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Article:
Thorne, S.J. orcid.org/0000-0003-0476-8466, Maathuis, F.J.M. and Hartley, S.E. (2023) Induction of silicon defences in wheat landraces is local not systemic and driven by mobilisation of soluble silicon to damaged leaves. Journal of Experimental Botany, 74 (17). pp. 5363-5373. ISSN 0022-0957

https://doi.org/10.1093/jxb/erad224

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Induction of silicon defences in wheat landraces is local not systemic and driven by mobilisation of soluble silicon to damaged leaves

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Running title: Mechanism of induction of silicon defences in wheat

Date of submission: 3/5/23

Number of tables: 0

Number of figures: 5

Word count: 4207

Supplementary tables: 3

Supplementary figures: 4

1. Highlight

Silicon is an important defence in crops. Here, a new mechanism involving the movement of soluble silicon in the phloem is proposed to explain localised induction of silicon defences.

2. Abstract

- In response to herbivory, many grasses, including crops such as wheat, accumulate significant levels of silicon (Si) as an antiherbivore defence. Damage-induced increases in Si can be localised in damaged leaves or more systemic, but the mechanisms leading to these differences in Si distribution remain untested.
- Ten genetically diverse wheat landraces (*Triticum aestivum*) were used to assess genotypic variation in Si induction in response to mechanical damage and how this was affected by exogenous Si supply. Total and soluble Si levels were measured in damaged and undamaged leaves, as were Si levels in the phloem, to test how Si was allocated to different parts of the plant after damage.
- Localised, but not systemic, induction of Si defences occurred, more pronounced when plants had supplemental Si. Damaged plants had significant increases in Si concentration in their damaged leaves, while the Si concentration in undamaged leaves decreased, such that there was no difference in the average Si concentration of damaged and undamaged plants.
- The increased Si in damaged leaves was due to the redirection of soluble Si, present in the phloem, from undamaged to damaged plant parts, potentially a more cost-effective defence mechanism for plants than increased Si uptake.

Keywords: damage, genotypic variation, localised response, phloem, reallocation, silicon, wheat (*Triticum aestivum*)

3. Introduction

Plants have many different types of defences against herbivores, including both physical and chemical defences (Howe and Jander, 2007). Particularly in grasses, which include many cereal crops, high silicon (Si) accumulation is an effective antiherbivore defence (Massey *et al.*, 2009; Han *et al.*, 2015; Singh *et al.*, 2020; Waterman *et al.*, 2021). Grasses deposit Si in structures such as phytoliths and silicified spines (Hartley *et al.*, 2015). Phytoliths increase leaf abrasiveness making tissues less palatable (Hall *et al.*, 2020), which deters both insect and mammalian herbivores. Furthermore, phytoliths can reduce herbivore digestive efficiencies and hence limit their growth and development (Massey and Hartley, 2006, 2009; Massey *et al.*, 2008, 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 (Coskun *et al.*, 2019; Singh *et al.*, 2020).

At least in grasses, Si is an inducible defence (Massey *et al.*, 2007): in response to herbivory, overall Si accumulation increases, and this is correlated with reduced herbivory (Reynolds *et al.*, 2012; Hartley *et al.*, 2015; Hall *et al.*, 2020). However, the Si response to herbivory varies significantly between both plant species and genotypes (Hartley and DeGabriel, 2016). For example, 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), and similar genotypic variation in Si uptake and deposition have been found in wheat (*Triticum aestivum*; Thorne *et al.*, 2021) and rice (Talukdar *et al.*, 2019). Soininen *et al*. (2013) reported both within and between species variation in Si accumulation among grasses in response to damage.

Plants accumulate Si in the form of silicic acid from the soil using a pathway that is relatively well characterised in rice. Si is transported through the roots by the serial action of two transporters, Lsi1 and Lsi2 (Ma and Yamaji, 2015). Silicic acid is then loaded into the xylem by the action of a third transporter, Lsi3 (Huang *et al.*, 2022). Subsequently, Si is translocated to the shoots *via* the transpiration stream (Ma and Yamaji, 2015). A fourth transporter, Lsi6, is required for xylem unloading of silicic acid (Yamaji *et al.*, 2008). Lsi6, Lsi3, and Lsi2 are also highly expressed in the node where they are involved in intervascular transfer to direct Si distribution within the plant (Yamaji *et al.,* 2015). High levels of silicic acid result in its autopolymerisation into immobile silica, which is the predominant form of Si in plants (Yoshida *et al.*, 1962). Thus, plant Si accumulation is affected by both the transpiration rate and the activity of Si transporters. Genotypic variation in Si accumulation has been linked to both differences in transpiration (McLarnon *et al.*, 2017) and differences in the abundance of Si transporters (Ma *et al.*, 2007*b*). However, a range of biotic and abiotic factors have been shown to influence genotypic variation in Si accumulation including damage (Soininen *et al.*, 2013), nitrogen availability and plant competition (de Tombeur *et al.*, 2022), and climatic factors (Johnson *et al.*, 2023).

Although damage-induced localised increases in tissue Si have been reported in several grasses (Hartley *et al.*, 2015; McLarnon *et al.*, 2017) and in cucumber (Islam *et al.*, 2020), the mechanism underpinning this localised increase in Si remains to be determined. One hypothesis is that the uptake of Si from the soil increases, and this additional Si is then directed specifically to damaged tissue. It is unlikely that differences in transpiration rate explain increased Si accumulation in

damaged leaves, as a localised damage response can be observed even in plants where transpiration rates were greatly reduced (McLarnon *et al.*, 2017). Instead, changes in Si transporter abundance and activity may be responsible, as has been suggested for rice (Ye *et al.*, 2013), though other studies have not found evidence for this mechanism (McLarnon *et al.*, 2017).

A second hypothesis proposes that part or all the "extra" Si gets relocated from undamaged tissue to damaged tissue. Si accumulation is an active process which involves significant energetic costs (de Tombeur *et al.*, 2023) and thus the mobilisation of the Si already present in the plant towards the sites where it is most needed to repel attack may be a more beneficial strategy than increased uptake from the soil. However, once Si is deposited, it cannot be remobilised (Samuels *et al.*, 1991), and thus this hypothesis could only pertain to the soluble Si fraction in plants. It has not yet been experimentally tested, nor has the interaction between genetic variation in patterns of Si accumulation, damage, and Si supply.

Here, the effect of mechanical damage on Si accumulation in a number of genetically diverse landraces of wheat (*Triticum aestivum*) was investigated and the following questions were addressed:

1. Is the induction of silicon-based defences in wheat a localised or systemic response, and how is the magnitude and pattern of induction affected by Si supply?
2. If the response is localised, by what mechanism is this achieved? Is the source of the increased Si in damaged leaves due to *de novo* uptake, or to reallocation of Si, in soluble form, to damaged leaves from other parts of the plant?

4. Methods

4.1. Genotype selection, growth conditions, and experimental treatments

To investigate whether there is genotypic variation in the effect of damage on Si accumulation, ten wheat landraces that differed significantly in their Si accumulation were selected: five high and five low Si-accumulating landraces (Thorne *et al.*, 2021, Supplementary Table S1). A balanced factorial experimental design was used with three plants per landrace per damage versus undamaged treatment. 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 half-strength Hoagland's solution supplemented with 1.8 mM dissolved sodium metasilicate ($Na_2SiO_3.9H_2O$). Plants were watered as required.

Two of the high and two of the low Si-accumulating landraces were then selected to examine the effect of Si availability on Si accumulation in response to damage. To control Si supply effectively, plants were grown hydroponically. Seeds were germinated in sand for 10-11 days, and then seedlings were transferred to 9 L plastic hydroponics boxes, filled with half-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. A balanced factorial experimental design was used with plants either damaged or not and supplemented with Si (+Si) or not (– Si). Plants supplemented with Si received Hoagland's solution containing 1.8 mM Si. Sodium chloride (NaCl) was used to balance sodium levels for plants not supplemented with Si. Three plants per landrace, per Si fertilisation level, and per damage treatment were used.

To identify the source of the additional Si leading to the localised induction in damaged leaves, one landrace (L1) was selected. A balanced factorial experimental design was used with plants either damaged or not. All plants were grown hydroponically with 1.8 mM Si for the first three weeks. Immediately prior to damage, half the plants were moved to a medium without Si supplementation (+/– Si) while the remaining plants continued to be grown with Si supplementation (+/+ Si). Plants were harvested three days after the first, second, and fourth damage. Three plants per treatment were used.

