A significance level of 0.05 was set after applying a FDR controlling procedure (Benjamini and Hochberg, 1995).

2.2.5 Differential gene expression analysis

Due to problems with model over-fitting for GWAS, differential expression (DE) analyses on subsets of high and low Si-accumulating landraces were performed to investigate whether there were any consistent differences in gene expression between the two groups. In contrast to GWAS and AT, DE uses only information on gene expression, and does not consider differences in gene sequence (SNP data). Additionally, DE compares gene expression for two groups, in this case high and low Si accumulators, rather than across all landraces.

Data normalisation and DE analysis was performed using the DESeq2 package (Love *et al*., 2014) in R (version 3.6.1, R Core Team, 2020). Transcriptomic count data from the leaves of 15-days-old wheat seedlings grown in glasshouse conditions without Si supplementation was provided by Dr Andrea Harper. Genes with low expression, defined as having a mean count of one across landraces, were removed prior to the analysis. A significance level of 0.05 was set after applying an FDR controlling procedure (Benjamini and Hochberg, 1995).

In total, four DE analyses were performed using different groupings of high and low Si accumulators:

- Group 1: 20 highest and 20 lowest Si accumulating landraces identified from experiment 1

- Group 2: Nine highest and seven lowest Si accumulators from experiment 1 for +Si plants only
- Group 3: Seven high and five low Si accumulating landraces identified from experiment 2
- Group 4: Five high and five low landraces used for subsequent experiments (see 3 and 4)

DE analyses were performed using different groups of high and low Si accumulators to reduce the likelihood of false positives. Such false positives most commonly occur when the contrasting groups are small (five or less). Thus, groups containing different numbers of high and low Si accumulating landraces were used. Shoot Si accumulation was significantly different between high and low Si accumulators for all groups (*t*-tests, $P < 0.05$ for all comparisons; Table 2.6). Only genes identified as being differentially expressed between high and low Si accumulators in all four analyses were considered as true hits. Gene annotation was done using Ensembl (Howe *et al*., 2021) and gene ontology enrichment analysis was performed using Panther (Mi *et al*., 2021).

2.2.6 Spine and stomatal density

For experiment 2, at harvest, approximately 5 cm of one leaf from each plant was cut with scissors and painted with clear nail varnish to make an epidermal peel. Once dry, the varnish was removed from the leaf using transparent sticky tape and stuck to a microscope slide. A Nikon Eclipse 50 I light microscope (Nikon Instruments, Kingston Upon Thames, Surrey) at 200 x magnification was used to count the number of stomata and spines for ten fields of view. The average spine/stomata density was calculated as spines/stomata mm^{-2}. Spine and stomatal density were only measured for two replicates.

2.2.7 Experiment 3: Scanning electron microscope and energy dispersive X-ray spectroscopy

To investigate whether Si is deposited differently in high and low Si-accumulating landraces, one high (H1) and one low (L4) Si-accumulating landrace was selected for scanning electron microscope and energy dispersive X-ray spectroscopy (SEM-EDX) analysis. Two replicate plants of each landrace were used. Plants were grown in compost, supplemented with 50 mL 1.5 mM sodium metasilicate ($Na_2SiO_3.9H_2O$) twice weekly and leaf samples were taken at 37-days-old. A rectangular section (~3 mm x 10 mm) of leaf

material was cut with a razor blade from a mature, expanded leaf blade on the main stem and immediately placed in deionised water. The samples were transferred to a fixative composed of 2.5 % glutaraldehyde and 4 % formaldehyde in 100 mM phosphate buffer. The samples were put in a vacuum chamber to remove the air and ensure leaves were completely under the fixative. An antistatic wetting agent and a cocktail stick were used for leaf segments that floated on top of the fixative. The samples were rotated for 6 h. Samples were washed twice in 100 µM phosphate buffer. An acetone graduated series was then used to dehydrate the samples by rotating samples for 30 min in 25, 50, 70, 90, 100, 100, and 100 % acetone. Samples were critical point dried and mounted on aluminium stubs using sticky carbon tape. Samples were coated in carbon and stored in a desiccator overnight. A JEOL 7800F Prime High Resolution Field Emission Scanning Electron Microscope coupled to an EDX (Thermo-Scientific) was used to image the samples and determine their elemental composition at an accelerating voltage of 15 kV.

2.2.8 Stomatal conductance

Transpiration is related to stomatal conductance (Lawson and Blatt, 2014). Therefore, stomatal conductance was used as a proxy for transpiration rate. For plants from experiment 2, after two weeks establishment in hydroponics, the stomatal conductance of six landraces was measured using an AP4-UM-3 porometer (Delta-T devices Ltd, Cambridge, United Kingdom). The three highest and three lowest Si-accumulating landraces based on the –Si screen from experiment 1 (2.2.1.1) were selected. However, these were later reclassified based on the results of experiment 2 (2.2.1.2), such that three high, two medium, and one low Si-accumulating landrace were used. Measurements were conducted in the glasshouse and the porometer was recalibrated before every use. Measurements were made at approximately midday. Large, healthy, new leaves from the top of the plant were used for measurements. Both sides of the leaf were measured and the average calculated. A minimum of five readings, on at least five different days, were taken for each landrace at each Si level for each replicate. The average stomatal conductance across all days for each plant was used for statistical analysis.

2.2.9 Experiment 4: Transpiration measurements

To further investigate whether differences in transpiration rate were correlated with differences in Si accumulation, an experiment was designed to measure transpiration

based on water loss over time. Seeds were germinated in sand for 10 d, then transferred to 50 mL falcon tubes filled with ½-strength Hoagland's solution. The nutrient solution was changed every two days. Half the plants were grown with 0.2 mM (low) Si and half the plants were grown with 1.8 mM (high) Si. A low Si treatment of 0.2 mM Si was selected instead of a no Si treatment due to the lack of variation in Si accumulation when plants are grown without Si. Dissolved sodium metasilicate ($Na_2SiO_3.9H_2O$) was used as a source of Si. Six landraces were selected, covering the full range of Si accumulating abilities and three plants per landrace per Si treatment were used.

After growing in falcon tubes for two weeks, the amount of nutrient solution in the falcon tubes was measured over three consecutive days to measure water loss. Tubes filled with nutrient solution but without plants were used to measure the evaporation rate. The transpiration and water uptake rates were calculated based on plant dry weight (DW) as:

Equation 2.1:

$$Transpiration\ (mL\ H_2O\ hr^{-1}\ g\ DW^{-1}) = \frac{Initial\ volume\ (mL) - Final\ volume\ (mL) - Evaporation\ (mL)}{Time\ (hr)\ \times Shoot\ dry\ weight\ (g)}$$

Equation 2.2:

$$Root\ water\ uptake\ (mL\ H_2O\ hr^{-1}\ g\ DW^{-1}) = \frac{Intial\ volume\ (mL) - Final\ volume\ (mL) - Evaporation\ (mL)}{Time\ (hr) \times Root\ dry\ weight\ (g)}$$

Due to the similarity of results between transpiration and water uptake, only the results for the transpiration rate are presented.

2.2.10 Measuring Si transporter gene expression

To investigate the possible causes of variation in Si concentration between landraces, the expression levels of the Si transporters *Lsi1, Lsi2, Lsi3,* and *Lsi6* were determined using reverse transcriptase quantitative polymerase chain reaction (RT-qPCR). Primers were designed to match all homoeologs, based on existing wheat sequences where available, or on homology to the barley sequence (Table 2.1). Ensembl (Howe *et al*., 2021) was used to

identify homoeologs and wheat-expression.com (Borrill *et al*., 2016; Ramírez-González *et al*., 2018) was used to check that putative genes were expressed. Primers were designed to match all homoeologs. Standard curves using serial 1:10 dilutions at 4 concentrations were used to determine primer efficiency for qPCR. Only primers with standard curve slopes between -3.0 and -3.6 were selected for subsequent qPCR.

The three highest and three lowest Si-accumulating landraces, and one medium Si-accumulating landrace were selected for RT-qPCR. Expression was measured for –Si plants and plants supplemented with 1.8 mM Si from experiment 2. Three biological replicates were used. Root tissue was collected and ground under liquid nitrogen using a mortar and pestle and RNA extracted using a Nucleospin RNA Plant and Fungi kit with DNase treatment (Macherey Nagel Bioanalysis), according to the manufacturer's instructions. RNA quality was checked using a NanoDrop 1000 (ThermoFisher).

Two cDNA synthesis reactions were performed using Reverse Transcriptase Superscript II M-MLV (Invitrogen). qRT-PCR was performed using fast SYBR green master mix (Applied Biosystems) with 2 µL cDNA and 350 nM primer on a QuantStudio3 Real Time PCR System instrument (ThermoFisher) with cycle conditions: denaturation: 95 °C for 20 s; cycling: 95 °C for 1 s, 60 °C for 20 s for 40 cycles; determination of melt curve to determine primer specificity by checking for single product amplifications. Two technical replicates of each of the two cDNAs were used (four technical replicates for each biological sample). After testing several potential housekeeping genes, actin was selected as a reference gene because it was expressed at a consistent level in all samples. A no template control was included. qPCR results were analysed using the method of Muller *et al*. (2002) and an adapted version of the Q-gene excel software (Simon, 2003). Primer efficiency was calculated as:

Equation 2.3:

$$Primer\ efficiency = 10^{\frac{1}{Standard\ curve\ gradient}}$$

Equation 2.4:

Normalised expression values were then calculated as:

$$Normalised\ expression = \frac{Actin\ primer\ efficiency^{C_t\ value}}{Si\ transporter\ primer\ efficiency^{C_t\ value}}$$

The average normalised expression for each of the four technical replicates per sample was used.

Table 2.1: List of primers used for qPCR.

Target Gene	Forward Primer	Reverse Primer	Product size	Ensembl gene name (all homoeologues)
Actin	TACTCCCTCACAACAACCGC	CTCCTAGCCGTTTCCAGCTC	104	TraesCS1A02G020500, TraesCS1B02G024500, TraesCS1D02G020000
Lsi1	CCTTCTCCAGCGAGATCCAC	CCTCCGACACCACCTTCTTG	129	TraesCS6A02G307300, TraesCS6B02G335900, TraesCS6D02G286400
Lsi2	TCATCGCCTTCAACAGCAAG	TCCTTCCAGTACATGCAGAGC	115	TraesCS5A02G529900, TraesCS4B02G361900, TraesCS4D02G354900
Lsi3	TGTTCAAGTACCTCGGCAAC	TTGAGGATGAACTCGGTGAGG	144	TraesCS4A02G412500, TraesCS4B02G312600, TraesCS4D02G310100
Lsi6	TACTCGAACGAGATCCACGAC	TCTCCGATATCACCTTCTTGCC	132	TraesCS7A02G187800, TraesCS7B02G092900, TraesCS7D02G188800

2.2.11 Statistical analysis

All statistical analyses were performed using R software (version 3.6.1, R Core Team, 2020). Summary statistics were calculated using the Rmisc package (Hope, 2013) and graphs were produced using the ggplot2 package (Wickham, 2016). Two-way analysis of variance (ANOVA) was used to test the effect of Si supplementation and landrace on Si concentration, dry weight, spine density, stomatal density, stomatal conductance, transpiration, and gene expression. Three-way ANOVA was used to test the effect of Si supplementation, landrace and time on Si concentration for plants that were harvested weekly. In all ANOVAs, temporal replicate was included as a factor to account for variation caused by plants being grown at different times.

Data normality was checked using Shapiro-Wilk tests and homogeneity of variance was tested using Levene's tests. To satisfy the test assumptions, Si concentrations were logit transformed, shoot dry weight and gene expression data was log transformed, and spine density was square root transformed. Untransformed values were used for stomatal density and stomatal conductance. A significance level of $P < 0.05$ was used for all analyses, except for gene expression analyses, where a Bonferroni correction to account for multiple testing was applied. Significant results were analysed by performing Tukey's Honest Significance Difference (HSD) *post-hoc* tests using the emmeans package (Lenth, 2021).

2.3 Results

2.3.1 Significant variation in Si accumulation among wheat landraces

Significant variation in Si accumulation across the 98 landraces used in experiment 1 was observed for both plants that received Si supplementation and those that did not (Figure 2.1; Table 2.3). Si supplementation increased the amount of variation in Si accumulation between landraces. There was a positive correlation between Si accumulation with and without Si supplementation ($r = 0.49$, $P < 0.001$). Increased Si supply resulted in 69.5 % to 155.7 % more Si being accumulated depending on the landrace, and on average, plants grown with supplementary Si accumulated 109 ± 0.002 % more Si compared to plants grown without additional Si.

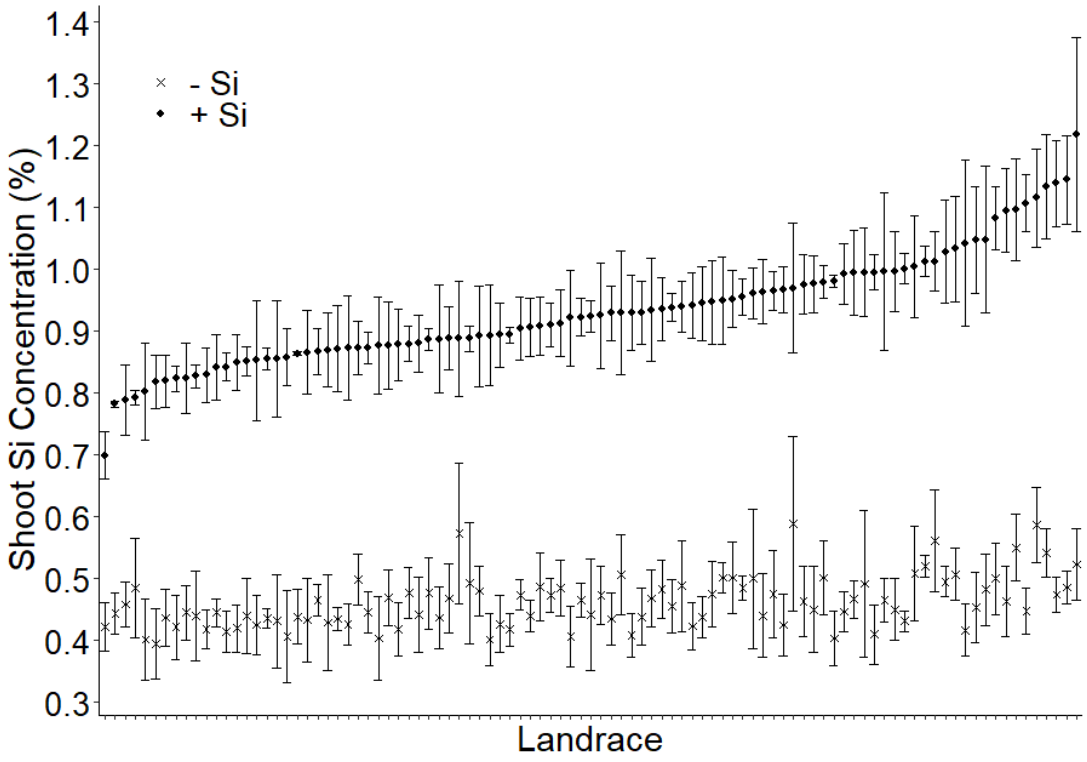

Figure 2.1: Variation in shoot Si concentration for a diversity panel of 98 wheat landraces (Experiment 1). –Si plants did not receive Si supplementation. +Si plants were supplemented 1.5 mM sodium silicate. Shown in order of increasing Si for +Si plants. Mean values ± standard error (SE) are shown. N = 3.

Based on shoot Si concentration when grown with and without Si fertilisation, ten landraces that accumulated high levels of Si, and ten landraces that accumulated low levels of Si were selected for further experiments. These two groups differed significantly in their Si accumulation (Table 2.4) and were designated as high (H) and low (L) Si accumulators, respectively. When grown with Si, high Si accumulators had an average shoot Si concentration of 1.05 ± 0.03 % compared to 0.90 ± 0.02 % for low Si accumulators.

2.3.2 Negative correlation between Si accumulation and growth

To determine whether there was an effect of Si on growth, the relationship between Si accumulation and shoot dry weight was investigated. There was a negative correlation between shoot dry weight and shoot Si concentration across the diversity panel (Figure 2.2; –Si: r = -0.84, P = < 0.001; +Si: r = -0.43, P < 0.001).

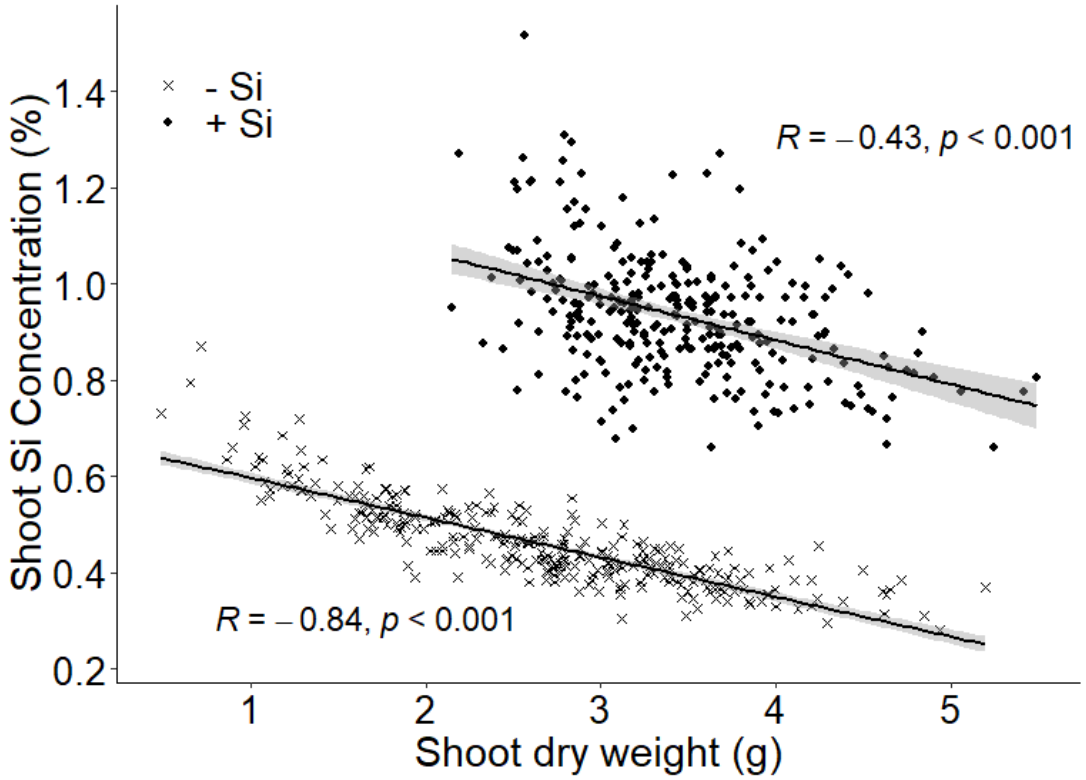

Figure 2.2: Correlation between shoot Si concentration and shoot dry weight for plants grown with and without Si supplementation (Experiment 1). Plotted are the results for each landrace for each replicate.

2.3.3 No evidence of genetic causes of variation in Si among wheat diversity panel

To specifically test whether SNP differences in putative Si transporter genes correlated with differences in Si accumulation among landraces, SNPs in putative Si transporters were identified and their effect on Si accumulation was tested using ANOVA. No SNPs were identified in putative *Lsi1* or *Lsi2* sequences. Across all homoeologs, 28 SNPs were identified in putative *Lsi3* sequences and 12 SNPs in putative *Lsi6* sequences. However, only eight of these SNPs had a minor allele frequency greater than 5 % and so were used to test for an association with Si accumulation. No significant associations were found (Table 2.5).

As no significant SNPs were identified in Si transporter genes, whether differences in Si accumulation between landraces correlated with differences in gene sequence and expression across the whole genome was investigated. AT analyses were performed using the Si concentrations for the diversity panel of 98 landraces when grown with Si. No

significant SNPs or GEMs were identified, although model over-fitting was a problem due to the low number of landraces used.

Differential expression (DE) analyses were performed to investigate whether there were differences in gene expression between high and low Si accumulators. No significant differences in gene expression were found for the Si transporter genes. However, 59 genes were identified as being consistently differentially expressed between groups of high and low Si accumulators. These genes related to a range of processes (Table 2.7). A gene enrichment analysis indicated that genes related to the biological processes of cold acclimation, water deprivation, and the response to abscisic acid (ABA) were significantly over-represented. This corresponded to a group of genes encoding putative dehydrins that were expressed at lower levels in high Si accumulators relative to low Si accumulators. Dehydrins are a group of hydrophilic proteins involved in protecting the plant during dehydration (Graether and Boddington, 2014). When focussing on molecular functions or cellular components, no pathways were identified as being significantly over- or under-represented.

2.3.4 Increasing Si availability increases variation in Si accumulation among selected landraces

To further characterise variation in Si accumulation among wheat landraces, the ten highest and ten lowest Si accumulators identified from the diversity panel for –Si plants were selected and grown hydroponically at different levels of Si availability (experiment 2). Increasing Si availability significantly increased both shoot and root Si accumulation in all landraces, although the increase was larger in the shoots (Figure 2.3). At all levels of Si availability, there was significant variation in shoot Si accumulation among landraces, but this variation was more pronounced at higher levels of Si availability (Table 2.8). There was no significant variation in root Si accumulation between landraces (Table 2.8).

To investigate the causes and consequences of variation in Si accumulation in subsequent experiments, this study aimed to identify landraces that consistently accumulated high or low levels of Si. It was hypothesised that landraces with contrasting Si accumulating abilities may respond differently to Si supplementation. Thus, one major aim of this study was to identify landraces that consistently accumulated either high or low levels of Si, in different growing conditions and with different levels of Si availability. However, slightly

different trends in Si accumulation ability were observed between plants from experiment 1 (in compost) and experiment 2 (hydroponics). In particular, there was a larger range in Si accumulating ability among the landraces in experiment 2, when plants were grown hydroponically with supplementary Si, suggesting that an extra category of Si accumulation ability was required. Thus, landraces were reclassified as high, low, and medium Si-accumulation types that were consistently ranked as having the highest, lowest, and middle Si concentration at a given level of Si availability, respectively (Figure 2.3).

To establish the accumulation types, landraces were ranked according to their Si accumulation in both experiment 1 and experiment 2, at each level of Si availability. Landraces that were consistently ranked as the highest or lowest Si accumulators at all levels of Si availability were classified as high (H) and low (L) Si accumulators respectively. However, some landraces showed variable levels of Si accumulating ability depending on the experiment and Si availability. Such landraces were classified as medium (M) Si accumulators. Among the medium Si accumulators, M1-M3 were identified as high Si accumulators in experiment 1 when grown without Si addition, while M4-M8 were identified as low Si accumulators. Landraces H1-H6 displayed high Si accumulation when grown both in compost and hydroponically, while landraces L2-L5 likewise displayed low Si-accumulating ability. Interestingly, landrace L1 was identified as a high Si accumulator in the –Si diversity panel screen, while H7 was identified as a low Si accumulator. For plants supplemented with 1.8 mM Si, the average shoot Si concentration was 2.35 ± 0.11 %, 2.80 ± 0.12 %, and 3.24 ± 0.10 % for low, medium, and high Si accumulators, respectively.

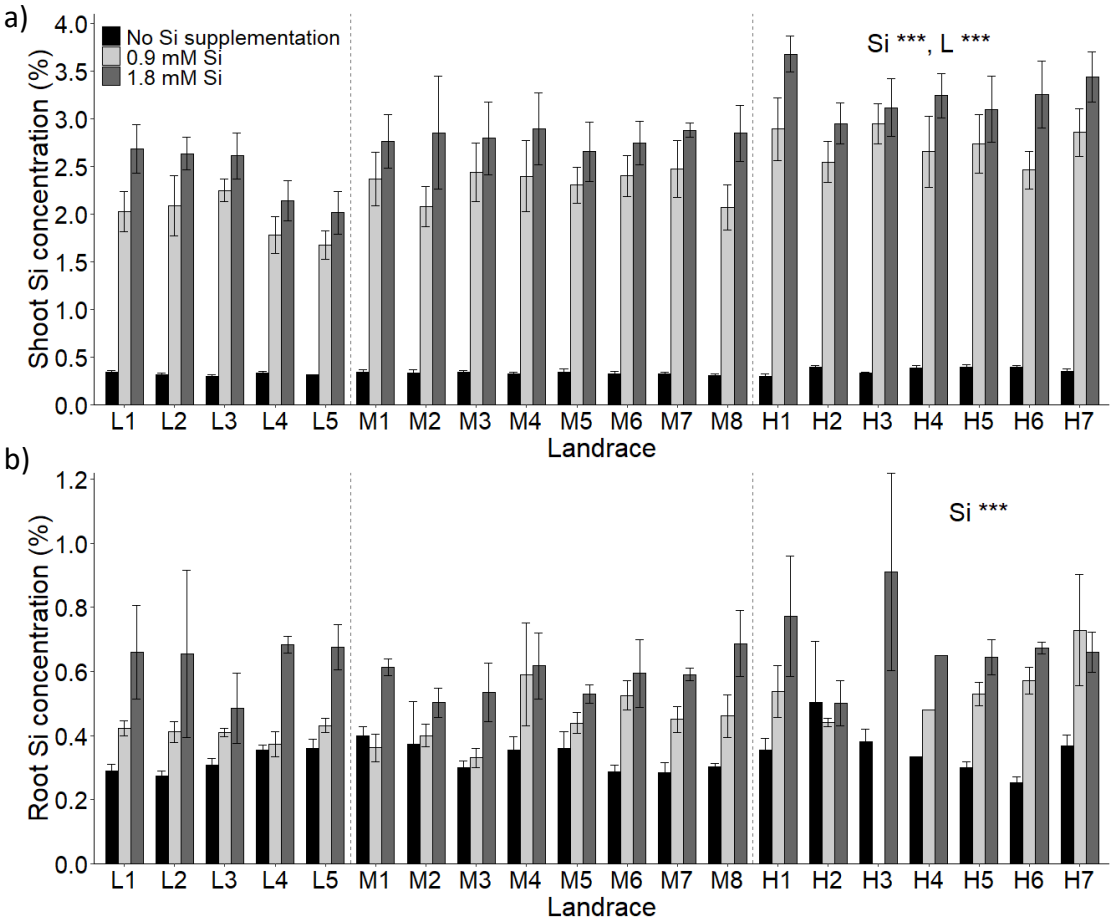

Figure 2.3: Variation in Si accumulation among selected wheat landraces grown with different levels of Si (Experiment 2). a) Shoot Si concentration. b) Root Si concentration. Note the different scales on the y-axis. Statistically significant impacts and interactions, determined by two-way ANOVA, are indicated in each panel, where *** $P < 0.001$, ** $P < 0.01$, and * $P < 0.05$. Mean values ± SE are shown. N = 5. L: landrace, Si: level of Si availability. Landraces were assigned as low (L), high (H), and medium (M) Si accumulators based on their Si accumulation in both experiments 1 and 2. For a small number of plants, the roots were too small to measure the root Si concentration, resulting in missing bars and error bars.

2.3.5 Limited variation in Si accumulation over time

Previous studies have found that plants take several weeks or longer to accumulate high levels of Si (Deshmukh *et al.*, 2020; Hodson and Sangster, 1998). To investigate whether landraces accumulate Si at different rates, plants were grown hydroponically at three levels

of Si supplementation and harvested weekly to measure their Si concentration (experiment 2). Three high and three low Si-accumulating landraces were selected based on the results from the –Si screen with 98 landraces, although two landraces were reclassified as medium Si accumulators based on the results of the hydroponics experiment (2.3.4).

Only small changes in root and shoot Si accumulation were observed over time (Figure 2.4). The ANOVA results indicated that there was a significant effect of time on shoot and root Si accumulation (Table 2.9). However, *post-hoc* testing indicated that this was driven by a significant decrease of 11.1 ± 3.2 % and 27.8 ± 15.6 % in shoot and root Si, respectively, for –Si plants when averaged across all landraces between weeks 3 and 7. Compared to three-weeks-old plants, there was a small but non-significant increase in shoot Si for seven-weeks-old plants of 4.7 ± 0.9 % and 15.0 ± 4.5 % for plants supplemented with 0.9 mM and 1.8 mM Si, respectively. For plants supplemented with 0.9 mM Si and 1.8 mM Si, respectively, root Si was 24.6 ± 9.0 % and 4.9 ± 2.1 % lower for seven-weeks-old plants compared to three-weeks-old plants, but this decrease was not significant.

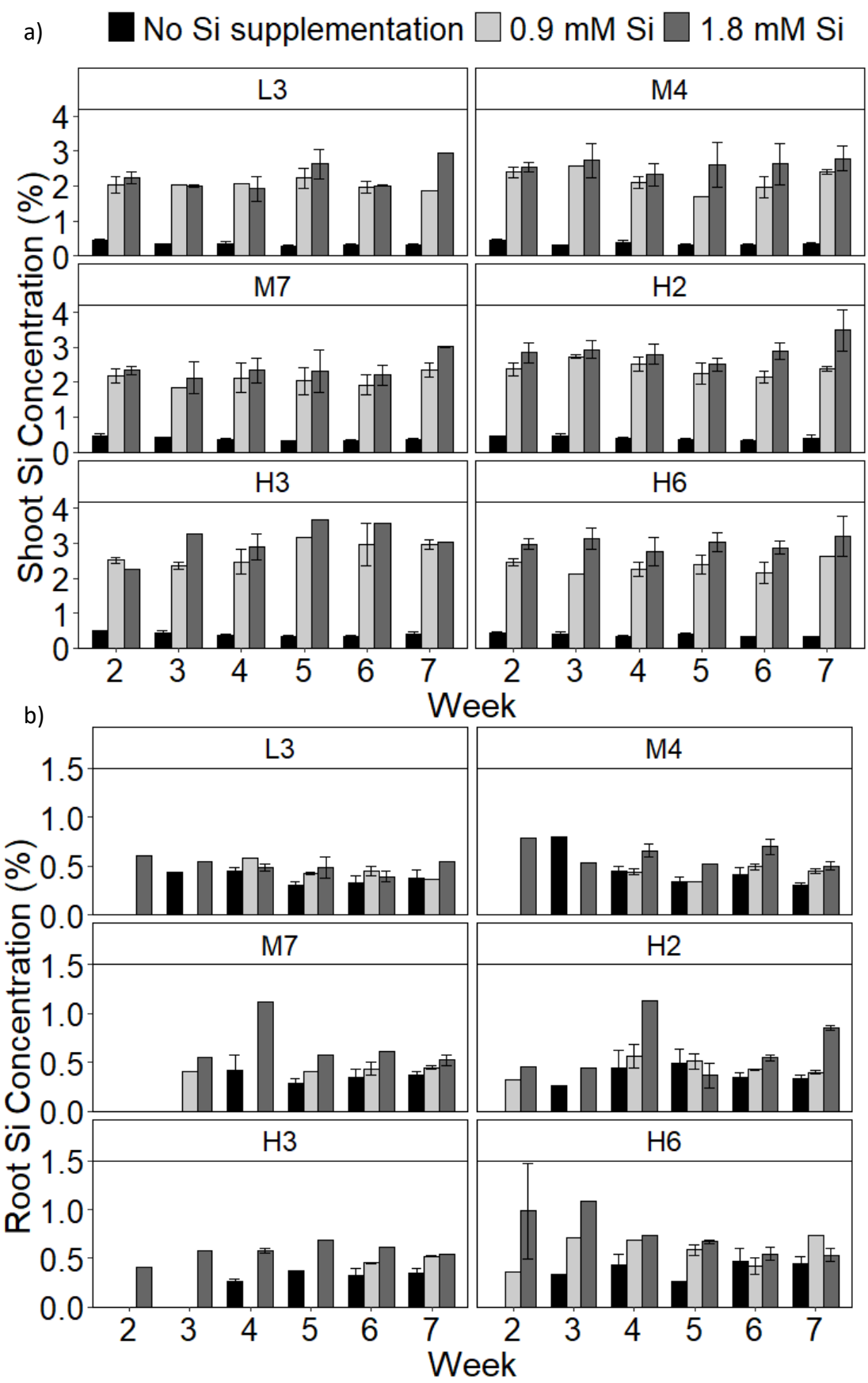
a)
No Si supplementation
0.9 mM Si
1.8 mM Si
L3
M4
M7
H2
H3
H6
Shoot Si Concentration (%)
0
1
2
3
4
2
3
4
5
6
7
Week
b)
Root Si Concentration (%)
0.0
0.5
1.0
1.5
Week

Figure 2.4: Variation in Si accumulation over time for landraces grown at different levels of Si availability (Experiment 2). a) Shoot Si concentration. b) Root Si concentration. Note different scales on y-axis. Mean values ± SE are shown. N = 3. Due to the small size of some plants, especially at earlier time-points, it was not possible to determine the Si concentration of all samples, resulting in missing bars and error bars. High (H), Low (L), and Medium (M) Si-accumulating landraces are indicated.

