RPTU Rheinland-Pfälzische Technische Universität Kaiserslautern Landau

Molecular and Physiological Analyses of Potassium and Silicon Nutrition for the Drought Response in Different Model and Crop Plant Species

vom Fachbereich Biologie

der Rheinland-Pfälzischen Technischen Universität Kaiserslautern-Landau

zur Verleihung des akademischen Grades „Doktor der Naturwissenschaften“ genehmigte

Dissertation

vorgelegt von

Li-Hsuan Ho, M.Sc., geboren am 01.07.1992

Wissenschaftliche Aussprache: 11.09.2023

Berichterstattende:

Herr Prof. Dr. H. Ekkehard Neuhaus (Betreuer)

Herr Prof. Dr. Matthias Hahn

Kaiserslautern im Jahr 2023

DE 386

Table of Contents

Summary 1

1. Introduction 3

  1.1. Water movement and transportation of photosynthates in plant 3

  1.2. Drought stress response 6

    1.2.1. Molecular response to drought stress 7

    1.2.2. Biochemical response to drought stress 8

    1.2.3. Physiological response to drought stress 14

  1.3. The role of silicon in plant 15

    1.3.1. Silicon uptake, transport system, and deposition in plant 15

    1.3.2. A key role of silicon in drought stress 18

  1.4. Objective of this work 21

2. Material and Method 22

  2.1. Plant material and cultivation 22

    2.1.1. Plants materials and plants growth 22

    2.1.2. Potassium or silicon fertilization in soil 23

  2.2. Soil substrate-base drought stress experiment 24

  2.3. Hydroponic system 25

  2.4. PAM assay 27

  2.5. Gas exchange analysis 27

  2.6. Metabolism measurements 27

    2.6.1. Metabolism extraction 27

    2.6.2. Sugar and starch measurement 28

    2.6.3. Ion chromatography (IC) 29

    2.6.4. High-performance liquid chromatography (HPLC) 29

  2.7. Subcellular metabolism analysis (non-aqueous fraction, NAF) 29

    2.7.1. Extraction 29

    2.7.2. Marker enzyme measurement 30

    2.7.3. Sugar measurement for NAF extraction 31

2.8. Molecular biological methods 31
2.8.1. RNA isolations and cDNA synthesis 31
2.8.2. Expression analysis by qRT-PCR 32
2.8.3. Expression analysis by RNA sequencing (RNASeq) 33
2.9. Software tools 34
2.10. Statistics 34
3. Results 35
3.1. Potassium effects on drought tolerance in Arabidopsis 35
3.1.1. Low potassium levels impact Arabidopsis shoots growth on soil under control and drought conditions 35
3.1.2. Potassium deficiency results in altered metabolite accumulation in leaves 38
3.1.3. K-dependent effects in shoots and root growth in the hydroponic growth system under drought stress 41
3.1.4. Tissue-specific alterations in metabolic accumulation under different levels of potassium supply 44
3.1.5. Potassium treatment alters expression of sugar and potassium transporters under drought stress 48
3.1.6. Potato plant growth and tuber yield under different potassium treatments 51
3.1.7. Potassium supply influences soluble sugars, starch, and potassium content in potato tubers 53
3.1.8. Expression of sugar transporters and potassium channels 55
3.2. The role of silicon during drought stress 58
3.2.1. Silicon promotes plant resistance to drought 58
3.2.2. Transcriptome analysis showed Si-dependent differences between early and long-term responses to drought 62
3.2.3. Si effects on photosynthesis and carbohydrate metabolism 65
3.2.4. Influence of silicon on subcellular sugar distribution 69
3.2.5. Si-regulated sugar transporters and enzymes involved in primary sugar metabolism under drought stress 70

3.2.6. Silicon increases tuber fresh weight at control but not drought conditions 76

3.2.7. The effects of silicon treatment on metabolites altering during drought stress in potato plants 78

3.2.8. Silicon induces the expression of sugar transporters under drought in leaves of WT but not NTT-antisense potato plants 81

4. Discussion 83

4.1. Potassium effects on sugar loading under drought stress 83

4.1.1. Severe potassium deficiency can lead to a slowing down of phloem mass transport, further impacting drought tolerance 84

4.1.2. The negative impact of high potassium fertilization on Arabidopsis under drought stress 85

4.1.3. Low amount of sodium may be a benefit for plant under drought stress 87

4.1.4. Potassium fertilization increases starch accumulation potato tubers 88

4.1.5. Potassium fertilization in altering sugar and K^+ loading in potato plants 90

4.2. The function of silicon in drought tolerance 92

4.2.1. *Arabidopsis thaliana* is a silicon excluder species 92

4.2.2. Silicon mitigates drought stress effects in Arabidopsis leaf tissue but not in root tissue 92

4.2.3. Silicon application increased the accumulation of sugars in leaf tissue via altering the primary carbohydrate metabolism processes 93

4.2.4. Silicon increases drought tolerance through increasing sugar accumulation in vacuole 98

4.2.5. Silicon increases sugar long distance transport via increase the hydraulic conductivity in vascular tissue 102

4.2.6. Silicon alleviates drought stress in leaves, but not in tubers 103

4.2.7. Silicon may regulate subcellular localization in drought-stressed potato leaves 105

4.3. Conclusion 106

4.4. Outlook 107

5. Reference... 109

6. Supplementary figures and table 120

7. Glossary 135

Summary

Drought is a significant environmental factor that can impair plant growth and development, leading to reduced crop productivity or even plant death. Maintaining sugar distribution from source to sink is crucial for increasing crop production under water limitation conditions. Numerous studies have suggested that nutrition fertilization, especially potassium (K), can enhance plant growth and yield production. To investigate the mechanism of K in sugar long-distance transportation under drought stress, we established a soil-grow system and a hydroponic-grow system with varying amounts of potassium supplementation and analyzed the biochemical and molecular responses in Arabidopsis and potato plants under drought stress conditions. Our findings showed that excess potassium fertilization limited sucrose metabolism, leading to lower drought tolerance in Arabidopsis in both grow systems. However, higher potassium supplementation altered sugar relocation and potassium movement, resulting in an increase in starch yield production in both potato plants with different sink strength capacities. We also proposed that a low amount of sodium increases Arabidopsis drought tolerance under low potassium conditions since a low amount of sodium can improve the control of osmotic potential, leading to more water being retained in plant cells.

Silicon (Si) has received considerable attention recently for its potential in mitigating drought stress, although the effects vary among different plant species. To investigate the mechanism of Si in drought stress tolerance, we applied monosilicic acid in hydroponic media and then applied PEG8000 to simulate drought stress. Our findings revealed that Si-dependent drought mitigation occurred more in the shoot than in the root of Arabidopsis, and we observed silicon accumulation in the shoot of Arabidopsis. In Si-treated plants, more glucose was accumulated in the vacuole, leading to better osmotic potential control under drought stress. RNA sequencing analysis showed that Si altered the activity of sugar transporters and the sugar metabolism process, and increased photosynthesis. However, Si-dependent regulation in sugar transporter showed different responses in potato. Understanding the mechanism of Si in potato requires further studies. Overall, our dissertation provides important information for clarifying the mechanism of Si in drought stress, which forms the basis for further investigation.

Zusammenfassung

Dürre beeinträchtigt das Pflanzenwachstum und kann zu Ertragseinbußen oder sogar zum Pflanzensterben führen. Eine optimale Verteilung von Zucker von der Quelle zur Senke ist entscheidend für eine höhere Ernteerzeugung bei Wasserknappheit. Kalium (K) als Nährstoffdünger kann das Pflanzenwachstum und den Ertrag verbessern. In unserer Untersuchung haben wir ein Boden- und ein Hydroponiksystem eingesetzt, um den Einfluss von K auf den Langstreckentransport von Zucker unter Dürrestress zu erforschen. Wir haben Arabidopsis- und Kartoffelpflanzen unter Dürrebedingungen analysiert. Die Ergebnisse zeigten, dass eine übermäßige Kaliumdüngung den Saccharosestoffwechsel bei Arabidopsis beeinträchtigte und die Dürretoleranz verringerte. Jedoch führte eine höhere Kaliumzufuhr zu einer Veränderung der Zuckerumverteilung und der Kaliumbewegung, was zu einer Steigerung der Stärkeerzeugung bei beiden Kartoffelpflanzen mit unterschiedlicher Speicherkapazität führte. Eine geringe Natriummenge erhöhte die Dürretoleranz bei Arabidopsis unter Kaliummangelbedingungen, da sie die Kontrolle des osmotischen Potenzials verbesserte und so zu einer verstärkten Wasserrückhaltung in den Pflanzenzellen führte.

Silicium wurde als vielversprechende Möglichkeit zur Linderung von Dürrestress betrachtet, aber seine Auswirkungen variieren bei verschiedenen Pflanzenarten. In unserer Studie haben wir den Einfluss von Silicium auf die Dürretoleranz in Arabidopsis untersucht. Die Ergebnisse zeigten, dass Silicium in der Sprossachse eine stärkere Dürreanpassung bewirkte, während in der Wurzel eine geringere Wirkung beobachtet wurde. Silicium führte zu einer Anreicherung von Glucose in der Vakuole, was zu einer besseren Kontrolle des osmotischen Potenzials unter Dürrestress führte. RNA-Sequenzierung ergab, dass Silicium die Aktivität von Zuckertransportern, den Zuckerstoffwechsel und die Photosynthese beeinflusste. Bei Kartoffeln zeigte die Si-abhängige Regulation der Zuckertransporter unterschiedliche Reaktionen. Für ein besseres Verständnis des Mechanismus von Silicium bei Kartoffeln sind weitere Untersuchungen erforderlich. Insgesamt liefert unsere Dissertation wichtige Informationen zur Klärung des Mechanismus von Silicium bei Dürrestress und bildet die Grundlage für weitere Untersuchungen.

