

**The ecological and genetic drivers of silicon accumulation in cereal crops**

by

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14 **1 Abstract**

15 As global agricultural production strains under degrading soil fertility and increasing losses  
16 due to climate change, there is growing research interest in new avenues for production  
17 improvement. New crop technologies must meet increasing public and regulatory demand  
18 for environmental sustainability, encouraging scientists to revisit overlooked or relatively  
19 unknown techniques that may unlock productivity gains. One of the promising developments  
20 to arise over the past 20 years is the potential of silicon to improve crop plant performance.  
21 With benefits to multiple dimensions of crop performance, silicon may be a key tool to  
22 guard crop production against uncertain future growing conditions. Our ability to mobilize  
23 silicon-based cropping strategies is dependent on a thorough understanding of the ultimate  
24 and proximate causes of silicon accumulation, including both the ecological and genetic  
25 interactions that can trigger increased uptake. In this thesis, I extend recent advances in our  
26 understanding of silicon ecology in cereal crops, testing for the presence of rapid silicification  
27 in common canadian crops, as well as using a genome-wide association study to identify  
28 genetic markers associated with high silicon content.

29 [to add: results, conclusion]

30 **2 Lay Summary**

31 Silicon provides tremendous benefits to plant health, but is not widely utilized in agriculture,  
32 despite it being highly abundance and naturally occurring in most soils. One of the main  
33 factors limiting it's application in agriculture is our poor understanding of the exact dynamics  
34 of how plants absorb and use silicon from the soil. I identified genetic traits associated with  
35 high silicon content in *Aegilops tauschii*, a relative of bread wheat, as well as demonstrated  
36 that cereal crops (e.g. wheat, barley, oats) have the ability to rapidly uptake silicon from  
37 the soil. This rapid uptake means that silicon may be a highly effective defence against  
38 insect pests. Combining these results with the genetic data, future research can aim towards  
39 creating breeding programs to develop cereal crops that can withstand insect damage based

on their silicon content. This development could provide an environmentally friendly strategy to maintain output to feed a growing human population.

### 3 Preface

(This was taken nearly verbatim from Matt’s thesis, so need to go back through to make sure I am not plagiarizing)

The research presented in this thesis is original and unpublished. Isaac Peetoom Heida and Dr. Juli Carrillo, with assistance from Dr. Jean-Thomas Cornelis, conceptualized and developed the experiment presented in Chapter Two. Isaac Peetoom Heida, Dr. Juli Carrillo and Dr. Gurcharn Singh Brar, with input from Dr. Jean-Thomas Cornelis, conceptualized and developed the experiment presented in Chapter Three. Isaac Peetoom Heida developed the question and methodology for Chapter Two. Dr. Aaron Beattie, Dr. Mazen Aljarrah, and Dr. Gurcharn Brar provided seeds for the experiment. Isaac Peetoom Heida designed and set up the experiment, processed and analysed the samples, and performed the statistical analysis. Dr. Shaun Barker and the Mineral Deposit Research Unit of the University of British Columbia provided facilities and expertise for the XRF analysis of the tissue samples. For Chapter Three, Isaac Peetoom Heida led plot set up and maintenance, with assistance from Grace Wang, Vincent Fetterley, Sara Salad, Katherine Buchanan, Martina Clausen, and Paul Fisher, and Matt Tsuruda. Isaac Peetoom Heida led the sample harvest, processing and analysis. Kelly Wang, Grace Wang, and Chelsea Gowton assisted with the sample harvesting. Dr. Daria Reshetniak, Paul Fisher, Lucas Friesen, Katie Pryer, Dr. Kinga Treder, Carly MacGregor, and Grace Wang all provided invaluable assistance with sample preparation. Chapters Two and Three of this thesis will be submitted to peer-reviewed journals for publication. For the purposes of this manuscript, actions are depicted in the singular first person.