2.3.6 No consistent effect of different levels of Si availability on growth

Shoot dry weight was highly variable between landraces and Si treatments (experiment 2; Figure 2.5). ANOVA results indicated that while dry weight varied significantly among landraces, there was no significant effect of Si (Table 2.8). Although the effect was not significant, there was a trend such that landraces L4, M5, M8, H4, H5, and H6 consistently exhibited larger shoot dry weight when grown in the presence of Si than when grown without Si. By contrast, landraces L3, M6, and H7 exhibited a lower shoot dry weight when grown at high levels of Si availability.

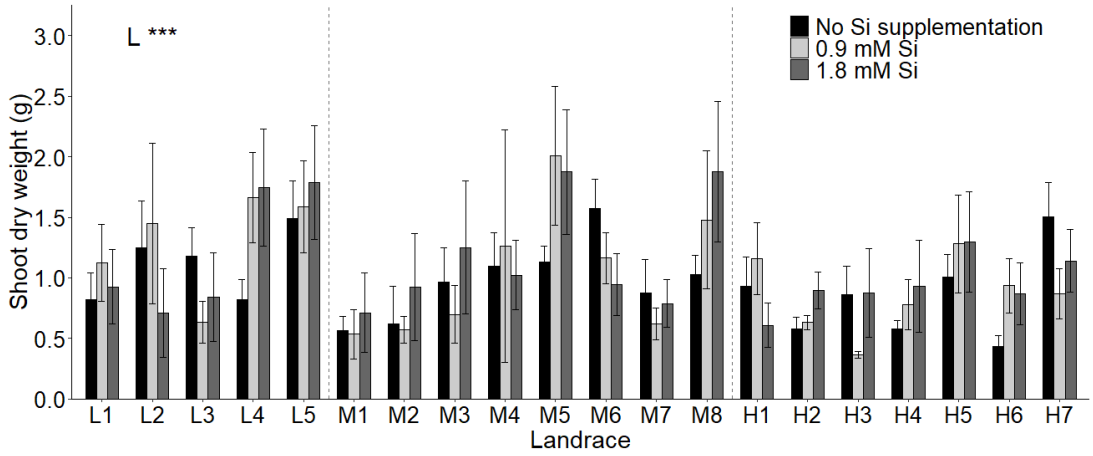

Figure 2.5: Variation in shoot dry weight for plants grown at different levels of Si availability (Experiment 2). Statistically significant impacts and interactions, determined by two-way ANOVA, are indicated in each panel, where *** $P < 0.001$, ** $P < 0.01$, and * $P < 0.05$. Mean values ± SE are shown. N = 5. L: landrace, Si: level of Si supplementation. Landraces were assigned as low (L), high (H), and medium (M) Si accumulators based on shoot Si when grown hydroponically with 1.8 mM and 0.9 mM Si.

2.3.7 Variable effect of Si on spine density

In the leaves, Si is often deposited in structures such as leaf hairs, trichomes, and spines (Figure 2.6; Hartley *et al*., 2015). It was hypothesised that differences in Si accumulation between landraces may correlate with differences in spine density. However, although spine density varied significantly among landraces, no significant effect of Si on spine density was observed in experiment 2 (Figure 2.7; Table 2.8). There was a positive correlation between shoot Si concentration and spine density for plants supplemented with 0.9 mM Si (0.9 mM Si: r = 0.36, P = 0.024), but not for plants grown with 1.8 mM Si (r = 0.29, P = 0.091), or for –Si plants (r = 0.14, P = 0.440).

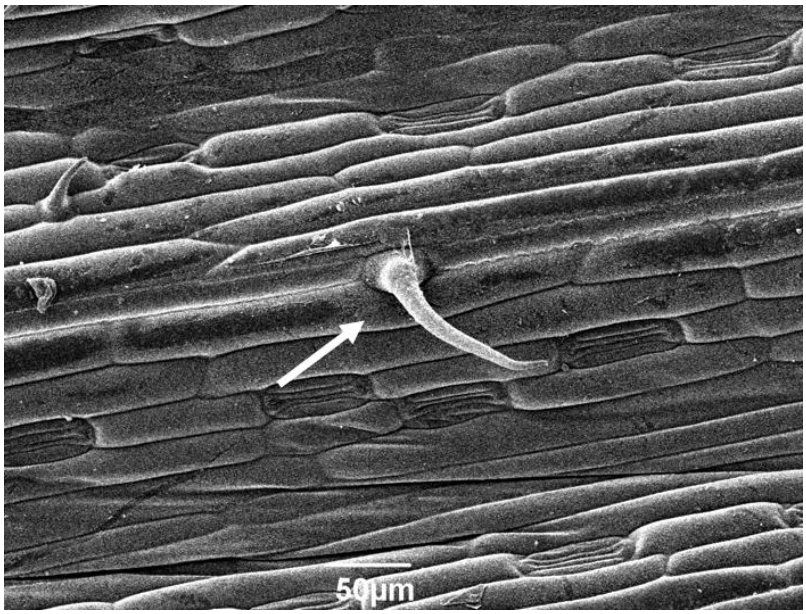

Figure 2.6: Scanning electron microscope (SEM) image of a silicified spine. 270 x magnification. White arrow indicates spine. Plant from experiment 3 (2.2.7).

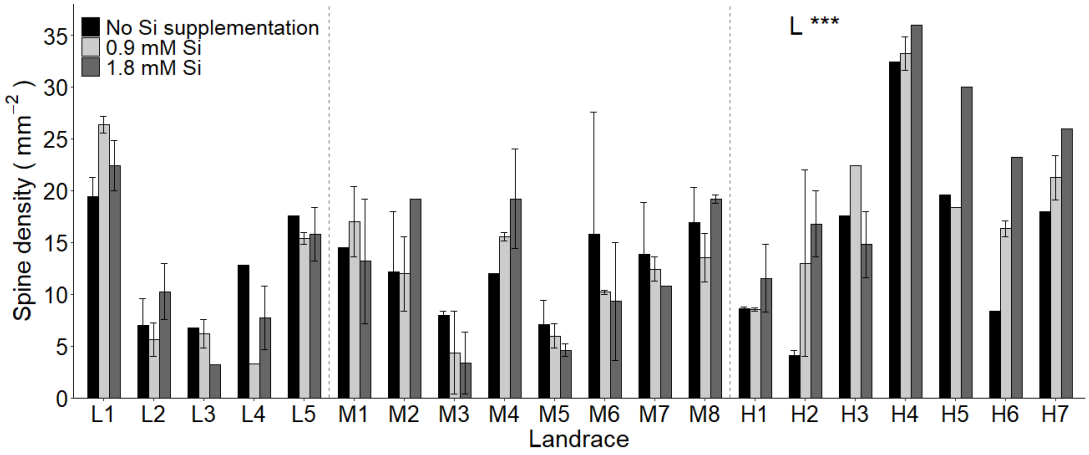

Figure 2.7: Variation in spine density for plants grown at different levels of Si availability (Experiment 2). Statistically significant impacts and interactions, determined by two-way ANOVA, are indicated in each panel, where *** $P < 0.001$, ** $P < 0.01$, and * $P < 0.05$. Mean values ± SE are shown. N = 2. L: landrace, Si: level of Si supplementation. Landraces were assigned as low (L), high (H), and medium (M) Si accumulators based on shoot Si when grown hydroponically with 1.8 mM and 0.9 mM Si.

To further examine differences in Si deposition between landraces, the leaf of one high (H1) and one low (L4) Si-accumulating landrace was selected and imaged using SEM-EDX (experiment 3; Figure 2.8). Si deposition was similar in both landraces, with high levels of Si deposited in silica cells and as silicified spines. Lower levels of Si deposition were observed across the leaf surface.

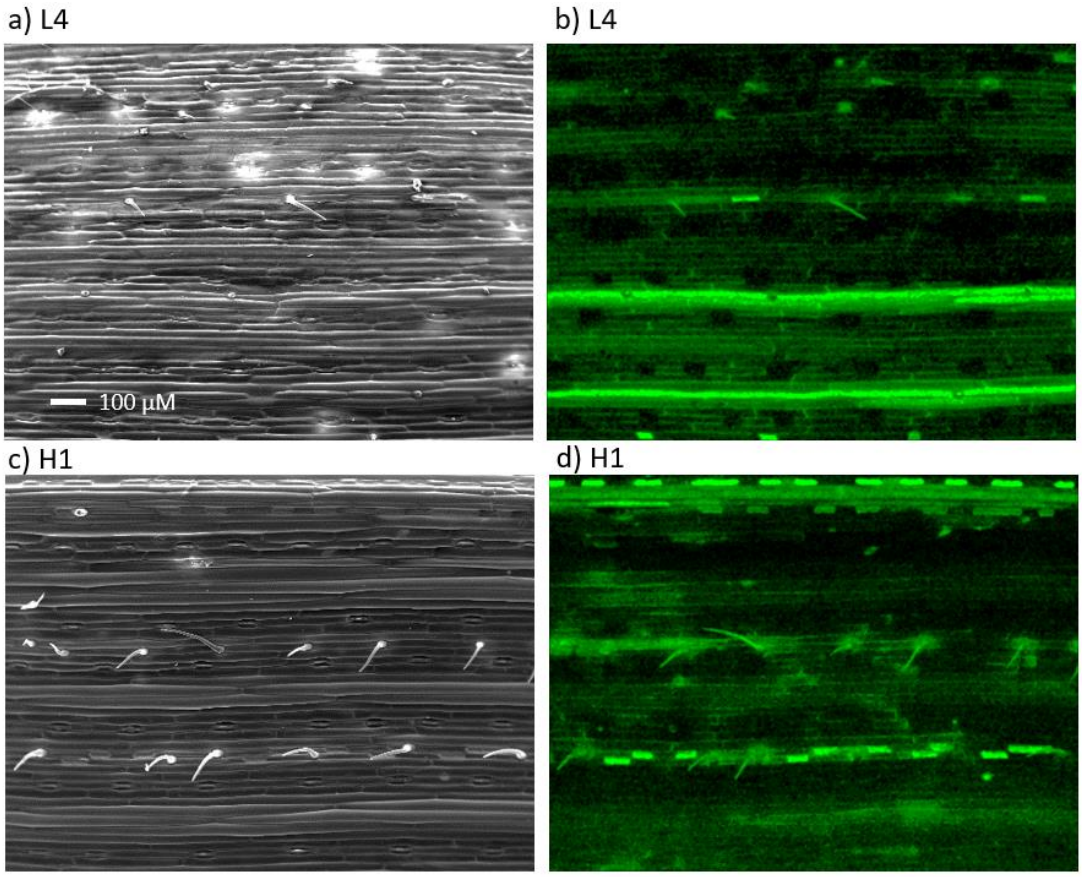

Figure 2.8: Variation in Si deposition between a high and low Si-accumulating landrace (Experiment 3). Representative scanning electron microscope (SEM) images (a and c) and energy dispersive X-ray (EDX) fluorescence of Si deposition (b and d) of a low Si-accumulating landrace (L4; a and b) and a high Si-accumulating landrace (H1; c and d). For EDX images, green intensity indicates Si concentration.

2.3.8 No correlation between shoot Si concentration and stomatal conductance

The extent to which Si accumulation relates to transpiration rate is currently debated (Exley, 2015; Kumar *et al*., 2017; McLarnon *et al*., 2017). Therefore, the relationship between Si accumulation and transpiration rate in the wheat landraces was investigated. Stomatal conductance is often correlated with transpiration rate (Lawson and Blatt, 2014). Thus, the stomatal conductance of six landraces was measured (experiment 2). Three high and three low Si-accumulating landraces were selected based on the results from the screen with 98 landraces, although two landraces were reclassified as medium (M) Si accumulators based on the results of the hydroponics experiment (2.3.4).

There was no significant effect of Si supplementation on stomatal conductance, although there was significant variation present among landraces (Figure 2.9; Table 2.8). There was a significant negative correlation between stomatal conductance and shoot Si concentration for plants supplemented with 0.9 mM Si (r = -0.46, P = 0.014), but not for –Si plants (r = 0.01, P = 0.996), or those supplemented with 1.8 mM Si (r = 0.07, P = 0.750). No effect of Si on the stomatal density was found, although there was significant variation between landraces (Table 2.8).

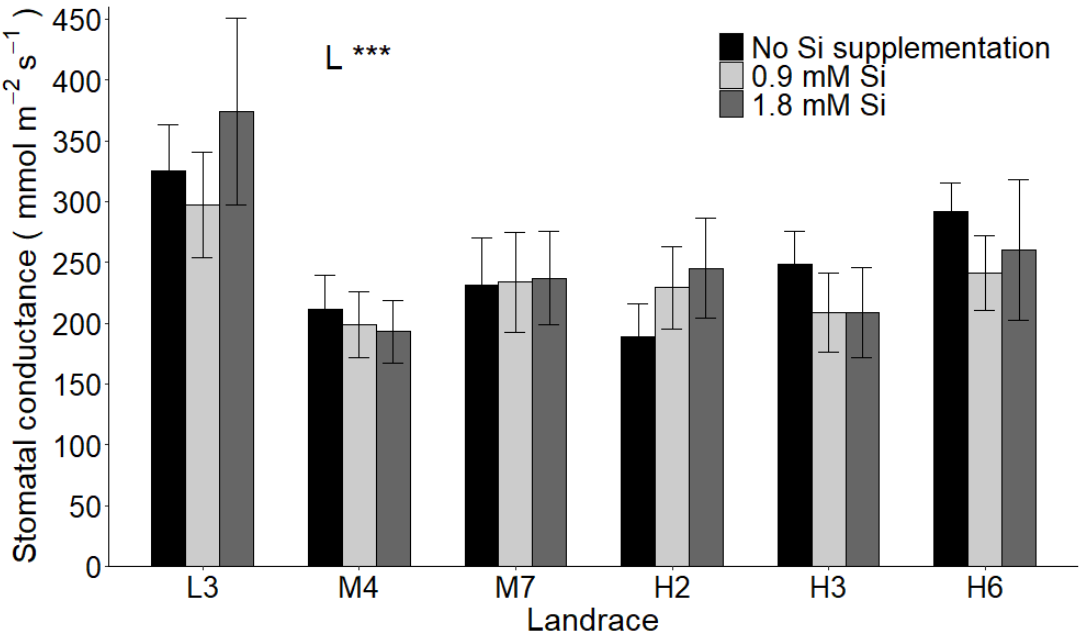

Figure 2.9: Variation in stomatal conductance for plants grown at different levels of Si supplementation (Experiment 2). Statistically significant impacts and interactions, determined by two-way ANOVA, are indicated in each panel, where *** P < 0.001, ** P < 0.01, and * P < 0.05. Mean values ± SE are shown. N = 5. L: landrace, Si: level of Si availability. Landraces were assigned as low (L), high (H), and medium (M) Si accumulators based on shoot Si when grown hydroponically with 1.8 mM and 0.9 mM Si.

2.3.9 Positive correlation between Si and transpiration at high level of Si availability

Due to the high variability associated with the stomatal conductance measurements, and to further investigate whether Si accumulation is correlated with transpiration rate, transpiration was measured based on water loss over time (experiment 4; Figure 2.10).

There was no significant effect of Si treatment on transpiration rate, although there was significant variation between landraces (Table 2.10). For high Si plants, there was a positive correlation between transpiration rate and shoot Si concentration (Figure 2.11, $r = 0.61$, $P = 0.011$), but the correlation with root Si concentration was not significant. There was no significant correlation between transpiration rate and shoot or root Si concentration for low Si plants (data not shown).

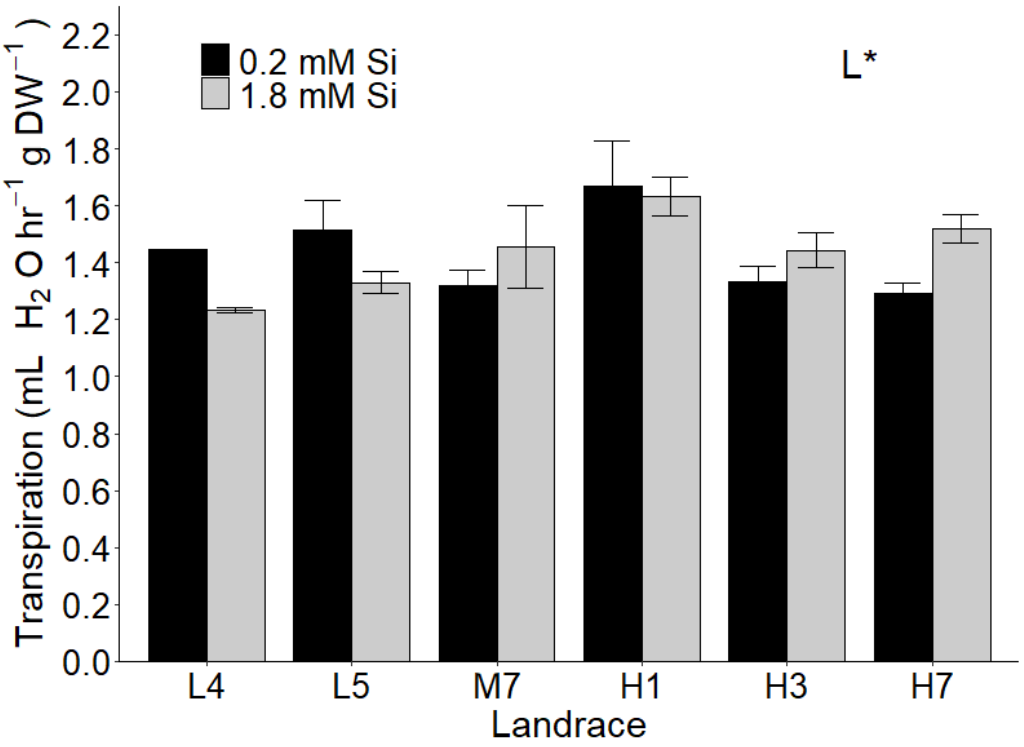

Figure 2.10: Variation in transpiration rate among wheat landraces grown at different levels of Si supplementation (Experiment 4). Statistically significant impacts and interactions, determined by two-way ANOVA, are indicated in each panel, where *** $P < 0.001$, ** $P < 0.01$, and * $P < 0.05$. Mean values ± SE are shown. N = 3. L: landrace, Si: level of Si availability. Landraces were assigned as low (L), high (H), and medium (M) Si accumulators based on shoot Si when grown hydroponically with 1.8 mM and 0.9 mM Si.

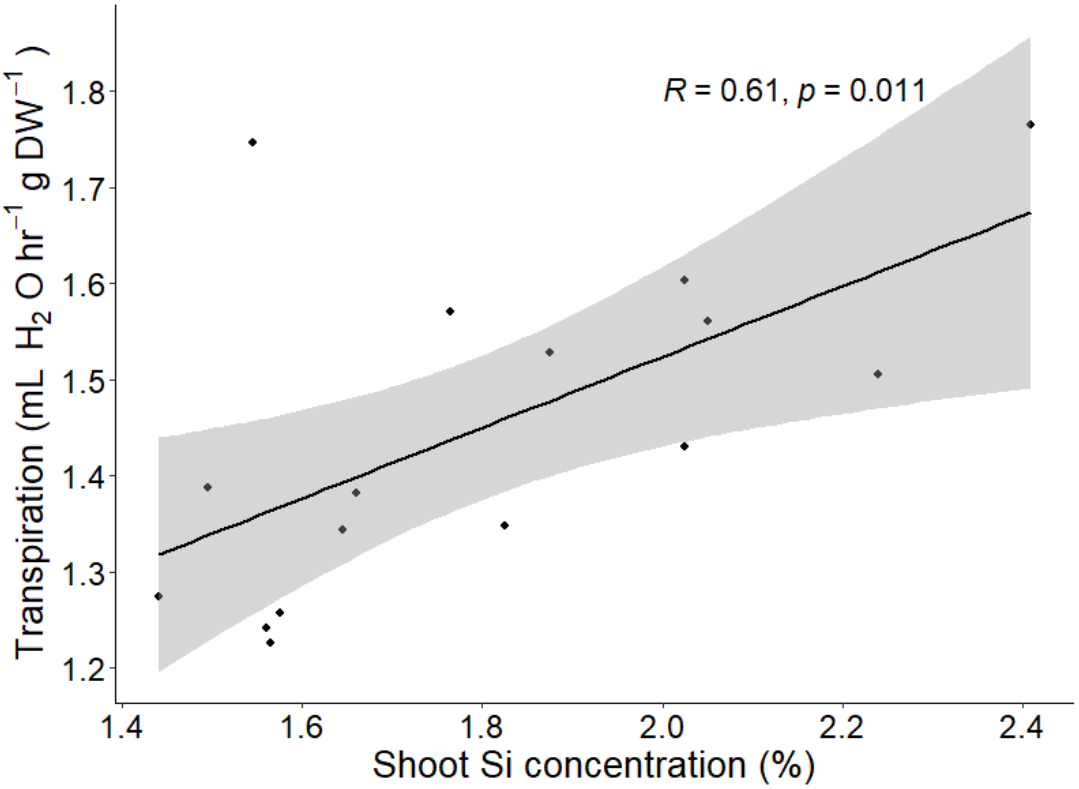

Figure 2.11: Correlation between transpiration and shoot Si concentration for plants grown with 1.8 mM Si (Experiment 4). Shown are the results for individual plants. Six landraces were used: L4, L5, M7, H1, H3, and H7, with three replicates per landrace.

2.3.10 No correlation between Si accumulation and Si transporter gene expression

There was no significant effect of Si treatment or landrace on Si transporter gene expression (experiment 2; Figure 2.12; Table 2.11). Additionally, there was no significant correlation between shoot or root Si concentration and gene expression for any Si transporter (data not shown). There was no significant difference in Si transporter gene expression between high and low Si accumulators at any level of Si supplementation (data not shown).

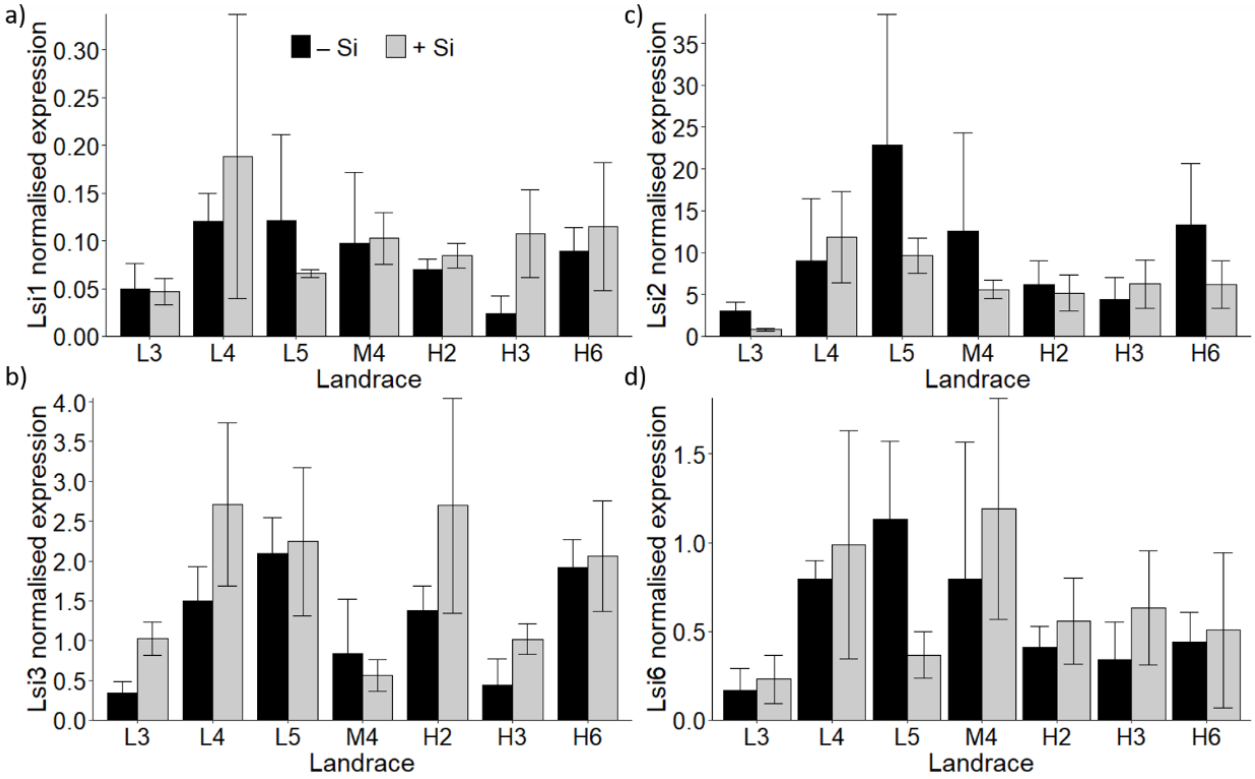

Figure 2.12: Variation in Si transporter gene expression between landraces supplemented (+Si) or not (–Si) with 1.8 mM Si (Experiment 2). a) *Lsi1*. b) *Lsi2*. c) *Lsi3.* d) *Lsi6*. Mean values ± SE are shown. N = 3. No statistically significant impacts or interactions were found. Landraces were assigned as low (L), high (H), and medium (M) Si accumulators based on shoot Si when grown hydroponically with 1.8 mM and 0.9 mM Si.

2.4 Discussion

2.4.1 Si accumulation varies significantly among wheat landraces

Significant variation in Si accumulation among a diversity panel of 98 wheat landraces was found. Previous studies have also reported significant variation in Si accumulation between cultivars of rice (Ma *et al*., 2007b; Mitani-Ueno *et al*., 2014; Talukdar *et al*., 2019), barley (Chiba *et al*., 2009; Ma *et al*., 2003), tall fescue (McLarnon *et al*., 2017), and pumpkin (Mitani-Ueno *et al*., 2011). However, this study is the most extensive to date examining variation in Si accumulation in wheat. While significant variation in Si accumulation was observed both when landraces were grown in compost and in hydroponics, the difference in Si accumulation between high and low Si accumulators was higher when plants were grown hydroponically. This is likely the result of higher Si availability in hydroponics compared to in compost. The increased range in Si accumulation ability when plants were grown hydroponically highlights the need to consider Si availability when assessing genotype Si accumulation potential.

In contrast to the high variation in shoot Si concentration, no significant variation in root Si between landraces was found. Root Si increased with increasing levels of Si availability, but this increase was similar for all landraces. Previous studies have focussed on shoot Si accumulation and have not measured root Si. However, root Si accumulation may be important, for example, in limiting the apoplastic bypass flow of toxic elements such as sodium, thus reducing their accumulation in the shoot (Yeo *et al*., 1999; Gong *et al*., 2006; Flam-Shepherd *et al*., 2018). In this study, for a small number of plants, the roots were too small to measure root Si concentration and this lack of data reduced statistical power. For plants treated with 1.8 mM Si, although the variation was not significant, root Si varied nearly two-fold among landraces. Further research is needed to confirm whether landraces vary in root Si accumulation as this could impact on whether landraces respond differently to Si fertilisation during stress conditions.

While some studies have suggested Si accumulation is a slow process occurring over several weeks (Deshmukh *et al*., 2020; Hodson and Sangster, 1998), other studies have demonstrated that significant Si accumulation can occur over a period of hours (Zexer and Elbaum, 2020; Waterman *et al*., 2021). In this study, only a very small increase in shoot Si concentration was observed when plants were grown continuously with Si over a five week

period, and Si concentration decreased over time in the roots and for –Si plants. Cotterill *et al*. (2007) reported similar results in wheat, with Si concentration significantly increasing between one-week- and three-weeks-old plants, but not between three- and ten-weeks-old plants. In rice, Si accumulated rapidly over a 12 h period when plants were moved from –Si to +Si media (Wu *et al*., 2006; Ma *et al*., 2007b). However, although Si continued to increase over a three month period, it was at a lower rate, and there was no increase in Si during the second month (Ma *et al*., 2006). Further work is needed to establish whether wheat landraces vary in their rate of Si accumulation over a period of hours or days rather than weeks.

Although Si supplementation significantly increased Si accumulation in all landraces, the effect of Si on spine density was less consistent. While there was a positive correlation between Si concentration and spine density for plants supplemented with 0.9 mM Si, this correlation was insignificant when plants were supplemented with 1.8 mM Si. In the grass *Festuca ovina,* Si supplementation increased the number of silicified spines, and in *Festuca arundinacea,* higher Si accumulation was correlated with higher spine density (Hartley *et al*., 2015). Similarly, in *Brachypodium distachyon,* increasing Si availability increased the number of spines (Hall *et al*., 2019). However, Rafi *et al*. (1997) reported that although Si increased the roughness of leaves and awns, there was no effect of Si on spine number or shape in wheat.

2.4.2 Evidence of a trade-off between Si and growth

In general, a positive effect of Si on growth is only observed during stress conditions (Coskun *et al*., 2019a). Walsh *et al*. (2018) reported no effect of Si on wheat when grown in optimal conditions . However, there are some reports of Si increasing wheat growth when grown in the absence of experimentally-imposed stress conditions (Maghsoudi *et al*., 2016a; Neu *et al*., 2017; Sienkiewicz-Cholewa *et al*., 2018). Here, although not significant, there was a trend for some landraces to respond positively to Si, while others exhibited a negative response. Previous studies have reported negative effects of Si when plants were grown at high levels of Si fertilisation in both rice (Flores *et al*., 2021; Ju *et al*., 2017) and wheat (Alzahrani *et al*., 2018). It is possible that different genotypes have different optimal levels of Si availability, and that high levels of Si reduce growth in some genotypes.