1. Introduction

Abiotic stress, such as drought, high soil salinity, heat, cold, and heavy metal toxicity, is a common unfavorable environmental condition that damages plant growth and the production of crops. Drought (water limitation) is one of the most critical environmental stresses threatening world food security and the economy; and occurs for several reasons, including low rainfall, high soil salinity, and high temperatures, factors which are increasingly precipitated by the global change of climate in many regions of the word. Therefore, plants have developed different strategies involving complex regulatory networks to either avoid or tolerate drought or efficiently recover from it during critical life cycle phases. Although several molecular response mechanisms of plants to drought have been revealed and drought resistance genes have been identified, it is challenging to transfer this knowledge into crops to improve stress resistance (Zhang *et al.*, 2022). So far, only one transgenic maize cultivar with a drought-resistance phenotype has been commercialized (Castiglioni *et al.*, 2008). Appropriately, new strategies for improving plant drought tolerance have been a goal in many studies.

1.1. Water movement and transportation of photosynthates in plant

Water, the most abundant solvent in living cells, directly participates in many biochemical reactions and makes up the medium for the movement of sugars and nutrients through the plant. Typically, water constitutes 80-95% of the mass of growing plant tissue (Taiz *et al.*, 2015). Proper water status ensures full turgescence of leaf mesophyll cells as well as stomatal conductance. Without adequate provision of water, the cell turgor is decreased, in consequence the stomata are closed, thereby reducing photosynthesis and the biosynthesis of carbohydrates which are serving as energy reserve and building blocks for plant metabolism. In addition, water pressure is essential to supply rigidity to non-lignified vascular tissue, allowing a plant to increase their height and support its weight. Plants absorb water through the root system and water movement in the plant is driven by transpiration at the leaf surface and root pressure. Water movement in soil and xylem depends on the the water potential (Ψ_w, $\Psi_w = \Psi_{solutes}$ +

$\Psi_{pressure}$) gradient from places with a higher water potential to the system with a lower water potential. The water potential of well-irrigated soils is about -0.3 to -0.5 MPa which is less negative than in root cells (-0.6 MPa).

The transpiration rate and root pressure work in tandem to regulate the water potential within the plant. Transpiration, driven by the opening of stomata, culminates in a decreased water potential on the leaf surface (Taiz *et al*., 2015). Simultaneously, root pressure is generated through the energy-consuming process of pumping ions against their concentration gradient, which leads to an elevated solute concentration in root tissue. It's noteworthy that an increase in solute concentration invariably lowers the solute potential (Ψsolutes), leading to a decrease in total water potential. In response to this gradient, water naturally migrates into the xylem and is propelled upwards to other plant organs **(Figure 1.1 (i))**.

However, water uptake and flow via the xylem is also important for the efficient flow of the second long-distance transport system in plants – the phloem – ensuring the provision of sink organs with sugars from photosynthetically active source tissues. This is because in source organs, sucrose is synthesized by photosynthesis and then exported into the apoplast by the SWEET11/12, the sucrose effluxers (Chen *et al.*, 2012). Subsequently sucrose is taken up actively by H^+-sucrose symporter SUT1/SCU2 (Riesmeier *et al.*, 1992, Stadler & Sauer, 1996)**(Figure 1.1 (ii))**. Sugar concentration in the phloem's Sieve Element/Companion cells lowers the water potential within the phloem by reducing solute potential. This change prompts water to migrate from the xylem into the phloem, generating a pressure that propels both sucrose and water towards the roots. The maintenance of a well-regulated vascular circulation, which includes both water transpiration and sucrose translocation, is crucial for robust plant development and the achievement of optimal productivity (Lemoine *et al.*, 2013).

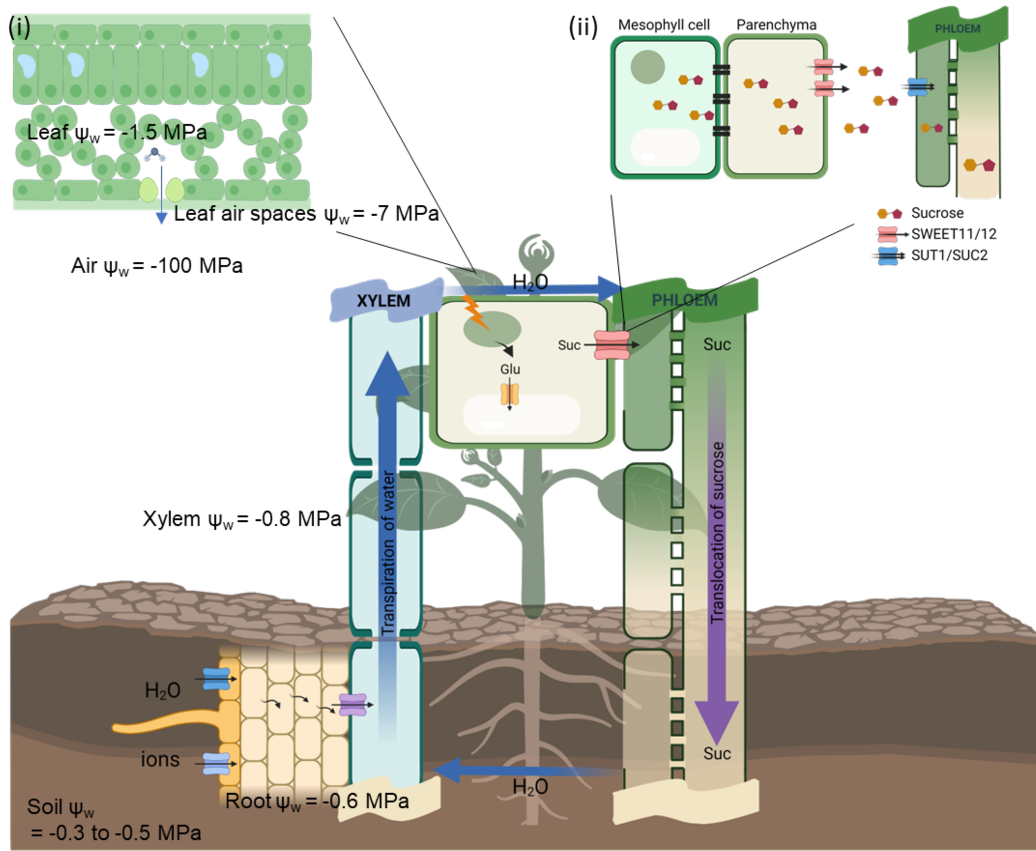


Figure 1.1 Plant vascular circulation. (i) Water evaporation from leaves causes a negative water potential gradient leading water to an upward movement from roots to through the xylem. (ii) Sucrose is uploaded into the phloem resulting in an increased osmotic pressure that causes water to move to the phloem.

1.2. Drought stress response

Drought is a major environmental factor impairing many physiological and metabolic processes in plants, which may lead to inhibition of plant growth and development, reducing crop productivity, or even leading to plant death (Pirasteh-Anosheh *et al.*, 2016). Under conditions of water limitation, the water potential in soil was decreases due to a reduction in hydrostatic pressure (Ψ_p) (Elizamar Ciríaco da *et al.*, 2013). This invalidates the water potential gradient between soil, root, and shoot, resulting in diminished vascular circulation and nutrition uptake leading to eventual growth arrest and, finally death of the plant organism (Cramer *et al.*, 2009, Billings & Phillips, 2011). For short-term drought stress, the responses of plants are restricted to the adjustment of stomatal conductance and water potential between tissues, while long-term drought stress is associated with metabolism alteration and nutrition allocation. In the following, the plant drought stress response will be introduced on three levels: (i) molecular responses, including the regulation of drought stress-related genes; (ii) biochemical mechanisms, including the accumulation, regulation, and re-localization of metabolites; (iii) physiological and morphological response, including stomatal movement, photosynthesis, leaf expansion, and root growth (**Figure 1.2**).