All 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 excess water was removed. Leaf, stem, and root fresh weight was recorded. Plants were oven-dried at 70 °C until constant mass was achieved, then dry weight was recorded.

4.2. Damage Treatment

A damage treatment was started four weeks after germination and continued for three weeks. In the case of the ten landraces grown in soil, plants were damaged three times a week, whereas plants grown hydroponically were damaged twice weekly. A damage treatment involved removing approximately half of a newly produced leaf along the midrib. Plants were harvested one day after the final damage event, seven weeks after germination unless otherwise stated. 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.

4.3. Si Measurements

Portable X-ray fluorescence spectroscopy (P-XRF) was used to measure the leaf Si concentration of all plants and the root Si concentration of plants grown hydroponically (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 commercial 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 (%). The Si concentration of damaged and undamaged leaves of damaged plants was analysed separately. Si content (mg) was calculated as:

$$Si\ content\ (mg) = \frac{Si\ concentration\ (\%)}{100} \times dry\ weight\ (mg)$$

4.4. Measuring soluble Si

To measure the amount of soluble Si present in the leaves, one landrace (L1) was grown hydroponically with 1.8 mM Si for seven weeks. Four leaves were damaged simultaneously (~17 % of the leaves) and plants were harvested five days after the damage. The soluble Si concentration was measured for ten damaged plants and six undamaged plants. Damaged and undamaged leaves of damaged plants were analysed separately.

Leaf soluble Si was measured using an adapted version of the molybdenum method of measuring plant available Si in the soil (Sauer *et al.*, 2006). Briefly, samples were oven-dried at 70 ˚C and ground in a ball mill. A total of 2 g of sample was added to a 50 mL polyethylene tube with 20 mL 0.01 M $CaCl_2$. Samples were shaken slowly at 30 rpm for 16 h then centrifuged at 2000 rpm for 10 min. The supernatant was passed through filter paper and the resulting sample was used for Si determination by the molybdenum assay. For the assay, 1 mL sample, 30 mL 20 % acetic acid, and 10 mL ammonium molybdate (54 g L^{-1}) were mixed in a 50 mL polyethylene tube. Samples were mixed and then left to stand for 5 minutes. 5 mL 20 % (w/v) tartaric acid and 1 mL of reducing solution were

added. The reducing solution comprised 8 g L^{-1} Na_2SO_3, 1.6 g L^{-1} 1-amino-2-napthol-4-sulfonic acid, and 100 g L^{-1} $NaHSO_3$ dissolved in deionised water. A final volume of 50 mL was made using 20 % acetic acid. Samples were left at room temperature for 30 min for colour development then the absorbance at 810 nm was measured. A standard curve was created using 0, 0.1, 0.5, 1, 5, and 10 µg Si mL^{-1} to determine the Si concentration of the samples. The amount of soluble Si measured using the molybdenum assay was compared to the total Si concentration measured using XRF to estimate the total soluble Si present.

4.5. Measuring soluble Si in phloem exudate

Phloem exudate was collected using the ETDA-mediated method as described in Xu *et al.* (2019) using plants grown hydroponically with 1.8 mM supplementary Si for three weeks. Three leaves per plant were damaged, then half the plants were moved to medium without supplementary Si. Phloem exudate was collected 24 h later using three leaves per plant. Phloem exudate was collected separately for damaged and undamaged leaves of damaged plants, and for undamaged plants. As a negative control, phloem exudate was collected in water (no EDTA) from undamaged plants. Three plants per treatment were used. The Si concentration of the phloem exudate was then analysed using the molybdenum method as described above.

4.6. Measuring expression of Si transporters

To investigate whether damage affected Si transporter gene expression, the expression levels of the Si transporters *Lsi3* and *Lsi6* were determined using reverse transcriptase quantitative polymerase chain reaction (RT-qPCR). These transporters were chosen as they are known to be expressed in the shoots, whereas the other Si transporters, *Lsi1* and *Lsi2*, are expressed predominantly in the roots (Yamaji *et al.*, 2015). Initial experiments on the leaves of wheat landraces confirmed this with only very low levels of expression of *Lsi1* and *Lsi2*, but significant expression of *Lsi3* and *Lsi6*, found. Plants of the landrace L1 were grown hydroponically with 1.8 mM Si for four weeks. Plants were then moved to – Si medium immediately prior to the first damage event and then subject to either one or two damage events (with the second damage event occurring 24 hours after the first damage event). Leaf samples were taken from the middle of the newest expanded leaf 4, 24, and 48 h after one or two damages. Initially two samples were analysed for each time point and subsequently up to five biological replicates from a given timepoint were analysed once the most relevant timepoints had been identified.

Primers were designed to match all homoeologs, based on existing wheat sequences where available, or on homology to the barley sequence (Supplementary Table 2). Leaf tissue was collected

and ground under liquid nitrogen using a mortar and pestle and RNA extracted using a RNeasy kit with DNase treatment (Qiagen), according to the manufacturer's instructions. cDNA synthesis was performed using Reverse Transcriptase Superscript II M-MLV (Invitrogen) on 500 ng RNA. RT-qPCR was performed using a QuantiNova SYBR green PCR kit (Qiagen) with 2 µL cDNA (diluted 1:10) and 700 nM primer on a Rotor-Gene Q PCR machine (Qiagen). Amplification was performed as follows: 95 ˚C for 2 min; 40 cycles of 95 ˚C 5 s and 60 ˚C for 10 s; determination of melt curve. *Actin* and *TEF1* were used as reference genes. The qPCR results were analysed using a variation of the 2^{DDCt} method as described in Muller *et al*. (2002) and an adapted version of the Q-gene excel software (Simon, 2003).

4.7. Statistical Analysis

All statistical analyses were performed using R software (version 4.2.0, R Core Team, 2022). Summary statistics were calculated using the Rmisc package (Hope, 2013) and graphs were produced using the ggplot2 package (Wickham, 2016). Two-way and three-way analyses of variance (ANOVA) were used to test the effect of Si supply, damage, number of damage events, and landrace on Si concentration, as relevant to each experiment. 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 concentration was logit transformed and Si content was log transformed. No transformation was applied to gene expression or soluble Si data. 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. Significant results were analysed by performing Tukey's Honest Significance Difference (HSD) *post-hoc* tests using the emmeans package (Lenth, 2021).

5. Results

5.1. Damage results in a localised increase in Si

Averaged across all ten landraces, repeated damage significantly increased Si accumulation in damaged leaves of damaged plants when compared to undamaged plants (Figure 1; $F_{1,40} = 67.9$, $P <$

0.001). The biggest percentage increase was 107.6 ± 28.4 % in the L3 landrace, compared to an increase of only 34.2 ± 9.3 % in H5. The localised induction in damaged leaves was significant in all landraces except landraces H5 and L2, though there was still a trend towards significance even in these landraces (P = 0.055 and 0.052 respectively). Overall, the interaction between landrace and damage was not significant ($F_{9,40}$ = 1.5, P = 0.171).

Si accumulation was lower in the undamaged leaves of the damaged plants, averaged across all ten landraces, when compared to the levels in undamaged plants (Figure 1; $F_{1,40}$ = 9.2, P = 0.004). However, post-hoc testing revealed that the decrease in undamaged leaves was specific to the low Si-accumulating landraces L1 (P = 0.013) and L5 (P = 0.026), with L3 and L4 showing a similar trend albeit marginally non-significant (P = 0.051 and 0.054 respectively). The lack of increase in Si in undamaged leaves on damaged plants, or even decreases in some landraces, means there was no overall increase in leaf Si in damaged plants compared to undamaged ones for any landrace (Supplementary Figure 1). Thus, the induction of Si defences occurred only locally and was not systemic.

On average, high Si-accumulating landraces had higher shoot Si concentrations compared to low Si accumulating landraces, with an average Si concentration of 2.97 ± 0.17 for undamaged high Si landraces compared to 1.89 ± 0.09 for low Si landraces. There was no significant correlation between leaf Si in undamaged plants and the relative increase in leaf Si due to damage (r = -0.32, P = 0.364).

5.2. The effect of damage on Si accumulation depends on Si availability

Repeated damage significantly increased the leaf Si accumulation in the damaged leaves of the four landraces tested (Figure 2; $F_{1,47}$ = 216.0, P < 0.001). This was the case in both the +Si (1.8 mM Si supplied to the hydroponic medium) and –Si (no exogenous Si supply) treatments, although this increase was significantly greater in the +Si treatment (Figure 2; Si availability x damage interaction: $F_{1,47}$ = 23.1, P < 0.001).