## 4 Introduction

### 4.1 A case for silicon in agriculture

As global agricultural production strains under degrading soil fertility and increasing losses due to climate change, researchers are leaving no stone unturned in the search for new technologies for sustainable improvement in crop production. New crop technologies must meet increasing public and regulatory demand for environmental sustainability, encouraging scientists to revisit overlooked or relatively unknown techniques that may unlock productivity gains. Over the past 20 years, plant-silicon relations has emerged as a promising field that may safeguard crop performance and security within a changing biosphere. With benefits to multiple dimensions of crop performance, silicon may be a key tool to guard crop production against uncertain future growing conditions.

### 4.2 Silicon in nature

Silicon abounds in the earth's crust, with various silicates, such as silicon dioxide ( $\text{SiO}_2$ ), comprising about 60% of the crust by mass. Nearly all terrestrial plants grow in soils containing silicon, and thus absorb nominal amounts through passive transport as water is absorbed into the plant. Silicates are found in a variety of forms, and vary in their plant availability. Crystalline forms, such as quartz, are highly resistant to weathering, and are poor sources of plant-available silicon, while amorphous forms of  $\text{SiO}_2$  are more available **fraysse'surface'2009**. As soils age, an increasing share of the plant-available silicon is derived from biogenic silicates, such as diatom testes or plant phytoliths **de'tombeur'plants'2020**. Uptake and usage of silicon is not uniform throughout the vascular plants. Plant clades vary in their expression of silicon transporter proteins and in the relative abundance of phytoliths in their tissues. Plant phytoliths are amorphous masses of silica, chemically similar to opal, that are found throughout the plant body and have highly variable geometries (**piperno'phytoliths'2006**). Phytolith morphologies are generally conserved within taxonomic groups, allowing their use as a tool for paleobotanical investigations. The highly variable morphology among clades, with the conservation of morphology within clades suggests active selection upon the structure of phytoliths. The morphology of phytoliths also varies between organs in the plant.

Stem phytoliths tend to be more elongate, with putative structural function, while leaf phytoliths are typically more orbicular, and are likely an adaptation to counter herbivory.

Silicon deposition may create apoplastic barriers that seal and toughen the plant tissue, which may benefit the plant in a multitude of ways (**coskun'controversies'2019**). Under this hypothesis, silicon deposits reduce water loss and radiation/temperature damage, and also limit the spread of effector compounds, dampening the effects of fungal pathogen and herbivore excretions designed to interfere with plant defensive physiology (**coskun'controversies'2019**). Empirical evidence shows that silicon is effective at limiting the growth and damage of insect and fungal pests (**fauteux'silicon'2005; massey'herbivore'2007**). The toughness of the depositions also serves a more direct mechanical role, as the hardened granules of silicon interrupt the chewing motions of herbivores, wearing down mandibles and teeth (**stromberg'functions'2016; waterman'short-term'2021-1**), and reduce the digestive efficiencies of herbivores (**johnson'silicon'2021**). Indeed, when supplemented with silicon plants are generally more resistant to a wide spectrum of stressors, including soil salinity, soil metal toxicity, cold and heat stress, UV stress, water deficits, and phosphorus deficiencies (**cooke'consistent'2016**). Continuing to untangle the various mechanisms through which silicon delivers beneficial effects to plants is key to fully realizing the potential of silicon in sustainable agriculture.

### 4.3 Silicon in soils and roots

Plants interact with silicon on a variety of levels, mobilizing it from soil aggregates, transporting it into and throughout their bodies, and finally precipitating it out of their xylem into solid masses in the leaves and stems. Within the soil environment, silicon commonly exists in both crystalline (geologic) and amorphous (biogenic) forms (**haynes'contemporary'2014**). Amorphous silicates can derive from previous plant material that has decayed in the soil, but also from marine and aquatic organisms such as diatoms. Globally, the silicon cycle involves silicates weathering out of terrestrial sediments, moving along water courses, and eventually being deposited in the sea, where it is incorporated into various plankton species, and eventually deposited in seafloor sediments. The continual exodus of silicon from terrestrial sediments over geologic timescales means as ecosystems age, plants become more and more