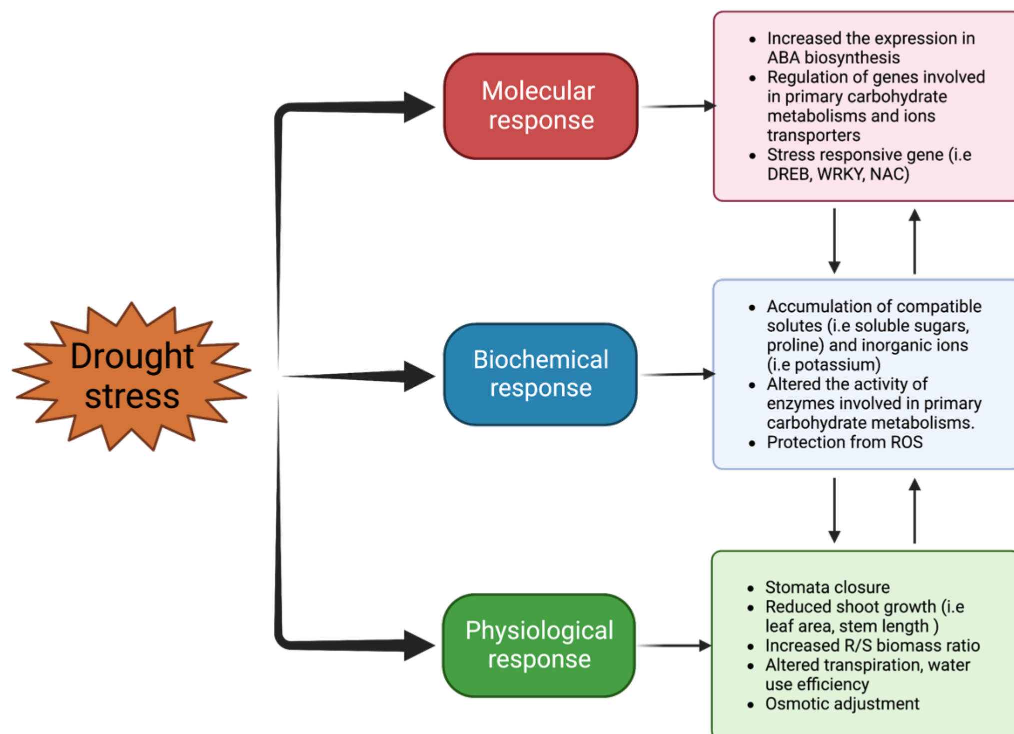


Figure 1.2 Overview of involved processes and responses of plants to drought stress (Kumar *et al.*, 2018).

1.2.1. Molecular response to drought stress

Indeed, the myriad of genes implicated in drought response, such as those encoding regulatory proteins (e.g., transcription factors, protein kinases) and functional genes (e.g., membrane transporters, osmoprotectants synthases), play a crucial role in facilitating the biochemical adaptions required to endure drought conditions (Kumar *et al.,* 2018). For example, ABA-dependent pathways involving transcription factors AREB/ABFs contribute significantly to osmotic adjustment, a critical biochemical response during drought (Fujita *et al.*, 2005, Fujita *et al.*, 2013). Similarly, the ABA-independent action of DREB2 transcription factors orchestrate the expression of genes involved in osmotic stress response, which influences biochemical changes such as the production of compatible solutes and enhancement of antioxidant enzymes (Sakuma *et al.*, 2002, Sakuma *et al.*, 2006a, Sakuma *et al.*, 2006b). The role of SnRK2 protein kinases is not just confined to the molecular level of regulating gene expression but extends to modulating biochemical processes. This is evident in its action on the potassium channel

KAT1, where SnRK2's negative regulation under drought stress leads to inhibition of stomatal opening, a clear example of the link between molecular signaling and biochemical response (Pilot *et al.*, 2001, Sato *et al.*, 2009). Moreover, SnRK2 also influences sugar transport during drought stress, further underlining its role in biochemical adaptations (Chen *et al.*, 2022). The involvement of SnRK3 kinases, also known as CIPKs (CBL-interacting protein kinase), in drought stress response is another key intersection of molecular and biochemical reactions to drought stress. Acting through calcium signals, CIPKs trigger a series of phosphorylation and dephosphorylation cascades that, in turn, regulate the expression of drought stress-responsive genes and stimulate biochemical adaptations (Asai *et al.*, 2002, D'Autreaux & Toledano, 2007, Hu *et al.*, 2013, Liu *et al.*, 2014b).

In sum, the molecular processes and genetic changes that occur during drought stress are inextricably linked to the plant's biochemical responses. These interactions underpin the plant's ability to survive and flourish under water-limited conditions.

1.2.2. Biochemical response to drought stress

As the soil dries, the water potential gradient between the environment and the cell becomes low. Osmotic adjustment (accumulation of solutes by cells) has been considered one of the processes in plant adaptation to drought. It has been shown that **compatible solutes** (including soluble sugars and amino acids) and **inorganic ions** (i.g., potassium) are accumulating in plant cells during stress response **(Figure 1.2)** (Hummel *et al.*, 2010, Takahashi *et al.*, 2020, Ilyas *et al.*, 2021). There is general acknowledgment that the major role of these metabolites is to serve as osmolytes to increase solute potential (Ψ_s, the osmotic component of Ψ_w), which in turn improves the ability of cells to retain water (Hoekstra *et al.*, 2001, Taiz *et al.*, 2015, Takahashi *et al.*, 2020). In addition, compatible solutes play a role in enzyme and protein stabilization, including maintaining membrane integrity and scavenging of ROS. Without the protection of compatible solutes, ROS attack proteins preferentially, leading to their degradation and impairment of cell functions (Hoekstra *et al.*, 2001).

Soluble sugars (glucose, fructose sucrose, etc.) have been known as major osmotic adjustment substances. The rise in soluble sugar contents is mainly facilitated by a breakdown of stored starch in source tissues. These sugars are also transported to sink organs (e.g., root) to support the energy demand for growth and other stress-responsive adaptive mechanisms (Durand *et al.*, 2016, Kaur *et al.*, 2021). Sucrose is the major form of carbohydrates transported and is synthesized by sucrose phosphate synthase (SPS) and sucrose phosphate phosphatase (SPP) from UDP-glucose and fructose 6-phosphate. While sucrose must be degraded into hexoses by sucrose synthase (SUS) or invertase (INV) for various metabolic and biosynthetic processes. SUS is involved in biosynthesis of starch and cellulose and degrades sucrose to yield UDP-glucose and fructose. INV is considered to be a key enzyme in cell osmotic regulation by hydrolyzing sucrose to yield hexoses (glucose and fructose)(Roitsch & González, 2004, Sergeeva *et al.*, 2006). These hexoses can be either transported into vacuoles for storage or be phosphorylated to hexose phosphates (including G6P and F6P) by hexokinase (HXK) or fructokinase (FRK), directed to starch synthesis in the plastid or to glycolysis (Ruan, 2014). The regulation of sucrose cleavage enzyme activity in plants is related to their drought stress response. Drought conditions usually increase SPS activity, which may increase the accumulation of sucrose, while increased SUS and INV activities may account for the accumulation of hexoses (Pinheiro *et al*., 2001; Praxedes *et al*., 2006). The alteration of these sucrose metabolic enzymes regulates the source-sink transitions, improving sucrose phloem loading and flowing into sink organs, such as roots and seeds. For example, activity of SPS increased and that of SUS decreased in rice leaves under drought stress, providing more sucrose for translocation to roots, thus increasing R/S ratio. In the same study, the activity of INV was increased in rice roots, resulting in increased sugar content in roots under drought stress (Xu *et al.*, 2015). Similarly, in soybean soluble sugar contents of leaves under drought conditions increased and starch content decreased via the regulation of the expression of *GmSPS1*, *GmSuSy2*, and *GmA-INV* genes and their corresponding enzymes (Du *et al.*, 2020b). The metabolism and translocation of soluble sugars under drought stress ultimately trigger pivotal morphological alterations as a coping mechanism in plants under environmental stressors. These changes, observable in various aspects, including root-to-shoot ratios, leaf characteristics,

and overall biomass allocation, are primarily driven by the adaptive redistribution of sugars to optimize plant resilience.

Drought conditions induce a complex cascade of biochemical changes in plants, impacting sugar accumulation, distribution, and metabolism. This intricate process heavily relies on a network of sugar transporters, which play essential roles in the plant's response to drought stress. One crucial aspect of sugar regulation is their long-distance distribution through the phloem. This process involves two steps: phloem loading and unloading. Cytosolic sucrose is first exported to the apoplast of phloem parenchyma transfer cells (PPTCs) by Sugar Will Eventually be Exported Transporters, SWEET11 and 12. Subsequently, the sucrose is symported with proton via an electrochemical gradient into the sieve element-companion cell (SE/CC) complex by the SUT1-type transporter (SUC2 in Arabidopsis) **(Figure 1.3)**. Under drought stress, the activity of sugar transporters, particularly SWEETs and SUTs, is affected. For instance, in Arabidopsis, the activity of SWEET11 and SWEET12 is enhanced through phosphorylation by SnRK2.2, SnRK2.3, and SnRK2.5 kinases under drought stress and after ABA treatment. This phosphorylation leads to an increased root-to-shoot sugar transport ratio, influencing plant adaptation to drought conditions (Chen et al., 2022). Similarly, Arabidopsis SUC2 is upregulated in response to drought and salt stress (Xu *et al.*, 2017), and similar responses have been observed in rice and maize (Ibraheem *et al.*, 2011a, Xu et al., 2017).