As with the ten landraces, damage resulted in only a localised induction of Si defences. Damage significantly decreased leaf Si accumulation in the undamaged leaves of damaged plants compared to undamaged plants in the +Si treatment ($F_{1,24}$ = 18.2, P < 0.001), but this did not occur in the –Si plants, although leaf Si in this treatment was already an order of magnitude lower ($F_{1,23}$ = 0.5, P = 0.503). There was no significant variation in the Si response to damage among landraces in either the –Si or +Si treatments. No significant effect of damage on root Si accumulation was found,

253 although, as in the leaf tissue, there was a positive effect of Si supply on Si concentrations
254 (Supplementary Figure 2).
255
256 **5.3. Si is redirected towards damaged leaves in soluble form**
257 To determine whether the observed increase in Si accumulation in damaged leaves was the result of
258 *de novo* Si uptake or due to reallocation of Si from undamaged leaves, the exogenous Si supply was
259 removed from plants from the L1 landrace prior to a series of damage events. Increasing the number
260 of damage events significantly increased Si accumulation in damaged leaves of damaged plants,
261 although this response was significantly higher in +/+Si (continuous Si supply) plants compared to
262 +/–Si (Si removed before damage) plants (Figure 3; Si x number of damage events interaction: $F_{2,24}$ =
263 21.6, $P < 0.001$). For +/+Si plants, a greater number of damage events led to a more pronounced Si
264 increase in damaged leaves. However, in the case of +/–Si plants, the opposite trend occurred: each
265 damage event was associated with a reduced localised induction, possibly because the total Si
266 concentration in these plants was "diluted" down by to continuing growth of plant tissue. Thus, after
267 four damage events, the increase in Si in damaged leaves in +/– Si plants was reduced to 81 % of the
268 increase occurring after a single damage, whereas for +/+ Si plants, the increase in Si after four
269 damage events was over three times as great (302 %) of that after a single damage.

270 The distribution of Si in the root, stem, and leaf tissues of damaged and undamaged plants, after
271 one, two, or four damage treatments, was calculated for both +/+Si and +/–Si plants. The total
272 amount of Si accumulated in the plants did not differ significantly between damaged and
273 undamaged plants (Figure 4; $F_{1,23}$ = 0.1, P = 0.778), regardless of the number of damage events or
274 the nature of the Si supply. Si content increased with an increasing number of damage events only
275 for +/+ Si plants, as would be expected in the case of continuous Si supply. There was no significant
276 difference in root or stem Si concentration between damaged and undamaged plants, although Si
277 supply significantly increased both root and stem Si concentration (Supplementary Figure 3). For +/–
278 Si plants, increasing the number of damage events significantly decreased the stem Si concentration
279 ($F_{2,24}$ = 20.5, $P < 0.001$).

280 In the absence of external Si supply (+/– Si plants) and given the similarity of total Si content
281 between damaged and undamaged plants, any increase in Si in damaged leaves most likely
282 originates from the relocation of Si from undamaged leaves. The soluble Si fraction of damaged and
283 undamaged leaves was measured in plants of the landrace L1 grown hydroponically for 7 weeks and
284 damaged once, in order to assess whether it is sufficiently large to account for the extra Si found in
285 damaged leaves, without the need for additional uptake. Undamaged plants had a soluble Si content

of 2.2 ± 0.1 mg g^{-1}, compared to 4.0 ± 0.1 mg g^{-1} for damaged leaves and 1.8 ± 0.1 mg g^{-1} for undamaged leaves of damaged plants. Based on the difference between undamaged leaves of damaged plants and undamaged plants, this means that on average there will be 0.4 mg g^{-1} soluble Si available to move from undamaged leaves to damaged leaves of damaged plants. The soluble Si from undamaged leaves needs to provide the 1.8 mg g^{-1} increase in soluble Si observed in damaged leaves compared to undamaged plants. Thus, as long as the amount of undamaged leaves is 4.5 times as the weight of damaged leaves on a plant, there is sufficient soluble Si to relocate from undamaged to damaged leaves and account for all the localised induction in Si defences observed.

It was hypothesised that the redistribution of Si from undamaged to damaged leaves is the result of soluble Si being moved *via* the phloem. Supporting this idea, significant concentrations of soluble Si were measured in the phloem exudate. For +/+ Si plants, soluble Si in the phloem was found to be: 1.9 ± 0.6 mM in undamaged plants, 1.1 ± 0.5 mM in undamaged leaves of damaged plants, and 1.9 ± 0.4 mM in damaged leaves. The phloem Si of +/– Si plants was lower at: 1.1 ± 0.5 mM in undamaged plants, 0.9 ± 0.7 mM in undamaged leaves of damaged plants, and 1.0 ± 0.4 mM in damaged leaves. The Si in the phloem of damaged leaves was significantly higher in the +/+ Si plants than in the +/– Si plants ($F_{1,8}$ = 9.4, P = 0.016) but there was no difference between the phloem Si levels in the undamaged leaves of damaged plants from the Si two treatments ($F_{1,8}$ = 2.0, P = 0.196). This suggests that soluble Si is being directed from undamaged to damaged leaves, particularly in plants with continuous Si supply, supporting the hypothesis that Si is being mobilised from undamaged to damaged leaves where Si can be deposited as a physical defence.

Soluble Si may be loaded into the phloem by the action of Si transporters. Specifically, the activity of *Lsi3* and *Lsi6,* which are known to be expressed in the leaves, may be upregulated in damaged plants. To test this hypothesis, RT-qPCR was applied to determine the expression levels of the *Lsi3* and *Lsi6* genes. However, no consistent significant differences in *Lsi3* or *Lsi6* gene expression between damaged and undamaged plants were found, irrespective of the time point (4, 24, or 48 h) or the number of damage events (one or two; Supplementary Figure 4).

6. Discussion

6.1. Is the induction of silicon-based defences a localised or systemic response, and how is this affected by Si supply?

Damage resulted in localised, but not systemic, induction of silicon defences. This response was observed across a range of landraces and at different levels of Si availability, although providing supplementary Si significantly increased the magnitude of the response. In contrast to the prevailing

hypothesis of Si being immediately deposited and thus rendered immobile, this study provides evidence that soluble Si can be moved from undamaged to damaged leaves to increase Si defences close to the site of wounding, where they are most needed. This appears to be the result of soluble Si being transported in the phloem sap to the stem, where it is then loaded into the xylem and transported to damaged leaves *via* the transpiration stream. However, this study found no evidence to suggest changes in Si transporter gene expression are involved.

In this study, artificial damage was used in place of herbivory to separate the effects of damage to tissue caused by the herbivore from the effects of molecules in the saliva and other excretions of the herbivore (Waterman *et al.*, 2019). Damage was found to significantly increase Si accumulation, a conclusion supported by previous studies which have found that mechanical damage is sufficient to significantly increase plant Si-based defences (McNaughton *et al.*, 1985; Kim *et al.*, 2014; Ryalls *et al.*, 2018), though it is also clear that actual herbivory can produce greater induction (Massey *et al.*, 2007).

This study found significant variation in Si accumulation among wheat landraces. Eight out of ten landraces responded to damage by significantly increasing Si accumulation in damaged leaves, and this increase ranged from 34.2 % to 107.6 %. Similar genotypic variation has been found in other species: Bañuelos and Obeso (2000) reported significant genotypic variation in response to damage in the grass species *Agrostis tenuis*. Likewise, 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. 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.

This study found only a localised, and not systemic, Si response to damage, with the Si concentration increasing only in the damaged leaves of damaged plants. Few previous studies have made a distinction between the Si concentration of damaged and undamaged leaves of damaged plants. In agreement with the results of this study, insect herbivory significantly increased Si accumulation in attacked leaves of two genotypes of cucumber (Islam *et al.*, 2020) and damage resulted in localised induction of Si defences in three grass species (Hartley *et al.*, 2015). 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.

6.2. By what mechanism is localised induction of Si defences achieved?

Overall, the Si concentration of damaged plants was not significantly different to that of undamaged plants. It was hypothesised that this was a result of the redirection 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. To test for this hypothesis, plants were grown with Si and then moved to –Si medium prior to damage. Increased Si accumulation in damaged leaves was still observed in these plants, as well as decreased Si accumulation in undamaged leaves, suggesting that Si redirection had occurred.