Further adding to the complex effects of Si on growth, it was hypothesised that there would be a positive correlation between high Si accumulation and growth. However, the opposite trend was found with a significant negative correlation between shoot Si concentration and shoot dry weight. Studies in other species have likewise reported a negative correlation between Si accumulation and biomass in non-stressed plants (de Tombeur *et al*., 2021; Johnson and Hartley, 2018). Simpson *et al*. (2017) suggested that there is a trade-off between Si and growth, with higher Si accumulation associated with a lower growth rate, especially for larger plants. Si deposition is an active process involving the use of active efflux transporters (Ma *et al*., 2007a; Ma and Yamaji, 2015) and thus there may be an energetic cost associated with high Si uptake (Simpson *et al*., 2017).

2.4.3 Mechanism of Si accumulation

Si is transported from the roots to the shoots in the transpiration stream (Ma and Yamaji, 2015). Consequently, in addition to relating to Si transporter abundance and activity, it has been suggested that Si accumulation will correlate with transpiration rate (Exley, 2015). However, subsequent studies in sorghum have found that although the transpiration stream is important for transporting silicic acid to the shoot, Si deposition is independent of transpiration rate (Kumar *et al*., 2017, 2020). Therefore, whether variation in Si accumulation among wheat landraces related to differences in transpiration was investigated.

There was a negative correlation between stomatal conductance and shoot Si concentration when plants were supplemented with 0.9 mM Si, although not at 1.8 mM Si. However, there was a positive correlation between transpiration and Si concentration for plants supplemented with 1.8 mM Si. The porometer readings were highly variable between days, and the presence of the porometer on the leaves may have affected the stomatal conductance (Clarke and Clarke, 1996; Idso *et al*., 1988). Measuring water loss rather than stomatal conductance may be a more accurate way of estimating transpiration rate.

Alternatively, a correlation between Si accumulation and transpiration may only occur when Si is abundant. By changing the humidity and wind level to manipulate the transpiration rate in cucumber, Faisal *et al*. (2012) found evidence that Si accumulation

occurred through entirely passive mechanisms at high levels of Si availability, but Si is actively accumulated when scarce. Supporting this, here, no correlation between transpiration and Si accumulation was observed when plants were grown with limited Si, while there was a positive correlation when plants were supplemented with a high level of Si.

Other studies have also provided evidence that plants, including wheat, accumulate Si through active processes (Gocke *et al*., 2013; Jarvis, 1987; Rains *et al*., 2006). Thus, variation in Si accumulation may relate to differences in Si transporter abundance and activity. Differences in Si accumulation between genotypes have been attributed to differences in Si transporter gene expression in rice (Ma *et al*., 2007b; Wu *et al*., 2006). In poinsettia, Hu *et al*. (2019) found consistent variation in *Lsi1* and *Lsi2* gene expression between cultivars, although the differences were not well correlated with differences in Si accumulation. In this study, Si transporter gene expression between landraces was highly variable and did not correlate with Si accumulation. However, primers were used that were able to bind to all homoeologous copies of Si transporter genes. It is possible that correlations between gene expression and Si accumulation would have been found had homoeologue-specific primers been used.

Si transporter gene expression varies both along the length of the root and with plant age (Soukup *et al*., 2017; Yamaji *et al*., 2008; Yamaji and Ma, 2007). In rice and cucumber, Si supply has been correlated with both increased (Ma *et al*., 2015; Wang *et al*., 2015a; Ye *et al*., 2013) and decreased (Holz *et al*., 2019; Ma *et al*., 2006; Mitani-Ueno *et al*., 2016) *Lsi1* gene expression. In ryegrass, there was a negative correlation between *Lsi1* expression and Si accumulation (Pontigo *et al*., 2021). However, in wheat, Si fertilisation had no effect on *Lsi1* expression (Montpetit *et al*., 2012). Overall, across studies, Si transporter gene expression is highly variable, and therefore it is perhaps unsurprising that no correlation between gene expression and Si accumulation was found in this study. Gene expression does not always correlate with protein abundance or function, and plant aquaporins have been shown to be post-translationally regulated (Verdoucq *et al*., 2014). Focusing on protein abundance rather than gene expression may provide more insight into the causes of variation in Si accumulation among wheat landraces.

2.4.4 No genetic differences between landraces

When applied to the diversity panel of 98 landraces, AT did not identify any significant SNPs or GEMs. This could be the result of model over-fitting, which was a problem with the SNP analysis, although it would be expected that strong results would still be apparent in this case. Two population structures were tested, using a PSIKO or a principal components analysis (PCA) model, but both models over-corrected the population structure; using more landraces may have improved the model fit. However, AT has been successfully used to identify genes associated with stem strength using only 100 wheat accessions (Miller *et al*., 2016). Alternatively, high Si accumulation may be a polygenic trait, with several common variants having only a small phenotypic effect, but the ability of association studies to identify such genetic markers is limited (Korte and Ashley, 2013). The SNP data used in this study was obtained from transcriptomic data. SNPs located within promoter regions, or other regions of the genome that are not transcribed, and thus not investigated here, could correlate with differences in Si accumulation.

It is also possible that SNP differences do not contribute to differences in Si accumulation between wheat landraces. Nevertheless, previous association studies have identified genetic differences that may influence variation in Si accumulation. Using 350 rice accessions, Talukdar *et al*. (2015) identified several quantitative trait loci (QTLs) related to germanium sensitivity which may affect Si accumulation, although no QTLs were associated with *OsLsi1* or *OsLsi6*. In a later study, SNPS in *OsLsi2* and *OsLsi3* were linked to differences in Si accumulation in a panel of only 50 rice accessions (Talukdar *et al*., 2019). However, as a diploid species with a relatively small genome, it may be easier to identify relevant genetic differences in rice compared to in a polyploid species such as wheat.

Previous studies have focussed on the effect of Si availability on gene expression, rather than whether there are differences in gene expression between genotypes. By focussing on differences between genotypes, this study aimed to investigate whether there were consistent differences in gene expression between landraces of varying Si-accumulating ability, rather than whether Si fertilisation is associated with changes in gene expression. As with the qPCR data, no significant differences in Si transporter gene expression were found. However, 59 genes were identified as being differentially expressed between groups of high and low Si accumulators. This included lower expression of five putative dehydrin

genes in high Si accumulators compared to low Si accumulators. If Si accumulation correlated with stress tolerance, it would be hypothesised that stress response-related gene expression would be higher in high Si accumulators. The lower dehydrin gene expression identified in this DE analysis is in contradiction with this hypothesis. However, the gene expression data used in this study was from 15-days-old plants grown without Si. As gene expression is highly variable, and does not always reflect protein abundance and activity, further investigation is needed to establish whether there are consistent differences in gene expression based on Si accumulation among wheat landraces.

2.5 Conclusion

Using a diversity panel of 98 wheat landraces, significant genotypic variation in Si accumulation was found. However, there was a negative correlation between Si accumulation and dry weight, suggesting that breeding wheat for increased Si accumulation may need to be balanced against the need to produce large plants with a high yield. Further work growing 20 landraces hydroponically allowed landraces that consistently accumulated high, medium, or low levels of Si to be identified. However, variation in Si accumulation between landraces was not related to differences in Si transporter gene expression or sequence. There was a positive correlation between transpiration and Si accumulation when plants were grown at a high availability of Si, suggesting that variation in Si concentration between landraces may partly be the result of differences in transpiration, at least at high levels of Si supplementation. Further research is needed to investigate the consequences of variation in Si accumulation among landraces, and to establish whether breeding wheat for increased Si accumulation is likely to have a beneficial effect on crop yield.

2.6 Appendix

Table 2.2: Shoot Si concentration for the 98-landrace diversity panel. Landraces in bold were used for subsequent hydroponics experiments.

Landrace	Collection	Plant ID	Plant Name	Origin	Shoot Si Concentration (%) (Mean ± SE)	
					–Si	+Si
YoGI_002	CIMMYT	BW 7112	RA SHIH PAI PÂ´I	China	0.47 ± 0.03	0.99 ± 0.07
YoGI_003	CIMMYT	BW7227	ARTEMOVKA	Former Soviet Union	0.41 ± 0.08	0.86 ± 0.05
YoGI_006	CIMMYT	BW 15958	V763.153	Pakistan	0.49 ± 0.12	1.00 ± 0.07
YoGI_011	**CIMMYT**	**CWI 2166**	**K7155.41**	**Kenya**	**0.52 ± 0.06**	**1.22 ± 0.16**
YoGI_013	CIMMYT	CWI 2168	K6995.4A	Kenya	0.47 ± 0.02	0.91 ± 0.05
YoGI_015	**CIMMYT**	**CWI 3909**	**OUBAARD**	**South Africa**	**0.51 ± 0.04**	**1.03 ± 0.08**
YoGI_021	CIMMYT	CWI 6075	KOELZ W 9375:AE	India	0.48 ± 0.07	0.97 ± 0.03
YoGI_022	CIMMYT	CWI 6076	KOELZ W 9376:AE	India	0.46 ± 0.06	1.10 ± 0.07
YoGI_028	CIMMYT	CWI 7129	KOELZ W 11192:AE	India	0.51 ± 0.08	1.00 ± 0.08
YoGI_034	CIMMYT	CWI 9915	ROOI KLEINKORING	South Africa	0.47 ± 0.05	0.94 ± 0.08
YoGI_038	CIMMYT	CWI 12335	AUSTRAL	Argentina	0.52 ± 0.02	1.01 ± 0.02

YoGI_047	CIMMYT	CWI 13432	WHITE FIFE	Japan	0.43 ± 0.05	0.85 ± 0.10
YoGI_051	CIMMYT	CWI 13629	AMERICANO	Uruguay	0.49 ± 0.08	0.79 ± 0.01
YoGI_052	CIMMYT	CWI 13644	RINK	United States	0.48 ± 0.04	0.88 ± 0.03
YoGI_054	**CIMMYT**	**CWI 13647**	**NEW ZEALAND**	**United States**	**0.54 ± 0.04**	**1.13 ± 0.08**
YoGI_059	CIMMYT	CWI 15005	LAGEADINHO	Brazil	0.47 ± 0.06	0.89 ± 0.05
YoGI_064	**CIMMYT**	**CWI 27304**	**TAIAN-KEN**	**Kenya**	**0.42 ± 0.04**	**0.85 ± 0.05**
YoGI_067	CIMMYT	CWI 31262	KENYA	Australia	0.45 ± 0.02	0.84 ± 0.05
YoGI_075	CIMMYT	CWI 52382	OAX93.21.34	Mexico	0.45 ± 0.07	0.98 ± 0.05
YoGI_079	CIMMYT	CWI 53414	NOVOMICHURINKA X	Former Soviet Union	0.44 ± 0.04	0.93 ± 0.04
YoGI_081	**CIMMYT**	**DW 4633**	**AKBUGDAY**	**Kyrgyzstan**	**0.57 ± 0.11**	**0.89 ± 0.09**
YoGI_085	Watkins	1190014	Sarajevo 4	Yugoslavia	0.44 ± 0.07	0.96 ± 0.05
YoGI_088	Watkins	1190032	Dehak	India	0.44 ± 0.05	0.82 ± 0.04
YoGI_098	**Watkins**	**1190045**	**Douchani**	**Syria**	**0.41 ± 0.05**	**1.00 ± 0.03**
YoGI_103	Watkins	1190091	Rodi Garamseli	India	0.50 ± 0.06	1.08 ± 0.05
YoGI_104	Watkins	1190092	Desi	India	0.43 ± 0.08	0.87 ± 0.06
YoGI_110	Watkins	1190105	Hâtif de Saône	France	0.50 ± 0.11	0.96 ± 0.04
YoGI_114	Watkins	1190126	Dolatkhani (white)	India	0.42 ± 0.05	0.82 ± 0.02
YoGI_118	Watkins	1190141	China 15	China	0.44 ± 0.02	0.87 ± 0.07
YoGI_130	Watkins	1190181	Kujawianka Wieclawicka	Poland	0.40 ± 0.07	0.88 ± 0.08

YoGI_133	Watkins	1190190	Roux de Presles	France	0.46 ± 0.06	0.98 ± 0.05
YoGI_134	**Watkins**	**1190191**	**Rouge des Ardennes**	**France**	**0.42 ± 0.03**	**0.89 ± 0.01**
YoGI_135	**Watkins**	**1190195**	**Gahu (Nepali) or Kyo (Sikkimese)**	**India**	**0.40 ± 0.04**	**0.98 ± 0.01**
YoGI_142	Watkins	1190218	Sbei Noir	Tunisia	0.42 ± 0.04	0.94 ± 0.05
YoGI_143	**Watkins**	**1190219**	**Alicante 1**	**Spain**	**0.55 ± 0.05**	**1.10 ± 0.08**
YoGI_144	Watkins	1190223	Shan wheat	Burma	0.44 ± 0.05	0.93 ± 0.05
YoGI_152	**Watkins**	**1190246**	**Soor Ghanum**	**India**	**0.59 ± 0.06**	**1.12 ± 0.08**
YoGI_156	Watkins	1190273	Seville 17	Spain	0.40 ± 0.06	0.82 ± 0.04
YoGI_162	Watkins	1190299	Smyrna 6	Turkey	0.49 ± 0.05	0.91 ± 0.05
YoGI_164	**Watkins**	**1190305**	**Sinai 1**	**Egypt**	**0.43 ± 0.05**	**0.97 ± 0.04**
YoGI_168	Watkins	1190319	China 15	China	0.43 ± 0.08	0.86 ± 0.09
YoGI_169	Watkins	1190320	China 2	China	0.45 ± 0.06	1.05 ± 0.09
YoGI_170	Watkins	1190323	China 14	China	0.44 ± 0.03	0.91 ± 0.05
YoGI_171	Watkins	1190324	China 20	China	0.49 ± 0.07	0.94 ± 0.04
YoGI_172	Watkins	1190325	1190325	United Kingdom	0.49 ± 0.05	0.91 ± 0.05
YoGI_178	Watkins	1190336	Hungary 2	Hungary	0.43 ± 0.02	1.00 ± 0.02
YoGI_181	Watkins	1190349	Golema Franga	Bulgaria	0.44 ± 0.09	0.92 ± 0.02
YoGI_182	Watkins	1190352	Sarajevo 8	Yugoslavia	0.45 ± 0.04	1.11 ± 0.05
YoGI_186	Watkins	1190374	Sarakhs	Iran	0.47 ± 0.03	1.00 ± 0.13

YoGI_189	**Watkins**	**1190396**	**Trigo Rietti**	**Portugal**	**0.40**	**±**	**0.04**	**0.89**	**±**	**0.08**
YoGI_190	Watkins	1190397	Trigo Ribeiro	Portugal	0.47	±	0.03	0.92	±	0.03
YoGI_191	Watkins	1190398	Abu Fashi	Palestine	0.48	±	0.06	1.05	±	0.12
YoGI_193	Watkins	1190406	Desi	India	0.51	±	0.06	0.93	±	0.10
YoGI_196	Watkins	1190420	Dhania	India	0.43	±	0.03	0.87	±	0.08
YoGI_197	Watkins	1190433	Soor Ghanum	India	0.48	±	0.06	0.89	±	0.02
YoGI_198	Watkins	1190436	China Sh108	China	0.41	±	0.03	0.93	±	0.06
YoGI_202	Watkins	1190450	Miercurea Ciucului	Romania	0.42	±	0.04	0.70	±	0.04
YoGI_203	Watkins	1190451	Samanta 117	Romania	0.46	±	0.04	0.79	±	0.06
YoGI_207	Watkins	1190468	Afghanistan 46	Afghanistan	0.48	±	0.04	0.89	±	0.08
YoGI_210	Watkins	1190474	Afghanistan 109	Afghanistan	0.43	±	0.07	0.87	±	0.07
YoGI_215	Watkins	1190483	Surka Oscista	Poland	0.42	±	0.04	1.04	±	0.13
YoGI_221	**Watkins**	**1190521**	**Dandi**	**India**	**0.50**	**±**	**0.06**	**0.95**	**±**	**0.05**
YoGI_227	**Watkins**	**1190568**	**China 19**	**China**	**0.43**	**±**	**0.05**	**0.89**	**±**	**0.05**
YoGI_231	**Watkins**	**1190605**	**Karabash**	**Greece**	**0.41**	**±**	**0.05**	**0.92**	**±**	**0.08**
YoGI_235	Watkins	1190627	Persia 45	Iran	0.48	±	0.05	0.95	±	0.07
YoGI_237	Watkins	1190636	Native hard	Tunisia	0.45	±	0.04	0.82	±	0.06
YoGI_240	Watkins	1190645	Mundia	India	0.45	±	0.05	1.00	±	0.06
YoGI_242	Watkins	1190652	China Sh107	China	0.44	±	0.03	0.95	±	0.06

YoGI_243	**Watkins**	**1190662**	**Samanta 1252**	**Romania**	**0.42**	**±**	**0.03**	**0.84**	**±**	**0.02**
YoGI_249	Watkins	1190670	Zlotka Miczynskiego	Poland	0.42	±	0.03	0.83	±	0.04
YoGI_255	Watkins	1190685	Trigo duros	Spain	0.44	±	0.04	0.86	±	0.00
YoGI_257	Watkins	1190694	Lyallpur 8A	India	0.49	±	0.02	0.96	±	0.03
YoGI_259	Watkins	1190700	Kaifeng 323-9	China	0.44	±	0.05	0.89	±	0.09
YoGI_261	Watkins	1190705	Kooseh	Iran	0.44	±	0.06	0.85	±	0.02
YoGI_265	Watkins	1190729	Gandum-i-Jiruft	Iran	0.44	±	0.03	0.78	±	0.01
YoGI_270	Watkins	1190740	Siberia W94421	USSR	0.49	±	0.10	0.89	±	0.02
YoGI_277	**Watkins**	**1190751**	**Armavir**	**USSR**	**0.40**	**±**	**0.07**	**0.80**	**±**	**0.08**
YoGI_280	Watkins	1190755	Crimea W94465	USSR	0.42	±	0.04	0.88	±	0.06
YoGI_285	Watkins	1190772	Yenisei W43320	USSR	0.50	±	0.04	0.87	±	0.04
YoGI_286	**Watkins**	**1190777**	**Finland 3**	**Finland**	**0.48**	**±**	**0.05**	**0.94**	**±**	**0.05**
YoGI_287	Watkins	1190779	Tulun 458	USSR	0.44	±	0.07	0.83	±	0.02
YoGI_291	Watkins	1190784	Oberdan	Italy	0.45	±	0.03	0.87	±	0.02
YoGI_298	Watkins	1190810	Turkestan W84532	USSR	0.47	±	0.05	0.93	±	0.08
YoGI_299	**Watkins**	**1190811**	**Algeria W7558**	**Tunisia**	**0.59**	**±**	**0.14**	**0.97**	**±**	**0.11**
YoGI_313	Prague	01C0200519	Eritrospermum 5755	Uzbekistan	0.47	±	0.03	0.87	±	0.04
YoGI_320	Prague	01C0203773	Jade	Saudi Arabia	0.50	±	0.06	0.98	±	0.03
YoGI_324	Prague	01C0202172	MCB 192	Peru	0.50	±	0.02	1.03	±	0.08

YoGI_328	Prague	01C0201384	Nohoean	Norway	0.44 ± 0.02	0.86 ± 0.02
YoGI_329	Prague	01C0201385	Nora	Norway	0.44 ± 0.06	0.88 ± 0.05
YoGI_330	**Prague**	**01C0201531**	**Orchon**	**Mongolia**	**0.56 ± 0.08**	**1.01 ± 0.05**
YoGI_334	Prague	01C0202818	Suwon 222	Korea	0.50 ± 0.02	0.95 ± 0.07
YoGI_336	IBTI	Unknown	Apache USA	Unknown	0.47 ± 0.03	1.14 ± 0.07
YoGI_343	IBTI	GedifluxRL (id#40001)	Muck	Germany	0.47 ± 0.03	0.91 ± 0.04
YoGI_345	IBTI	GedifluxRL (id#39977)	STAMM-101	Austria	0.45 ± 0.03	0.99 ± 0.05
YoGI_348	IBTI	GedifluxRL (id#39779)	Vilmorin-27	France	0.46 ± 0.04	0.94 ± 0.02
YoGI_349	IBTI	GedifluxRL (id#40037)	Shamrock	United Kingdom	0.49 ± 0.03	1.15 ± 0.07
YoGI_350	IBTI	Unknown	Paragon control	Unknown	0.47 ± 0.04	0.88 ± 0.07

Table 2.3: ANOVA results for Si, landrace, and their interaction on shoot Si concentration for a diversity panel of 98 wheat landraces. Replicate was included as a factor to account for differences between plants grown at different times. Statistically significant results are highlighted in bold.

	Shoot Si (%)		
	df	*F*	*P*
Landrace	**96**	**1.85**	**< 0.001**
Si	**1**	**3542.47**	**< 0.001**
Replicate	**2**	**44.88**	**< 0.001**
Landrace x Si	96	0.61	0.998

Table 2.4: ANOVA results for Si, accumulation type, and their interaction on shoot Si concentration for 20 landraces selected as being either high or low Si accumulators. Replicate was included as a factor to account for differences between plants grown at different times. Statistically significant results are highlighted in bold.

	Shoot Si (%)		
	df	*F*	*P*
Accumulation type	**1**	**52.54**	**< 0.001**
Si	**1**	**639.00**	**< 0.001**
Replicate	**2**	**7.98**	**< 0.001**
Accumulation type x Si	1	3.83	0.053

Table 2.5: ANOVA results for the effect of single nucleotide polymorphisms (SNPs) in Si transporter genes on Si accumulation. The number of landraces with each allele is also indicated.

SNP	No. landraces with dominant allele	No. landraces with minor allele	No. landraces with N/ mixed allele	*df*	*F*	*P*
Lsi3_A4	83 (A)	12 (G)	2 (R)	2	0.41	0.666
Lsi3_A7	84 (T)	11 (C)	2 (Y)	2	0.40	0.669
Lsi3_A10	85 (G)	10 (T)	2 (K)	2	0.41	0.668
Lsi6_D3	75 (G)	21 (N)	1 (R)	2	0.39	0.677
Lsi6_A3	87 (C)	10 (T)	NA	1	0.03	0.873
Lsi6_B1	52 (C)	26 (T)	18 (N), 1 (Y)	3	0.27	0.847
Lsi6_B4	71 (A)	20 (T)	6 (W)	2	0.20	0.817
Lsi6_B6	87 (T)	9 (N)	1 (Y)	2	2.53	0.085

Table 2.6: Average shoot Si concentrations and t-test results for the groups of high and low Si accumulators used for DE analyses. The average shoot Si was calculated using the results for plants that received Si supplementation in experiment one (mean ± SE). Statistically significant results are highlighted in bold.

Group used for DE analysis	Average Shoot Si concentration (%)			
	Low Si accumulators	High Si accumulators	*t*	*P*
Group 1	**0.83 ± 0.01**	**1.06 ± 0.01**	**3.89**	**< 0.001**
Group 2	**0.79 ± 0.02**	**1.13 ± 0.02**	**3.20**	**0.002**
Group 3	**0.82 ± 0.04**	**1.08 ± 0.04**	**3.01**	**0.005**
Group 4	**0.82 ± 0.04**	**1.06 ± 0.05**	**2.71**	**0.010**

Table 2.7: Genes identified as being differentially expressed between high and low Si accumulators using differential expression (DE) analysis. The analysis was repeated using four groups of high and low Si accumulating landraces (2.2.5). The log fold change and *P*-value for each gene for each group are indicated. The five genes identified by GO enrichment analysis as being over-represented are highlighted in bold. These corresponded to five dehydrin genes that are involved in the response to abiotic stress.

		Group 1		Group 2		Group 3		Group 4	
Ensembl gene name	Gene annotation	Fold change	*P*	Fold change	*P*	Fold change	*P*	Fold change	*P*
TraesCS1A02G431200	Leucine-rich repeat receptor-like protein kinase family protein	-0.02129	2.40E-13	-0.04559	1.03E-15	-0.09702	5.44E-15	-0.09742	9.41E-15
TraesCS1B02G093100	F-box protein	0.00674	2.56E-13	0.035422	5.57E-13	0.069214	2.86E-12	0.097195	4.09E-10
TraesCS1B02G219500	RING/U-box superfamily protein	-0.01828	1.07E-16	-0.04553	8.52E-14	-0.09691	2.25E-13	-0.09731	1.08E-13
TraesCS1B02G274500	Pectinesterase	0.013698	1.47E-13	0.009886	1.74E-11	0.069281	1.07E-12	0.097276	4.94E-13
TraesCS1D02G056900	S-locus lectin protein kinase family protein	-0.02128	9.78E-14	-0.04552	1.06E-13	-0.09692	2.31E-13	-0.09731	1.06E-13
TraesCS2B02G333700	Glutamate decarboxylase	0.014478	4.80E-13	0.035412	1.03E-10	0.069188	2.79E-12	0.097198	8.28E-12
TraesCS2B02G365800	Translation initiation factor IF-2	-0.04732	0.000568	-0.04554	6.93E-14	-0.09691	2.43E-13	-0.0973	1.24E-13

TraesCS2B02G384900	Serine/threonine-protein kinase	-0.00901	1.07E-14	-0.04556	9.53E-14	-0.09688	3.44E-12	-0.09728	1.69E-13
TraesCS2B02G444200	Alcohol dehydrogenase, putative	-0.02128	1.21E-14	-0.04556	4.05E-14	-0.09685	4.75E-13	-0.09724	2.62E-13
TraesCS2B02G451900	Myosin heavy chain-like protein	0.011692	3.85E-14	0.035447	1.21E-13	0.069185	1.37E-12	0.097256	8.28E-12
TraesCS2B02G482800	BLT14.1 protein	-0.39383	0.017725	-0.47478	0.031105	-0.97193	2.01E-07	-1.4854	0.001718
TraesCS2D02G052900	Kinase family protein	0.001055	9.34E-14	-0.04542	6.51E-13	-0.09671	1.51E-12	-0.0971	8.81E-13
TraesCS2D02G357200	Tuftelin-interacting protein 11	0.018401	4.20E-14	0.026386	1.12E-11	0.071081	0.023095	0.097355	0.023883
TraesCS2D02G579700	Myb/SANT-like DNA-binding domain protein	0.019887	1.27E-14	0.035461	2.45E-13	0.069258	2.91E-13	0.097356	1.39E-12
TraesCS3A02G030000	E3 ubiquitin-protein ligase	-0.00792	1.16E-13	-0.04548	2.20E-13	0.069143	2.93E-12	0.097192	1.74E-11
TraesCS3A02G044300	ARM repeat superfamily protein	0.020719	4.11E-19	0.035472	8.85E-16	0.069323	1.19E-16	0.09745	1.42E-15
TraesCS3A02G047600	NBS-LRR disease resistance protein-like protein	-0.00788	1.12E-13	-0.04541	7.62E-13	-0.09668	1.88E-12	-0.09707	1.07E-12
TraesCS3A02G401800	Pleckstrin homology domain-containing family A member 8	-0.01573	1.29E-16	-0.04559	7.84E-15	-0.09696	1.01E-13	-0.09735	5.87E-14
TraesCS3B02G197900	VQ motif family protein	-0.02126	1.08E-13	-0.04541	7.62E-13	-0.0969	2.79E-13	-0.09729	1.51E-13
TraesCS3D02G059200	F-box family protein	-0.01764	5.59E-13	-0.04553	9.16E-14	-0.09683	5.57E-13	-0.09722	3.06E-13

TraesCS3D02G346800	Tetratricopeptide repeat protein 7A	0.016811	4.91E-14	0.035451	4.00E-12	0.069278	1.62E-13	0.097384	5.59E-13
TraesCS3D02G439400	Non-specific serine/threonine protein kinase	-0.02127	7.73E-14	-0.04554	7.22E-14	-0.0969	2.79E-13	-0.09729	1.50E-13
TraesCS3D02G469200	Disease resistance protein (NBS-LRR class) family	-0.02373	8.07E-16	-0.0454	2.28E-12	-0.0968	7.62E-13	-0.09719	4.46E-13
TraesCS4A02G162100	Stearoyl-[acyl-carrier-protein] 9-desaturase, chloroplastic	-0.02799	3.21E-22	-0.04549	2.00E-13	-0.09675	1.04E-12	-0.09715	6.21E-13
TraesCS4A02G479100	Disease resistance protein (NBS-LRR class) family	-0.02126	6.59E-14	-0.0455	1.40E-13	-0.09683	5.57E-13	-0.09723	3.06E-13
TraesCS4B02G288000	Transcription factor protein	-0.00865	1.57E-13	-0.04535	1.91E-12	-0.09681	6.47E-13	-0.09721	3.54E-13
TraesCS4B02G308900	Alanine--tRNA ligase	-0.52278	0.000795	-0.61662	0.01157	-0.91123	0.021824	-0.87532	0.006137
TraesCS4B02G309000	Alanine--tRNA ligase	-0.51011	0.000171	-0.5416	0.009433	-0.93242	0.021382	-0.79574	0.002295
TraesCS4B02G376100	Gibberellin 2-beta-dioxygenase	0.009933	3.76E-18	0.035441	2.04E-13	0.069251	4.30E-12	0.09731	2.78E-12
TraesCS4D02G197700	Low temperature and salt responsive protein family	-0.37623	0.035357	-0.51419	0.022283	-1.16324	0.002588	-1.01565	6.80E-05
TraesCS5A02G077300	Pathogenesis-related thaumatin superfamily protein	-0.03086	2.36E-27	-0.05482	0.00492	-0.09701	0.008039	-0.0974	0.006244

TraesCS5A02G298600	Transmembrane protein, putative	-0.01158	2.08E-14	-0.04547	4.50E-13	-0.09667	2.00E-12	-0.09706	1.16E-12
TraesCS5B02G020000	Leucine-rich repeat receptor-like protein kinase family protein	0.021279	8.78E-14	0.035416	2.74E-11	0.069983	0.036266	0.097314	0.031804
TraesCS5B02G256700	SKP1-like protein 4	0.007247	3.89E-14	0.03544	3.24E-12	0.069249	7.20E-13	0.097303	1.90E-12
TraesCS5B02G399000	GDSL esterase/lipase	-0.0034	8.58E-13	0.035444	2.74E-11	0.069202	1.04E-12	0.097275	1.52E-11
TraesCS5B02G536100	RNA binding protein	0.001695	3.12E-13	0.003262	6.70E-12	-0.0968	7.20E-13	-0.09719	4.10E-13
TraesCS5D02G265400	Mechanosensitive ion channel	-0.01	1.30E-17	-0.04534	1.91E-12	-0.15516	0.000919	-0.15538	0.000587
TraesCS5D02G291000	RING/U-box superfamily protein	-0.00953	1.25E-14	-0.04553	1.09E-13	0.069164	2.00E-12	0.097224	2.77E-10
TraesCS5D02G355200	transmembrane protein, putative (DUF594)	-0.01074	1.98E-14	-0.04552	1.17E-13	-0.09687	3.84E-13	-0.09726	4.59E-13
TraesCS5D02G369400	Low temperature and salt responsive protein	-0.473	0.006105	-0.72173	0.001241	-1.51329	6.50E-09	-1.53465	2.50E-10
TraesCS6A02G042000	Serpin-like protein	-0.00601	9.34E-14	-0.04542	6.51E-13	-0.09683	5.57E-13	-0.09723	3.06E-13
TraesCS6A02G255200	Aluminum-activated malate transporter-like	0.013516	2.92E-13	0.035434	1.74E-11	0.069237	4.91E-13	0.097325	3.64E-11
TraesCS6A02G350100	**Dehydrin**	**-0.40898**	**0.000134**	**-0.62547**	**3.67E-06**	**-1.10155**	**0.000247**	**-0.98042**	**9.77E-06**
TraesCS6A02G350300	**Dehydrin**	**-0.37151**	**0.032689**	**-0.46866**	**0.047809**	**-1.52889**	**1.94E-06**	**-1.39759**	**1.42E-08**

TraesCS6B02G044600	2-oxoglutarate and Fe(II)-dependent oxygenase superfamily protein, putative	0.002036	3.77E-13	0.01588	1.04E-11	0.069223	6.80E-13	0.097306	2.70E-11
TraesCS6B02G061100	Cytochrome P450 family protein, expressed	0.018364	6.65E-14	0.035466	3.41E-12	0.069315	1.88E-12	0.097231	1.07E-11
TraesCS6B02G101300	F-box domain containing protein, expressed	0.0107	5.11E-13	0.035414	6.13E-13	0.069182	1.10E-11	0.097055	9.44E-12
TraesCS6B02G247100	DNA double-strand break repair rad50 ATPase, putative isoform 1	0.009285	1.25E-13	-0.04551	1.21E-13	0.069241	4.65E-13	0.097334	2.45E-12
TraesCS6B02G383200	**Dehydrin**	**-0.46841**	**7.88E-05**	**-0.62909**	**2.37E-05**	**-1.04909**	**0.001418**	**-0.94315**	**0.000145**
TraesCS6D02G040300	Histone H2A	-0.01705	9.29E-15	-0.04551	1.21E-13	-0.0969	0.041866	-0.0973	0.036877
TraesCS6D02G092800	Disease resistance protein (NBS-LRR class) family	0.011283	1.33E-13	0.035434	3.19E-13	0.069049	1.10E-11	0.097057	2.94E-09
TraesCS6D02G166300	Argonaute family protein	0.00396	2.24E-13	0.035445	1.74E-11	0.069131	3.57E-12	0.097174	2.07E-11
TraesCS6D02G332500	**Dehydrin**	**-0.21932**	**0.013932**	**-0.44781**	**3.92E-05**	**-1.05319**	**1.27E-05**	**-1.06874**	**2.44E-06**
TraesCS7A02G245400	Receptor-like kinase	-0.0047	5.35E-14	-0.04551	1.20E-13	0.069182	4.48E-12	0.097253	6.05E-11
TraesCS7A02G400800	ATP binding microtubule motor family protein	0.016805	1.71E-13	0.035426	4.89E-13	0.069201	1.04E-12	0.097273	6.45E-12
TraesCS7D02G549900	**Dehydrin**	**-0.3996**	**0.008798**	**-0.44297**	**0.035011**	**-1.00727**	**0.001745**	**-1.00712**	**0.000594**

TraesCSU01G069100	Glycerol-3-phosphate acyltransferase 3, putative	-0.00594	3.00E-13	-0.04552	1.11E-13	0.069132	3.49E-12	0.097176	2.02E-11
TraesCSU01G136200	Protein kinase, putative	-0.0067	6.17E-14	-0.04545	2.07E-13	-0.09689	2.96E-13	-0.09728	1.58E-13
TraesCSU01G216900	ARM repeat superfamily protein	0.021356	2.10E-17	0.035415	2.51E-11	0.069201	1.04E-12	0.097273	6.45E-12

Table 2.8: ANOVA results for Si, landrace, and their interaction, on Si concentration, dry weight, spine density, and stomatal conductance for plants grown hydroponically at different levels of Si supplementation. Replicate was included as a random effect to account for variability between plants grown at different times, apart from the models examining spine density and stomatal density where the two replicates were carried out at the same time. Statistically significant results are highlighted in bold.