Another critical aspect of sugar transport is their movement within the vacuole. The central vacuole, which takes 90% of total cell volume, is vital for cellular functions, particularly turgor maintenance. Tonoplast Sugar Transporters (TSTs) have been identified as major facilitators of monosaccharide import into the vacuole **(Figure 1.3)**. TSTs mediate glucose and fructose accumulation under cold stress, and the loss of TST1 and TST2 reduces glucose and fructose levels in cold-treated Arabidopsis leaves, resulting in compromised cold resistance. However, the regulation of TSTs under drought stress differs. Under severe drought stress, *At*TST1 expression is reduced, while *At*TST2 expression is increased, although their specific roles in drought stress responses are not yet fully understood. In cotton, the activity of TST2 is regulated by phosphorylation through CBL2/CIPK6, promoting glucose accumulation in the vacuole. Several

sugar transporters have been identified for glucose export from the vacuole to the cytosol. Among them, Early Responsive to Dehydration6-Like transporters, such as AtERD6 and AtESL1, play important roles in sugar transport **(Figure 1.3)**. AtERD6 is a putative sugar transporter triggered by dehydration and cold stress, while AtESL1 functions as a low-affinity facilitator with multiple substrate preferences and is influenced by salinity and drought. Additionally, specific SWEET transporters, including SWEET16 and SWEET17, are localized to the tonoplast and facilitate the flux of glucose, fructose, and sucrose **(Figure 1.3)**. AtSWEET17, in particular, is a fructose-specific sugar porter localized to the vacuolar membrane, modulating root development and enhancing drought stress tolerance. Transgenic tomatoes expressing MdSWEET17 from apple exhibit increased fructose accumulation and improved drought tolerance.

Proline, an important compatible solute, plays a significant role in plant cells during drought stress. Its functions go beyond being an osmotic adjustment substance. Proline also contributes to the stabilization of membrane and protein structures, acts as a scavenger for reactive oxygen species (ROS), and helps buffer cellular redox potential under challenging conditions (Verbruggen & Hermans, 2008, Dien *et al.*, 2019). The accumulation of proline has been proposed as a valuable biochemical marker for assessing the severity of drought stress in various plant species, including bean, potato, maize, sorghum, and rapeseed (Blum & Ebercon, 1976, Ibarracaballero *et al.*, 1988, Crusciol *et al.*, 2009, Arteaga *et al.*, 2020). Its use as a marker has provided insights into the response and adaptation of plants to water scarcity, as demonstrated by these and other studies.

As stated above, not only the accumulation of sugars, but also of ions in the plant cell mediate plant drought tolerance, as the stabilize cellular turgor during water loss. **Potassium (K)** is the most abundant cation in plants, and it is closely related to water homeostasis and water use efficiency (Kuchenbuch *et al.*, 1986, Tanguilig *et al.*, 1987). Increased uptake of K^+ is an important response of drought-stressed plants (Andersen *et al.*, 1992, Wang *et al.*, 2004, Mahouachi *et al.*, 2006). K is highly mobile in plants and plays an essential role for the regulation of stomatal movement, osmotic potential, sugar transport, and cation-anion balancing (Marschner, 2011, Oosterhuis *et al.*, 2014, Kaiser, 1982). K^+ uptake in

plants is regulated by a diverse set of channels and transporters that play a crucial role in maintaining potassium balance. Key components include, HAK5, AKT1, and CNGC channels are key components involved in efficient K^+ uptake across a range of substrate concentrations. The outward-rectifying SKOR channel is responsible for releasing K^+ into xylem vessels, facilitating nutrient delivery to different plant parts. Additionally, K^+ uptake and efflux mechanisms contribute to the regulation of cell water potential and turgor, crucial for osmotic regulation. K^+ also influences osmotic pressure in the root xylem, which drives long-distance flow from the roots to the shoots (Lebaudy *et al.*, 2007). It has been proposed that K functions as a mobile energy source in plant vascular tissue (Gajdanowicz *et al.*, 2011). K^+ is released via the phloem by the channel protein AKT2 to the apoplast creating an electrochemical potential. Therefore, more energy can be used for sucrose reloading via the sucrose/H^+ symporter, especially in an energy-limiting condition. Mutants lacking AKT2 exhibit half of the sucrose content in phloem sap and a lower phloem potential when compared to wild type plants (Deeken *et al.*, 2002). Physiological studies have also proved that K transport is under phytohormone control. For example, ABA decreased the expression of the K channel SKOR, a transport protein responsible for loading K^+ to the xylem, in roots (Gaymard *et al.*, 1998) and, simultaneously, increased that of AKT2 in shoots. This regulation of AKT2 and SKOR channel activity during drought stress results in decreased K transport into the xylem sap and increased K recirculation in the phloem, improving K accumulation in roots, osmotic adjustment, and finally root growth (Lebaudy *et al.,* 2007). Large amounts of K are recirculated from the shoots to the roots via the phloem and subsequently returned to the shoots via the xylem. Biochemical analyses have proved that activity of K transporters is regulated via Calcineurin B-like protein and CBL-interacting protein kinase (CBL/CIPKs). For example, the AKT1 channel is activated via phosphorylation by CBL1/CIPK23 complex, resulting. The CBL1 gene can be induced by drought (Albrecht *et al.*, 2003), and the protein level increases by ABA, mannitol , and NaCl treatment (Cheong *et al.*, 2003).

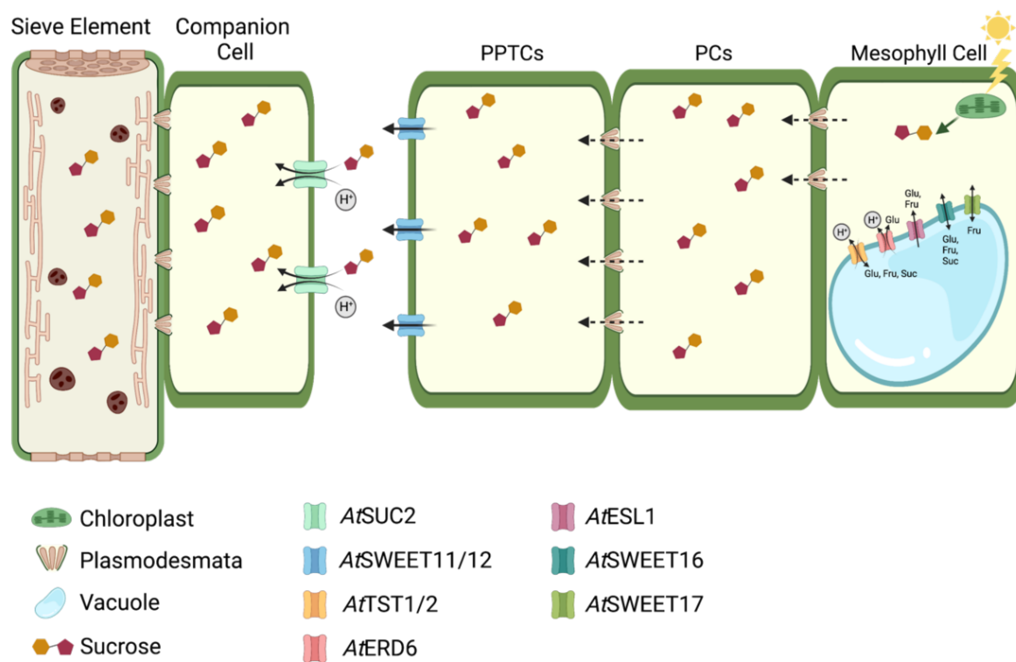


Figure 1.3 Sugar transporters for phloem loading and accumulation in plant cells. Sucrose synthesized via photosynthesis in the mesophyll cell is transported symplastically into parenchyma cells (PCs) and then to phloem parenchyma transfer cells (PPTCs). Sucrose is next transported via *At*SWEET11/12 transporters to the apoplast. *At*SUC2 transporters actively uptake these sugars from the apoplast into companion cells and subsequently to the sieve element for long-distance transport in the phloem. A part of sucrose is transported vis vacuole transporters into vacuole for storage. The import of monosaccharides is mediated via TST (tonoplast sugar transporter) protein with exchanging of a proton H^+. TSTs can also mediate sucrose. The export of monosaccharides is mediated via members of the Early-Responsive to Dehydration 6-Like proteins (i.g., ERDL6 and ESL1). SWEET16 or 17 belonging to SWEET family facilitate sugars on the vacuole membrane.

1.2.3. Physiological response to drought stress

Plant water relations are influenced by several crucial characteristics, including relative water content (RWC), leaf water potential, stomatal conductance, transpiration rate, and leaf temperature. Drought stress often leads to a decrease in RWC across various plant species, resulting in a decline in cell turgor and inhibition of cell elongation (Ings *et al.*, 2013). In response to this decline in cell turgor, stomatal closure is a rapid and common reaction to drought stress in most plants **(Figure 1.2)**. By closing their stomata, plants can effectively reduce water loss and prevent rapid dehydration. However, this protective mechanism also leads to a decrease in CO_2 fixation and can impact overall photosynthetic activity. Such situation favors accumulation of NADPH and limitation of $NADP^+$. Under such conditions, oxygen (O_2) is a replacement acceptor for electrons overflowing from the thylakoid electron transport chain, resulting in the formation of reactive oxygen species (ROS, including singlet oxygen (O2·) and H_2O_2), thus causing photoinhibition (Vass *et al.*, 2007, Fischer *et al.*, 2013). Photoinhibition negatively affects photosynthetic capacity, therefore, damaging plant growth and diminishing crop yield (Takahashi & Murata, 2008). Although plant growth is in general reduced when the soil water supply is decreased, shoot growth (such as leaf expansion and the number of leaves) is often inhibited to a greater extent than root growth. In fact, the root-to-shoot (R/S) biomass ratio of plants in drying soil may increase since drought stress also alters signaling and biosynthesis pathways of phytohormones (e.g., of ABA, cytokinin, and auxin) **(Figure 1.2)** (Kurepa & Smalle, 2022). On the other hand, drought stress increases R/S ratio via alteration of carbohydrate distribution and enzymatic activity in sink organs. Water limitation not only causes a reduction of plant growth but can also induce early leaf senescence. Drought-induced leaf senescence contributes to nutrient remobilization in source tissues, thus ensuring the survival of the rest of the plant (i.e. of storage sink organs) (Munné-Bosch & Alegre, 2004).