It has widely been reported in the literature that once deposited as silica, Si cannot be remobilised (Yoshida *et al.*, 1962; Jones and Handreck, 1967; Samuels *et al.*, 1991; Liu *et al.*, 2019; Frick *et al.*, 2020). However, it is likely that the Si was redirected into damaged leaves prior to deposition as silica, when it was still in a soluble form. Earlier studies have indicated that there may be relatively high levels of soluble Si present in the cytoplasm (Gartner *et al.*, 1984; Hodson and Evans, 1995), which could be used to increase the levels of Si in damaged leaves even after the removal of Si from the growth medium.

To examine whether there was sufficient soluble Si to explain the increased Si localised in damaged leaves, leaf soluble Si was measured. The soluble Si concentration of undamaged plants was taken as a baseline for the amount of soluble Si predicted to be present in plants before damage. The undamaged plants had an average total leaf Si concentration of 1.4 %, of which 15.7 % was soluble Si. Thus, undamaged plants comprised approximately 0.22 % soluble Si. However, this is likely to be an underestimate as the formation of silicomolybdate complexes in the molybdenum-based assay depends on the size of the silicates present. While monomeric and dimeric silicates react quickly, higher oligomers may not have fully reacted over the course of the 30-minute assay (Coradin *et al.*, 2004). Despite this, it was calculated that there is sufficient soluble Si present in undamaged leaves to explain the increase in Si in damaged leaves. Thus, it was concluded that the increase in Si in damaged leaves can be explained by the movement of soluble Si from undamaged leaves into damaged leaves of damaged plants (Figure 5).

It was hypothesised that the increase in Si in damaged leaves would be the result of differences in Si transporter gene expression. In rice, Si transporters are used to preferentially allocate Si to the panicle and away from the flag leaf (Yamaji and Ma, 2009; Yamaji *et al.*, 2015) and it is possible that a similar mechanism results in the preferential allocation of Si to damaged leaves. To test for this, several time points, after one and two damage events, were used to investigate whether the

localised increase in Si in response to damage was the result of changes in Si transporter gene expression. However, no significant differences in Si transporter gene expression were found, despite the numerous timepoints tested meaning that it is unlikely any increase in gene expression was overlooked. Differences in Si transporter gene expression have been found in rice (Ye *et al.*, 2013) but not in other species (McLarnon *et al.*, 2017). It remains possible that post-transcriptional processes affect the activity of Si transporters as gene expression is not always indicative of protein activity. Post-translational regulation has been found to be important for aquaporins (Verdoucq *et al.*, 2014). Alternatively, yet to be identified Si transporters may be involved, or the activity of transcriptional regulators may be involved, as has been found in rice (Wang *et al.*, 2017).

To the best of our knowledge, no previous studies have been carried out to directly measure Si in the phloem, and it is generally suggested that Si may not be phloem-mobile (Raven, 1983). However, xylem-to-phloem Si transfer has been suggested to occur during grain filling in rice, though this was not actually tested (Zhou *et al.*, 2021). Likewise, Yang et al. (2017) speculated that Si in the phloem may deter aphid feeding in rice but could not rule out the possibility that their results were due to Si affecting phloem sap composition. Both boron and arsenic, which share many chemical similarities to Si, have been observed in the phloem (Carey *et al.*, 2011; Zhao *et al.*, 2012; Umemura and Takenaka, 2014), although previous studies have not investigated whether this is also the case for Si. In the case of boron, it must bind to sugars before being transported in the phloem (Umemura and Takenaka, 2014) and it is possible that a similar mechanism is involved in Si redirection, with *in vitro* studies supporting the idea that various forms of Si can bind to biologically relevant polymers (Annenkov *et al.*, 2017). Our study is the first to report significant levels of soluble Si present in the phloem exudate.

The data presented here suggest a model of soluble Si being redirected from undamaged to damaged leaves *via* the phloem. While it is noted that contamination is often an issue associated with ETDA-facilitated phloem exudate collection, alternative methods including aphid stylectomy would not provide sufficient yield for soluble Si analysis (Gaupels *et al.*, 2008). Another issue with EDTA-facilitated phloem exudate collection is that the amount of exudate released is unknown, meaning that quantitative comparisons between samples are not possible. Nevertheless, the presence of Si in the phloem exudate of +/– Si plants which have been grown without exogenous Si for 24 hr strongly supports the hypothesis that Si is being transported in the phloem as the presence of soluble Si in such +/– Si plants cannot be explained by *de novo* uptake from the hydroponic medium. Furthermore, as localised Si induction was observed even nine days after the removal of

external Si supply, it is unlikely that there is still enough Si present in the xylem to explain the observed induction of Si.

Due to the low levels of plant-available Si found in many soils, it has recently been suggested that there are significant costs associated with high Si accumulation and that these are much higher than previously recognised (de Tombeur *et al.*, 2023). Negative correlations between Si accumulation and biomass have been reported in several grass species, and in a number of studies (Simpson *et al.*, 2017; Johnson and Hartley, 2018; de Tombeur *et al.*, 2021), suggesting that there is a trade-off between Si uptake and growth. This could reflect the fact that Si deposition is an active process involving the use of active efflux transporters (Ma *et al.*, 2007*a*; Ma and Yamaji, 2015), so there may be an energetic cost associated with high Si uptake (Simpson *et al.*, 2017). Thus, redirection of soluble Si from undamaged leaves to sites of damage might be a more energetically favourable way of increasing defences against herbivores than the more costly mechanism of increasing Si uptake.

6.3. Conclusions

By separately measuring Si accumulation in damaged and undamaged leaves, this study has demonstrated that damage results in only a localised, and not systemic, induction of Si defences. This localised induction was observed in multiple landraces, although it varied between them, and the response was stronger when plants were grown at high levels of Si availability. The evidence presented here suggests that this localised induction is the result of the redirection of soluble Si from undamaged to damaged leaves *via* the phloem. To the best of our knowledge, this is the first demonstration of soluble Si being redirected towards the sites of wounding within damaged plants to increase their defences, a mechanism which has implications for the cost-effectiveness of these defences, as well as for wider understanding of the fitness benefits of Si accumulation.

7. Supplementary data

The following supplementary data are available at JXB online.

Table S1. List of landraces used for this study.

Table S2. List of primers used for this study.

Table S3. ANOVA results for the effect of damage and Si supply on Si accumulation.

Fig. S1. Average leaf Si concentration for ten wheat landraces.

Fig. S2. Root Si concentration for four landraces grown hydroponically with and without Si supplementation and subject to damage.

Fig. S3. Stem and root Si concentrations for plants grown without Si supplementation after damage.

Fig. S4. Si transporter gene expression after damage.

8. Acknowledgements

We would like to thank the horticulture team at York for assistance with plant growth. For the purpose of open access, the author has applied a Creative Commons Attribution (CC BY) licence to any Author Accepted Manuscript version arising.

9. Author contributions

Conceptualization, S.J.T., S.E.H. and F.J.M.M.; methodology, S.J.T., S.E.H. and F.J.M.M.; formal analysis, S.J.T.; investigation, S.J.T.; data curation, S.J.T.; writing—original draft preparation, S.J.T.; writing—review and editing, S.E.H. and F.J.M.M.; funding acquisition, S.E.H. and F.J.M.M. All authors have read and agreed to the published version of the manuscript.

10. Conflict of interest

The authors declare no competing interests.

11.Funding statement

This work was supported by the Biotechnology and Biological Sciences Research Council (Award ref 1949569). The University of Sheffield provided additional financial support.

12. Data availability

All data generated and analysed in this study are available upon request.