	Shoot Si (%)			Root Si (%)			Shoot dry weight (g)			Spine density (mm^{-2})			Stomatal density (mm^{-2})			Stomatal conductance (mmol m^{-2} s^{-1})		
	df	*F*	*P*	*df*	*F*	*P*	*df*	*F*	*P*	*df*	*F*	*P*	*df*	*F*	*P*	*df*	*F*	*P*
Si	**2**	**4919.06**	**< 0.001**	**2**	**133.88**	**< 0.001**	2	1.18	0.309	2	0.60	0.555	2	2.06	0.139	2	0.57	0.571
Landrace	**19**	**7.24**	**< 0.001**	19	1.47	0.106	**19**	**8.64**	**< 0.001**	**19**	**7.39**	**< 0.001**	**19**	**2.20**	**0.015**	**5**	**1.00**	**< 0.001**
Replicate	**3**	**95.83**	**< 0.001**	3	1.13	0.339	**3**	**182.70**	**< 0.001**							**3**	**27.94**	**< 0.001**
Si x Landrace	38	1.14	0.275	37	1.11	0.327	38	0.97	0.520	38	0.75	0.817	38	0.84	0.713	10	0.92	0.524

Table 2.9: ANOVA results for Si, landrace, time, and their interactions, on shoot Si concentration. Replicate was included as a random effect to account for variability between plants grown at different times. Statistically significant results are highlighted in bold.

	Shoot Si (%)			Root Si (%)		
	df	*F*	*P*	*df*	*F*	*P*
Si	**2**	**4287.93**	**< 0.001**	**2**	**54.29**	**< 0.001**
Time	**5**	**5.64**	**< 0.001**	**5**	**2.37**	**0.047**
Landrace	**5**	**13.83**	**< 0.001**	**5**	**2.77**	**0.024**
Replicate	**2**	**36.84**	**< 0.001**	**2**	**11.53**	**< 0.001**
Si x Time	**10**	**3.19**	**< 0.001**	9	0.83	0.594
Si x Landrace	10	1.40	0.187	10	0.76	0.666
Time x Landrace	25	0.60	0.931	24	1.04	0.434
Si x Time x Landrace	50	0.55	0.992	32	1.32	0.166

Table 2.10: ANOVA results for Si, landrace, and their interaction, on transpiration. Statistically significant results are highlighted in bold.

	Transpiration (mL H_2O hr^{-1} g DW^{-1})		
	df	*F*	*P*
Si	1	0.02	0.893
Landrace	**5**	**2.98**	**0.039**
Si x Landrace	5	1.74	0.176

Table 2.11: ANOVA results for Si, landrace, and their interaction, on Si transporter gene expression (Normalised expression values). Replicate was included as a random effect to account for variability between plants grown at different times. A Bonferroni correction for multiple testing was applied setting the significance level to 0.0125. Statistically significant results are highlighted in bold.

	Lsi1			*Lsi2*			*Lsi3*			*Lsi6*		
	df	*F*	*P*	*df*	*F*	*P*	*df*	*F*	*P*	*df*	*F*	*P*
Si Level	2	0.66	0.523	2	0.10	0.903	2	1.56	0.225	2	2.04	0.146
Landrace	7	0.85	0.554	7	1.56	0.180	7	2.46	0.036	7	0.67	0.694
Replicate	2	3.55	0.039	**2**	**7.09**	**0.003**	2	0.25	0.782	**2**	**22.18**	**< 0.001**
Si x Landrace	10	1.05	0.425	10	0.35	0.962	10	0.83	0.604	10	0.73	0.693

3 Chapter 3: The effect of damage on Si accumulation among wheat landraces

3.1 Introduction

Wheat is an important food crop worldwide, but significant yield losses are caused by biotic stress. Pests and pathogens are estimated to cause global wheat yield losses of 21.5 %, with aphids and armyworms the pests causing the greatest losses (Savary *et al*., 2019). Climate change is expected to exacerbate yield losses due to herbivory as higher temperatures increase the metabolic rate of insects, resulting in increased food consumption (Petersen *et al*., 2000; Dillon *et al*., 2010). Additionally, increased temperature will affect insect population growth rates, with increases expected outside of tropical regions (Deutsch *et al*., 2008). Overall, a warming of 2 °C is predicted to increase wheat yield losses from insect pests by as much as 46 % (Deutsch *et al*., 2018).

Pesticides are commonly applied to reduce the impacts of herbivory. However, despite such mitigation strategies, pests still reduce annual wheat yields by up to 7.9 % (Oerke, 2006). Moreover, pesticides are associated with significant negative environmental impacts, including reduced biodiversity (Beketov *et al*., 2013) and impacts on human health (Kim *et al*., 2017). Thus, alternative methods of reducing wheat losses due to herbivory are required.

Plants have many different types of defences against pests, including both physical and chemical defences (reviewed in Howe and Jander, 2007; Singh *et al*., 2020). Particularly in grasses, high silicon (Si) accumulation is an effective antiherbivore defence (Massey *et al*., 2006; Massey and Hartley, 2009). Grasses deposit Si in structures such as phytoliths and silicified spines (Hartley *et al*., 2015). Phytoliths increase leaf abrasiveness, which deters both insect and mammalian herbivores, as well as reducing their digestive efficiencies and growth (Massey and Hartley, 2006; Massey *et al*., 2008; Massey and Hartley, 2009; Massey *et al*., 2009). In addition, Si deposited in the apoplast may act as a physical barrier, potentially preventing the release of insect oral secretions and oviposition fluids, known as effectors, which are used by herbivores to recognise compatible host plants, into plant cells (Coskun *et al*., 2019a; Singh *et al*., 2020).

At least in grasses, Si is an inducible defence (Massey *et al*., 2007): in response to herbivory, Si accumulation in grasses increases, and this is correlated with reduced herbivory

(Reynolds *et al*., 2012; Hartley *et al*., 2015; Islam *et al*., 2020). In wheat, Si has been found to reduce slug feeding (Griffin *et al*., 2015), greenbug infection (Goussain *et al*., 2005), and rabbit herbivory (Cotterill *et al*., 2007). Si has been proposed as a less ecologically damaging alternative to conventional pesticides (Alhousari and Greger, 2018). However, there is currently a lack of knowledge regarding whether Si fertiliser is an economically viable method of reducing herbivory in crop species.

In particular, several factors affect the ability of Si to reduce herbivory in grasses. The intensity of herbivory appears to be an important factor in the induction of Si defences, with a study on the tussock grass *Deschampsia caespitosa* indicating that a threshold level of herbivory is required for triggering increased Si accumulation (Reynolds *et al*., 2012). Furthermore, at least in *D. caespitosa*, there is a time lag between the occurrence of herbivory and the induction of Si defences (Massey *et al*., 2008; Reynolds *et al*., 2012). Additionally, multiple damage events have been shown to be required to induce Si defences in the grasses *Lolium perenne* and *Festuca ovina* (Massey *et al*., 2007). However, previous studies have focussed on wild grass species, and whether repeated damage events are also required to induce Si defences in crops such as wheat has not yet been investigated.

The Si response to herbivory varies significantly between both plant species and genotypes (Hartley and DeGabriel, 2016). Different patterns of Si accumulation and deposition were found in three species of *Festuca* in response to artificial damage and Si supply (Hartley *et al*., 2015). Similarly, in the grass *Agrostis tenuis,* Si accumulation in response to damage varied between genotypes (Bañuelos and Obeso, 2000). Soininen *et al*. (2013) reported both within and between species variation in Si accumulation among grasses in response to artificial damage. However, whether there is a correlation between baseline Si accumulation and the induction of Si defences in response to herbivory has not yet been investigated.

In addition to acting as a physical defence, there is increasing evidence that Si accumulation also affects plant biochemistry and physiology (Singh *et al*., 2020). In rice, there is evidence that Si increased resistance to leaf folder both by increased cell silicification acting as physical barrier, and by increasing the activity of antioxidative enzymes (Han *et al*., 2016). Likewise, Yang *et al*. (2017) concluded that improved resistance to brown plant hopper in rice was the result of Si acting both as a mechanical defence barrier and by interacting with

defence-associated signalling pathways. In wheat, Si increased the activity of enzymes involved in the synthesis of defence compounds, which was correlated with a reduction of the number of aphids on the plant (Gomes *et al*., 2005).

The phytohormone jasmonic acid (JA) is important in mediating the plant response to herbivory (Howe and Jander, 2007). Various studies have shown that Si accumulation and the JA response can mutually influence each other. Ye *et al*. (2013) suggested that there is a positive feedback loop such that Si accumulation promotes the JA pathway, and the JA pathway promotes Si accumulation. Likewise, further evidence of Si promoting the JA pathway comes from a study in rice by Lin *et al*. (2019) using an *Oslsi1* mutant deficient in Si transport. Infected wild-type plants treated with Si had higher transcript levels of genes related to JA biosynthesis and perception than infected plants not treated with Si, but this difference was not seen in the *Oslsi1* mutant (Lin *et al*., 2019). However, Kim *et al*. (2014), Hall *et al*. (2019), and Johnson *et al*. (2020) suggested that while JA acts to promote Si accumulation, Si inhibits the accumulation of JA. Further work is needed to understand the interaction between Si accumulation and JA signalling. In particular, it is currently unknown whether the extent to which a plant accumulates Si directly correlates with changes in JA signalling.

The ability of plants to use Si to reduce herbivory is not universal (Bañuelos and Obeso, 2000; Kvedaras *et al*., 2009). In particular, Si defences may be ineffective against phloem-feeding pests such as aphids (Massey *et al*., 2006; Rowe *et al*., 2020). Moreover, herbivory does not always induce increased Si accumulation (Quigley and Anderson, 2014). There may be genotypic variation in the effect of herbivory on Si accumulation. Further research is needed to establish during which conditions Si fertiliser will be most beneficial and economically feasible as a mechanism to improve crop resistance to herbivory (Singh *et al*., 2020). To improve understanding about the induction of Si defences and the underlying mechanisms, experiments where damage levels can be controlled, and the signals for induction can be identified, are required. Artificial damage can be used in place of herbivory to separate the effects of damage induced by the herbivore from the effect of molecules in the saliva and other excretions of the herbivore (Waterman *et al*., 2019).

In this chapter, a series of experiments was conducted to improve knowledge regarding the role of Si as an antiherbivore defence in wheat. Genetically diverse landraces were used to

examine whether there is genotypic variation in the effect of damage on Si accumulation. Specifically, the following questions were addressed:

1. Does the increase in Si accumulation in response to damage vary among wheat landraces, and how is this influenced by Si supply?
2. Does damage induce systemic Si defences throughout damaged plants, or only a localised response close to the site of wounding?
3. Does increased Si accumulation result in increased silicified spine density on the leaves of damaged plants?
4. Is increased Si accumulation the result of changes in Si transporter gene expression?
5. Does Si affect the expression of JA-related genes, and does this differ as a function of landrace and Si supply?
6. How many damage events are needed to induce an increase in Si, and how quickly does Si accumulation take place after plants are damaged?

3.2 General methods

3.2.1 Growth conditions

For all experiments, plants supplemented with Si received ½-strength Hoagland's solution containing 1.8 mM dissolved sodium metasilicate ($Na_2SiO_3.9H_2O$). Sodium chloride (NaCl) was used to balance sodium levels for plants not supplemented with Si. Plants were grown under controlled glasshouse conditions (16 h daylight; 20 °C /15 °C day/night). At harvest, roots were washed in deionised water and then leaf and root fresh weights were recorded. Plants were oven-dried at 70 °C until constant mass was achieved, then dry weight was recorded. For damaged plants, the weights of damaged and undamaged leaves were recorded separately.

For plants grown hydroponically, seeds were germinated in sand for 10-11 days, and then seedlings were transferred to 9 L hydroponics boxes, filled with ½-strength Hoagland's solution. The pH was adjusted to 5.6-6.0 using 1 M HCl or 0.1 M KOH. The nutrient solution was changed every 3-4 days. The hydroponics solutions were aerated throughout the experiment.

3.2.2 Damage treatment

A damage treatment was started four weeks after germination and continued for three weeks. Plants were damaged two times per week (three for experiment 1) by removing approximately half of a leaf along the midrib. Plants exhibiting slow growth that had not produced new leaves since the previous damage event were damaged only by removing more of an already damaged leaf. Plants were harvested one day after the final damage event, seven weeks after germination. Plants that were not mechanically damaged were labelled as undamaged plants. The weight and Si concentration of damaged and undamaged leaves of damaged plants were analysed separately.

3.2.3 Si measurements

Leaf and root Si concentration was measured by portable X-ray fluorescence spectroscopy (P-XRF) as described in section 2.2.2.

3.2.4 Measuring expression of Si transporters and JA-related genes

To measure gene expression, primer design and qPCR was performed as described in section 2.2.10. Primers were designed to match all homoeologs (Table 3.1). Primers for a peroxidase (*POX*) were taken from Bozkurt *et al*. (2010). A primer concentration of 200 nM (not 350 nM) was used for *allene oxide cyclase* (*AOC*). To investigate the effect of damage on the expression of JA-related genes, log ratios were used to calculate the fold change in gene expression between damaged and undamaged plants.

Table 3.1: List of primers used for RT-qPCR.

Target Gene	Forward Primer	Reverse Primer	Product size	Ensembl gene name (all homologues)
Actin	TACTCCCTCACAACAACCGC	CTCCTAGCCGTTTCCAGCTC	104	TraesCS1A02G020500, TraesCS1B02G024500, TraesCS1D02G020000
Lsi1	CCTTCTCCAGCGAGATCCAC	CCTCCGACACCACCTTCTTG	129	TraesCS6A02G307300, TraesCS6B02G335900, TraesCS6D02G286400
Lsi2	TCATCGCCTTCAACAGCAAG	TCCTTCCAGTACATGCAGAGC	115	TraesCS5A02G529900, TraesCS4B02G361900, TraesCS4D02G354900
Lsi3	TGTTCAAGTACCTCGGCAAC	TTGAGGATGAACTCGGTGAGG	144	TraesCS4A02G412500, TraesCS4B02G312600, TraesCS4D02G310100
Lsi6	TACTCGAACGAGATCCACGAC	TCTCCGATATCACCTTCTTGCC	132	TraesCS7A02G187800, TraesCS7B02G092900, TraesCS7D02G188800
POX	CAAGGTGAACTCGTGATGGA	TTGAGGATTCAACCGTCGTT	176	TraesCS2A02G107600, TraesCS2B02G125300, TraesCS2D02G107900
AOC	ATCTGCATCCTGATCCAGCAC	TCGCCGAAGTAGATGCTGTAG	77	TraesCS6A02G334800, TraesCS6B02G365200, TraesCS6D02G314300
AOS	ACAAGGCGCTGTACAAGTAC	GAACAGCAGGTTGTGGCATG	104	TraesCS4A02G061900, TraesCS4B02G237600, TraesCS4D02G238800
COI1	TGGCATGCAAGAAGAGGAAG	TTTGCAGAACGTTCCGATGG	142	TraesCS1A02G279100, TraesCS1B02G288100, TraesCS1D02G278400
FPS	TGGAGACGGCATTTCACAAG	TCTGAACAGCTTTGCTTGGC	87	TraesCS3A02G254300, TraesCS3B02G286300, TraesCS3D02G255200

3.2.5 Statistical analysis

All statistical analyses were performed using R software (version 3.6.1, R Core Team, 2020). Summary statistics were calculated using the Rmisc package (Hope, 2013) and graphs were produced using the ggplot2 package (Wickham, 2016). Three-way analysis of variance (ANOVA) was used to test the effect of Si supply, damage, and landrace on Si concentration, spine density, and gene expression. The effect of Si supply, damage, and time on Si concentration was tested using a three-way ANOVA with temporal replicate included as a factor to account for variation caused by plants being grown at different times. Due to the lack of independence between damaged and undamaged leaves from damaged plants, ANOVAs were performed separately comparing undamaged plants to either damaged or undamaged leaves of damaged plants. Additionally, the average leaf Si concentration of damaged plants was calculated by averaging the Si concentration of damaged and undamaged leaves, accounting for differences in the proportion of the leaves that were damaged or undamaged. ANOVA was then used to compare the average leaf Si concentration of damaged plants to undamaged plants.

Data normality was checked using Shapiro tests and homogeneity of variance was tested using Levene's tests. To satisfy the test assumptions, Si concentrations were logit transformed. The expression of JA-related genes was log transformed. No transformation was applied to spine density. Paired *t*-tests were used to test for localised induction of Si defences between damaged and undamaged leaves of damaged plants. A significance level of $P < 0.05$ was used for all analyses, except for gene expression analyses, where a Bonferroni correction to account for multiple testing was applied. Significant results were analysed by performing Tukey's Honest Significance Difference (HSD) *post-hoc* tests using the emmeans package (Lenth, 2021).

3.3 Experiment 1: The effect of Si supply on Si accumulation and Si transporter gene expression after damage in contrasting landraces

3.3.1 Experiment 1: Methods

3.3.1.1 Experimental design

The two highest and two lowest Si-accumulating landraces, as characterised in 2, were selected to examine the effects of damage on Si accumulation (H1, H3, L4, L5, respectively). A balanced factorial experimental design was used with plants either damaged or not and

supplemented with Si (+Si) or not (–Si). Three plants per landrace per Si supplementation level per damage treatment were used, giving a total of 48 plants. Plants were grown hydroponically. At harvest, epidermal peels were made to measure spine density (3.3.1.2) and leaf samples were taken to measure Si transporter gene expression (3.3.1.3). Root and leaf Si concentration was determined using P-XRF (2.2.2; 3.2.3).

3.3.1.2 Spine density estimation

Leaf spine density was measured using epidermal peels as described in section 2.2.6. For damaged plants, a damaged and undamaged leaf was used. Three leaves per landrace per treatment were measured.

3.3.1.3 Si transporter gene expression

To investigate the possible causes of variation in Si concentration between landraces, the expression levels of the Si transporters *Lsi1, Lsi2, Lsi3,* and *Lsi6* were determined using RT-qPCR, as described in section 3.2.4. For each landrace, RNA was extracted from the leaves of one undamaged plant and two damaged plants grown with 1.8 mM Si. RNA was extracted from undamaged plants, and damaged, and undamaged leaves of damaged plants, separately.

3.3.2 Experiment 1: Results

3.3.2.1 Damage increases leaf Si accumulation

Repeated damage significantly increased leaf Si accumulation in damaged leaves in both +Si and –Si plants, although this response was larger in +Si plants (Figure 3.1). For +Si plants, damage significantly decreased Si accumulation in the undamaged leaves of damaged plants compared to undamaged plants. For –Si plants, there was no significant difference in leaf Si concentration between undamaged leaves of damaged plants and undamaged plants (Table 3.2). There was no significant variation in the Si response to damage among landraces, as indicated by the lack of significant interaction between damage and landrace in the ANOVA model. Damaged leaves had higher Si concentrations than undamaged leaves of the same plant, but this localised induction was more pronounced for +Si plants than –Si plants (Table 3.3). In contrast to the strong effect of damage on leaf Si accumulation, no significant effect of damage on root Si accumulation was found (Figure 3.1; Table 3.2).

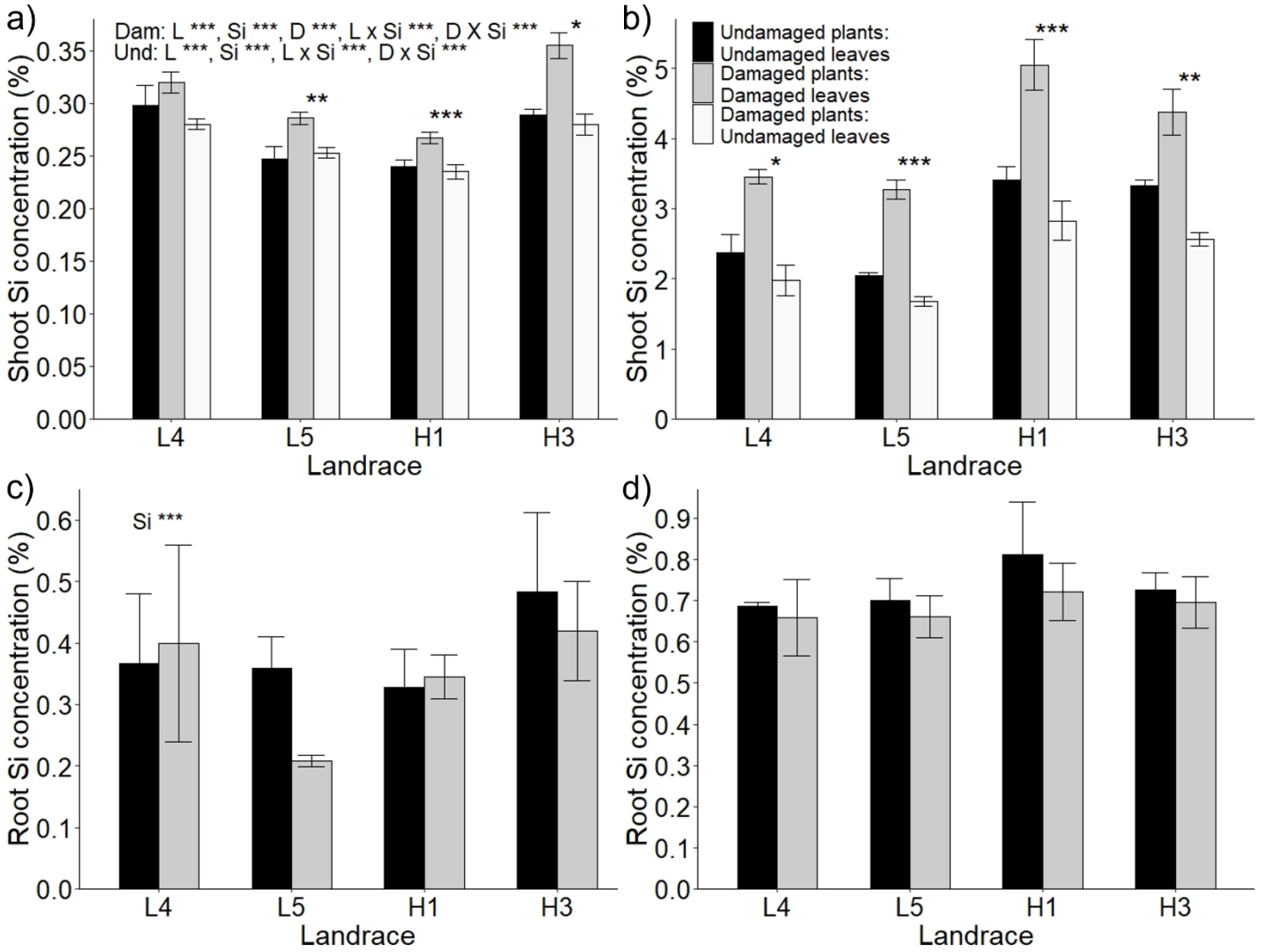
a)
Dam: L ***, Si ***, D ***, L x Si ***, D X Si ***
Und: L ***, Si ***, L x Si ***, D x Si ***
Shoot Si concentration (%)
0.00
0.05
0.10
0.15
0.20
0.25
0.30
0.35
**
***
*
L4
L5
H1
H3
Landrace
b)
Undamaged plants:
Undamaged leaves
Damaged plants:
Damaged leaves
Damaged plants:
Undamaged leaves
Shoot Si concentration (%)
0
1
2
3
4
5
*
***
***
**
L4
L5
H1
H3
Landrace
c)
Si ***
Root Si concentration (%)
0.0
0.1
0.2
0.3
0.4
0.5
0.6
L4
L5
H1
H3
Landrace
d)
Root Si concentration (%)
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
L4
L5
H1
H3
Landrace

Figure 3.1: Effect of Si supply and damage on Si accumulation. a) Leaf Si of –Si plants. b) Leaf Si of +Si plants. c) Root Si of –Si plants. d) Root Si of +Si plants. Note the different scales on the y-axis. Statistically significant impacts and interactions, determined by three-way ANOVA, are indicated where *** $P < 0.001$, ** $P < 0.01$, and * $P < 0.05$. The Si concentration of leaves from undamaged plants, and the damaged and undamaged leaves from damaged plants was analysed separately. Dam: lists significant results from the ANOVA comparing damaged leaves of damaged plants to undamaged plants and Und: lists significant results from the ANOVA comparing undamaged leaves of damaged plants to undamaged plants. L: landrace, Si: level of Si supplementation. D: damage treatment. Significant differences between damaged and undamaged leaves of damaged plants, as determined by paired *t*-tests, are shown. Mean values ± standard error (SE) are shown. N = 3. H indicates a high Si-accumulating landrace. L indicates a low Si-accumulating landrace.

3.3.2.2 No effect of damage on spine density

There was no significant effect of damage or Si supplementation on silicified spine density, although there was significant variation between landraces (Figure 3.2; Table 3.2). Similarly, there was no significant difference in spine density between damaged and undamaged leaves of the same plant (*t*-tests, $P > 0.05$ for all comparisons).

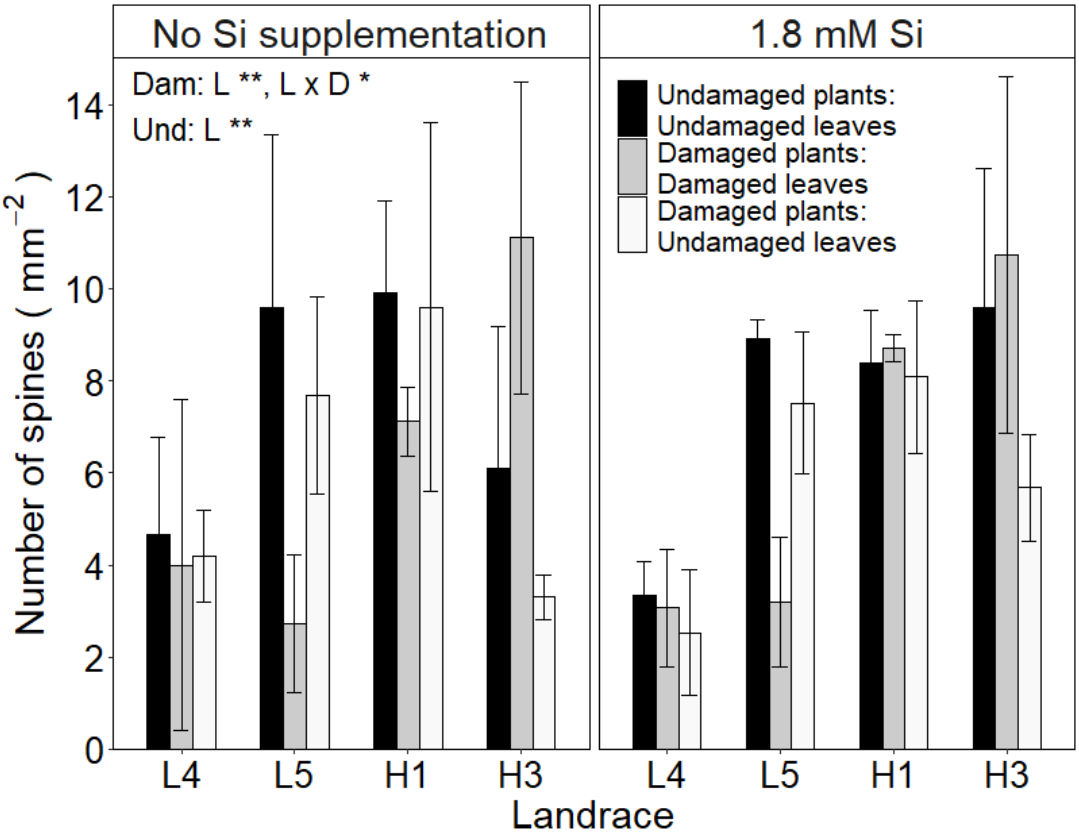

Figure 3.2: Variation in silicified spine density between landraces subject to damage treatment. Mean values ± SE are shown. N = 3. The spine density of leaves from undamaged plants, and the damaged and undamaged leaves from damaged plants was analysed separately. Statistically significant impacts and interactions, determined by three-way ANOVA, are indicated where *** $P < 0.001$, ** $P < 0.01$, and * $P < 0.05$. Dam: lists significant results from the ANOVA comparing damaged leaves of damaged plants to undamaged plants and Und: lists significant results from the ANOVA comparing undamaged leaves of damaged plants to undamaged plants. L: landrace, Si: level of Si supplementation. D: damage treatment. There were no significant differences between damaged and undamaged leaves of damaged plants. Mean values ± SE are shown. N = 3. H indicates a high Si-accumulating landrace. L indicates a low Si-accumulating landrace

3.3.2.3 No effect of damage on Si transporter gene expression

There was no consistent induction in Si transporter genes due to damage, although *Lsi6* expression was significantly lower in damaged plants than undamaged plants (Figure 3.3; Table 3.4). There was significant variation in *Lsi1* and *Lsi3* expression between landraces. *Lsi2* expression was too low to be detected in several samples and thus not analysed.