Additionally, drought is also linked to changes in cell wall structures. A number of studies report that cellulose biosynthesis can be altered in response to drought stress in several different species including Arabidopsis, tobacco cell, wheat roots, and grape leaves (Bray, 2004, Iraki *et al.*, 1989, Piro *et al.*, 2003, Sweet *et al.*, 1990). However, in other studies, an increased level of UDP-Glc and the

expression of *SUSs* (sucrose synthase) and *UDP-glucose pyrophosphorylase* (UGPase) was observed in cotton under drought stress, suggesting a higher cellulose biosynthesis (Zheng *et al.*, 2014). Increased cellulose synthesis could be a way by which cell wall elasticity (CWE) and cell turgor pressure are maintained, thus keeping cell growth under low water potential (Martínez *et al.*, 2007).

1.3. The role of silicon in plant

Proper mineral nutrition is basic for plants. Essential plant nutrients are either classified as macronutrients or micronutrients. Macronutrients include nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg) and sulfur (S). Micronutrients are zinc (Zn), copper (Cu), iron (Fe), manganese (Mn), boron (B), molybdenum (Mo), chlorine (Cl) and nickel (Ni). In recent research, silicon (Si) has been considered as non-essential but beneficial plant nutrient (Waraich *et al.*, 2011, Huber *et al.*, 2012). Si is the second most abundant element in the earth crust after oxygen and comprises 50–70% of the soil mass (Epstein, 1994, Ma & Yamaji, 2006). The benefits of Si accumulation include tolerance to biotic and abiotic stresses, such as drought, low temperature, salinity, metal toxicity, diseases and pests, enhancement of secondary metabolites in various medicinal plants, prevention of nutrient imbalances, and stimulation of photosynthesis (Ma, 2004, Zhu & Gong, 2014, Adrees *et al.*, 2015). The beneficial effects of Si have mainly attributed to its deposition in plants, and variation of the quantity and deposition patterns between species have been observed (Guerriero *et al.*, 2020). Plant species have different abilities to accumulate Si. Active accumulators, accumulate around 1.5% to 10% Si content in shoots, e.g., rice, wheat, maize, and sorghum. The passive accumulators are plants with a shoot Si content of only 0.5–1.5%, e.g., cucumber, bitter gourd, or melon. The plants with a Si content of less than 0.2% are classified as Si excluders, which was associated with most dicots, such as tomato, potato, canola, and lentil (Takahashi *et al.*, 1990)

1.3.1. Silicon uptake, transport system, and deposition in plant

Silicon (Si) is taken up by roots as monosilicic acid [$Si(OH)_4$], which is the soluble form present in the soil at pH values lower than nine and concentrations below 2

mM. After uptake and transportation to the shoots, as a result of transpiration, Si concentrates and polymerizes into colloidal silica gel ($SiO_2 \cdot nH_2O$) (Yoshida *et al.*, 1962). The uptake mechanism of Si from soil into root requires specific transmembrane proteins identified as Si transporters to mediate the whole process. The first Si transporter (named Lsi1) was identified as belonging to nodulin 26-like intrinsic proteins (NIPs) subfamily in the aquaporin (AQP) family in rice, and corresponding loss of function mutants showed a lower biomass and decreased quality of yield (Ma *et al.*, 2006). Lsi2 is an efflux transporter that actively pumps Si out of the cell with the help of a proton gradient (Ma *et al.*, 2007). In rice, Si is taken up as monosilicic acid by Lsi1, and then be pumped out in aerenchyma by Lsi2 at the proximal side of the cell. The silicic acid moves upwards to the shoot via the transpiration stream, where Lsi6 (an influx transporter, homolog of Lsi1) unloads Si from the xylem and facilitates its transport to the different parts of the plant **(Figure1.4)**(Mandlik *et al.*, 2020). The molecular mechanism of Si uptake varies amongst species. Homologs of both Lsi1 and Lsi2 have been identified in monocot crops such as wheat, barley, sorghum, and maize with variations in their localization (Mitani *et al.*, 2009). In maize, Si deposition is mediated by two genes, *ZmLsi1* and *ZmLsi6.* The function of ZmLsi1 is the uptake of Si through roots, whilst ZmLsi6 is located in the parenchyma cells of the leaves and is for xylem unloading (Bokor *et al.*, 2015). Homologs of *OsLsi2* responsible for efflux have been found in barley (*HvLsi2*) and maize (*ZmLsi2*) and their proteins function in a similar manner, but they are only present in the root endodermis (Mitani *et al.,* 2009). Monocots such as rice can accumulate Si up to 10% of the plant mass while most of the dicots accumulate much less. Only few information is available on the mechanism of Si transport in dicots. There, Si transportation involves concentration-independent (passive) and metabolically active process which are inhibited by low temperature (Liang *et al.*, 2005). The first gene encoding an influx Si-transporter in dicots was identified as Pumpkin *Lsi1* (CmLsi1), localizing in the root cells (Mitani *et al.*, 2011). The *CmLsi2-1* and *CmLsi2-2*, Si efflux transporters, have been isolated from two pumpkin cultivars (Mitani *et al.,* 2011). These transporters show an efflux transport activity for Si and are expressed in both roots and shoots (Mitani-Ueno *et al.*, 2011). Similarly, *GmNIP2-1* and *GmNIP2-2,* belonging to the NIP2 subfamily of AQPs, have been cloned from soybean and identified and characterized as putative influx Si-

transporter genes (Deshmukh *et al.*, 2013). Although the identification of Si transporters has been defined the Si uptake at the root level and its subsequent transport to the tissues, the transporter involved in Si loading in the xylem is not yet known. Also, the mechanism of the accumulation of Si in cells are not known.

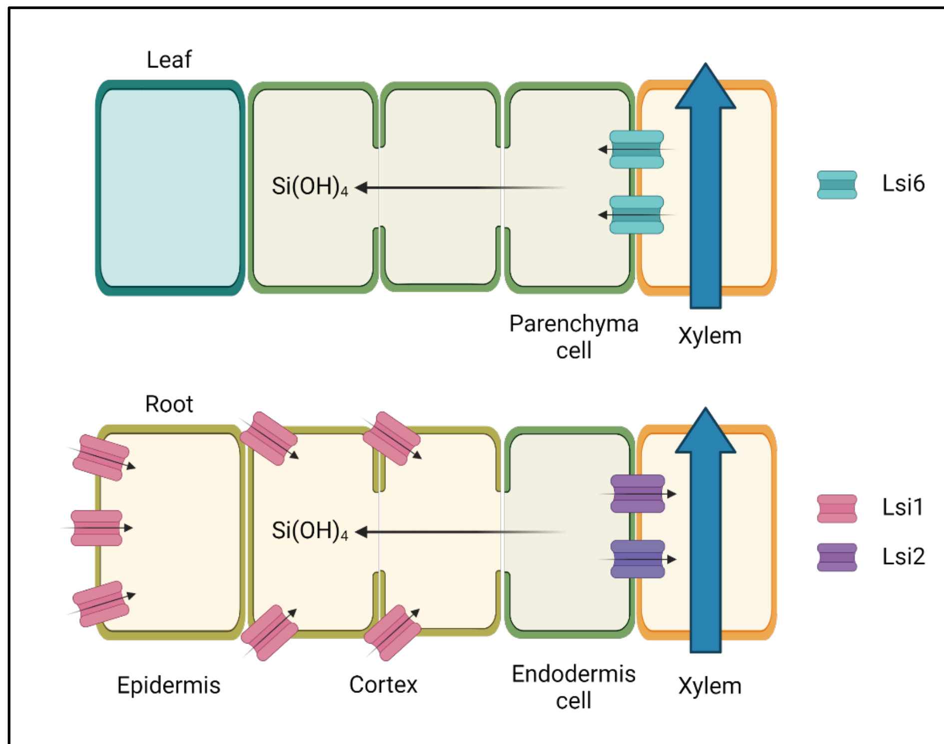


Figure 1.4 Si transport in a typical grass species. Silicic acid from the soil is transported into the root cells by Lsi1 transporter. The silicic acid then diffuses across the root into the endodermis. At the endodermis, Lsi2 transports silicic acid into the stelar apoplast from where it diffuses into the xylem and is transported to the shoot in the transpiration stream. In rice, the presence of aerenchyma means that Lsi2 is localized at both the exodermis and endodermis. In the shoot, silicic acid is unloaded from the xylem by further Lsi6 and deposited in the cells.