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14. Figures

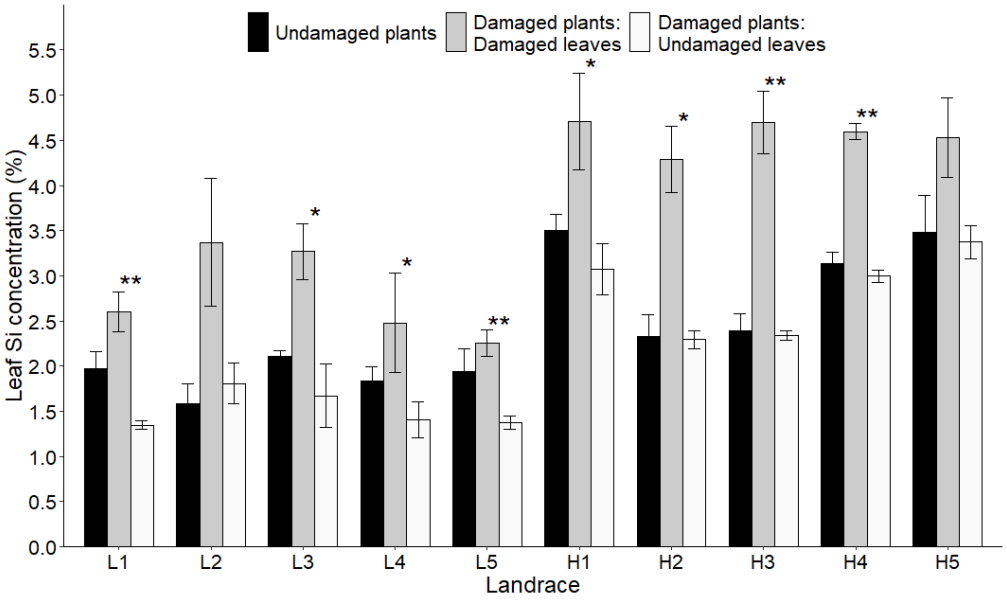


Figure 1: Variation in Si accumulation following damage in ten wheat landraces. Leaf Si concentration for damaged and undamaged leaves of damaged plants, and undamaged plants. L1-L5 are low Si-accumulating landraces; H1-H5 are high Si-accumulating landraces. Mean values ± SE are shown. N = 3. ANOVA damaged leaves of damaged plants vs undamaged plants: Landrace: $F_{9,40}$ = 12.0, P < 0.001, Damage treatment: $F_{1,40}$ = 67.9, P < 0.001. ANOVA undamaged leaves of damaged plants vs undamaged plants: Landrace $F_{9,40}$ = 19.5, P < 0.001, Damage treatment: $F_{1,40}$ = 9.2, P = 0.004. Statistically significant differences between damaged and undamaged leaves of damaged plants, determined by paired *t*-tests, are indicated: *** *P* < 0.001, ** *P* < 0.01, and * *P* < 0.05.

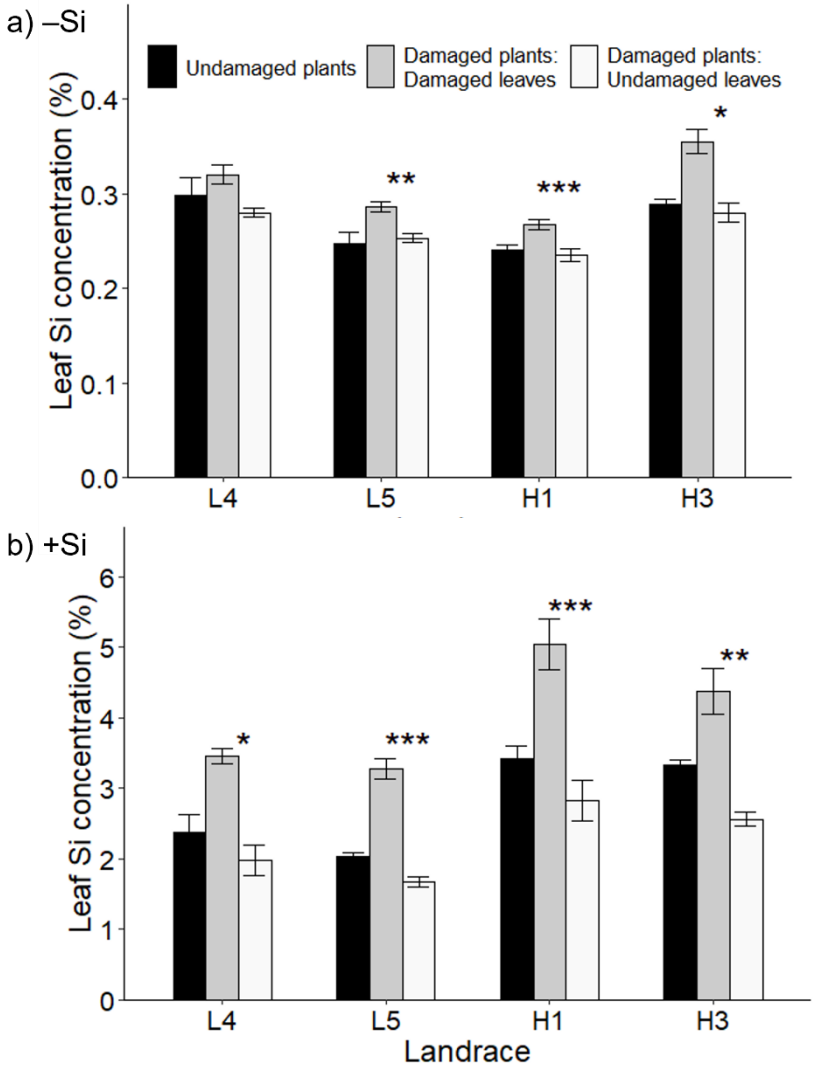


Figure 2: Effect of Si supply and damage on Si accumulation. a) Leaf Si concentration of –Si plants. b) Leaf Si concentration of +Si plants. Note the different scales on the y-axis. Mean values ± standard error (SE) are shown. N = 3. L4 and L5 are low Si-accumulating landrace. H1 and H3 are high Si-accumulating landraces. L indicates a low Si-accumulating landrace. ANOVA for Landrace, Si availability, Landrace x Si availability, and Damage treatment x Si availability at $P < 0.001$ for both damaged and undamaged leaves of damaged plants vs undamaged plants, see

Supplementary Table **3** for full results. Statistically significant differences between damaged and undamaged leaves of damaged plants, determined by paired *t*-tests, are indicated: *** $P < 0.001$, ** $P < 0.01$, and * $P < 0.05$.

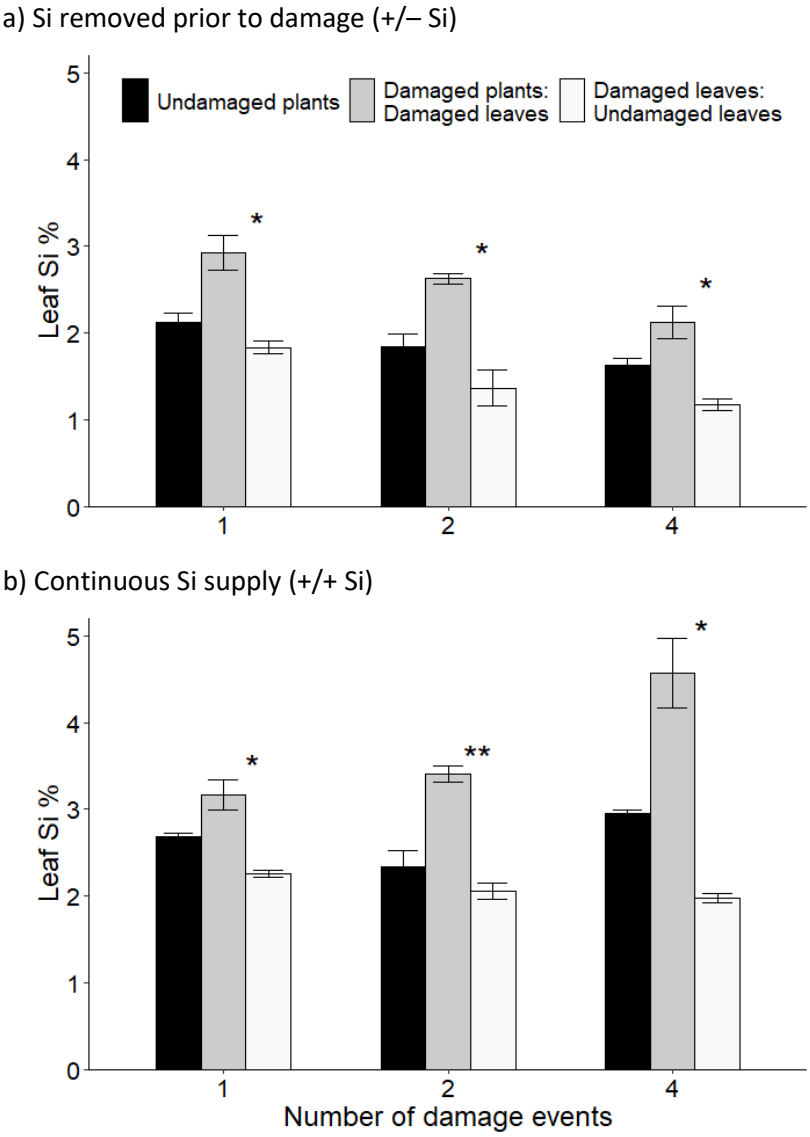


Figure 3: Effect of reducing Si availability on Si accumulation after damage. Leaf Si concentration for damaged and undamaged leaves of damaged plants, and undamaged plants. a) Plants moved to medium without Si supplementation when damage was started (+/–Si plants). b) Plants grown with continuous Si supplementation (+/+ Si plants). The low Si-accumulating landrace, L1, was used. Mean values ± SE are shown. N = 3. ANOVA for Si availability, Damage treatment, and Number of damage events x Si availability at $P < 0.01$ for both damaged and undamaged leaves of damaged plants vs undamaged plants, see

Supplementary Table **3** for full results. Statistically significant differences between damaged and undamaged leaves of damaged plants, determined by paired *t*-tests, are indicated: *** $P < 0.001$, ** $P < 0.01$, and * $P < 0.05$.