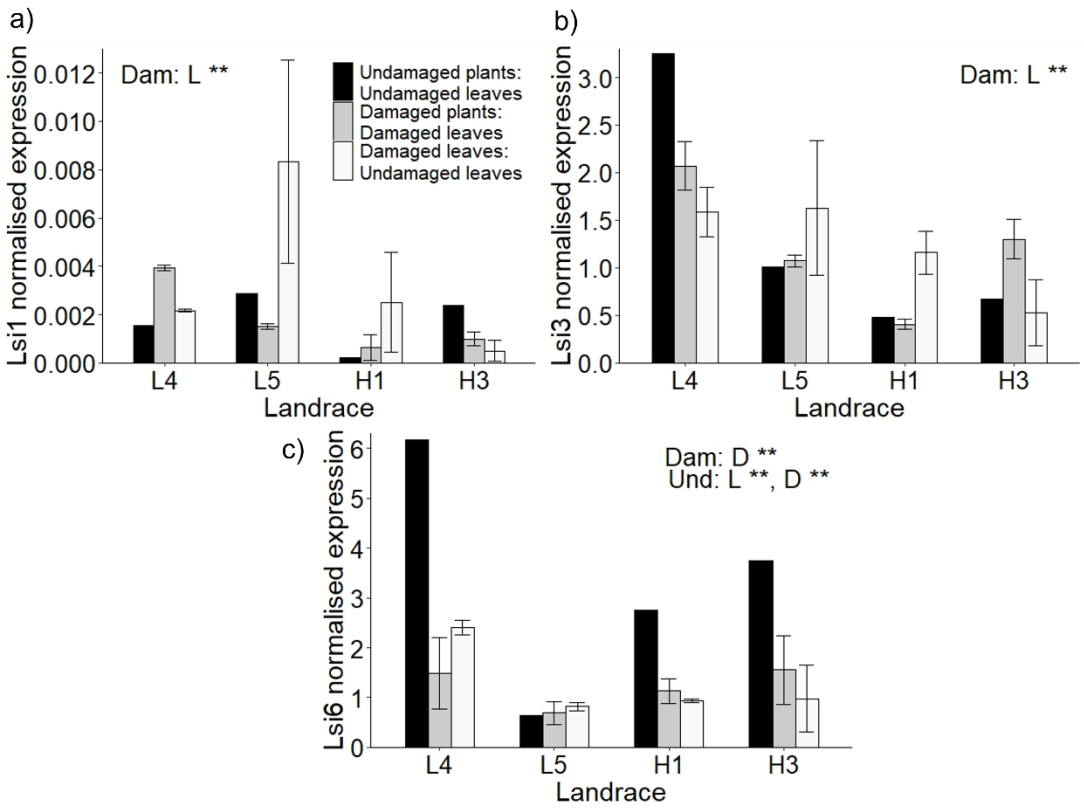

Figure 3.3: Variation in Si transporter gene expression due to damage in wheat landraces. a) *Lsi1*. B) *Lsi3.* C) *Lsi6*. Note the different scales on the y-axis. The gene expression of leaves from undamaged plants, and the damaged and undamaged leaves from damaged plants was measured separately. *Lsi2* gene expression was too low to be detected for several samples and is not shown. Statistically significant impacts and interactions, determined by three-way ANOVA, are indicated where *** $P < 0.001$, ** $P < 0.01$, and * $P < 0.05$. Dam: lists significant results from the ANOVA comparing damaged leaves of damaged plants to undamaged plants and Und: lists significant results from the ANOVA comparing undamaged leaves of damaged plants to undamaged plants. L: landrace, Si: level of Si supplementation. D: damage treatment. There were no significant differences between damaged and undamaged leaves of damaged plants. Mean values ± standard error (SE) are shown. N = 1 for undamaged leaves of undamaged plants, N = 2 for damaged and undamaged leaves of damaged plants. H indicates a high Si-accumulating landrace. L indicates a low Si-accumulating landrace.

3.4 Experiment 2: Variation in Si accumulation and expression of JA-related genes in response to damage among landraces

3.4.1 Experiment 2: Methods

3.4.1.1 Experimental design

To further investigate whether damage induced different Si responses among landraces, five high and five low Si-accumulating landraces were used (as characterised in 2: H1, H3, H4, H5, H7, L1, L2, L3, L4, L5). A balanced factorial experimental design was used with plants either damaged or undamaged. Three plants per landrace per treatment were used, giving a total of 60 plants. Two seeds were planted in 1 L pots filled with a 2:1 mix of sand and terragreen. One week after germination, seedlings were thinned to one plant per pot. After thinning, all plants were fed twice weekly with 200 mL ½-strength Hoagland's solution supplemented with 1.8 mM Si. Plants were watered as required. At harvest, leaf samples were taken from undamaged plants and from undamaged leaves of damaged plants to measure the expression of JA-related genes. Leaf Si concentration was determined using P-XRF (2.2.2; 3.2.3).

3.4.1.2 Expression of JA-related genes

To investigate whether there is a correlation between Si accumulation and variation in JA signalling among landraces, the expression levels of two JA biosynthesis genes, *allene oxide cyclase* (*AOC*) and *allene oxide synthase* (*AOS*), and the JA receptor, *coronatine-insensitive1* (*COI1*) were determined. Additionally, the expression levels of *farnesyl pyrophosphate synthetase* (*FPS*), involved in terpene synthesis, which occurs as part of the defence response, and a *peroxidase* (*POX*), which encodes an antioxidative enzyme that shows increased expression upon herbivory, were measured.

It was hypothesised that any effect of Si on the expression of JA-related genes may vary over time, thus gene expression was measured at four time-points. Undamaged leaves of damaged and undamaged plants were sampled at 6 h and 24 h after the first and final damage event and used for RNA extraction. The same two high Si landraces (H1, H3) and one low Si landrace (L4) that were used for Si transporter expression were used. Additionally, the low Si landrace, L1 was used. Gene expression was measured for the undamaged leaves of two damaged plants and leaves of two undamaged plants. A pilot study found no effect of Si supply on the expression of JA-related genes; therefore gene expression was only measured for +Si plants (data not shown).

3.4.2 Experiment 2: Results

3.4.2.1 Damage significantly increased Si accumulation

As no significant effect of damage on Si accumulation was found for –Si plants, or in the roots (Experiment 1; 3.3), the effect of damage on Si accumulation in the full range of ten landraces was only investigated for the leaves of +Si plants (Figure 3.4). Consistent with experiment 1, repeated damage significantly increased Si accumulation in damaged leaves (Table 3.5). However, the extent of this increase did not vary significantly among landraces, as indicated by the lack of significant interaction in the ANOVA results between landrace and damage (Table 3.5). There was no significant correlation between leaf Si in undamaged plants and the increase in leaf Si due to damage (r = -0.32, P = 0.364). Interestingly, damage significantly decreased Si accumulation in undamaged leaves (Table 3.5).

There was a localised response to damage, such that damaged leaves had higher Si concentrations than undamaged leaves of each damaged plant. This response was significant in all landraces except landraces H5 and L2, where it was almost significant (Table 3.6). Although not significant, the extent of this localised response varied across landraces, with the biggest increase in Si of 107.6 ± 28.4 % in L3, compared to an increase of only 34.2 ± 9.3 % in H5. However, when using the average leaf Si concentration for all leaves of damaged plants, there was no significant difference between undamaged and damaged plants for any landrace (Figure 3.5; t-tests, $P > 0.05$ for all comparisons). Overall, Si accumulation was slightly, but significantly, lower in damaged plants compared to undamaged plants (Table 3.5). Thus, induction of Si defences occurred only locally and was not systemic.

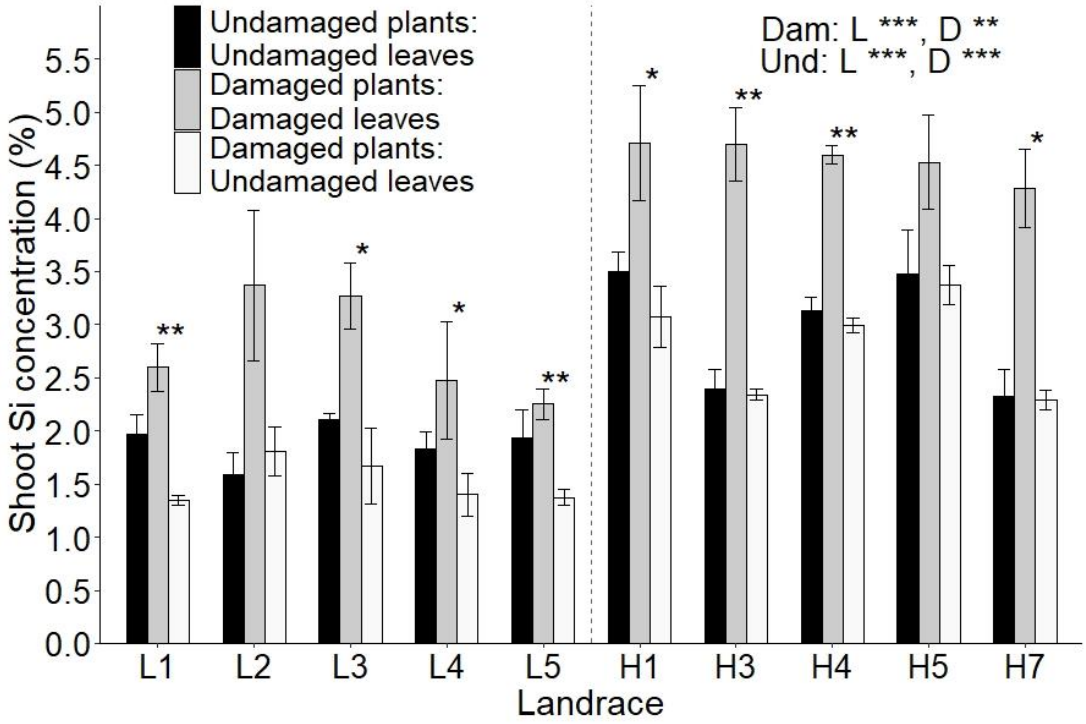

Figure 3.4: Variation in Si accumulation due to damage among wheat landraces. Leaf Si concentration for damaged and undamaged leaves of damaged plants, and undamaged plants. Statistically significant impacts and interactions, determined by two-way ANOVA, are indicated where *** $P < 0.001$, ** $P < 0.01$, and * $P < 0.05$. Dam: lists significant results from the ANOVA comparing damaged leaves of damaged plants to undamaged plants and Und: lists significant results from the ANOVA comparing undamaged leaves of damaged plants to undamaged plants. L: landrace, D: damage treatment. Significant differences between damaged and undamaged leaves of damaged plants, as determined by paired *t*-tests, are shown. Mean values ± SE are shown. N = 3. The dotted line separates high (H) Si-accumulating landraces and low (L) Si-accumulating landraces.

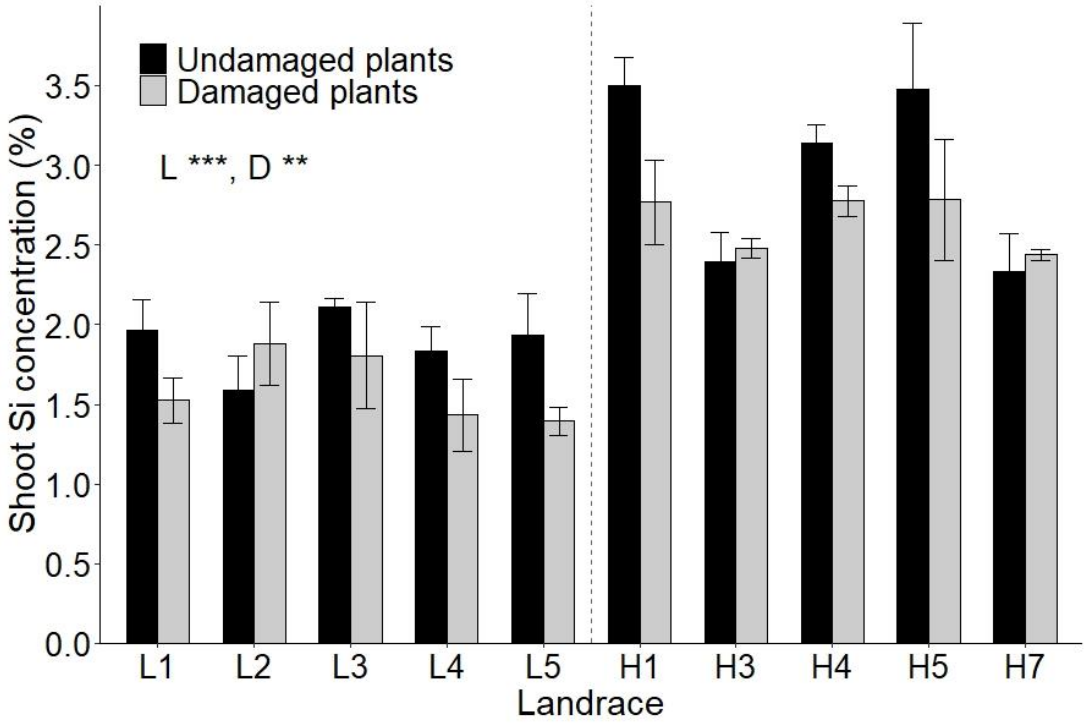

Figure 3.5: Variation in Si accumulation between damaged and undamaged plants for ten wheat landraces. The average leaf Si concentration for damaged and undamaged leaves of damaged plants was calculated. Statistically significant impacts and interactions, determined by two-way ANOVA, are indicated where *** $P < 0.001$, ** $P < 0.01$, and * $P < 0.05$. L: landrace, D: damage treatment. Mean values ± SE are shown. N = 3. The dotted line separates high (H) Si-accumulating landraces and low (L) Si-accumulating landraces.

3.4.2.2 No effect of damage on the expression of JA-related genes

In a preliminary study, no effect of Si supply on the expression of JA-related genes was found (data not shown). Thus, gene expression was only measured for +Si plants. There were no consistent changes in the expression of JA-related genes due to damage, with expression found to be highly variable between landraces at all time-points (Figure 3.6; Table 3.7). There was no significant correlation between Si concentration at harvest and the expression of JA-related genes at any time-point for any gene studied (data not shown).

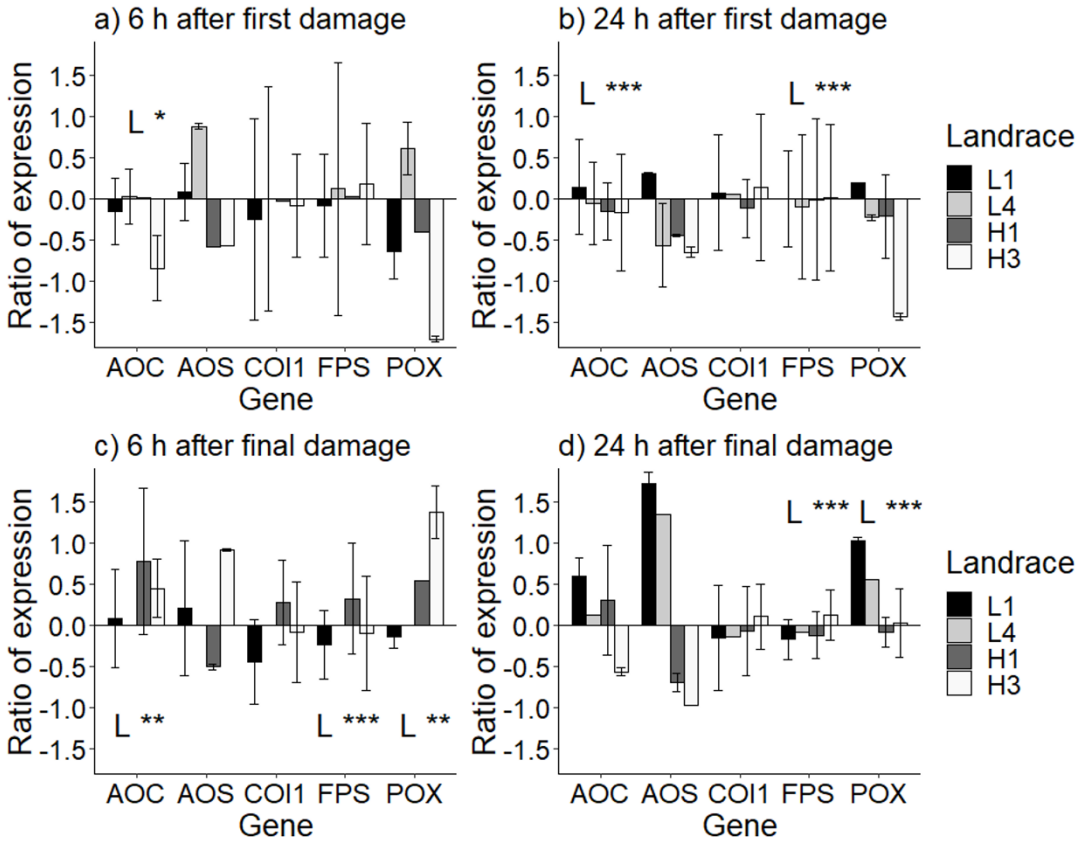

Figure 3.6: Effect of damage on the expression of JA-related genes. The log ratio of gene expression for undamaged leaves of damaged plants compared to undamaged leaves of undamaged plants was calculated for each time-point: a) 6 h after the first damage event; b) 24 h after the first damage event; c) 6 h after the final damage event; d) 24 h after the final damage event. A ratio greater than zero indicates that gene expression was increased, and a ratio less than zero indicates expression was decreased, in undamaged leaves of damaged plants relative to undamaged plants. Statistically significant impacts and interactions, determined by two-way ANOVA, are indicated above the relevant gene where *** $P < 0.001$, ** $P < 0.01$, and * $P < 0.05$. L: landrace, D: damage treatment. Mean values ± SE are shown. N = 2. L denotes low Si-accumulating landraces and H denotes high Si-accumulating landraces. Insufficient RNA was obtained to measure gene expression for landrace L4 6 h after the final damage event.

3.5 Experiment 3: Number of damage events required to induce a Si response

3.5.1 Experiment 3: Methods

3.5.1.1 Experimental design

To investigate both long- and short-term Si accumulation in response to damage, a time-course experiment using the high Si-accumulating landrace H1 was performed. Three temporally separate replicates were performed, with a minimum of two weeks between replicates, giving a total of three plants per treatment. Plants were grown hydroponically (3.2.1). Damage treatment was performed as described in 3.2.2. One plant from each treatment was harvested 0, 4, 10, 24, 48, and 72 h after the first damage event, and 96 h after the second, fourth, and sixth damage events. Leaf and root Si concentration was determined using P-XRF (2.2.2; 3.2.3).

3.5.2 Experiment 3: Results

3.5.2.1 Si accumulation increased after two damage events

It has been suggested that a single herbivory or damage event is insufficient to induce increased Si accumulation (Massey *et al*., 2007). This conclusion was supported by a preliminary experiment that found no increase in leaf Si after a single damage event in two high Si-accumulating landraces (data not shown). Thus, here it was investigated whether a threshold number of damage events is required to increase Si accumulation.

The high Si landrace H1 was used to examine Si accumulation after multiple damage events (Figure 3.7; Table 3.8). As hypothesised, no significant increase in leaf Si in damaged leaves after a single damage event was observed. However, after two damage events, in +Si plants, damage significantly increased Si accumulation (Tukey's HSD: $P < 0.05$ at 10 d, 17 d, and 24 d sampling). While –Si plants also increased their Si accumulation in response to multiple damage events, this increase was not significant at any time-point (Tukey's HSD, $P > 0.05$ for all comparisons). No difference in root Si accumulation between damaged and undamaged plants was observed at any time-point (Figure 3.7; Table 3.8).

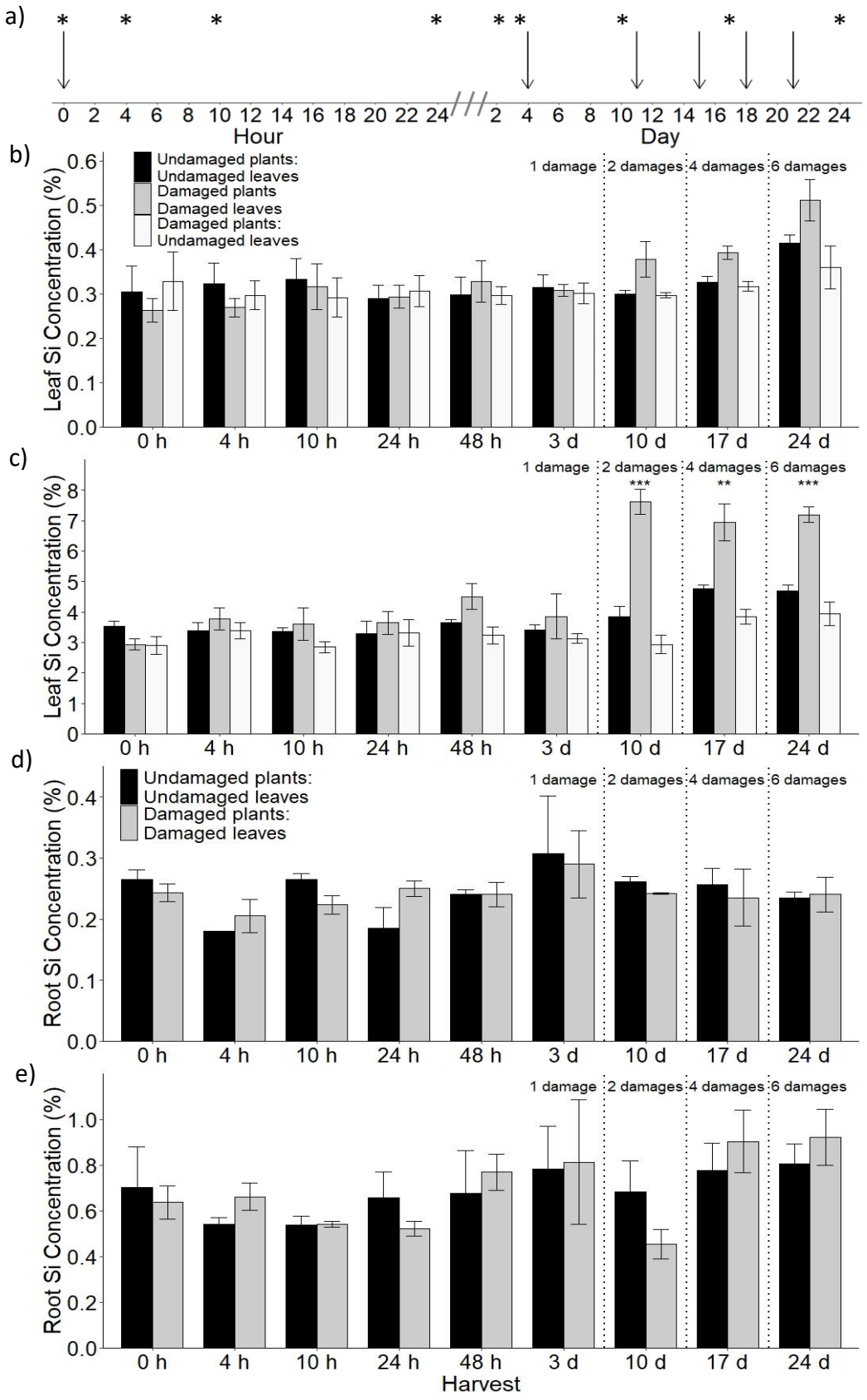
a)
0 2 4 6 8 10 12 14 16 18 20 22 24 2 4 6 8 10 12 14 16 18 20 22 24
Hour
Day
b)
Undamaged plants:
Undamaged leaves
Damaged plants
Damaged leaves
Damaged plants:
Undamaged leaves
1 damage
2 damages
4 damages
6 damages
Leaf Si Concentration (%)
0 h
4 h
10 h
24 h
48 h
3 d
10 d
17 d
24 d
c)
***
**
***
d)
Undamaged plants:
Undamaged leaves
Damaged plants:
Damaged leaves
Root Si Concentration (%)
e)
Harvest

Figure 3.7: Variation in Si accumulation over time after damage. a) Timeline showing when damage events occurred (arrows) and when plants were sampled (asterisks). B) Leaf Si of –Si plants. c) Leaf Si of +Si plants. d) Root Si of –Si plants. e) Root Si of +Si plants Si. Note the different scales on the y-axis. The Si concentration of leaves from undamaged plants, and the damaged and undamaged leaves from damaged plants was analysed separately. Time-points where the Si concentration was significantly higher in damaged leaves of damaged plants compared to undamaged leaves of undamaged plants, determined by *post-hoc* Tukey's HSD tests, are indicated where *** $P < 0.001$, ** $P < 0.01$, and * $P < 0.05$. Mean values ± SE. N = 3. The high Si-accumulating landrace, H1, was used.

3.6 Discussion

3.6.1 Does Si induction in response to damage vary among wheat landraces, and how is this influenced by Si supply?

Numerous studies have demonstrated that Si accumulation increases leaf abrasiveness and thus deters herbivores (e.g. Cotterill *et al*., 2007; Massey and Hartley, 2009; Griffin *et al*., 2015). It is also known that herbivory increases Si accumulation (Massey and Hartley, 2006; Massey *et al*., 2007). However, whether there is variation in the extent to which herbivory increases Si accumulation between genotypes has not yet been extensively investigated. To test this, mechanical damage can be used as a form of simulated herbivory to separate the effects of damage induced by the herbivore from the effect of molecules released by the herbivore (Waterman *et al*., 2019). Such mechanical damage has indeed been found to increase plant Si (McNaughton *et al*., 1985; Kim *et al*., 2014; Ryalls *et al*., 2018). Here, it was shown that wheat also responds to simulated herbivory, in the form of mechanical damage, by increasing leaf, but not root, Si accumulation.

Although there was significant variation in Si accumulation among wheat landraces, the landraces did not vary significantly in the extent to which they increased Si content in response to herbivory. In contrast, Bañuelos and Obeso (2000) reported significant genotypic variation in response to damage in the grass species *Agrostis tenuis*. Likewise, using six genotypes for each of four grass species, Soininen *et al*. (2013) found significant genotypic variation in Si induction in response to damage in only two of the species examined. França *et al*. (2019) reported genotype-specific effects of Si in rice, such that Si reduced stem damage by stink bugs in only two out of three genotypes investigated. In this

study, the percentage increase in Si due to damage was larger for +Si plants than for –Si plants, but in neither case was the variation between landraces significant.

3.6.2 Does damage induce systemic Si defences throughout damaged plants, or only a localised response close to the site of wounding?

Although Si accumulation increased in wheat landraces in response to damage, this was a localised response, such that the Si concentration only increased in the damaged leaves of damaged plants. Overall, the Si concentration of damaged plants was not significantly different to that of undamaged plants. It is hypothesised that this is a result of preferential allocation of Si into damaged leaves, with the Si content of undamaged leaves of damaged plants being significantly lower than that of undamaged leaves of undamaged plants. In rice, Si transporters are used to preferentially allocate Si to the panicle and away from the flag leaf (Yamaji *et al*., 2015) and it is possible that a similar mechanism results in the preferential allocation of Si to damaged leaves. Alternatively, damage may increase transpiration in damaged leaves, resulting in damaged leaves having higher Si contents compared to undamaged leaves in damaged plants.

Increased Si concentration only in damaged leaves is in agreement with the results reported in Hartley *et al*. (2015), who reported localised induction of Si defences after damage in three grass species. However, although damaged leaves had higher Si compared to undamaged plants for all genotypes, McLarnon *et al*. (2017) found significantly increased Si in damaged leaves compared to undamaged leaves in only one out of three tall fescue genotypes investigated. In this study, all landraces responded to damage by increasing Si accumulation in damaged leaves compared to undamaged leaves, although this increase was only significant in eight out of the ten landraces studied. Despite ranging from 34.2 % to 107.6 %., the extent of this increase did not vary significantly among landraces.

Previous studies have reported increased leaf Si in response to both mechanical damage (Kim *et al*., 2014; McNaughton and Tarrants, 1983) and herbivory (Massey and Hartley, 2006; Johnson *et al*., 2019), but have not made a distinction between the Si concentration of damaged and undamaged leaves of damaged plants. Massey *et al*. (2007) reported that in *Lolium perenne,* locust and vole grazing increased Si accumulation, but artificial damage alone was insufficient to increase Si. In *Festuca ovina*, the increase in Si in response to herbivory was much greater than that in response to artificial damage (Massey *et al*., 2007). Molecules within herbivore secretions that get released into the plant may affect

the Si response, explaining why plants respond differently to mechanical damage compared to herbivory. Additionally, a more frequent damage treatment was used by McNaughton and Tarrants (1983) compared to Massey *et al*. (2007), which may explain the contrasting results regarding the ability of mechanical damage to induce increased Si accumulation.

3.6.3 Does increased Si accumulation result in increased spine density on the leaves of damaged plants?

Silicon structures including spines, trichomes, and phytoliths are important antiherbivore defences in plants (reviewed in Hartley and DeGabriel, 2016). A lack of silicified trichomes has been associated with increased herbivore susceptibility in rice (Andama *et al*., 2020). It was hypothesised that herbivory would increase spine density, however, no effect of Si or damage on spines was found. Likewise, Hartley *et al*. (2015) reported that damage had little effect on spine formation in three grass species. No effect of Si supplementation or herbivory on trichome density was found in maize or soybean, although combined herbivory and Si treatment increased trichome density in tomato (Acevedo *et al*., 2021).

Alternatively, it is possible that spine morphology, rather than density, was affected by damage treatment, but this was not measured in this study. In *Braychpodium distachyon*, increased silicified trichome size was correlated with decreased herbivore growth (Hall *et al*., 2020a). However, Hartley *et al*. (2015) found only slight morphological changes in silica structures in response to damage in three grass species. Another possibility is that, rather than being deposited as spines, additional Si accumulated as a result of damage is deposited in the cell wall, which may inhibit the entry of effectors released by insect herbivores, and therefore prevent insects from identifying the plant as a palatable target (Coskun *et al*., 2019a). In this study, only silicified spines were counted, but Si can also be deposited in other structures such as phytoliths and macrohairs (Hartley *et al*., 2015).