central in the local silicon cycle, with much of the silicon in living plant tissue being recycled from previous plant material decaying in the soil(de'tombeur'plants'2020). In highly weathered soils with low nutrient availabilities, plants take a more active role in liberating nutrients, including silicon, for uptake. Organic acids and chelating agents, exuded from plant roots, pry tightly bound nutrients such as phosphorus and silicon from soil aggregates, increasing their availabilities for uptake into the root system (de'tombeur'silicon'2021-1). This active scavenging for silicon remains poorly understood, but it may be an important mechanism in plant defence (allowing for increased uptake during a defensive response) and breeding for increased root exudation may improve crop plant performance and nutrient use efficiency (de'tombeur'silicon'2021).

#### 4.4 Silicon transporters

One of the most important advances in plant-silicon research was understanding the mechanisms through which silicon is acquired and transported into the plant. Silicon's most common form in soil solution is silicic acid ( $H_2SiO_4$ ), which has a maximum solubility of around 2 mM (haynes'contemporary'2014). While there is some evidence that small amounts of silicic acid can be transported during water uptake, this method of transport is insufficient to explain the larger amounts of silicon found in some plant families. Research in rice has identified four gene products that transport silicon into and through the plant body. Two of these (LSi1, LSi2) transport silicic acid from the soil into the roots, while the other two (LSi3, LSi6) act to unload silicic acid from the xylem into leaves and inflorescences (yamaji'orchestration'2015). Orthologs of these proteins have been identified in other cereal crops, and additional analogous silicon transporter proteins have been discovered in the Cucurbitaceae (reynolds'silicon'2016). Though not identified, there is a hypothesized fifth protein responsible for loading silicic acid into the xylem (farooq'silicon'2015). The expression of these genes, or lack-there-of, can not only influence the total amount of silicon accumulated by the plant, but also its relative distribution, as knockout of LSi6 increases leaf silicon content while decreasing the silicon content of seed husks in rice yamaji'transporter'2008. Breeding for silicon content and use-efficiency in crop plants may be crucial to improving crop performance under a changing climate christian'breeding'2022. However, we still have a

relatively poor understanding surrounding the how genetics influence the silicon phenotype of a plant. Further investigations into how genotypic variation is reflected in the silicon content of plants can aid in the discovery of new genes involved in silicon accumulation, and may provide targets for silicon breeding programs.

## 4.5 Silicon in leaves

Once inside the plant, silicon is deposited in specialized silica cells, forming phytoliths (waterman'short-term'2021). Silicon deposits show consistent and taxa-specific morphologies, suggesting evolutionary pressure selecting for these structures to yield certain functions to the plant (piperno'phytoliths'2006). In stems, these phytoliths are often long and narrow, oriented parallel with the shoot, and seem to increase structural rigidity(stromberg'functions'2016). The use of silicon as a structural component represents a highly energetically efficient strategy, as silicon is 10x cheaper on an energy unit basis to produce than lignin (stromberg'functions'2016). Stem silicification has been investigated as it relates to lodging resistance in cereal crops, and silicon supplementation has been found to reduce the prevalence of lodging in rice and wheat (dorairaj'influence'2017; muszynska'mechanistic'2021). In leaves, phytoliths are typically more stout, though they still increase the mechanical toughness of the leaf (simpson'still'2017). This overall toughness, and abundance of phytoliths in leaves likely evolved to limit herbivore damage, rather than improve the growth characteristics as in stem phytoliths (stromberg'functions'2016). Interestingly, even in the absence of silicon, plants develop silica cells, and rapidly fill them when silicon becomes available (waterman'short-term'2021-1). As phytoliths develop in the leaves, polymerization of monosilicic acid is aided by interactions with proteins in the cell wall, which act as sites of nucleation (nawaz'phytolith'2019). Silicon deposition in the leaves can happen on relatively short time scales, outpacing the accumulation of other defensive compounds such as phenolics (waterman'short-term'2021). Thus, silicon-based defences in crop plants may be one of the first lines of active anti-herbivore defence, providing rapid and sensitive responses to herbivory.