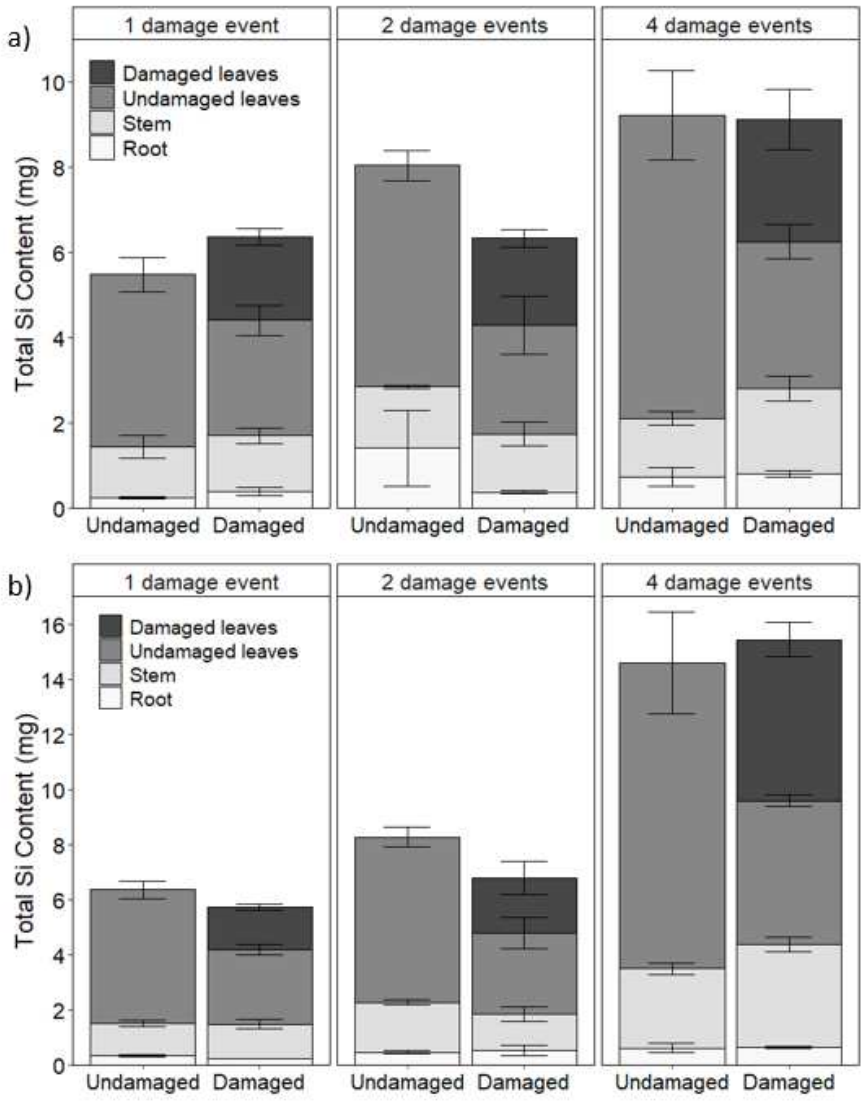


Figure 4: Total leaf Si content was unaffected by damage. Allocation of total Si accumulated to different plant tissues in damaged and undamaged plants after successive damage events. a) +/– Si plants sampled after 1, 2, and 4 damage events. b) +/+ Si plants after 1, 2, and 4 damage. Mean values ± SE are shown. N = 3. Note different scales of y-axis. Significant ANOVA effects: Number of damage events: $F_{2,23} = 36.0$, $P < 0.001$, Si availability $F_{1,23} = 13.8$, $P = 0.001$, Number of damage events x Si availability $F_{2,23} = 8.1$, $P = 0.002$.

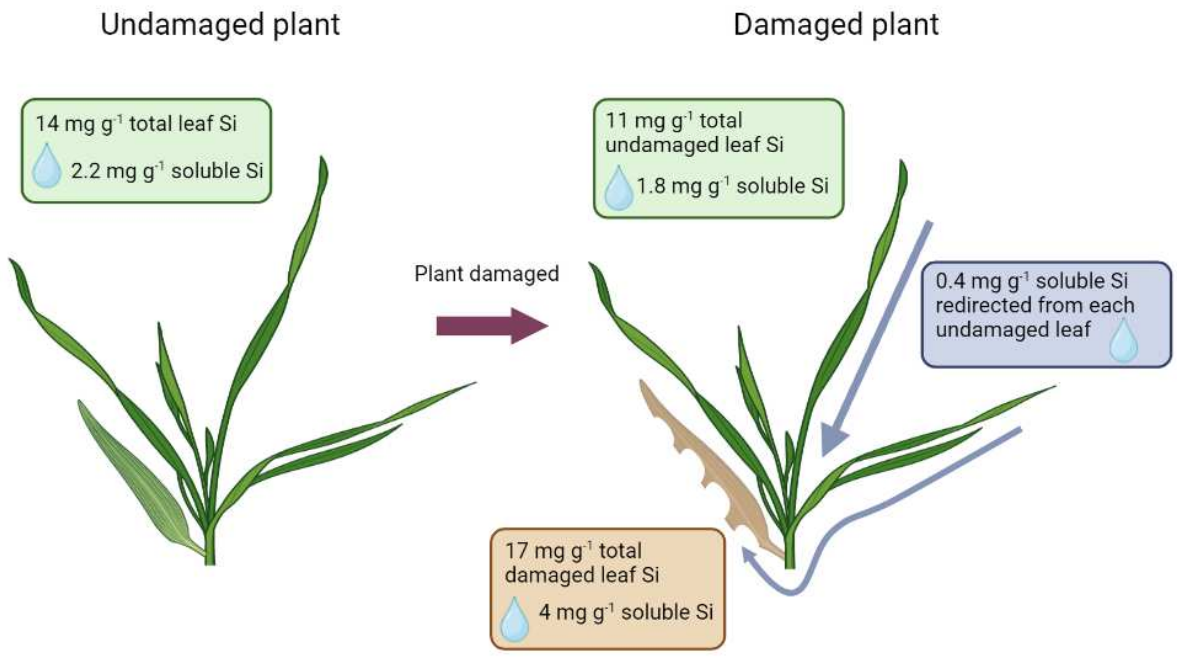


Figure 5: The localised increase in Si in damaged leaves can be explained by the redirection of soluble Si from undamaged leaves of damaged plants. An undamaged plant weighing 1 g DW contains a total of 14 mg Si g^{-1} leaf on average, of which 2.2 mg g^{-1} is soluble. After damage, soluble Si is moved from undamaged leaves to damaged leaves. This movement of soluble Si increases the total Si concentration in damaged leaves to 17 mg Si g^{-1}, of which 4 mg g^{-1} is soluble. Provided the weight of undamaged leaves is at least 4.5 times more than the weight of damaged leaves, the increase in Si in damaged leaves can be explained by the redirection of soluble Si from undamaged to damaged leaves of damaged plants. This was the case for all the experiments presented here. *Figure created using BioRender (https://www.biorender.com).*

Supplementary Information

Supplementary Table 1: List of landraces used in this study. H1-H5 are high Si-accumulating landraces while L1-L5 are low Si-accumulating landraces, as characterised in Thorne *et al.* (2021).