3.6.4 Is increased Si accumulation the result of changes in Si transporter gene expression?

While Si was consistently higher in damaged leaves, it was not possible to relate this to differences in Si transporter gene expression. *Lsi6* expression was significantly lower in both the damaged and undamaged leaves of damaged plants compared to undamaged plants, which is in contradiction with the observed increase in Si concentration in damaged leaves of damaged plants compared to undamaged leaves of undamaged plants. By contrast, Ye *et al*. (2013) reported increased Si transporter gene expression in response to

both methyl jasmonate treatment and leaf folder infection. In a similar experiment, Lin *et al*. (2019) also reported increased *Lsi1* expression in rice supplemented with Si and infected with leaf folder.

In this study, leaf samples to measure Si transporter gene expression were taken at the end of the experiment, after nine damage events. However, this study also showed that Si accumulation significantly increases after only two damage events. As changes in gene expression are often relatively short-lived, it is possible that in this study changes in Si transporter gene expression occurred in periods when no sampling took place. Increased *Lsi2* expression was found in the roots of tall fescue after eight weeks of damage (McLarnon *et al*., 2017), but whether similar expression changes occur in the leaves or in crop species such as wheat had not previously been examined. Alternatively, rather than changes in gene expression, post-transcriptional regulation may be important for regulating the activity of Si transporters.

3.6.5 Does Si affect the expression of JA-related genes, and does this differ as a function of landrace and Si supply?

No significant correlation between Si accumulation and JA-related gene expression was found in this study. Additionally, a preliminary study found no significant effect of Si on the expression of JA-related genes. Contrasting effects of Si on JA concentrations and signalling have also been reported in the literature. Ye *et al*. (2013) and Xue *et al*. (2021) suggested that Si primes the JA response, whereas Kim *et al*. (2014) and Hall *et al*. (2020b) reported that Si inhibited the JA response. Using mutants defective in JA signalling, it has been suggested that Si acts through the JA signalling pathway to influence plant volatile production, and thus improve the attractiveness of the plant to parasitoids (Liu *et al*., 2017).

In this study, the expression of JA-related genes was not significantly induced by damage, and variation in expression between landraces was not correlated to their Si accumulation. By contrast, in rice, during unstressed conditions, Jang *et al*. (2018) found that Si application increased JA levels at all time-points tested, although the extent of the increase varied depending on the concentration of Si used and the time-point. It is possible that differences in sample timing explain these different results, with the results presented by Ye *et al*. (2013) also showing lower levels of JA 24 h after herbivore infection but no difference between +Si and –Si plants until 6 h after infection. Previous studies have

focussed on damaged leaves, but here undamaged leaves were selected to measure gene expression, which may explain why no significant differences were seen between treatments. Additionally, it is possible that differences in gene expression would have been observed if homoeologue-specific primers had been used.

3.6.6 How many damage events are needed to induce an increase in Si, and how quickly does Si accumulation take place after plants are damaged?

Previous studies have indicated that a single damage event is insufficient to increase Si accumulation (Massey *et al*., 2007). However, to date, no research has examined the minimum number of damage events needed to induce this increase in Si. Massey *et al*. (2007) used only two damage treatments, with plants subject to 16 damage events over a 12 month period compared to plants subject to a single damage event. In this study, plants were sampled after one, two, four, or six damage events in order to investigate the number of damage events needed to increase Si accumulation. It was found that two damage events were required to induce a significant increase in Si accumulation in damaged leaves. Interestingly, the magnitude of the increase in Si accumulation did not increase significantly following further damage events, suggesting that there is a simple threshold number of damage events needed to increase Si accumulation. This also suggests that increasing Si accumulation after damage incurs a cost to the plant, for example, causing a slower growth rate.

Nevertheless, it remains unknown whether it is the number or the extent of damage that is important for increasing Si accumulation. High levels of vole grazing have been shown to be necessary to increase Si accumulation in the grass species *Deschampsia caespitose* (Hartley and DeGabriel, 2016; Reynolds *et al*., 2012). In this study, the amount of damage was not measured, although it was positively correlated with the number of damage events, and thus it was not possible to separate the effects of these two variables.

3.7 Conclusion

To conclude, damage significantly increased Si accumulation, although the extent of this Si induction did not vary significantly among landraces. There was a localised response such that damage increased Si accumulation only in the damaged leaves of damaged plants. Further research is needed to understand the consequences of localised induction of Si defences on the plant response to herbivory. No effect of damage on spine density or Si transporter gene expression was observed. Likewise, there was no significant effect of Si on

the expression of JA-related genes. A minimum of two damage events were needed to induce increased Si accumulation, but subsequent damage events did not further increase Si. This suggests that there is a threshold amount of damage needed for induction of Si defences and that there is a cost to the plant associated with increasing Si accumulation.

3.8 Appendix

Table 3.2: ANOVA results for the effect of landrace, damage, and Si supply on Si concentration and spine density in damaged and undamaged leaves of damaged plants compared to undamaged plants. Statistically significant results are highlighted in bold.

	Leaf Si (%)						Root Si (%)			Silicified spines (mm^{-2})					
	Undamaged leaves			Damaged leaves						Undamaged leaves			Damaged leaves		
	df	*F*	*P*	*df*	*F*	*P*	*df*	*F*	*P*	*df*	*F*	*P*	*df*	*F*	*P*
Landrace	**3**	**22.39**	**< 0.001**	**3**	**24.81**	**< 0.001**	3	1.71	0.179	**3**	**4.25**	**0.010**	**3**	**4.35**	**0.009**
Damage	1	0.09	0.764	1	**215.99**	**< 0.001**	1	0.79	0.378	1	1.83	0.183	1	1.46	0.233
Si	**1**	**6188.77**	**< 0.001**	**1**	**9076.47**	**< 0.001**	**1**	**88.54**	**< 0.001**	1	0.01	0.945	1	0.09	0.768
Landrace x Damage	3	0.20	0.897	3	0.54	0.66	3	0.71	0.550	3	0.27	0.847	**3**	**3.02**	**0.039**
Landrace x Si	**3**	**23.73**	**< 0.001**	**3**	**27.41**	**< 0.001**	3	1.19	0.326	3	0.87	0.466	3	0.16	0.924
Damage x Si	**1**	**12.32**	**< 0.001**	**1**	**23.10**	**< 0.001**	1	0.16	0.691	1	0.01	0.932	1	0.02	0.890
Landrace x Damage x Si	3	0.16	0.923	3	1.39	0.26	3	0.86	0.466	3	0.03	0.994	3	0.41	0.744

Table 3.3: Paired *t*-test results comparing Si concentration in damaged and undamaged leaves of damaged plants for each landrace. Significant results are highlighted in bold. The percentage increase in Si of damaged leaves of damaged plants compared to undamaged plants is also indicated (mean ± SE).

	–Si			+Si		
Landrace	Change due to damage (%)	*t*	*P*	Change due to damage (%)	*t*	*P*
L4	7.3 ± 7.2	2.71	0.225	45.7 ± 11.4	**5.82**	**0.028**
L5	15.6 ± 5.1	**7.08**	**0.002**	60.2 ± 4.8	**14.19**	**< 0.001**
H1	11.3 ± 3.3	**9.07**	**< 0.001**	47.8 ± 8.9	**10.68**	**< 0.001**
H3	22.9 ± 4.0	**3.65**	**0.036**	31.7 ± 7.9	**5.22**	**0.006**

Table 3.4: ANOVA results for the effect of landrace, damage, and their interaction, on Si transporter gene expression for plants from experiment one. A Bonferroni correction for multiple testing was applied setting the significance level to 0.0125. *Lsi2* expression was too low to be detected for several samples and thus is not shown. Statistically significant results are highlighted in bold.

Gene		Damaged			Undamaged		
	Factor	*df*	*F*	*P*	*df*	*F*	*P*
Lsi1	Landrace	**3**	**17.70**	**0.009**	3	1.67	0.310
	Damage	1	0.002	0.965	1	0.64	0.470
	Landrace x Damage	3	10.58	0.023	3	0.57	0.6661
Lsi3	Landrace	**3**	**38.76**	**0.002**	3	3.71	0.119
	Damage	1	0.92	0.393	1	0.12	0.749
	Landrace x Damage	3	6.66	0.049	3	2.13	0.240
Lsi6	Landrace	3	5.48	0.067	**3**	**18.45**	**0.008**
	Damage	**1**	**21.57**	**0.010**	**1**	**45.39**	**0.003**
	Landrace x Damage	3	4.63	0.087	3	7.70	0.039

Table 3.5: ANOVA results for the effect of landrace and damage on leaf Si concentration in damaged and undamaged leaves of damaged plants compared to undamaged plants for plants from experiment two. Averaged shows the results when the average Si of undamaged and damaged leaves of damaged plants (accounting for differences in weight) was compared with undamaged plants. All plants were supplied with Si. Statistically significant results are highlighted in bold.

	Leaf Si (%)								
	Damaged leaves			Undamaged leaves			Averaged		
	df	*F*	*P*	*df*	*F*	*P*	*df*	*F*	*P*
Landrace	**9**	**19.53**	**< 0.001**	**9**	**12.05**	**< 0.001**	**9**	**13.81**	**< 0.001**
Damage	**1**	**9.24**	**0.004**	**1**	**67.90**	**< 0.001**	**1**	**8.43**	**0.006**
Landrace x damage	9	1.43	0.208	9	1.53	0.171	9	1.25	0.291

Table 3.6: Paired *t*-test results comparing the Si concentration in damaged and undamaged leaves of damaged plants for plants from experiment two. Statistically significant results are highlighted in bold. The percentage increase in Si of damaged leaves compared to undamaged plants is also indicated (mean ± SE). N = 3 for each landrace.

Landrace	Change due to damage (%)	*t*	*P*
L1	**92.5 ± 11.4**	**10.97**	**0.008**
L2	82.9 ± 23.7	4.20	0.052
L3	**107.6 ± 28.4**	**5.50**	**0.032**
L4	**75.2 ± 20.1**	**4.56**	**0.045**
L5	**63.9 ± 0.03**	**23.57**	**0.002**
H1	**53.6 ± 11.7**	**5.32**	**0.033**
H3	**100.4 ± 12.8**	**10.87**	**0.008**
H4	**53.6 ± 3.2**	**20.79**	**0.002**
H5	34.2 ± 9.2	4.07	0.055
H7	**88.9 ± 22.8**	**4.76**	**0.041**

Table 3.7: ANOVA results for the effect of damage, landrace, and their interaction on the expression of JA-related genes at different time-points for plants from experiment two. A Bonferroni correction for multiple testing was applied setting the significance level to 0.01. Statistically significant results are highlighted in bold.

Gene	Damage event	Time	Factor								
			Landrace			Damage			Landrace x Damage		
			df	*F*	*P*	*df*	*F*	*P*	*df*	*F*	*P*
POX	First	6 h	3	3.78	0.067	1	1.32	0.289	3	0.57	0.653
		24 h	3	1.28	0.353	1	0.008	0.932	3	0.11	0.952
	Final	6 h	**3**	**8.71**	**0.007**	1	5.76	0.043	2	2.10	0.185
		24 h	**3**	**8.54**	**0.010**	1	0.62	0.457	3	0.43	0.741
AOS	First	6 h	3	1.34	0.348	1	0.00	0.992	3	1.62	0.280
		24 h	3	3.50	0.063	1	0.36	0.562	3	0.52	0.681
	Final	6 h	3	0.71	0.574	1	0.80	0.400	2	1.43	0.302
		24 h	3	1.02	0.440	1	0.59	0.469	3	2.52	0.141
AOC	First	6 h	**3**	**9.67**	**0.010**	1	4.39	0.081	3	2.26	0.182
		24 h	**3**	**88.78**	**< 0.001**	1	0.33	0.579	3	0.87	0.493
	Final	6 h	**3**	**11.01**	**0.003**	1	8.22	0.021	2	1.92	0.208
		24 h	3	0.81	0.528	1	0.58	0.470	3	0.55	0.662
FPS	First	6 h	3	3.77	0.067	1	0.38	0.557	3	0.79	0.537
		24 h	**3**	**17.67**	**< 0.001**	1	0.19	0.674	3	0.49	0.696
	Final	6 h	**3**	**26.45**	**< 0.001**	1	0.24	0.640	2	2.45	0.148
		24 h	**3**	**9.874**	**0.007**	1	0.173	0.690	3	0.326	0.807
COI1	First	6 h	3	8.22	0.011	1	3.66	0.097	3	1.33	0.338
		24 h	3	0.96	0.456	1	0.43	0.529	3	0.57	0.648
	Final	6 h	3	2.28	0.157	1	1.21	0.304	2	2.99	0.107
		24 h	3	0.50	0.695	1	0.51	0.499	3	0.51	0.690

Table 3.8: ANOVA results for the effect of Si supply, damage, time-point, and their interactions on Si accumulation for plants from experiment three. Damaged and undamaged leaves of damaged plants were compared, separately, to undamaged plants. Statistically significant results are highlighted in bold.

	Si (%)								
	Damaged leaves			Undamaged leaves			Roots		
	df	*F*	*P*	*df*	*F*	*P*	*df*	*F*	*P*
Si	**1**	**7137.76**	**< 0.001**	**1**	**7023.76**	**< 0.001**	1	**166.7**	**< 0.001**
Damage	**1**	**10.56**	**0.002**	1	13.27	**< 0.001**	1	0.47	0.494
Time-point	**8**	**19.04**	**< 0.001**	8	5.66	**< 0.001**	8	1.22	0.304
Replicate	**2**	**8.37**	**< 0.001**	2	8.48	**< 0.001**	2	0.29	0.747
Si x Damage	**1**	**8.14**	**0.006**	1	3.32	0.073	1	0.09	0.770
Si x Time-point	**8**	**2.60**	**0.014**	8	0.88	0.541	8	1.83	0.090
Damage x Time-point	**8**	**4.59**	**< 0.001**	8	0.62	0.763	8	0.78	0.620
Si x Damage x Time-point	8	0.76	0.643	8	0.50	0.874	8	1.25	0.285

4 Chapter 4: The effect of silicon on osmotic and drought stress tolerance in wheat landraces

4.1 Introduction

Drought causes annual global wheat yield losses of around 20 % (Daryanto *et al*., 2016). Moreover, anthropogenic climate change is predicted to induce changes in global precipitation patterns, with droughts likely to become more frequent in some areas, further exacerbating yield losses (IPCC, 2014, 2019). The impacts of abiotic stresses like drought may be exacerbated by the focus of crop domestication on optimising yields at the expense of reduced stress tolerance and genetic diversity (Kahiluoto *et al*., 2019). Current mitigation strategies include crop irrigation and breeding for increased stress tolerance, particularly by using the genetic diversity of landraces to breed cultivars with improved drought tolerance (Dwivedi *et al*., 2016). However, irrigation can cause water shortages (Wichelns, 2015; Shen *et al*., 2013) and soil salinisation (Martínez-Alvarez *et al*., 2016), while breeding approaches are slow, labour intensive, and complicated by genotype-environment interactions (Bhat *et al*., 2020).

Plants may use Si to improve drought tolerance, although the exact underpinning mechanisms are largely unknown (Thorne *et al*., 2020). Drought stress induces oxidative damage (Osakabe *et al*., 2014), and plant Si accumulation has been shown to reduce oxidative damage, notably by increasing antioxidative enzyme activity (Gong *et al*., 2005; Tale Ahmad and Haddad, 2011; Alzahrani *et al*., 2018). Additionally, plants use Si to improve water use efficiency during drought stress (Alzahrani *et al*., 2018; Ibrahim *et al*., 2018; Merwad *et al*., 2018), for example *via* an increase in stomatal conductance, which in turn improves the photosynthetic rate (Sonobe *et al*., 2009; Yin *et al*., 2014; Wang *et al*., 2019). Nevertheless, other studies have reported that Si decreases stomatal conductance (Gao *et al*., 2006) or has no effect (Yang *et al*., 2019; Gengmao *et al*., 2015). Alternatively, improved water use efficiency may be linked to lower transpiration, which may occur by reducing the cuticular water conductance (Vandegeer *et al*., 2021b; Agarie *et al*., 1998).

Most studies in wheat report that Si increases growth and yield during drought stress (Ahmad *et al*., 2016; Gong *et al*., 2003; Alzahrani *et al*., 2018; Othmani *et al*., 2020), although there are others reporting no significant effect either in wheat (Sattar *et al*., 2019) or in other species (Hosseini *et al*., 2017; Maillard *et al*., 2018). The inconsistent nature of observed Si effects may reflect variation in plant species and genotype, as has been found

for understanding the Si effect on herbivory tolerance (Massey *et al*., 2009; Hartley *et al*., 2015; Soininen *et al*., 2013). For example, genotypic variability in Si accumulation is likely to influence the response to Si. While many studies have assessed the effect of Si on cultivars that differ in drought tolerance (Ouzounidou *et al*., 2016; Parveen *et al*., 2019; Maghsoudi *et al*., 2019), there is a clear lack of studies examining the effect of Si on drought tolerance in a larger range of genotypes, particularly those that vary in Si accumulation.

Although there is increasing interest in the use of local landraces in crop breeding programs to improve stress tolerance (Lopes *et al*., 2015; Dwivedi *et al*., 2016), to date, only a few studies have used landraces to investigate the effect of Si availability on stress tolerance in wheat (Merah *et al*., 1999; Simpson *et al*., 2017). Using wheat landraces that consistently differed in Si accumulation, this study examined whether the effect of Si on osmotic and drought stress varied among landraces. Additionally, whether there is variation in the Si effect on transpiration was examined. It was hypothesised that the impact of Si on stress tolerance in landraces would depend on their capacity to accumulate Si, and this would correlate with Si-induced changes to transpiration.

4.2 Methods

4.2.1 Experimental design and plant growth conditions

Previous work identified wheat landraces that varied widely in their Si accumulation (2). Five high (H1, H3, H4, H5, H7) and five low (L1, L2, L3, L4, L5) Si-accumulating landraces were selected to examine the effects of Si on osmotic and drought stress. In total, four experiments were used:

- Experiment 1 examined the effect of Si availability on plant growth and Si accumulation during osmotic stress.
- Experiment 2 examined the effect of Si availability on plant growth and Si accumulation during short-term drought stress
- Experiment 3 examined the effect of Si availability on plant growth and total plant grain weight (yield) during long-term drought stress
- Experiment 4 examined the effect of Si availability on transpiration during drought stress

For all experiments, a balanced factorial design was used with plants grown under control (no experimentally imposed stress) or stress conditions, with (+Si) or without Si supplementation (–Si). For all experiments, seeds were placed onto filter paper in Petri dishes and left in the dark at 4 °C for 48 h before moving to a 20 °C growth chamber with 12 h day/night lighting for germination. Plants were grown under controlled glasshouse conditions (16 h daylight; 20 °C /15 °C day/night). At harvest, shoot fresh weight was recorded and plants were oven-dried at 70 °C for 72 h to obtain shoot dry weights. Root fresh and dry weight was obtained only for plants grown hydroponically.

4.2.1.1 Experiment 1: Effect of Si during osmotic stress

Germinated seeds were transplanted into sand and grown for 10-11 days. Seedlings were then transferred to 9 L hydroponics boxes filled with ½-strength Hoagland's solution and aerated throughout the experiment. Half the plants were grown with 1.8 mM dissolved sodium metasilicate ($Na_2SiO_3.9H_2O$) and the salt level was balanced for the remaining plants using 3.6 mM sodium chloride (NaCl). Osmotically stressed plants were grown with 5 % (w/v) polyethylene glycol (PEG)-6000. The pH was adjusted to 5.6-6 using 1 M HCl or 0.1 M KOH. The nutrient solutions were changed weekly. Transpiration rate was measured after four weeks of osmotic stress (4.2.3). Plants were harvested after transpiration was measured. The experiment was composed of four temporally separate replicates, with a minimum of two weeks between replicates.

4.2.1.2 Experiment 2: Effect of Si on growth during short-term drought stress

Osmotic stress imposed using chemical agents such as PEG is frequently applied to plants to mimic physiological drought. Such hydroponics-based assays have the advantage of exposing plants to a more controlled, less complex growth substrate than soil, and allow access to roots. However, genuine drought stress, i.e., water deficit, much better simulates real field conditions. Furthermore, responses to osmotic and drought stress can be very different (Chen and Kao, 1993; Whalley *et al*., 1998). The study was therefore repeated using compost-grown wheat where water deficits could be applied.

One-week-old seedlings were transplanted into 9 L boxes filled with F2+S compost (Levington) and treated with Calypso insecticide (Bayer). All plants were watered as required. Three days after transplanting, half the plants were assigned to drought treatment by withholding watering until 40 % field capacity (FC) was achieved. All plants were then watered as required to maintain the soil moisture at either control (100 % FC) or

drought (40 % FC) levels. The soil moisture content was checked using a soil moisture probe (ML3 ThetaProbe Soil Moisture Sensor, delta-T). Twice weekly, instead of using tap water to maintain the FC, half the plants from each watering treatment received 150 mL 1.8 mM dissolved sodium metasilicate ($Na_2SiO_3.9H_2O$); the remaining plants received the same amount of 3.6 mM sodium chloride (NaCl) to balance the sodium level. Replacing the tap water with Si ensured that plants were able to receive additional Si without compromising the drought treatment. Plants were harvested after one month of drought treatment. Four temporally separate replicates were performed, with a minimum of two weeks between replicates. Two plants per landrace per treatment per replicate were grown.

4.2.1.3 Experiment 3: Effect of Si on yield during long-term drought stress

One-week-old seedlings were transplanted into 9 L plastic boxes filled with F2+S compost (Levington) and treated with Calypso insecticide (Bayer). After the emergence of 1-2 tillers, the seven winter landraces were vernalised in a 4 ˚C vernalisation chamber for two months. After vernalisation, the remaining three spring landraces (H1, H3, and H5) were transplanted into the box. Treatments were started one week after vernalisation. Control plants were maintained at 100 % FC, while drought-stressed plants were maintained at 40 % FC. For the first week, half the plants from each watering treatment received 200 mL 1.8 mM dissolved sodium metasilicate ($Na_2SiO_3.9H_2O$) twice weekly. This was then increased to 300 mL Si twice weekly for two weeks, and finally to 400 mL three times per week until the end of grain filling (Zadok's growth stage 87; Zadoks *et al*., 1974), when Si treatments were stopped. The remaining plants received the same amount of 3.6 mM sodium chloride (NaCl) to balance the sodium levels. Plants were harvested when the ear on the main stem reached Zadok's growth stage 92 (Zadoks *et al*., 1974) and yield, defined as total plant grain weight, was recorded. Four temporally separate replicates were performed, with a minimum of two weeks between replicates.

4.2.1.4 Experiment 4: Effect of Si on transpiration during drought stress

Five-days-old seedlings were transplanted to 350 mL pots filled with F2+S compost (Levington) and treated with Calypso insecticide (Bayer). Treatments were started one week after transplanting. Control plants were maintained at 100 % FC, while drought-stressed plants were maintained at 40 % FC. Half the plants received 40 mL 1.8 mM dissolved sodium metasilicate ($Na_2SiO_3.9H_2O$) three times per week while the remaining plants received the same amount of 3.6 mM sodium chloride (NaCl) to balance the sodium level. Transpiration was measured after four weeks of drought stress (4.2.3) and plants

were harvested immediately after the transpiration measurements. Three temporally separate replicates were performed, with a minimum of two weeks between replicates.

4.2.2 Si measurements

Shoot and root Si concentration was measured by portable X-ray fluorescence spectroscopy (P-XRF) as described in section 2.2.2.

4.2.3 Transpiration measurements

Transpiration rate was calculated based on weight loss over time over two consecutive days, accounting for differences in plant dry weight (DW; Equation 4.1). Plants were weighed in the morning and evening of the first day, and the morning of the second day to estimate the rate of transpiration during both the day and night. There was a strong positive correlation between day and night transpiration rates, hence only the transpiration results from the total experimental period are presented. For plants grown hydroponically, root water uptake was calculated based on root dry weight (Equation 4.2). The results for root water uptake and transpiration were similar, thus only the results for transpiration are presented.

Equation 4.1:

$$Transpiration\ (g\ H_2O\ hr^{-1} g\ DW^{-1}) = \frac{Initial\ weight\ (g) - Final\ weight\ (g) - Evaporation\ (g)}{Time\ (hr) \times Shoot\ dry\ weight\ (g)}$$

Equation 4.2:

$$Water\ uptake\ (g\ H_2O\ hr^{-1} g\ DW^{-1}) = \frac{Initial\ weight\ (g) - Final\ weight\ (g) - Evaporation\ (g)}{Time\ (hr) \times Root\ dry\ weight\ (g)}$$

For plants grown hydroponically (Experiment 1), transpiration was measured after four weeks of osmotic stress. Plants were transferred into 50 mL falcon tubes filled with 30 mL of the relevant nutrient solution (+/ –PEG, +/–Si). Filled tubes, without plants, were used to estimate the underlying evaporation rate. After weighing in the evening of the first day, the tubes for plants grown without PEG were refilled to 30 mL to ensure that plant water uptake was not limited by a lack of medium.

For plants grown in compost (Experiment 4), transpiration was measured after four weeks of drought stress. Prior to measurements, the pots were covered with tinfoil to reduce evaporative water loss. To estimate the underlying evaporation rate, filled pots without plants and covered with tinfoil were used. No water was supplied to the pots while transpiration measures were being taken.

4.2.4 Determination of soil Si

Three pots filled with compost and treated with Si, but without plants, were used to determine plant-available Si in the compost using a variation of the method described in Sauer *et al*. (2006). Air-dried compost was sieved (2 mm mesh) and 2 g of compost was then mixed with 20 mL 0.01 M $CaCl_2$ and shaken at 20 rpm for 24 h. The mixture was centrifuged for 10 min at 2000 rpm, filtered, and the supernatant used to determine the Si level of the compost.

Plant-available Si was determined using the molybdenate method described in Liang *et al*. (2015). A volume of 30 mL 20 % acetic acid and 10 mL 54 g L^{-1} ammonium molybdate ($H_{24}Mo_7N_6O_{24}.4H_2O$) solution was added to 1 mL sample. The mixture was shaken and rested for 5 min then 5 mL 20 % tartaric acid, 1 mL reducing solution, and 3 mL 20 % acetic acid was added. The reducing solution contained 8 g L^{-1} sodium sulphite (Na_2SO_3), 1.6 g L^{-1} 1-amino-4-sulfonic acid, and 100 g L^{-1} sodium bisulfite ($NaHSO_3$). After allowing 30 min for colour development, the absorbance was measured with a spectrophotometer (Jasco V-560) at 810 nm. Plant-available Si in hydroponics medium was determined using the same method. PEG was added at 0, 2, 4, 6, 8, and 10 % (w/v) to investigate whether the presence of PEG affected plant-available Si.

4.2.5 Statistical analysis

All statistical analyses were performed using R software (version 3.6.1, R Core Team, 2020). Summary statistics were calculated using the Rmisc package (Hope, 2013) and graphs were produced using the ggplot2 package (Wickham, 2016). Three-way analysis of variance (ANOVA) was used to test the effect of Si availability, stress treatment, and landrace or accumulation type on Si concentration, dry weight, grain weight, and transpiration. In all ANOVAs, temporal replicate was included as a factor to account for variation caused by plants being grown at different times. Data normality was checked using Shapiro tests and homogeneity of variance was tested using Levene's tests. To satisfy the test assumptions, Si concentrations were logit transformed and grain weights were square root transformed.

Dry weights were log transformed, apart from for the long-term drought experiment where a square root transformation was used. Transpiration was square root transformed for plants grown hydroponically, but no transformation was used for plants grown in compost. In all analyses, a significance level of $P < 0.05$ was used. Significant results were analysed by performing Tukey's Honest Significance Difference (HSD) *post-hoc* tests using the emmeans package (Lenth, 2021).

4.3 Results

4.3.1 Si slightly increased growth during osmotic stress

Shoot dry weight was significantly affected by stress treatment, Si availability, and landrace (Experiment 1; Figure 4.1). There was a significant interaction between osmotic stress and Si availability, such that while supplying plants with Si did not increase growth during control conditions, there was a small but significant positive effect of Si on shoot dry weight during osmotic stress (Table 4.2). However, there was no significant interaction between osmotic stress or Si availability and landrace, suggesting landraces responded similarly to Si supply and osmotic stress. When analysing landraces separately using pairwise comparisons of estimated marginal means, Si significantly increased growth during osmotic stress only for landraces L2, L3, and H5. Across all landraces, osmotic stress decreased growth by an average of 83.7 ± 1.4 %. However, Si supplementation increased shoot dry weight during osmotic stress by an average of 13.7 ± 10.2 % compared to –Si plants.

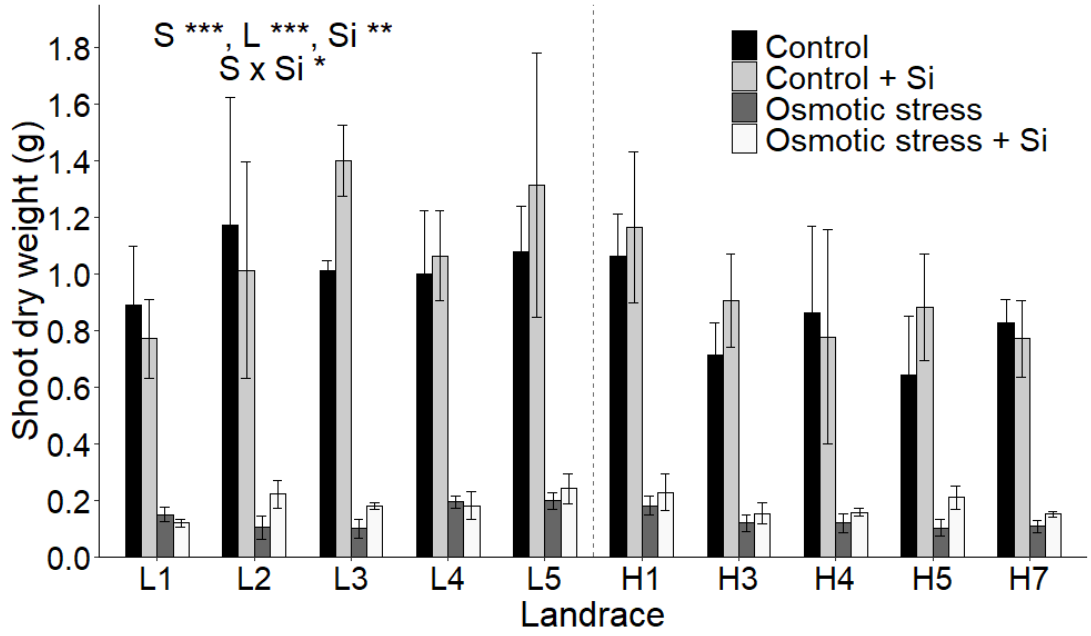

Figure 4.1: The effect of Si on shoot dry weight during osmotic stress (Experiment 1). Statistically significant impacts and interactions, determined by three-way ANOVA, are indicated in each panel, where *** $P < 0.001$, ** $P < 0.01$, and * $P < 0.05$. Mean values ± standard error (SE) are shown. N = 4. Control plants were grown without PEG-6000 while osmotically stressed plants were grown with 5 % PEG-6000. +Si plants were grown with Si supplementation. L: landrace, Si: level of Si availability, S: osmotic stress treatment. H indicates a high Si-accumulating landrace. L indicates a low Si-accumulating landrace.