1.3.2. A key role of silicon in drought stress

The alleviating effects of Si on drought stress has been observed in a variety of crop plants species, including both monocots (e.g., rice, wheat, maize, and sorghum) and dicots (e.g., tomato, cucumber, sunflower, soybean, cotton, mango, and canola) (Ming *et al.*, 2012, Gong *et al.*, 2005, Kaya *et al.*, 2006, Liu *et al.*, 2014a, Shi *et al.*, 2016, Ma, 2004, Gunes *et al.*, 2008, Shen *et al.*, 2010, Farooq *et al.*, 2013, Helaly *et al.*, 2017, Habibi, 2014). Si enhances plant drought tolerance via the following mechanisms:

(i) Increasing the root to shoot ratio: It was suggested that Si application regulates polyamine (PA) and 1-aminocyclopropane-1-carboxylic acid (ACC) levels in sorghum under drought stress conditions to increase root growth and the root/shoot ratio, thus improving root water uptake (Yin *et al.*, 2014). The effects of Si in enhancing root/shoot ratios in rice have also been reported (Ming *et al.,* 2012, Fleck *et al.*, 2011). These Si-mediated changes in root development also increase root endodermal silicification and suberization, therefore enhancing the capability of water retention to overcome the effects of drought stress (Lux *et al.*, 2002, Fleck *et al.,* 2011).

(ii) Promoting the osmotic driving force: The accumulation of compatible solutes in the cell results in a favorable osmotic gradient between the plant roots and the growth medium to facilitate water uptake (Javot & Maurel, 2002, Hsiao & Xu, 2000). Si applied promotes osmolyte accumulation in many plant species, especially Si accumulators, such as rice, wheat, maize, and sorghum under drought stress, thus improving the osmotic driving force for water uptake. Moreover, Si regulates the activities of enzymes involved in carbohydrate metabolism and affects the lignification of cell walls, consequently regulating assimilate synthesis and transport efficiency (Ming *et al.,* 2012, Gong *et al.,* 2005, Kaya *et al.,* 2006, Sonobe *et al.*, 2010). Although many studies have reported that Si application increased plant drought tolerance by regulating osmotic adjustments, little is known about the mechanisms of Si-mediated osmotic adjustment in plants.

(iii) Increasing aquaporin (AQP) activity: Aquaporins belong to the major intrinsic protein (MIP) family and regulate the transport of water and small solutes

across membranes (Kruse *et al.*, 2006, Wang *et al.*, 2016), contributing to root water uptake, especially under drought stress condition (Kaldenhoff *et al.*, 2008, Liu et al., 2014a). In sorghum plants, Si application enhances aquaporin activity via upregulation of the *SbPIP1;6*, *SbPIP2;2*, and *SbPIP2;6* genes, consequently increasing root water uptake under drought stress (Liu *et al.*, 2015).

(iv) Maintaining nutrient balance: Mineral nutrient uptake and homeostasis can be disturbed by environmental stimuli, especially drought stresses. It has been reported that the uptake of nitrogen (N), phosphate (P), potassium (K), calcium (Ca), magnesium (Mg), iron (Fe), copper (Cu), and manganese (Mn) increases in response to Si application under drought stress (Kaya *et al*., 2006, Gunes *et al*., 2008, Chen *et al.*, 2011), which not only enhances plant growth but also improves plant drought tolerance. For example, K and Ca contents were increased in maize in response to Si application under drought stress (Kaya *et al.,* 2006), where K supports plant growth, osmotic adjustment, and drought tolerance, and Ca is critical for improving plant growth, maintaining the integrity of plant membranes and regulating ion permeability and selectivity (Marschner, 2011).

(v) Inhibiting reactive oxygen species (ROS) and hydrogen peroxide (H_2O_2): High exogenous hydrogen peroxide (H_2O_2) levels can inhibit root hydraulic conductance due to membrane electrolyte leakage and ROS levels (Benabdellah *et al.*, 2009). H_2O_2 is involved in the formation of suberin lamellae, which form a hydrophobic barrier in the endodermis and exodermis of roots (Razem & Bernards, 2002). In tomato plants under drought stress, Si application reduces H_2O_2 production and suberin lamella formation and further increases water permeability (Shi *et al*., 2016).

(vi) Regulating leaf water loss: Si-induced reduction in transpiration was considered by physical blockade via cuticle layer thickening from silica deposits, which contributes to the maintenance of leaf water potential under water-deficient conditions (Agarie *et al.*, 1998, Savant *et al.*, 1996, Savant *et al.*, 1999). On the other hand, Si-regulated transpiration is also due to the loss of guard cell turgor resulting in stomatal closing (Gao *et al.*, 2005, Gao *et al.*, 2006). However, Si-regulated transpiration is dynamic and depends on root

water status, environmental conditions, plant species, and genotype. In some cases, Si application increased the leaf transpiration rate in some crop plants, such as rice, tomato, pepper, and sorghum, under drought stress. (Liu et al., 2014a, Shi et al., 2016, Chen et al., 2011, Soares Pereira *et al.*, 2013). This increased transpiration was due to an improvement in leaf water status via increased water uptake, enhanced leaf xylem sap flow, and increased leaf water potential. However, it has also been reported that Si has no effect on the transpiration rates of cucumber and rose plants under drought stress conditions (Hattori *et al.*, 2008, Savvas *et al.*, 2007).

(vii) Modification of signaling pathway: Si application has been reported to increase plant tolerance by regulating endogenous plant phytohormone balance and associated signaling events. In barley plants, Si application decreased ABA homeostasis via transcriptional regulation of ABA biosynthesis and degradation pathways, thus improving stress tolerance (Hosseini *et al.*, 2017). Si also mediates the modulation of multiple genes involved in stress-responsive pathways via the ABA pathways. In rice, Si regulates the transcription factors *OsNAC5* and *OsDREB2A*, which trigger the expression of stress-responsive genes that improve drought stress tolerance via ABA-dependent and ABA-independent pathways, respectively (Dubouzet *et al.*, 2003, Hussain *et al.*, 2011).

1.4. Objective of this work

This project's main aim was to investigate whether and how the nutritional status of potassium and silicon influences drought response and tolerance in terms of growth, phloem long-distance transport, and gene expression. To achieve this aim, two focuses are studied:

(i) To clarify the question of how potassium application might stimulate sucrose flow and overcome drought-induced slowdown of vascular circulation the following questions should be answered:

- Does potassium fertilization promote sugar transport to sink organs?
- How does potassium mediate sugar transport?

(ii) To analyze the molecular and physiological role of silicon on drought tolerance, studies were focused on following questions:

- Does Si mitigate drought effects in Arabidopsis plants?
- Does Si application improve drought tolerance by increasing potassium uptake?
- What is the mechanism of Si in improving drought tolerance?

In this study, we first aim to answer those questions by using the model plant *Arabidopsis thaliana*, and second aim to apply to the crop plant potato (*Solanum tuberosum*).

2. Material and Method

2.1. Plant material and cultivation

2.1.1. Plants materials and plants growth

This study was composed of two primary components: one focused on Arabidopsis, and the other on potato plants. The *Arabidopsis thaliana* component involved wild-type plants of the Columbia (Col-0) variety. These plants were initially grown in ED73 type soil substrate (Patzer, www. einheitserde.de) with a 15% sand mix, pre-cultured at 4°C for 48 hours, and then transferred to a short-day growth chamber. This chamber was maintained at 22°C with a light intensity of 120 µmol photons m^{-2} s^{-1} in a 10-hour day/14-hour night cycle for seven days. After this pre-culturing period, the plants were transferred to eight cm pots filled with soil containing varying amounts of potassium (K soil substrate, see Method 2.1.2) for further analysis.

The potato component of the study involved both wild-type and NTT-antisense transgenic lines of the Désirée variety. The NTT-antisense transgenic line, characterized by constitutive down-regulation of the *St*NTT gene in potato tubers, has been previously described (Tjaden *et al.,* 1998). Fresh potato tubers were stored at 4°C for approximately one month to induce germination, after which they were planted in 20 cm pots (one tuber per pot) filled with 1.7 kg of K soil substrate. These potato plants were grown in a greenhouse at 25°C with a light intensity of 320 µmol m^{-2} s^{-1}. PAM measurements (see Method 2.4) were taken 28 days after tuber germination. Potato leaf samples were collected on day thirty after germination, with each sample consisting of two to three mature leaves (greater than three cm in length). Tuber harvest occurred three months post-germination. All subsequent molecular and metabolomic analyses were performed on freeze-dried samples.

For the analysis of silicon fertilization on potato plants, tubers were initially grown in nine cm square pots using an ED75 soil substrate. After two weeks, the young potato plants were transferred to 20 cm pots, each containing 1.7 kg of soil substrate, and were treated with 5 mmol kg^{-1} of $Si(OH)_4$ (see Method 2.1.2).

These plants were then grown for an additional two weeks. Following this period, a drought treatment was applied by reducing water supply (see Method 2.2). Potato leaf samples for phloem exudate extraction were taken 30 days after the initiation of the drought treatment. Potato leaf and tuber samples for metabolomic and transcriptional analyses were collected 47 days after the drought stress commenced. Each sample comprised of three leaves or three tubers. For the assessment of potato shoot growth, the shoots from each pot were harvested and their fresh weight was measured

2.1.2. Potassium or silicon fertilization in soil

A nutrition-poor soil substrate, with an extremely low nutritional value (Zero-Soil, Hawita, Vechta, Germany), was used in this study. This substrate contained background levels of K lower than 0.1 $g{\cdot}kg^{-1}$ (Ho *et al.*, 2020). For growing Arabidopsis plants, the nutrition-poor type of soil was mixed with sand (5:1). For growing potato plants, the nutrition-poor type of soil was mixed with sand and stones (5:1:1).