Landrace	Collection	Plant ID	Plant Name	Origin
H1	CIMMYT	CWI 2166	K7155.41	Kenya
H2	Watkins	1190195	Gahu (Nepali) or Kyo (Sikkimese)	India
H3	CIMMYT	CWI 3909	OUBAARD	South Africa
H4	Watkins	1190777	Finland 3	Finland
H5	Prague	01C0201531	Orchon	Mongolia
L1	Watkins	1190521	Dandi	India
L2	Watkins	1190568	China 19	China
L3	Watkins	1190605	Karabash	Greece
L4	Watkins	1190662	Samanta 1252	Romania
L5	Watkins	1190751	Armavir	USSR

Supplementary Table 2: List of primers used for RT-qPCR.

Target Gene	Forward Primer	Reverse Primer	Product size	Ensembl gene name (all homologues)
Actin	AATGGTCAAGGCTGGTTTCG	ATCACCGACATAGGCATCCTTC	124	TraesCS1A02G020500, TraesCS1B02G024500, TraesCS1D02G020000
Tef1	TTGGTGGCATTGGAACTGTG	TTGACCTCAGTTGTCAGACCAG	103	TraesCS5B02G418200 TraesCS5D02G423400
Lsi3	TGTTCAAGTACCTCGGCAAC	TTGAGGATGAACTCGGTGAGG	144	TraesCS4A02G412500, TraesCS4B02G312600, TraesCS4D02G310100
Lsi6	TACTCGAACGAGATCCACGAC	TCTCCGATATCACCTTCTTGCC	132	TraesCS7A02G187800, TraesCS7B02G092900, TraesCS7D02G188800

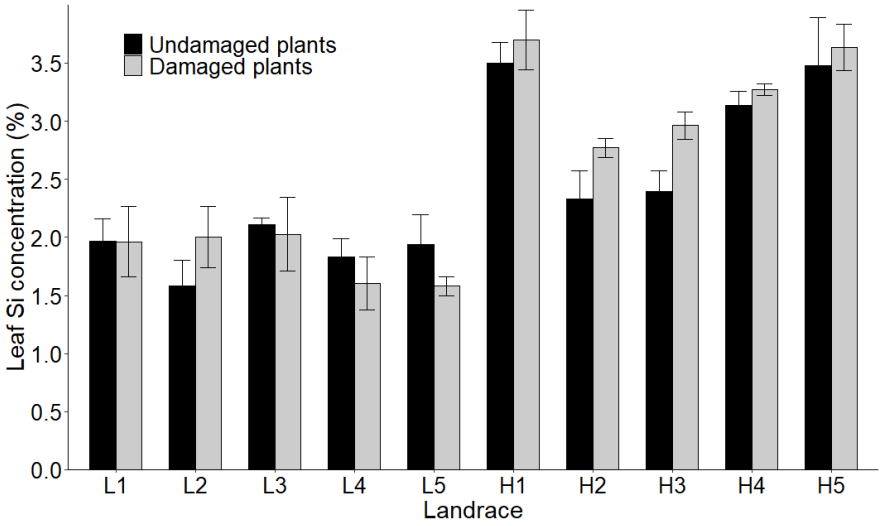
Supplementary Table 3: ANOVA results for Figures 2 and 3. Statistically significant results are highlighted in bold. N = 3 for each factor combination.

a) The effect of landrace, damage, and Si supply on Si concentration in damaged and undamaged leaves of damaged plants compared to undamaged plants.

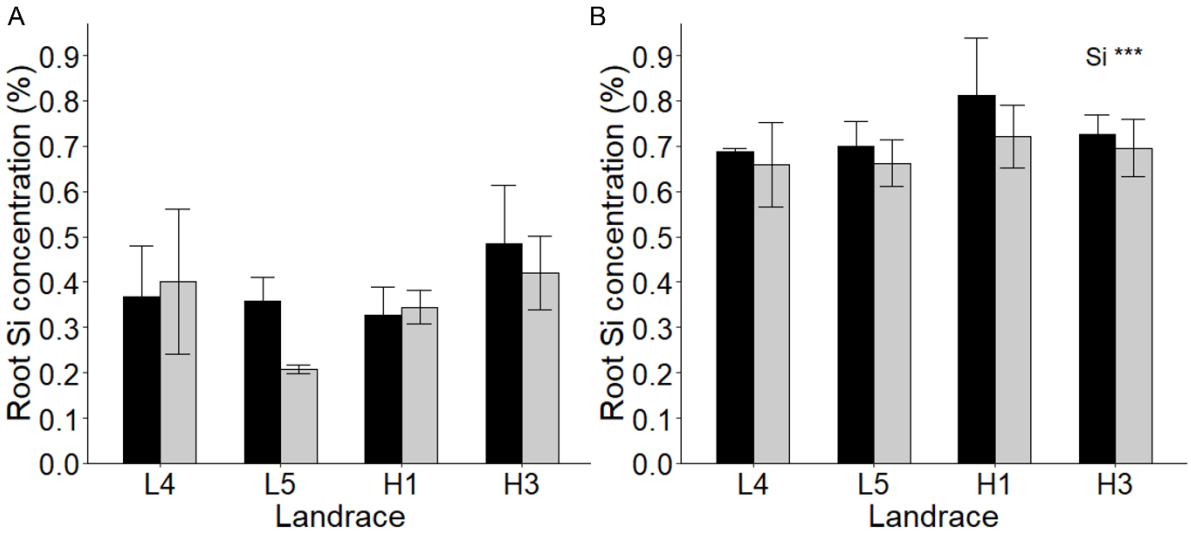
	Leaf Si (%)						Root Si (%)		
	Undamaged leaves			Damaged leaves					
	df	*F*	*P*	*df*	*F*	*P*	*df*	*F*	*P*
Landrace	**3**	**22.39**	**< 0.001**	**3**	**24.81**	**< 0.001**	3	1.71	0.179
Damage	1	0.09	0.764	1	**215.99**	**< 0.001**	1	0.79	0.378
Si	**1**	**6188.77**	**< 0.001**	**1**	**9076.47**	**< 0.001**	**1**	**88.54**	**< 0.001**
Landrace x Damage	3	0.20	0.897	3	0.54	0.66	3	0.71	0.550
Landrace x Si	**3**	**23.73**	**< 0.001**	**3**	**27.41**	**< 0.001**	3	1.19	0.326
Damage x Si	**1**	**12.32**	**< 0.001**	**1**	**23.10**	**< 0.001**	1	0.16	0.691
Landrace x Damage x Si	3	0.16	0.923	3	1.39	0.26	3	0.86	0.466

b) The effect of damage, Si supply, and number of damage events on total plant Si content and leaf Si concentration in damaged and undamaged leaves of damaged plants compared to undamaged plants.