4.3.2 Si slightly increased transpiration during osmotic stress

The transpiration rate was measured after four weeks of osmotic stress. There was a significant interactive effect between Si and osmotic stress such that Si had no effect on transpiration during control conditions, but marginally increased transpiration during osmotic stress (Experiment 1; Figure 4.2; Table 4.2). While the effect of osmotic stress on transpiration differed significantly among landraces, there was no significant variation in the effect of Si on transpiration among landraces. When analysing landraces separately using estimated marginal means, Si significantly increased transpiration during osmotic stress for landraces L3 and H4. There was no significant correlation between shoot Si concentration and transpiration rate (data not shown).

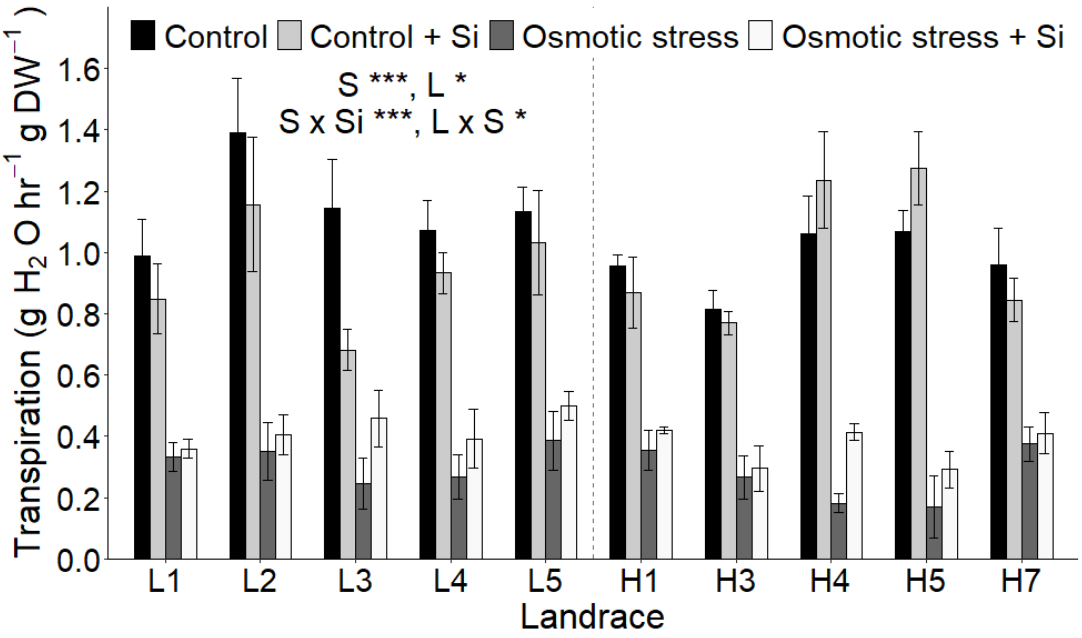

Figure 4.2: The effect of Si on transpiration during osmotic stress (Experiment 1). Statistically significant impacts and interactions, determined by three-way ANOVA, are indicated in each panel, where *** $P < 0.001$, ** $P < 0.01$, and * $P < 0.05$. Mean values ± SE are shown. N = 4. Control plants were grown without PEG-6000 while osmotically stressed plants were grown with 5 % PEG-6000. +Si plants were grown with Si supplementation. L: landrace, Si: level of Si availability, S: osmotic stress treatment.

4.3.3 Osmotic stress decreased Si accumulation

For +Si plants, osmotic stress significantly decreased both shoot and root Si accumulation. There was significant variation in shoot Si concentration among landraces, but the decrease in shoot Si accumulation due to osmotic stress was similar for all landraces (Experiment 1; Figure 4.3; Table 4.2). Across all landraces, osmotic stress decreased Si accumulation by 60.2 ± 2.3 % for +Si plants and by 15.7 ± 4.4 % for –Si plants. However, while for +Si plants, osmotic stress decreased root Si concentration, for –Si plants, root Si concentration was generally higher in osmotically stressed plants, although this increase was not significant for any landrace (Tukey's HSD, $P > 0.05$). There was no significant correlation between the increase in shoot dry weight or transpiration with Si and root or shoot Si concentration during stress.

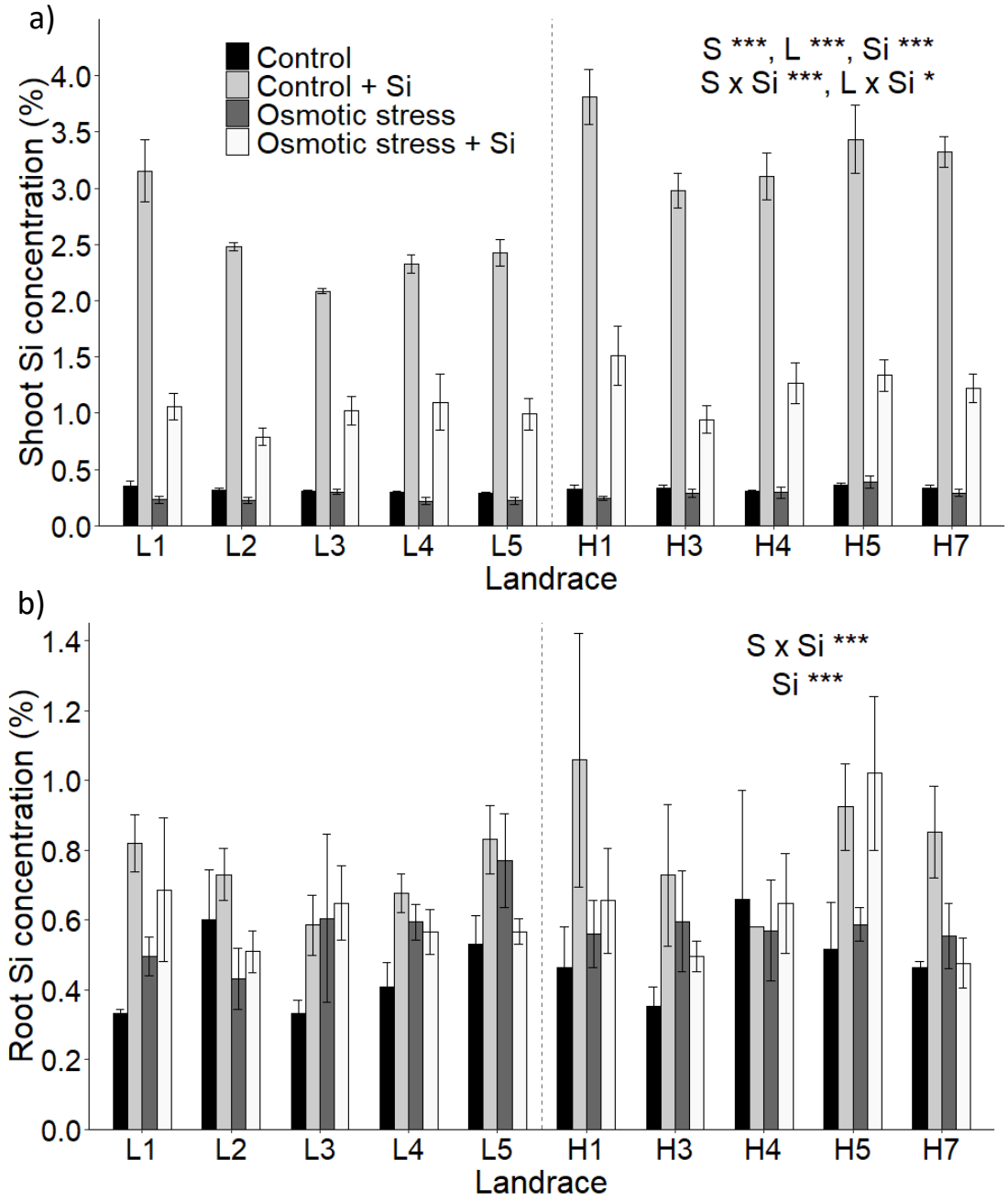

Figure 4.3: Variation in Si accumulation among wheat landraces due to osmotic stress (Experiment 1). a) Shoot Si concentration. b) Root Si concentration. Statistically significant impacts and interactions, determined by three-way ANOVA, are indicated in each panel, where *** $P < 0.001$, ** $P < 0.01$, and * $P < 0.05$. Mean values ± SE are shown. N = 4. Control plants were grown without PEG-6000 while osmotically stressed plants were grown with 5 % PEG-6000. +Si plants were grown with Si supplementation. L: landrace, Si: level of Si availability, S: osmotic stress treatment. H indicates a high Si-accumulating landrace. L indicates a low Si-accumulating landrace.

4.3.4 No effect of Si on plant growth or yield during drought stress

For the short-term drought experiment (Experiment 2; 4.2.1.2), drought significantly decreased shoot dry weight, and the extent of this decrease was similar for all landraces (Figure 4.4; Table 4.3). There was no significant effect of Si availability on shoot dry weight. Across all landraces, for –Si plants, drought stress reduced shoot dry weight by 40.2 ± 2.6 % compared to 43.6 ± 2.5 % for +Si plants.

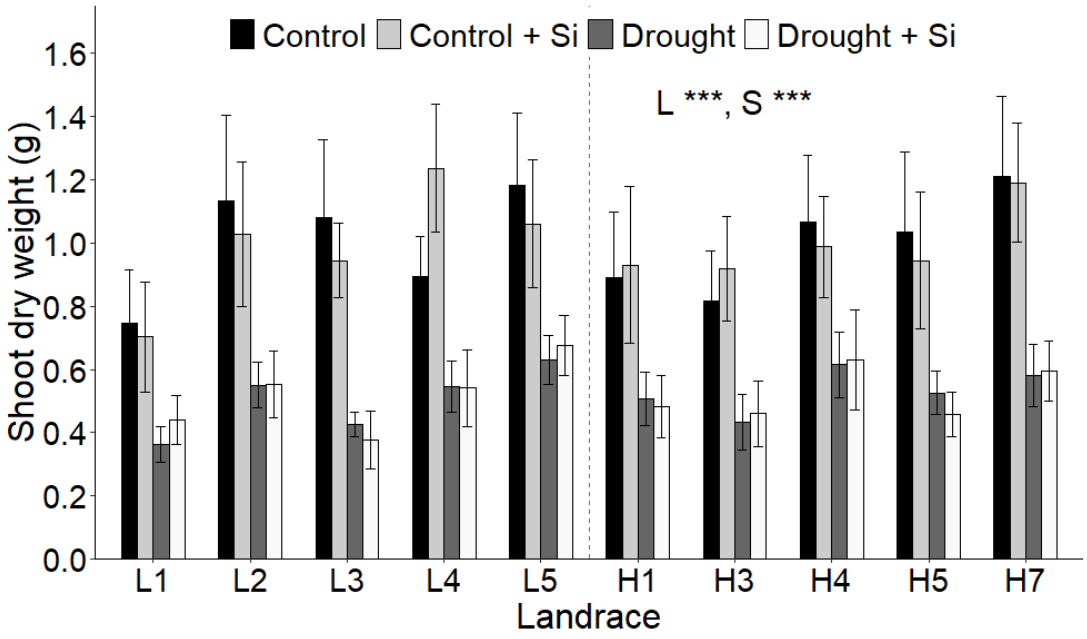

Figure 4.4: The effect of Si on shoot dry weight during short-term drought stress (Experiment 2). Statistically significant impacts and interactions, determined by three-way ANOVA, are indicated in each panel, where *** $P < 0.001$, ** $P < 0.01$, and * $P < 0.05$. Mean values ± SE are shown. N = 4. Control plants were grown in the absence of drought stress at 100 % FC while drought stressed plants were grown at 40 % FC. +Si plants were grown with Si supplementation. L: landrace, S: drought stress treatment. H indicates a high Si-accumulating landrace. L indicates a low Si-accumulating landrace.

Likewise, for the long-term drought experiment (Experiment 3; 4.2.1.3), drought stress significantly reduced shoot dry weight, but there was no effect of Si (Figure 4.5; Table 4.3). Across all landraces, long-term drought stress reduced shoot dry weight in –Si plants by an average of 43.2 ± 15.8 % compared to 51.9 ± 5.5 % in +Si plants. Across all treatments, the shoot dry weight of spring landraces (H1, H3, and H5) was lower than for winter landraces.

Overall, both long-term and short-term drought stress had similar impacts on shoot dry weight, irrespective of the presence of Si.

Drought stress significantly reduced total plant grain yield, and this effect varied among landraces, such that landraces H1, L1, and L5 showed the largest reduction in grain yield due to drought stress. However, as was observed for shoot dry weight, Si had no significant effect on grain weight during long-term drought stress.

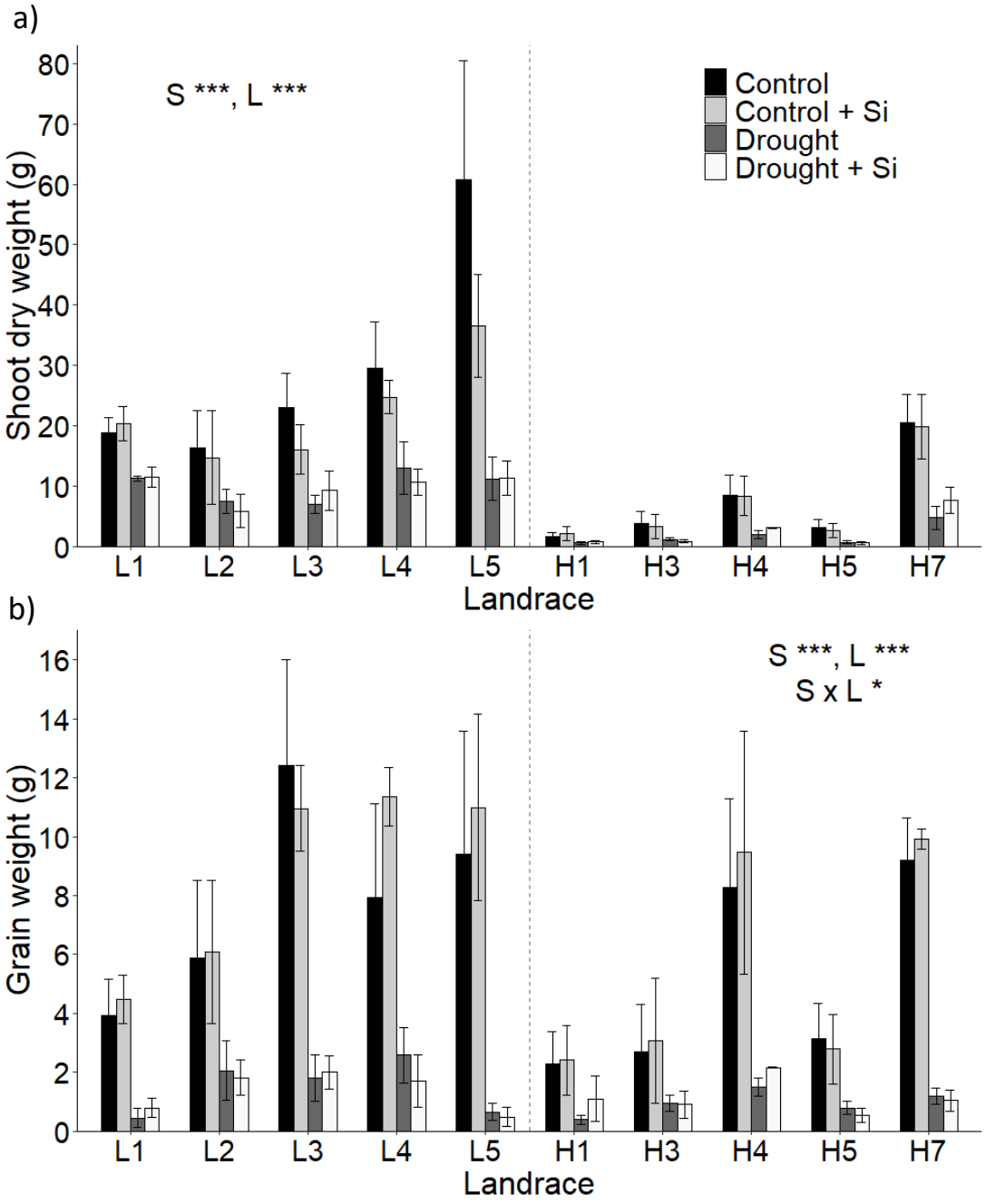

Figure 4.5: The effect of Si on a) shoot dry weight and b) total plant grain weight during long-term drought stress (Experiment 3). Statistically significant impacts and interactions, determined by three-way ANOVA, are indicated in each panel, where *** $P < 0.001$, ** $P < 0.01$, and * $P < 0.05$. Mean values ± SE are shown. N = 4. Control plants were grown in the absence of drought stress at 100 % FC while drought stressed plants were grown at 40 % FC. +Si plants were grown with Si supplementation. L: landrace, S: drought stress treatment. H indicates a high Si-accumulating landrace. L indicates a low Si-accumulating landrace.

4.3.5 No effect of Si on transpiration during drought stress

Drought stress significantly reduced transpiration, but in contrast to what was observed during osmotic stress conditions, there was no significant effect of landrace on transpiration rate (Experiment 4; Figure 4.6; Table 4.3). There was no effect of Si on transpiration rate. There was a positive correlation between shoot Si concentration and transpiration rate for both –Si and +Si plants grown during control conditions (–Si: $r = 0.62$, $P < 0.001$; +Si: $r = 0.50$, $P = 0.006$), and for +Si plants during drought stress ($r = 0.59$, $P < 0.001$). However, there was a significant negative correlation between transpiration rate and shoot Si for drought-stressed, –Si plants ($r = -0.43$, $P = 0.02$).

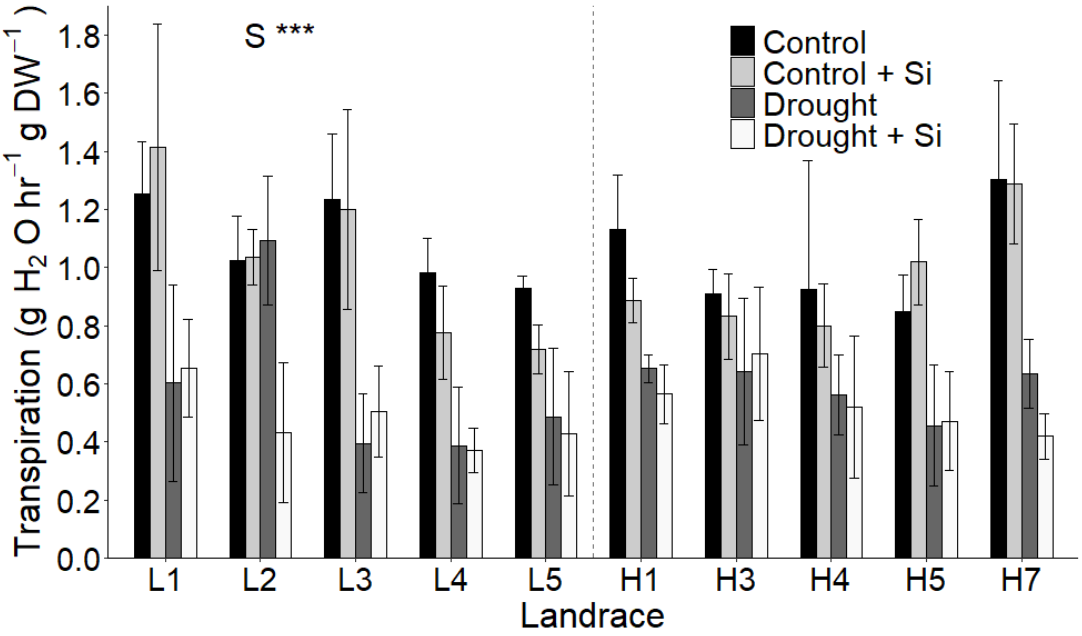

Figure 4.6: The effect of Si on transpiration during drought stress (Experiment 4). Statistically significant impacts and interactions, determined by three-way ANOVA, are indicated in each panel, where *** $P < 0.001$, ** $P < 0.01$, and * $P < 0.05$. Mean values ± SE are shown. N = 3. Control plants were grown in the absence of drought stress at 100 % FC while drought stressed plants were grown at 40 % FC. +Si plants were grown with Si supplementation. L: landrace, Si: level of Si availability, S: drought stress treatment. H indicates a high Si-accumulating landrace. L indicates a low Si-accumulating landrace.

4.3.6 Drought stress increased Si accumulation

For +Si plants, drought stress increased shoot Si accumulation by 8.7 ± 1.9 % (Experiment 2; Figure 4.7). There was no significant effect of drought stress on Si accumulation for plants

that did not receive Si supplementation. While landraces exhibited variable increases in Si accumulation in response to increasing Si availability, the increase in Si due to drought stress was similar for all landraces (Table 4.3). Due to the lack of Si effect during drought stress, it was not possible to correlate changes in Si concentration with changes in drought stress tolerance.

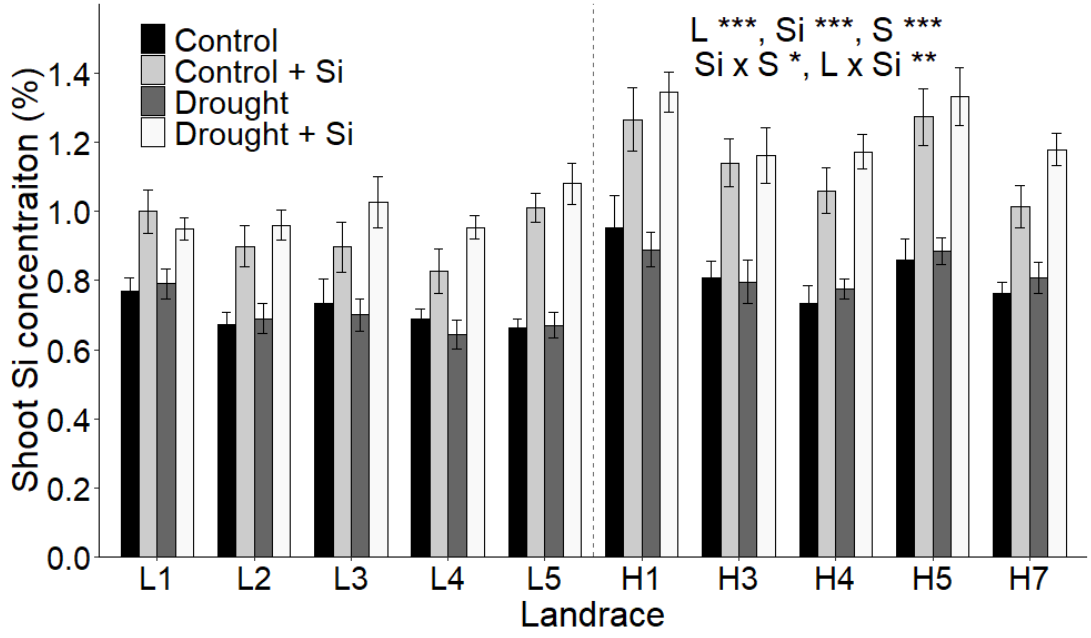

Figure 4.7: Variation in Si accumulation among wheat landraces due to drought stress (Experiment 2). Statistically significant impacts and interactions, determined by three-way ANOVA, are indicated in each panel, where *** $P < 0.001$, ** $P < 0.01$, and * $P < 0.05$. Mean values ± SE are shown. N = 4. Control plants were grown in the absence of drought stress at 100 % FC while drought stressed plants were grown at 40 % FC. +Si plants were grown with Si supplementation. L: landrace, Si: level of Si availability, S: drought stress treatment. H indicates a high Si-accumulating landrace. L indicates a low Si-accumulating landrace.

The increase in shoot Si accumulation due to Si supplementation was similar to the increase in plant-available Si in the compost due to Si supplementation. For control plants, Si supplementation increased plant shoot Si concentration by 37.5 ± 2.5%, while the plant-available Si in the compost increased by 43.3 ± 10.0 %. Without Si supplementation, the compost had a $CaCl_2$-extractable Si content of 0.17 ± 0.02 mM, compared to 0.25 ± 0.02 mM for Si-treated compost.

4.4 Discussion

Increasing soil Si availability by Si fertilisation could be a cost-effective manner to mitigate water stress in crops. However, reports on its efficacy vary widely and uncertainty regarding its mechanisms remains. An important question in this regard is whether and how variation in plant Si accumulation relates to the impact of Si on plant growth during water stress. Previous studies have investigated differences in Si accumulation between genotypes (Ma *et al*., 2007b; Chiba *et al*., 2009; Hartley *et al*., 2015; Murozuka *et al*., 2015), and also the effect of Si accumulation on growth, including yield. However, to our knowledge, no studies have correlated differences in Si accumulation with differences in stress tolerance. To address this question, we examined whether there is a different effect of Si on osmotic and drought stress in landraces varying in their Si accumulation.

4.4.1 Limited effect of Si on growth during osmotic stress

Si supplementation resulted in a significant increase in shoot dry weight for osmotically stressed plants. However, this increase was small and, furthermore, did not correlate with tissue Si levels of the various landraces. The recorded increase was smaller than those reported in other studies, both in wheat (Pei *et al*., 2010), and other species (Shi *et al*., 2016; Sonobe *et al*., 2010; Ming *et al*., 2012), although these studies used shorter stress periods and so reported less severe effects of osmotic stress on dry weight.

Decreased Si accumulation during osmotic stress contrasts with the positive effect of Si during osmotic stress, and also argues against the existence of a correlation between tissue Si and a mitigating effect of Si on stress. This effect was most pronounced in the shoots but also occurred to a lesser extent in the roots. PEG is commonly taken up by plants and thus can cause effects in addition to osmotic stress (Lawlor, 1970; Yaniv and Werker, 1983; Raggi, 1992). It is possible that PEG decreases Si availability, as suggested by the decreased absorbance reported in this study (Table 4.1). Decreased Si accumulation in response to osmotic stress imposed using PEG has been previously reported (Pei *et al*., 2010; Meunier *et al*., 2017; Xu *et al*., 2017; Maillard *et al*., 2018). Contrary to the findings from this study, in the case of barley, osmotic stress led to a rise in tissue Si but it did not alter biomass (Hosseini *et al*., 2017), and a similar result was reported in tall fescue (Vandegeer *et al*., 2021b).

4.4.2 Si did not increase growth during drought stress

In contrast to the small effect of Si during osmotic stress, no effect of Si on plant growth or yield during drought stress was found in this study. This contrasts with the widely reported increased growth in wheat in the literature (Ahmad *et al*., 2016; Gong *et al*., 2003; Alzahrani *et al*., 2018; Othmani *et al*., 2020). Likewise, Si has been found to improve grain yield during drought stress in both rice (Ibrahim *et al*., 2018) and maize (Marques *et al*., 2021). Nevertheless, there are other studies on osmotic- and drought-stressed wheat that did not report a significant increase in shoot dry weight (Sattar *et al*., 2019; Xu *et al*., 2017). Furthermore, a lack of response to Si has also been reported for other crop species. For example, Ruppenthal *et al*. (2016) reported that Si did not improve growth during drought in soybean, although Si did reduce membrane damage and increased peroxidase activity.

As reported in this study, drought stress often induces increased Si accumulation (Chen *et al*., 2011; Merwad *et al*., 2018). However, other studies have reported decreased Si accumulation during drought stress both in wheat (Alzahrani *et al*., 2018) and other species (Ibrahim *et al*., 2018; Grašič *et al*., 2019; Yang *et al*., 2019). Ahmad *et al*. (2007) found a positive correlation between Si uptake and shoot dry weight in wheat, although drought stress decreased Si accumulation.

4.4.3 Limited effect of Si on transpiration

It has been proposed that Si may improve stress tolerance by decreasing transpiration, particularly cuticular transpiration, although most studies have reported that Si increases transpiration during both osmotic and drought stress (Thorne *et al*., 2020). In this study, the presence of Si caused a small, but significant increase in transpiration for most landraces during osmotic stress, but no effect of Si on transpiration was found during drought stress. There was a positive correlation between shoot Si and transpiration for plants grown in compost, but not for plants grown hydroponically. Previous studies have generally reported that Si increases transpiration in wheat (Alzahrani *et al*., 2018; Gong and Chen, 2012; Sattar *et al*., 2019; Maghsoudi *et al*., 2016b), although decreased transpiration has also been reported (Bukhari *et al*., 2020).

4.4.4 Contrasting effects of Si during osmotic stress and drought

The contradicting behaviours seen in response to PEG-induced hyperosmotic conditions and drought imposed *via* a reduction in field capacity strongly suggest that findings from osmotic stress experimentation cannot reliably represent those obtained from soil-based conditions where realistic, physiologically relevant drought is applied. These disparities may partly be due to methodological aspects; for instance, many studies use sodium- or potassium-silicate as a Si treatment but fail to correct cation concentrations in the control treatment. Hence it is possible that the observed Si response is in fact due to extra sodium or potassium fertilisation.

Interpretational divergence is another potential source of confusion; many studies report a positive impact of Si on tolerance to osmotic or drought stress, when in reality the effects of Si are already obvious in control treatments and thus are not stress specific. Ahmad *et al*. (2016) reported a small, but significant effect of Si on wheat yield. However, the effect of Si on grain yield was similar under both drought and control conditions, and the potassium levels were not reported to be balanced.

Kuhla *et al*. (2021) suggested that Si increases soil water availability and thus improves growth during drought stress. This is in contradiction with the findings of this study where Si had a positive effect only for osmotically stressed plants. However, here, drought-stressed plants were grown in compost, which has different properties compared to the soil used by Kuhla *et al*. (2021).

4.4.5 Genotypic variation in the response to Si

Genotype specific Si responses could be another important factor influencing the effect of Si on drought and osmotic stress tolerance. Hu *et al*. (2019) found that the positive effect of Si on growth in poinsettia during control conditions was cultivar dependent. Similarly, in sugarcane, the effect of Si under water deficit conditions varied among cultivars, with a significant positive effect on dry weight observed in only one out of four cultivars tested (de Camargo *et al*., 2019). In wheat, Sapre and Vakharia (2017) found variation in both the physiological response to osmotic stress and Si accumulation among ten wheat cultivars. Bukhari *et al*. (2020) reported a significant increase in the yield of two wheat cultivars during drought with various Si application methods, but this effect was much larger in one cultivar than the other. In this study, Si caused a very small increase in growth during osmotic stress, but this did not correlate with changes in Si accumulation.

As increasing Si availability did not change drought stress tolerance, this study found no evidence that the beneficial effect of Si was correlated with Si accumulation. In the absence of experimentally-imposed stress conditions, Merah *et al*. (1999) found no significant correlation between Si content and grain yield among ten durum wheat genotypes. Likewise, there was no correlation between the beneficial effect of Si on growth during drought stress and Si accumulation among seven lentil genotypes (Biju *et al*., 2021). In this study, for osmotically stressed plants, there was no correlation between the small increase in dry weight with Si supplementation and shoot or root Si accumulation.

4.5 Conclusions

In this study, only a limited positive effect of Si on growth during osmotic stress was found, and there was no effect of Si during drought, irrespective of the data in previous chapters of this thesis, which demonstrated significant variation in Si accumulation between landraces. Furthermore, osmotic stress reduced Si accumulation, while drought stress increased Si accumulation. It remains unknown why studies report contrasting effects of Si, but the results presented here suggest that for wheat, Si fertilisation is likely to result in only limited mitigation of the impacts of water stress.