## 4.6 Looking back and looking forward

Much of today's plant silicon work is indebted to the pioneering work of Jones and Handreck (jones'silica'1967) and the subsequent mapping of silicon across the plant kingdom by Takahashi et al. (takahashi'possibility'1990). Epstein's seminal epstein'silicon'1999 paper provided a comprehensive review of the state of knowledge in plant silicon, and has spurred a generation of researchers to extend the preliminary findings of the 20th century out across crop production systems and plant ecologies around the world. Silicon is best studied in the grass family (Poaceae) due to the comparatively high silicon content found in most members of the family (often over 1% of dry weight), as well as the economic importance of domesticated species within the clade (reynolds'silicon'2016). Rice, maize, wheat, and barley alone account for one-third of the worlds' total cultivated land area (faostat), and are all domesticated grass species. Silicon supplementation as an agricultural practice has been extensively studied in rice and sugar cane, as these crops tend to deplete soil silicon stocks, necessitating replenishment by application of silicon-rich amendments (haynes'contemporary'2014; meena'case'2014). Due to the overall high silicon content of soils globally, Si is rarely truly limiting in soils, though certain forms of silicon are much more plant available than others (fraysse'surface'2009). Thus, the applicability and importance of silicon supplementation is unlikely to be realized in more temperate dry-land production systems, particularly in wheat and barley. This does not however nullify the utility of silicon research in these systems, as great work can still be done to improve the manner and efficiency in which these temperate crops utilize the ample silicon available in their soils. Our ability to integrate silicon as a tool for production improvement in crop production is currently limited by a poor understanding of the genetic controls over silicon accumulation, as well as a limited understanding about the extent to which crops utilize silicon in pest-protection.



# Chapter 1: Identifying rapid silicon accumulation in cereal crops

## 5.1 Introduction

To address acute damage from herbivores, plants have developed a host of defensive strategies, ranging from changes to the body plan down to the development of novel compounds to poison those that would try to eat the plant (**agrawal'plant'2006**). In the broadest of terms, plant defenses can be categorized as either tolerance or resistance strategies. Resistance strategies involve attempts to limit tissue loss through interference with herbivory, while tolerance strategies result in increased regrowth after tissue loss. Due to the vastly different nature and ontogeny of various defensive strategies in plants, plant defences operate across a range of intensities and time scales, from short-term temporary activation, to long-lasting changes in the morphology of the plant (**agrawal'plant'2006; karban'induced'1989**). In most scenarios, induced plant defences are activated in response to an external cue, and build in intensity over time, with defensive hormone signals peaking approximately five hours after the initial induction event (**schmelz'quantitative'2003**). Despite this rapid hormonal response, actual defensive phenotypes are slower to emerge, often operating on the scale of days or generations (**karban'induced'1989**). Many defensive responses are also context dependent, where the identity of the damaging actor, the severity of damage, and a host of other factors interact to determine the final defensive response (**waterman'simulated'2019**). The most effective defensive strategies should be those that can either prevent herbivory outright, or can mount a rapid response to limit damage. These same strategies are also the most promising for crop production, where pest damage represents both an economic and food security cost. Integrating better natural plant defences into crop production systems may be key to reducing the environmental impact of agriculture, but hinges upon a thorough understanding of plant defensive physiology.