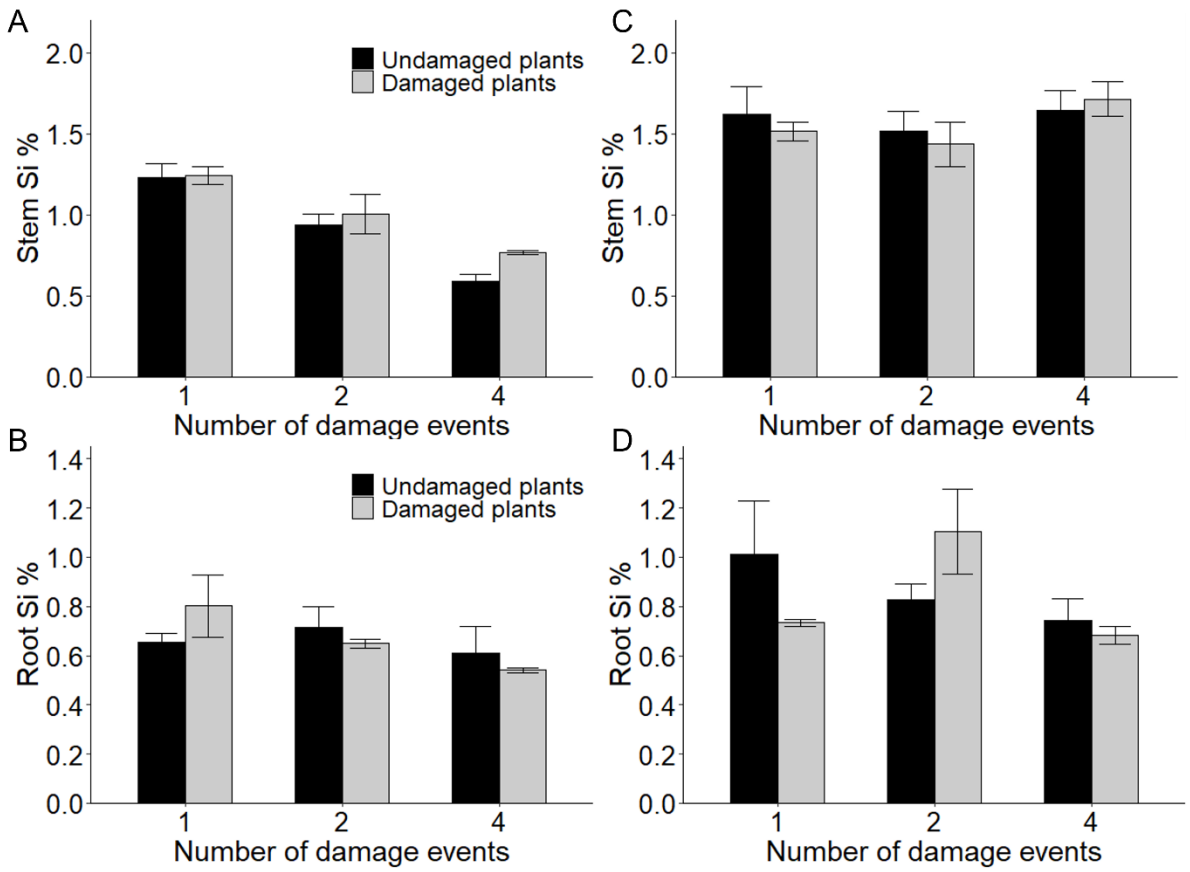
	Leaf Si (%)						Plant Si content (mg)		
	Undamaged leaves			Damaged leaves					
	df	*F*	*P*	*df*	*F*	*P*	*df*	*F*	*P*
Number of damage events	**2**	**10.30**	**< 0.001**	2	1.75	0.196	**2**	**36.01**	**< 0.001**
Damage	**1**	**42.81**	**< 0.001**	**1**	**84.23**	**< 0.001**	1	0.08	0.778
Si	**1**	**95.99**	**< 0.001**	**1**	**108.24**	**< 0.001**	**1**	**13.80**	**0.001**
Number x Damage	2	2.62	0.094	2	1.34	0.281	2	0.37	0.698
Number x Si	**2**	**6.79**	**0.005**	**2**	**21.64**	**< 0.001**	**2**	**8.11**	**0.002**
Damage x Si	1	0.15	0.701	1	0.07	0.800	1	0.16	0.694
Number x Damage x Si	2	1.21	0.316	2	1.87	0.176	2	0.35	0.709



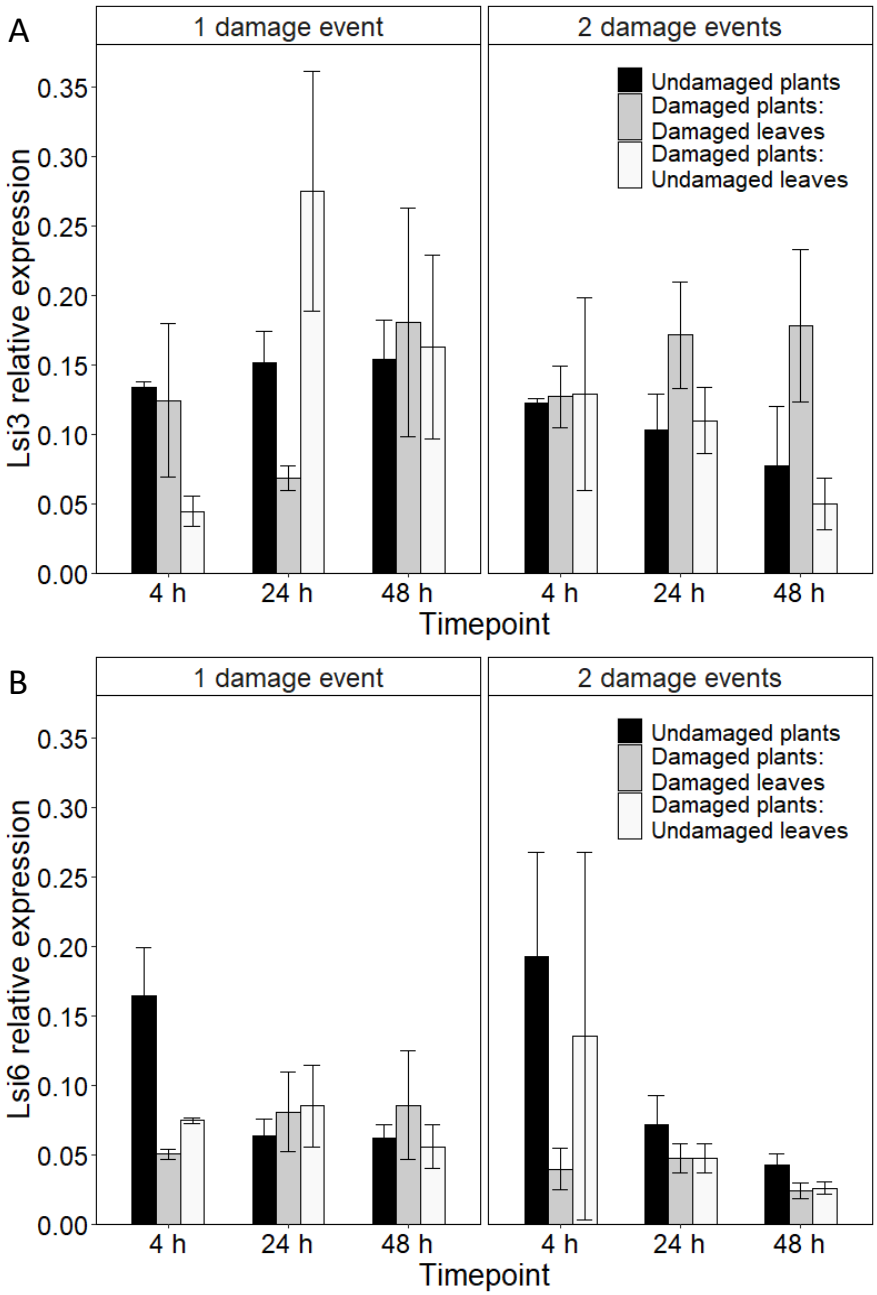
Supplementary Figure 1: Variation in leaf Si accumulation between damaged and undamaged plants for ten wheat landraces. The average leaf Si concentration for damaged and undamaged leaves of damaged plants was calculated. L1-L5 are low Si-accumulating landraces; H1-H5 are high Si-accumulating landraces. Mean values ± SE are shown. N = 3. Statistically significant ANOVA effect: Landrace: $F_{9,40} = 18.7$, $P < 0.001$.



Supplementary Figure 2: Effect of damage and Si supply on root Si accumulation. (A) Root Si of –Si plants. (B) Root Si of +Si plants. L4 and L5 are low Si-accumulating landraces; H1 and H3 are high Si-accumulating landraces. Mean values ± standard error (SE) are shown. N = 3. Statistically significant ANOVA effect: Si: $F_{1,47} = 88.5$, $P < 0.001$.



Supplementary Figure 3: Effect of reducing Si availability on Si accumulation after damage. (A) Stem Si concentration for plants moved to medium without Si supplementation when damage was started (+/– Si plants). (B) Stem Si concentration for plants grown continuously with Si supplementation (+/+ Si plants). (C) Root Si concentration for +/– Si plants. (D) Root Si concentration for +/+ Si plants. Mean values ± SE are shown. N = 3. The low Si accumulating landrace, L1, was used. Significant ANOVA effects for stem Si: Si availability: $F_{1,24} = 138.5$, $P < 0.001$; Number of damage events: $F_{2,24} = 12.2$, $P < 0.001$; Number of damage events x Si availability: $F_{2,24} = 20.5$, $P < 0.001$; for root Si: Si availability: $F_{1,23} = 12.7$, $P = 0.002$; Number of damage events: $F_{2,23} = 5.2$, $P = 0.013$.



Supplementary Figure 4: Si transporter gene expression at different time points. (A) *Lsi3* expression. (B) *Lsi6* expression. The low Si-accumulating landrace, L1, was used. Mean values ± SE are shown. N = 2-5. Dam: number of damage events. No significant differences between damaged and undamaged plants, and between damaged and undamaged leaves of damaged plants, were found.