4.6 Appendix

4.6.1 PEG treatment may decrease the plant-available Si

To determine whether the reduction in plant Si concentration observed during osmotic stress was due to plants accumulating less Si, or the result of PEG decreasing the plant-available Si in the nutrient solution, the Si availability of Hoagland's solution with 1.8 mM dissolved sodium metasilicate ($Na_2SiO_3.9H_2O$) and variable levels of PEG was measured. Adding PEG to the Hoagland's significantly decreased the absorbance, but this decrease was similar for all levels of PEG used (Table 4.1; $F_{5,12} = 3.32$, $P = 0.04$). However, it must be noted that, after several hours, a precipitate formed in the samples with PEG which may have affected the spectrophotometer readings.

Table 4.1: Absorbance at 810 nm for 1.8 mM ½-strength Hoagland's solution spiked with varying levels of PEG-6000. Shown is the mean and standard error of three replicates.

PEG (w/v %)	Absorbance
0	1.15 ± 0.10
2	0.55 ± 0.09
4	0.60 ± 0.05
6	0.72 ± 0.06
8	0.59 ± 0.04
10	0.62 ± 0.06

4.6.2 Statistical analysis results

Table 4.2: ANOVA results for osmotic stress, Si, landrace, and their interactions, on shoot dry weight, Si concentration, and transpiration. Replicate was included as a random effect to account for variability between plants grown at different times. Statistically significant results are highlighted in bold.

	Shoot dry weight (g)			Transpiration (g H_2O hr^{-1} g DW^{-1})			Shoot Si (% dry weight)			Root Si (% dry weight)		
	df	*F*	*P*	*df*	*F*	*P*	*df*	*F*	*P*	*df*	*F*	*P*
Landrace	**9**	**3.99**	**< 0.001**	**9**	**2.49**	**0.012**	**9**	**6.77**	**< 0.001**	9	1.40	0.200
Stress treatment	**1**	**726.19**	**< 0.001**	**1**	**519.77**	**< 0.001**	**1**	**306.88**	**< 0.001**	1	0.004	0.951
Si availability	**1**	**9.61**	**0.002**	1	1.56	0.214	**1**	**2842.89**	**< 0.001**	**1**	**27.32**	**< 0.001**
Replicate	**3**	**29.24**	**< 0.001**	**3**	**2.99**	**0.034**	**3**	**3.62**	**0.015**	**3**	**3.65**	**0.015**
Landrace x Stress	9	0.36	0.951	**9**	**2.26**	**0.022**	9	1.67	0.104	9	0.82	0.595
Landrace x Si	9	1.24	0.277	9	1.23	0.285	9	2.38	0.017	9	0.82	0.602
Stress x Si	**1**	**5.74**	**0.018**	**1**	**15.16**	**< 0.001**	**1**	**138.38**	**< 0.001**	**1**	**18.70**	**< 0.001**
Landrace x Stress x Si	9	1.13	0.348	9	1.29	0.252	9	0.61	0.791	9	0.53	0.851

Table 4.3: ANOVA results for drought stress, Si, landrace, and their interactions, on shoot and grain weight, transpiration, and shoot Si concentration. Replicate was included as a random effect to account for variability between plants grown at different times. Statistically significant results are highlighted in bold. Short-term experiment (experiment 2): 4.2.1.2; Long-term experiment (experiment 3): 4.2.1.3.

	Shoot dry weight (g) (short-term experiment)			Shoot dry weight (g) (long-term experiment)			Grain weight (g) (long-term experiment)			Transpiration (g H_2O hr^{-1} g DW^{-1})			Shoot Si (%)		
	df	*F*	*P*	*df*	*F*	*P*	*df*	*F*	*P*	*df*	*F*	*P*	*df*	*F*	*P*
Landrace	**9**	**6.33**	**< 0.001**	**9**	**49.47**	**< 0.001**	**9**	**5.31**	**< 0.001**	9	1.37	0.217	**9**	**33.67**	**< 0.001**
Stress treatment	**1**	**224.93**	**< 0.001**	**1**	**135.43**	**< 0.001**	**1**	**184.27**	**< 0.001**	**1**	**56.80**	**< 0.001**	**1**	**11.32**	**< 0.001**
Si availability	1	0.15	0.70	1	0.32	0.57	1	0.46	0.501	1	0.90	0.345	**1**	**707.36**	**< 0.001**
Replicate	**3**	**125.22**	**< 0.001**	**3**	**16.42**	**< 0.001**	**3**	**6.46**	**< 0.001**	2	0.63	0.538	**3**	**126.35**	**< 0.001**
Landrace x Stress	9	1.06	0.40	9	1.46	0.173	**9**	**2.25**	**0.025**	9	1.01	0.441	9	0.87	0.549
Landrace x Si	9	0.62	0.78	9	0.55	0.832	9	0.13	0.999	9	0.42	0.921	**9**	**2.59**	**0.007**
Stress x Si	1	0.28	0.60	1	1.03	0.312	1	0.27	0.606	1	0.02	0.892	**1**	**6.65**	**0.010**
Landrace x Stress x Si	9	0.41	0.93	9	0.59	0.804	9	0.34	0.958	9	0.41	0.925	9	0.88	0.544

5 Chapter 5: General discussion

5.1 Summary of aims

Numerous studies have reported that plants supplied with Si are more tolerant to abiotic and biotic stresses than plants grown with no or minimal Si (reviewed in Debona *et al*., 2017). Thus, Si fertilisation has been proposed as a method of improving crop yields. However, species vary in their ability to accumulate Si, and this is predicted to affect the benefits that they get from Si fertilisation. In addition to species-level variation, genotypes within a species are also hypothesised to vary in their ability to accumulate Si, although to date this possibility has not been extensively investigated. This thesis aimed to improve understanding regarding variation in Si accumulation among genotypes, and whether this impacts on the effects of increasing Si availability on growth (Table 5.1). It is possible that while some genotypes show positive responses to Si supply, others show no response, or may even be negatively impacted by Si supply. Another important question pertains to whether shoot or root Si accumulation is critical for improving growth. Knowledge about variation in Si accumulation, and the impact of this on the response to Si, is critical to determining whether increasing soil Si availability using Si fertiliser could be an economically viable means of improving crop yields.

Presently, most Si research has focussed on rice, but it is important to investigate whether Si has similar beneficial effects in other species. Rice is typically grown in very wet conditions in the form of rice paddies, whereas wheat and many other crop species are grown in much drier conditions. It is possible that the factors underlying Si uptake differ between rice paddies and drier field conditions. Likewise, the benefits of high Si supply may be different in rice paddies. However, research into whether the effects of Si differ between rice and other species, and among different growth systems, is currently lacking. Wheat is one of the most important crop species worldwide and has previously been found to accumulate significant levels of Si (i.e. over 1 % Si by dry weight; Ma and Takahashi, 2002). For example, when grown for 30 days with 20 ppm Si, wheat accumulated 1.75 % Si in the leaves (Deshmukh *et al.,* 2020). Hence, wheat was selected as a study species for this thesis.

Due to their high genetic diversity and adaptation to suboptimal conditions, wheat landraces are being used in wheat breeding programs to breed elite cultivars with higher stress tolerance (Lopes *et al*., 2015). In this thesis, wheat landraces were used to

investigate firstly, the extent to which there is genotypic variation in Si accumulation (Chapter 2). Secondly, the effect of damage on Si accumulation was examined (Chapter 3). Finally, whether variation in Si accumulation impacts on the effects of increasing Si availability on growth was examined. As Si fertiliser is often reported to only impact on growth during stress conditions (Coskun *et al*., 2019a), drought stress was used to investigate the effect of Si on growth (Chapter 4). By using landraces with varying Si accumulation abilities, it was possible to test whether Si had a consistent impact on all landraces, or whether the effect of Si correlated with root or shoot Si accumulation. Moreover, due to the considerable genetic diversity within wheat landraces, it was possible to investigate the potential mechanisms underpinning the effects of Si.

Table 5.1: The main findings from this thesis, their potential implications, and possible targets for future research.

Chapter	Main findings	Implications	Targets for future research
2	Significant variation in Si accumulation among landraces May relate to transpiration at high Si supply No evidence that variation relates to differences in Si transporter sequence or expression Negative correlation between Si accumulation and growth	Potential to breed wheat for altered Si accumulation, but first need to better understand the causes Breeding for increased Si may decrease growth	Does the abundance of Si transporters differ between landraces? What is the relationship between Si accumulation and yield? Does it vary among genotypes?
3	Si supply increases accumulation only in damaged leaves Extent of increase in Si accumulation due to damage similar for all landraces No evidence that damage or Si supply increases spine density No effect of Si supply on JA-related gene expression Multiple damage events needed to increase Si accumulation	When breeding for increased Si, need to consider how stress will affect Si accumulation	Is the number of events or extent of damage important for inducing localised Si accumulation? Under what conditions, and in which species, does systemic Si induction occur? How effective is localised Si induction in reducing herbivory?
4	Small positive effect of Si supply on growth and transpiration during osmotic stress No effect of Si supply on growth or transpiration during drought Variable effects of stress on Si accumulation	Breeding for increased Si accumulation won't necessarily improve yield or stress tolerance Need to determine mechanism of Si to understand whether Si beneficial	During which drought stress conditions is Si beneficial? Why do wheat landraces not benefit from Si in the same way as elite cultivars? What is the mechanism underpinning the effects of Si on plant growth and physiology during drought?

5.2 Wheat landraces vary in Si accumulation ability

The first part of this study was focussed on identifying landraces that varied significantly in their ability to accumulate Si. In Chapter 2, significant variation in shoot Si accumulation among a diversity panel of 98 wheat landraces was found. Using a subset of twenty landraces of varying Si accumulating ability, it was further demonstrated that variation in Si accumulation is present both when plants are grown hydroponically and in compost, and is persistent across different levels of Si availability. However, Si accumulation of plants not supplied with additional Si (–Si) was not always a good predictor of potential Si accumulation after Si supplementation (+Si). Compared to other landraces, the relative amount of Si accumulated by two landraces was different when grown hydroponically with Si compared to when grown in compost without Si supply, and the range of tissue Si concentrations was higher for +Si plants (Chapter 2). This suggests that soil Si availability should be considered when assessing genotype Si accumulation potential. Previous studies where plants have been grown hydroponically at very high levels of Si may not reflect Si accumulation in the field where Si fertiliser is typically applied at lower levels. If the beneficial effect of Si is related to Si accumulation, this suggests that such high Si experiments may overestimate the potential benefits of Si fertilisation in an agricultural context.

In contrast to the significant variation in shoot Si concentration observed across landraces, no significant variation in root Si accumulation between landraces was observed in this study. Many previous studies did not measure root Si accumulation (e.g. Silva *et al.*, 2010; Moldes *et al.*, 2016; Farooq *et al.*, 2019; Lekklar *et al.*, 2019), despite evidence that Si deposited in the root is important for reducing the accumulation of toxic nutrients and controlling water flux (Fleck *et al*., 2015; Gong *et al*., 2006). In a study that did measure root Si (Tahir *et al*., 2010), similar levels of variation in root and shoot Si accumulation were found for five wheat genotypes when grown with supplemental Si. However, without Si fertilisation, the extent of variation in root Si was an order of magnitude lower than for shoot Si (Tahir *et al*., 2010).

5.3 Correlation between Si accumulation and effect of Si

While several previous studies have examined genotypic variation in Si accumulation in barley (Ma *et al*., 2003), rice (Talukdar *et al*., 2019), and wheat (Cotterill *et al*., 2007), they have not attempted to correlate this with the (beneficial or otherwise) effect of Si

accumulation. In Chapter 2, a negative correlation between shoot Si concentration and shoot biomass was found. Previous studies have reported similar negative correlations (de Tombeur *et al*., 2021; Johnson and Hartley, 2018; Simpson *et al*., 2017). A similar trade-off between Si accumulation and yield could exist. However, studies in both wheat (Neu *et al*., 2017) and rice (Flores *et al*., 2021) have indicated that an intermediate level of Si supply can increase grain yield in the absence of experimentally-imposed stress conditions, although both high and low Si supply resulted in lower grain yield. In this thesis, no correlation was detected between Si accumulation and yield, and there was no significant increase in yield for any landrace due to Si fertilisation in control (non-stressful) conditions (Chapter 4). It appears that there are costs associated with Si accumulation and further investigations are needed to understand these costs. Too much Si fertiliser may have detrimental impacts on plant growth. Thus, when considering using Si fertiliser in agriculture, the optimal amount of Si fertilisation must first be determined.

Whereas the above points to no or little Si effect on plants that are growing in non-stressful (control) conditions, this may not be the case for sub-optimal conditions. To examine whether there is a correlation between Si accumulation and growth when plants were subjected to stress, osmotic stress and drought were applied (Chapter 4). There was a small but significant improvement in growth for osmotically stressed plants supplemented with Si, but this coincided with decreased Si accumulation compared to plants that were not stressed. By contrast, drought stress significantly increased Si accumulation, but Si supplementation did not improve growth. For +Si plants, the shoot Si concentration was similar for plants subjected to osmotic and drought stress, suggesting that there is not simply a minimum level of Si that plants must accumulate to exhibit a beneficial effect of Si fertilisation. However, as root Si was not determined for drought-stressed plants, it was not possible to establish whether differences in root Si correlated with the differing effects of Si during osmotic stress compared to drought. Tahir *et al.* (2010) found that while Si fertilisation improved growth during salt stress to different extents among five wheat genotypes, this was not correlated with root or shoot Si accumulation. Further research is needed to investigate whether a threshold level of tissue Si is required for Si to have a beneficial effect on plant growth.

5.4 Causes of variation in Si accumulation

To understand the potential consequences of widespread Si fertilisation in agriculture, as well as to inform how plants use Si currently available in the soil, it is necessary to establish

how plants accumulate Si. There is some evidence that Si accumulation is partially related to transpiration rate (McLarnon *et al*., 2017; Henriet *et al*., 2006; Cornelis *et al*., 2010). Thus, it was hypothesised that there would be a positive correlation between transpiration and Si accumulation. Supporting this hypothesis, in Chapter 2, there was a positive correlation between shoot Si concentration and transpiration rate for plants grown with at a high level of Si availability. However, this correlation was not found for plants grown at a low level of Si availability. This is in agreement with results in cucumber, which also suggested that the role of transpiration in Si accumulation depends on its availability (Faisal *et al*., 2012). However, in Chapter 4, no correlation between transpiration and shoot Si was found for plants grown hydroponically, although transpiration was measured based on pot weight loss in Chapter 4 rather than falcon tube water loss, as was used in Chapter 2. The plants used in Chapter 4 were older than the plants used in Chapter 2. It was also demonstrated in Chapter 2 that the Si concentration remained unchanged after two and seven weeks of Si treatment. Therefore, it is possible that the relationship between Si accumulation and transpiration changes over time.

Interestingly, in Chapter 4, a positive correlation between shoot Si and transpiration was found for both +Si and –Si unstressed plants grown in compost. Plant-available soil Si for –Si plants grown in compost was higher than for –Si plants grown hydroponically, but how Si availability affects the role of transpiration in Si accumulation remains unknown. Moreover, during drought and salt stress, Si has been observed to increase transpiration in numerous plant species (Thorne *et al*., 2020), which may impact on Si accumulation. A role for passive, transpiration mediated-mechanisms in causing cultivar differences in Si accumulation has previously been suggested (McLarnon *et al*., 2017), and this thesis provides further evidence that variation in transpiration rate may partly explain differences in Si accumulation.

5.5 Differences in Si accumulation are not linked to genetic variation

In addition to relating to transpiration rate, variation in Si may also be due to the activity of specific Si transporters (reviewed in Ma and Yamaji, 2015). In this thesis, no evidence was found to support the hypothesis that variation in Si accumulation among wheat landraces was due to differences in Si transporter gene sequence, or genetic differences elsewhere in the genome. This is in contrast to studies in rice that have associated genetic differences with differences in Si accumulation (Talukdar *et al*., 2019, 2015). The SNPs used in this thesis were identified from transcriptome data and it remains possible that there are

genetic differences elsewhere in the genome, for example in promoter sequences, which correlate with differences in Si accumulation. With the recent assembly of the wheat genome (IWGSC, 2018), it should be possible in the future to perform whole-genome GWAS in wheat to establish whether there are any genetic differences associated with differences in Si accumulation. Additionally, as gene expression is not always related to protein activity, comprehensive proteomic studies in a range of genotypes of varying Si accumulating ability would help to identify specific proteins that may be involved in Si accumulation (Thorne *et al*., 2020).

Once genomic and proteomic studies have identified putative genes or proteins involved in Si accumulation, their expression and activity can be measured to confirm their involvement in Si accumulation. In this thesis, no consistent differences in putative Si transporter gene expression between landraces were identified (Chapters 2 and 3). This is in contrast to several studies in rice (Ma *et al*., 2007b; Wu *et al*., 2006) and barley (Mitani *et al*., 2009a) that have suggested that differences in transporter expression are correlated with differences in Si accumulation. Similarly, studies in rice have suggested that changes in gene expression cause changes in Si accumulation during stress conditions (Abdel-Haliem *et al*., 2017; Gupta *et al*., 2021). Gene expression is highly variable across time (Yamaji and Ma, 2007), and thus differences in Si transporter gene expression may only be observed at time-points different to those used in this study. Additionally, gene expression does not always correlate with protein abundance or activity, and post-translational regulation has been found to be important for aquaporins (Verdoucq *et al*., 2014).

Wang *et al.* (2017) identified a transcriptional regulator of Si transporter genes in rice. Genomic, transcriptomic, and proteomic studies could be used to identify similar regulators in wheat. Mutant studies could then help to establish the role of such regulators in Si accumulation. Such knowledge regarding regulators of Si accumulation would assist in establishing whether breeding for increased Si is likely to negatively impact on other plant processes. For example, several studies have suggested that there are interactions between Si and plant hormones (reviewed in Khan *et al.*, 2021). In Chapter 3, there was no evidence that Si affected JA-related gene expression, but whether JA signalling affected Si accumulation was not investigated. Omics approaches could be used to further understand the interactions between Si and other plant processes.

5.6 Variation in Si deposition

In addition to accumulating Si differently, landraces may also differ in how they deposit Si. Si is deposited in an array of forms in the leaves, including as silicified spines (Hartley *et al*., 2015). In contrast to the increase in spine density with increasing Si availability reported in previous studies in forage grass species and *Brachypodium distachyon* (Hartley *et al*., 2015; Hall *et al*., 2019), in this study, no effect of Si on spine density was found, both for undamaged and damaged plants. Si can also be deposited as phytoliths or in the cell wall (reviewed in Mandlik *et al.*, 2020). In tall fescue, increasing Si supply increased phytolith density, but decreased trichome density (Vandegeer *et al*., 2021a).

In Chapter 3, repeated damage induced localised Si accumulation, such that the Si concentration increased only in damaged leaves. Previous studies have found systemic increases in Si due to damage (McNaughton *et al*., 1985; Kim *et al*., 2014; Ryalls *et al*., 2018). It is plausible that differences in plant species and experimental design explain these different results. Initially, in response to damage, plants may increase Si only in damaged leaves, whereas more extensive damage may result in systemic increases in Si. Nevertheless, the failure of previous studies to measure Si separately for damaged and undamaged leaves of artificially damaged plants means that it is not certain whether the plants in these studies did induce systemic increases in Si, or whether the increase in Si in damaged leaves was simply sufficiently large to increase the overall Si concentration. Hartley *et al.* (2015) reported only localised induction of Si defences in three grass species. Future studies should therefore analyse the damaged and undamaged leaves of damaged plants separately.

5.7 Calculating the economic feasibility of Si fertiliser

Numerous previous studies have reported beneficial effects of Si on plant growth during stress conditions (Cooke and Leishman, 2016; Johnson *et al*., 2020; Li *et al*., 2018). To determine the economic feasibility of Si fertiliser, it is important to establish whether increased Si accumulation results in a stronger beneficial effect of Si, or whether there is a saturation level of Si accumulation, beyond which plants will not gain additional benefits. However, this thesis did not find a significant effect of Si fertilisation on growth during non-stressed (Chapter 2) or drought conditions (Chapter 4), although there was a small positive effect of Si during osmotic stress (Chapter 4). Limitations of previous studies may explain the disparity. In many studies, potassium-, sodium-, or calcium-silicate are used as Si

sources and in the absence of proper control experiments, these silicates increase cation concentrations and thus alter plant nutrition (Thorne *et al*., 2020). Additionally, variation in experimental conditions may explain the contrasting findings regarding the potential benefits of Si fertiliser. It has been suggested that the beneficial effect of Si depends on both the stress severity and type (Cooke and Leishman, 2016; Li *et al*., 2018).

To determine whether Si fertilisation is economically viable, it is essential that a cost-benefit analysis is performed. Feasibility will depend on a large range of parameters such as the source of Si, crop species, the amount of Si available prior to fertilisation, the yield increase due to Si, production costs, and any potential negative impacts of Si fertiliser (Thorne *et al*., 2020). In addition to the beneficial effects of Si on plant stress tolerance, additional beneficial effects of Si fertilisers should be considered when assessing their feasibility. For example, Si fertilisers can contain other beneficial nutrients such as potassium and have potential to capture carbon dioxide from the atmosphere (Beerling *et al*., 2018).

An important issue is that, to date, studies have focussed predominantly on the benefits of Si and have neglected to consider the potential negative consequences of Si fertilisation. For example, negative impacts of Si on growth have been reported in some species, and thus the availability of Si in the soil prior to Si fertilisation must be considered (Zhang *et al*., 2017; Kang *et al*., 2016; Dehghanipoodeh *et al*., 2018; Trejo-Téllez *et al*., 2020). Furthermore, cheaper forms of Si fertiliser can be contaminated with toxic metals, which will slowly build up in the soil and could create future yield losses and health issues (Ito, 2015). Although silicate rocks can be used to capture carbon dioxide, the mining and transport of such Si fertilisers could nevertheless have potential negative environmental impacts (Beerling *et al*., 2018). Finally, high Si content may reduce straw digestibility, preventing its use as a feedstock for livestock (Cougnon *et al*., 2020) or as a biofuel (Gressel, 2008).

Overall, cost-benefits analyses to date have concluded that Si fertiliser is likely to be economically viable in some cases, but these have only been performed for rice. In the absence of experimentally-imposed stress, Flores *et al.* (2021) suggested that foliar applications of intermediate levels of Si may be economically viable for rice. Likewise, Alvarez and Datnoff (2001) concluded that Si fertilisation would likely be economically viable in most rice-producing countries. Thorne *et al.* (2020) concluded that yield gains

greater than 10 % during drought or salt stress in rice would be required to justify the cost of Si fertiliser. Similar analyses are required for other crop species. This should involve large-scale field trials, using multiple crops and genotypes, with different levels of Si fertilisation, during different stress conditions. Such field trials would establish the minimum level of Si fertilisation required as well as the potential yield gains from Si fertilisation. As was found in this thesis, such field trials may highlight circumstances when Si fertilisation would not confer benefits.

5.8 Future directions

To establish the full potential of Si fertiliser in agriculture, particularly for species such as wheat where Si fertiliser is not currently commonly used, it is important to understand the mechanisms underpinning the effects of Si, and thus whether Si fertilisation could lead to any negative unintended effects. Increasing soil Si availability by Si fertilisation may not be a panacea for maintaining crop yields despite abiotic and biotic stresses. The negative correlation between plant biomass and Si accumulation observed in chapter 2 suggests that Si accumulation may incur costs to the plants, and this possibility should be investigated further.

Moreover, further investigation is needed to establish whether the positive effects of Si are only the consequence of Si deposition within the cell wall, or whether there is a biochemical role for Si. Presently, only weak evidence is available to support such a biochemical role of Si, with most omics studies identifying few genes or proteins as being affected by Si (e.g. Watanabe *et al.*, 2004; Fauteux *et al.,* 2006; Chain *et al.*, 2009; Jang *et al.*, 2018). Strong evidence supporting a biochemical role for Si, showing that Si directly affects gene expression, requires reproducible, short timescale (minutes to hours) studies, and the use of more tractable study systems such as cell cultures. If specific genes or proteins are identified, mutational studies could then be used to unravel any putative biochemical role for Si.

Si fertiliser is already used on a variety of crops in countries such as India to protect plants against herbivory (Murali-Baskaran *et al.,* 2021). However, there remain several important questions regarding the ability of plants to use Si as a deterrent against herbivory. For example, how long the rate of Si uptake remains increased after herbivory remains to be determined. In the perennial grass species *Deschampsia caespitosa*, herbivore-induced increases in Si defences gradually decreased after the cessation of herbivory, returning to

levels observed in plants not subject to herbivory around one year later (Reynolds *et al*., 2012). However, similar studies in annual crop species such as wheat have not yet been conducted. Similarly, it remains unknown whether there are herbivore conditions that induce systemic rather than localised induction of Si defences. Further experiments exploring whether there is variation in deposition of Si between genotypes would also be useful for establishing whether Si fertiliser could be used more widely to improve herbivory tolerance.

5.9 Conclusions

It has been suggested that Si fertilisation could be a panacea for improving crop yields, by being able to mitigate against the negative impacts of biotic and abiotic stress in a wide range of plants species. However, results in this thesis show that increased Si availability is not always advantageous and further research is needed to determine the conditions during which Si fertilisation may be beneficial (Figure 5.1). In Chapter 2, significant variation in Si accumulation among wheat landraces was found, which was partially attributed to differences in transpiration rate. In Chapter 3, it was shown that repeated damage induced localised Si accumulation, but the mechanism underpinning this, and the effects of this on herbivore tolerance, remain to be determined. The complete lack of beneficial effect of Si during drought discussed in Chapter 4 was surprising and suggests further investigation is needed before the widespread adoption of Si fertilisation to improve drought tolerance in wheat. Overall, genotypic variation is likely to be an important influence on the effects of Si and should be considered in future studies examining the role of Si in plant biology.

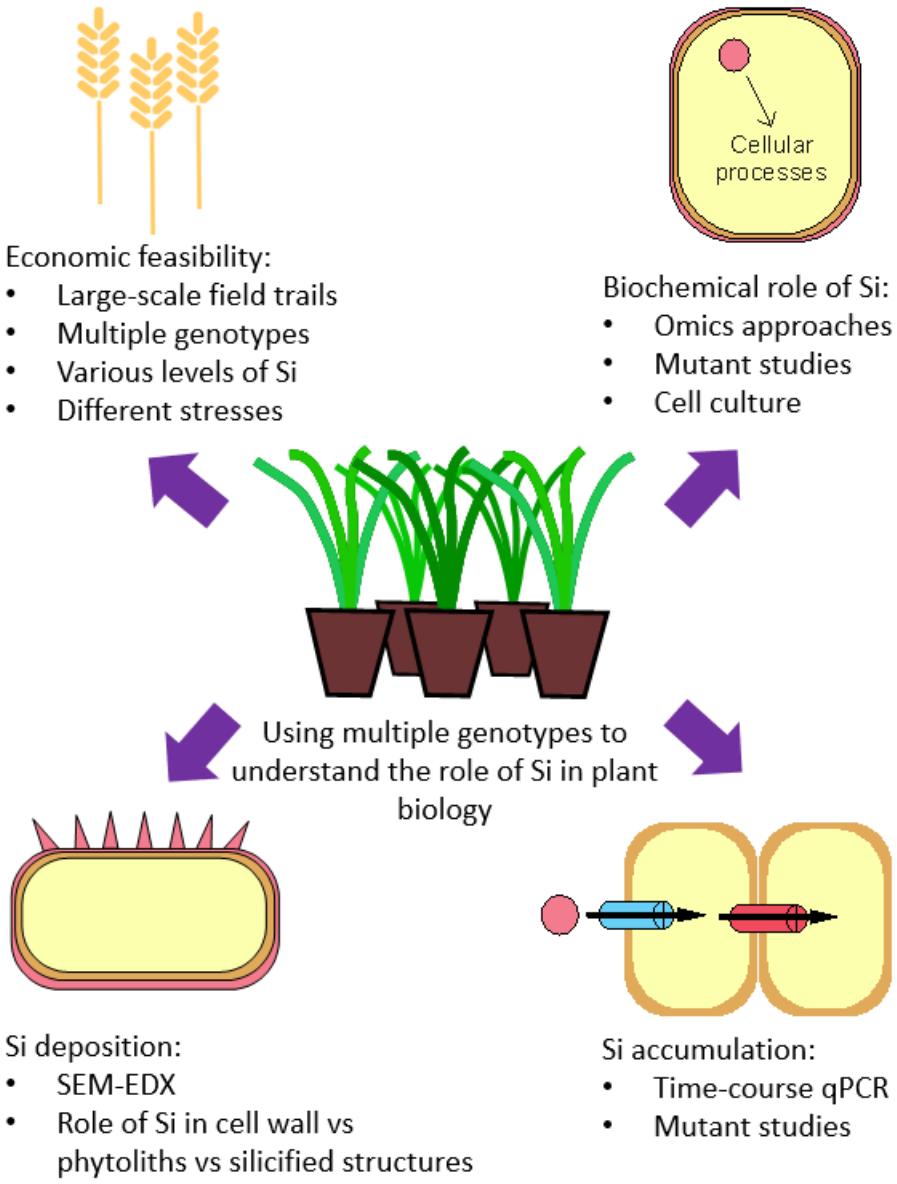

Figure 5.1: Potential future experiments using multiple genotypes to understand the role of Si in plant biology

6 Abbreviations

ABA	Abscisic acid
ANOVA	Analysis of variance
ASZ	Active silicification zone
AOC	Allene oxide cyclase
AOS	Allene oxide synthase
Ar/R	Aromatic/arginine selectivity filter
AT	Associative transcriptomics
$CaCl_2$	Calcium chloride
CAT	Catalase
CCMV	Cowpea chlorotic mottle virus
cDNA	Complementary deoxyribonucleic acid
CIMMYT	International Maize and Wheat Improvement Centre
CMMV	Cowpea mild mottle virus
COI1	Coronatine-insensitive 1

DE	Differential expression analysis
DW	Dry weight
EDX	Energy dispersive X-ray spectroscopy
FAO	Food and Agricultural Organisation of the United Nations
FC	Field capacity
FDR	False discovery rate
FPS	Farnesyl pyrophosphate synthetase
GEM	Gene expression markers
GSGR	Glycine-Serine-Glycine-Arginine
GWAS	Genome wide association study
H	Used to denote high Si-accumulating landrace
HCl	Hydrochloric acid
IPCC	Intergovernmental Panel on Climate Change
ITPS	Intergovernmental Technical Panel on Soils
JA	Jasmonic acid

KOH	Potassium hydroxide
L	Used to denote low Si-accumulating landrace
Lsi	Low silicon
M	Used to denote medium Si-accumulating landrace
MLM	Mixed linear model
NaCl	Sodium chloride
NIP	Nodulin-26 like intrinsic proteins
NPA	Asparagine-Proline-Alanine
NPK	Nitrogen-Phosphorus-Potassium fertiliser
PCA	Principal components analysis
PEG	Polyethylene glycol
POX	Peroxidase
PSIKO	Population structure inference using kernel-PCA and optimisation
P-XRF	Portable X-ray fluorescence
QTL	Quantitative trait loci

RNA	Ribonucleic acid
ROS	Reactive oxygen species
RPKM	Reads per kb per million aligned reads
RT-qPCR	Reverse transcriptase quantitative polymerase chain reaction
SA	Salicylic acid
SE	Standard error
SEM	Scanning electron microscopy
Si	Silicon
SNP	Single nucleotide polymorphism
SOD	Superoxide dismutase
TMV	Tobacco mosaic virus
TRSV	Tobacco ringspot virus
Tukey's HSD	Tukey's honest significant difference test
WUE	Water use efficiency

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