One of the most promising avenues for new crop defence is the harnessing of silicon (**reynolds'silicon'2016**). Silicon acts on multiple temporal and physiological scales, delivering broad spectrum resistance to pests, pathogens, and abiotic stressors (**cooke'consistent'2016; coskun'controversies'2019**). Soluble silicon taken up from the soil is deposited predomi-

nantly in the leaf epidermis, where it forms solid granules that increase the toughness of the tissue, reducing herbivore digestive efficiency (**cooke'is'2011**). Plant silicon is expressed latently, but also increases in response to herbivory (**takahashi'possibility'1990**). Multiple studies have demonstrated lasting elevated silicon in response to real and simulated herbivory (**massey'are'2008**; **hartley'ecology'2016**), and recent evidence points to silicon accumulation as being a relatively rapid response, even preempting some chemical defences (**waterman'short-term'2021**). This rapid action makes silicon accumulation a promising trait for future crop development. Despite the novel results, this pattern has so far been observed in just one species, and only under artificial herbivory via the application of methyl-jasmonate. Though a useful tool for herbivory research, methyl-jasmonate application fails to reproduce a complete herbivory signal for the plant, thus observed changes to plant defence may not be representative of a true herbivory scenario (**strauss'direct'2002**). Testing for this rapid silicon accumulation across a variety of grain crops, and under both simulated (methyl-jasmonate) and real herbivory is a crucial first step towards integrating rapid silicification into our understanding of plant defence and crop protection.

Plant silicon research has mostly focused on members of the grass family (Poaceae) due to their exceptional silicon content within the plant kingdom, as well as the economic importance of domesticated grass species (**reynolds'silicon'2016**). Domesticated crops differ significantly from their wild relatives, due to effects of strong selective pressure imposed by humans (**chen'crop'2015**). Most domesticated crops show much lower genetic diversity than their wild ancestors (**hafeez'creation'2021**; **smith'domestication'2019**). Initial selection for a few individuals with favourable traits creates a genetic bottleneck, and the majority of allelic diversity is lost. Subsequent selection by humans for agronomically relevant traits can result in concurrent losses of adaptations to natural environments, as the traits that maximize human value (eg. yield, ease of harvest) can come at the cost of ecologically relevant traits such as defence (**whitehead'domestication'2017**; **chen'crop'2015**). Indeed, in the context of silicon, we can detect clear signals of domestication across the Poaceae family, where wild ancestors consistently have higher baseline silicon content than their domesticated descendants (**simpson'still'2017**). Due to the effects of selection on plant defence it becomes crucial to test new developments in the silicon-defence literature in

modern crop species, both to validate their utility towards agricultural production, and to gather further observations on the dynamics of silicon-based defences in the first hours after herbivory.

In this study, we tested four globally important cereal crop species for rapid silicon accumulation under artificial and real herbivory. In a glasshouse environment, we grew bread wheat (*Triticum aestivum*), oats (*Avena sativa*), barley (*Hordeum vulgare*) and Triticale ( $\times$  *Triticosecale*), and tested the following hypotheses:

1. Rapid silicon accumulation is a conserved trait in the Poaceae, and the tested species silicon content would show a significant increase in silicon content within 18 hours of the herbivory treatment applications.
2. Due to different phylogeny and domestication history, the tested species would vary in the strength of their silicon accumulation response to herbivory.
3. Due to the different cues involved when comparing true herbivory damage and methyl-jasmonate induced defensive induction, the tested species would show different patterns of short-term silicon accumulation in response to cricket (*Acheta domesticus*) herbivory and methyl-jasmonate application.

This study is a thematic replication of Waterman et al.’s **waterman’s short-term’2021** paper, but attempts to extend the findings to commercially important grain crops. The findings of this study will refine our understanding of the prevalence of rapid silicification in the Poaceae, and will help to inform the value of potential applications of silicon-based defences into grain crops.

## 5.2 Methods

### 5.2.1 Plant growth and experimental treatments

To test the prevalence of rapid silicon accumulation in canadian cereal crops, we selected three cultivars for each of oats, bread wheat, triticale, and barley. We selected cultivars on the basis of minimizing shared pedigree, and no cultivars shared more than one common ancestor within the last two crossing generations. At the start of the experiment, we germinated seeds