Four different soil substrate mixtures (K0 to K3) were used in this study (**Table 2.1**). The K2 condition was used as a standard condition, with the following stock solutions were used: NH_4NO_3 (60 $g{\cdot}L^{-1}$), KH_2PO_4 (40 $g{\cdot}l^{-1}$), K_2SO_4 (6 $g{\cdot}l^{-1}$), $MgSO_4$ (20 $g{\cdot}l^{-1}$), H_3BO_3 (1.4 $g{\cdot}l^{-1}$), FeNa-EDTA (0.7 $g{\cdot}l^{-1}$), $CuSO_4{\cdot}5H_2O$ (0.8 $g{\cdot}l^{-1}$), $ZnSO_4{\cdot}7H_2O$ (0.9 $g{\cdot}l^{-1}$), $MnCl_2{\cdot}4H_2O$ (1.8 $g{\cdot}l^{-1}$), Na_2MoO_4 (7.5 $mg{\cdot}l^{-1}$). Per 20 kg of nutrition-poor soil, 200 ml of each of the stock solutions was added. A total of 1 L suspension of each CaO (60 $g{\cdot}l^{-1}$) and $CaCO_3$ (80 $g{\cdot}l^{-1}$) were directly mixed into 20 kg soil substrate. For K0 soil substrate preparation, both KH_2PO_4 and K_2SO_4 were replaced by the same concentration of NaH_2PO_4 and Na_2SO_4. For the K1 soil substrate, only KH_2PO_4 was replaced. For the K3 soil substrate, 200 ml of KCl stock (71.6 $g{\cdot}l^{-1}$) was added into 20 kg of K2 soil.

The soil for silicon fertilization analysis was prepared with ED73 type of soil substrate (Patzer, www. einheitserde.de) and 5 mmol kg^{-1} of Si $(OH)_4$ was added.

Table 2.1 Ground fertilization kg^{-1} soil substrate (dry mass)

Macro elements

Fertilizer	Stock-solution ($g \cdot l^{-1}$)	Element	$mg \cdot kg^{-1}$ soil substrate
NH_4NO_3	60	N	200
KH_2PO_4	40	K	115
		P	93
K_2SO_4	6	K	27
		S	11
$MgSO_4$	20	Mg	40
		S	44
$CaCO_3$	4 (g kg^{-1} soil substrate)*1	Ca	1835
CaO	3 (g kg^{-1} soil substrate)*1	Ca	2143
KCl	71.6	K	358
Micro elements			
Fertilizer	**Stock-solution (g l^{-1})**	**Element**	**mg kg^{-1} soil substrate**
$CuSO_4$*5H2O	0.8	Cu	2
$MnCl_2$*4H2O	1.8	Mn	5.2
$ZnSO_4$*7H2O	0.87	Zn	2
H_3BO_3	1.43	B	2.503
FeNa-EDTA	0.7	Fe	0.92
$NaMoO_4$*2H2O	0.0088	Mo	0.03

*1 Mix calcium carbonate and calcium oxide directly into dry soil substrate
*2 Potassium fertilizers were added dependent on the conditions

Table 2.2 Concentration of potassium and sodium in four conditions

Fertilization treatment	K (mmol kg^{-1} soil)	Na (mmol kg^{-1} soil)
K0	0	3.577
K1	0.687	2.89
K2	3.59	0
K3	9.18	0

2.2. Soil substrate-base drought stress experiment

To perform drought stress on Arabidopsis, plants were grown on the well-watered soil for a week and then the Arabidopsis seedlings were transferred into K soils substrate (see Method 2.1.2) for growing for two weeks (see Methos 2.1.1). The drought treatment was performed by stopping water supplied. The total weight of each pot was confirmed before drought treatment and every day after performing

drought treatment. When the soil field capacity (FC) is reduced to 50%, a process that took approximately one week, the FC was then maintained at this level for one week.

The soil field capacity was determined using the following formula (Gupta *et al.*, 2016):

$$FC (\%) = (WW - DW)/ DW \times 100$$

WW: completely wet soil weight

DW: completely dry soil weight

To perform drought stress on the potato, the tubers were first grown in nine centimeters square pot with ED75 type of soil substrate for two weeks and then the potato plants were transferred into the twenty centimeters pots with 1.7 kg of soil with silicon fertilizer (see Method 2.1.2) for growing for two weeks. Stop watering until the leaves of the plants were half wilted. 30% of water was given to plants every two days for 47 days.

The amount of water was calculated by the following formular:

$$(WW - DW) \times 30\%$$

WW: completely wet soil weight

DW: completely dry soil weight

2.3. Hydroponic system

Hydroponic media in this study was Hoagland solution with modification, included macronutrients (2mM $Ca(NO_3)_2$, 1mM $MgSO_4$, 0.88 K_2SO_4, 0.25mM KH_2PO_4) and micronutrients (20uM Fe-EDTA, 10uM NaCl, 10uM H_3BO_3, 1uM $ZnSO_4$, 1 uM $MnSO_4$, 0.1 uM $CuSO_4$, 0.01 $(NH_4)_6Mo_7O_{24}$) were supplied as control media (K2). For the K0 condition, both of K_2SO_4 and KH_2PO_4 were replaced with Na_2SO_4 and NaH_2PO4 in the same concentration. For the K1 condition, same concentration of Na_2SO_4 replaced with K_2SO_4. For the K3 condition, 2.5 mM of KCl was added in background of the K2 condition (**Table 2.3**). The hydroponic media with silicon (Si media), the K1 media was used as the background, and then 2mM of Si (OH)4 was added into the K1 media.

To supply drought stress under the hydroponic system, PEG8000 was added into the corresponding culture medium to create an osmotic pressure. -0.4 MPa was performed in this study.

Water potential (Ψ) was calculated by the equation following (Michel, 1983):

$$\Psi = 1.29[PEG]^2T - 140[PEG]^2 - 4.0[PEG]$$

Ψ: Solution water potential [bar; -0.1 MPa=1bar]

PEG: Concentration of PEG8000 [g/ gWater]

T: Temperature [°C]

Table 2.3 Nutrients in Hydroponic media

Conditions	K (mmol l-1)	Na (mmol l-1)
K0	0	1.1
K1	0.25	0.88
K2	1.1	0
K3	4	0

The hydroponic setup in this study followed the protocol as described (Conn *et al.,* 2013). In general, the Arabidopsis seeds were first germinated and grown on control media (K2) for ten days and then the Arabidopsis seedlings were transferred to the media with different potassium concentrations or Si media for growing for three weeks. The osmotic stress was performed by adding the PEG8000. Plant samples for transcript analyses and metabolism analyses were harvested six hours and eight days after PEG8000 applied **(Figure 2.1)**. The fresh weight of samples was first checked, and the dry weight of the same samples was confirmed after freeze-drying. Freeze-dried samples were used for metabolism analysis.

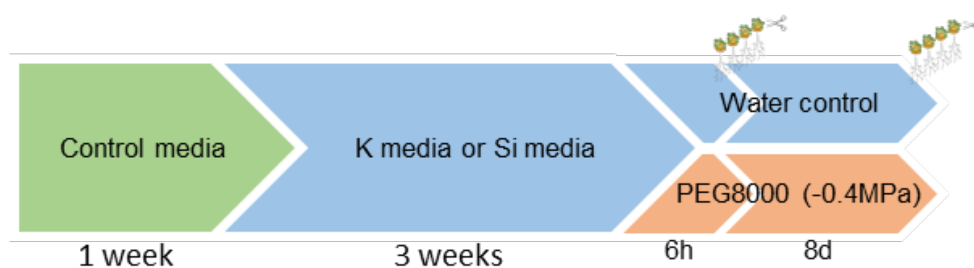


Figure 2.1 Growth scheme of Arabidopsis plants under hydroponic for analysis of gene expression or metabolism.

2.4. PAM assay

Photosynthetic activity of three individual plants per genotype was measured using an Imaging-PAM M-Series-System (Heinz Walz, Effeltrich, Germany) which was carried out as described (Keller *et al.*, 2021). Plants were darkened for 10 minutes to deplete photosystem II (PSII) energy. Capacity of PSII was measured upon repeated administration of light pulses with photosynthetic active radiation (PAR) of 76 µmol photons m^{-2} s^{-1} every 20 seconds until saturation was reached after 14 cycles. The measured fluorescence values (Fm, Fo, Fm', Fo') were used to calculate the effective quantum yield of photosynthesis [Y(II)] as well as the quantum yield of regulated non-photochemical energy loss [Y(NPQ)] and non-regulated non-photochemical energy loss [Y(NO)] (Ritchie *et al.*, 2010). The calculation of the corresponding parameters was performed by the associated software ImagingWinGigE (Heinz Walz, Effeltrich, Germany).

2.5. Gas exchange analysis

The measurements were carried out with the GFS-3000 (Walz, Erlangen-Eltersdorf). Air and photosynthetic parameters supply were controlled by the machine. For Arabidopsis*,* the whole plant (with pot) was put into a chamber (Arabidopsis Chamber 3010-A, Walz) with constant air flow and air composition. For the potato plants, a leaf was put into measure head (Cuvette 3010-S, Walz). CO_2 assimilation, CO_2 respiration and stomatal conductance were measured by examining the composition of entered air and released air. All parameters from Arabidopsis were divided by the weight of each measured plant.

2.6. Metabolism measurements

2.6.1. Metabolism extraction

Soluble metabolites were extracted from ten milligrams of freeze-dried plant material with 1 ml 80% EtOH in sealed 1.5 ml screw-lid reaction tubes at 80 °C for 30 minutes. Extracts were dried by vacufuge concentrator (Eppendorf, Germany)

and resolved in $_{dd}H_2O$. The pellets, were used for starch isolation, washed with 1 ml of $_{dd}H_2O$. 200 µl of $_{dd}H_2O$ was added into pellets and then autoclaved for 40 minutes. 200 µl of enzyme mix (5 U α-amylase and 5 U amyloglucosidase in 200 mM sodium acetate, pH4.8) was added and then incubated at 37°C for four hours for hydrolyzing to glucose. The reaction was stopped by heating at 95°C for five minutes.

2.6.2. Sugar and starch measurement

Soluble sugars (glucose, fructose, and sucrose) and hydrolyzed starch were measured using enzymatic assay base on conversion of glucose-6-phosphate to 6-phosphogluconolacton by NAD^+ (Stitt *et al.*, 1989). Briefy, 20 µl of extraction was mix with 190 µl of premix reagent (100 mM HEPES, pH7.5; 10 mM $MgCl_2$; 2 mM ATP; 0.8 mM NAD; 0.5 U glucose-6-phosphate-dehydrogenase) and the extension was measured at 340 nm in a Microplate Reader Infinite® (Tecan, Switzerland) as blank. The extension of soluble sugars was measured respectively after addition of 1.2 µl enzymes (1:10 dilution in 33 mM HEPES, pH7.5) hexokinase (Roche, Germany), phosphoglucoisomerase (Roch, Germany), and invertase (Sigma-Aldrich, Germany). For starch analysis, the extension was measured after adding hexokinase.

Sugar contents were calculated according to the formular:

$$C\ (\mu mol\ mL^{-1}) = \frac{\Delta E \cdot V_{total} \cdot F \cdot V_{extraction}}{\varepsilon_{NADH} \cdot d \cdot V_{probe} \cdot W_{sample}}$$

C = Sugar concentration

ΔE = Extension of sugar measurement – extension of blank

V_{total} *= Total volume in a well of 96-well [ml]*

F = Dilution factor

$V_{extraction}$ *= Extraction volume [ml]*

ε_{NADH} *=The molar extinction coefficient for NADH [*$6.22\ \frac{ml}{\mu mol \cdot cm}$*]*

d = layer thickness [0.51 cm]

V_{probe} *= measured sample volume [ml]*

Wsample = sample weight [g]

2.6.3. Ion chromatography (IC)

Ions in the extract of soluble metabolites (see Method 2.6.1) were measured in a 761 Compact IC system (Metrohm, Herisau, Switzerland) and separated using the Metrosep C4-150/4.0 column for cations and a Metrosep A supp 4-250/4.0 column (both Metrohm, Herisau, Switzerland). 3.4 mmol l^{-1} HNO_3 and 1.6 mmol l^{-1} were used as eluent for cation analysis and 1.8 mM Na_2CO_3 and 1.7 mM $NaHCO_3$ as eluent, 50mM H_2SO_4 as counterion for anion measurement. The corresponding software Metrodata IC Net 2.3 (Metrohm, Herisau, Switzerland) was used for peak evaluation.

2.6.4. High-performance liquid chromatography (HPLC)

Amino acid concentrations in extracts of soluble metabolites (see 2.6.1) were measured by high-performance liquid chromatography in a Dionex system (Dionex Softron, Germering, Germany) consisting of an automatic sample injector Dionex ASI-100, a Dionex P680 HPLC pump, and a Dionex RF2000 fluorescence detector. An AminoPac® PA1 column (Dionex Softron, Germering, Germany) was used for the separation of amino acids. 0.1 M Na-acetate with 7 mM triethanolamine (pH 5.2) was used as eluent. 20 µl of extraction was prepared for measurement by adding 60 µl boric acid buffer (0.2 M; pH 8.8) and 20 µl 6-aminoquinolyl-Nhydroxysuccinimidyl carbamate (AQC; 3 mg dissolved in 1.5 milliliter acetonitrile) were mixed. Samples were vortexed and incubated at 55°C for ten minutes to derivatize with AQC. Peaks were analyzed using Chromeleon software (Thermo Fisher Scientific, Waltham, Massachusetts, USA).

2.7. Subcellular metabolism analysis (non-aqueous fraction, NAF)

2.7.1. Extraction

The method was described in Fürtauer *et al.* (2016) with modification. For the most part, 8-10 mg of fresh sample was grinded with liquid nitrogen and then freeze-dried. All following steps were performed on ice. One milliliter of heptane

(C_7H_{16}; "7H", d: 0.684 g cm^{-3})- tetrachlorethylene (C_2C_{l4}; "TCE", d: 1.623 g cm^{-3}) mixture with a density of ρ = 1.3 g cm^{-3} was added into freeze-dried samples and then those samples were sonicated with ice bath for at least 45 min and then filtered with nylon gauze (200 µm porous) to remove particles. Centrifuge at 21,100g, 4°C for 10 min. Supernatant was transferred into new tube, meaning the sample from first gradient density. The pellet was resuspended with the next gradient density of "7H-TCE" (Table 2.4). The samples were sonicated again with ice bath for 20 min. Centrifuge at 21,100g, 4°C for 10 min. Supernatant was transferred into a new tube. Repeat this step with following gradient density of "7H-TCE" (**Table 2.4**) until the fraction 1.5 was added. At the least fraction, the pellet from fraction 1.5 (indicated as ρ = 1.5 3 g cm^{-3}) was resuspended with one ml 7H (ρ > 1.6 g cm^{-3}). All the supernatants from different fractions were split equally into three tubes and then those samples were dried by a vacuum concentrator (Eppendorf, Germany).

Table 2.4 The ratio of heptane and tetrachlorethylene (7H-TCE)

Fraction [cm^{-3}]	1.3	1.35	1.4	1.45	1.5
Ratio (ml) [Heptane:Tetrachlorethylen]	0.52:1	0.41:1	0.311:1	0.22:1	0.15:1

2.7.2. Marker enzyme measurement

To determine the proportion of vacuole, cytosol as well as plastids in the individual fractions, specific marker enzymes were used. The dried extraction of an aliquot was dissolved with 0.5 milliliter ice-cold extraction buffer (50 mM Tris-HCl, pH7.3; 5 mM MgCl2; 1 mM DTT, fresh added) and stored on ice for 15 min and then centrifuged for 5 min at 21100 g and 4°C. The enzyme-containing supernatant was used for further measurements.

Acid phosphatase was used as vacuolar marker enzyme. The samples were prepared for blank and assay for each gradient fraction. 75 µl of extractions were incubated with 225 µl of Ink B buffer (0.125% Triton™X-100 (w/v); 125 mM sodium acetate, pH 4.8) at 37 °C for 5 min in dark. To assay samples, 100 µl of substrate solution (1 mg/mL p-nitrophenylphosphate in Ink B buffer) were added and incubated at 37 °C for 25 min. Subsequently, the reactions were stopped by

adding 400 µl stopping solution (1 M Na_2CO_3) and the yellow color change was measured spectrophotometrically at 410 nm in the Infinite® M Nano microplate reader (Tecan). To the blanks, 400 µl Stopping solution was added before mixing with 100 µL substrate solution as a reference.

UDP-glucose pyrophosphorylase (UGPase) was used as cytosolic marker enzyme. Prepare UGP buffer: 100 mM Tris/HCl, pH 8.0; 2 mM $MgCl_2$; 2 mM NaF. 70 µl extractions from gradient fractions were mixed with 140 µl assay solution (1 reaction: 0.36 µl of 100 mM $NADP^+$; 0.56 µl 500 mM UDP-glucose; 14 µl 10 mM glucose-1,6-bisphosphate; 3 U/ml phosphoglucomutase; 1 U/ml glucose-6-phosphate dehydrogenase in UGPase buffer) in 96well at room temperature. The reaction was started by adding 7 µl of 100 mM Sodium pyrophosphate (NaPPi, $Na_4P_2O_7$). The increase of optical density at 334 nm was recorded for 6 min in Tecan. The slope of a linear regression was proportional to UGPase activity.

Alkaline Pyrophosphatase (PPase) was used as a plastid marker enzyme. For each gradient fraction, 20 µl of extractions were mixed with 800 µl assay buffer (50 mM Tris–HCl, pH 8.0; 10 mM $MgCl_2$; 1.3 mM Na-pyrophosphate, fresh prepared) and incubated at room temperature for 10 min. Assay buffer without extract was taken as reference. The reactions were stopped by incubation at 95 °C for 5 min. 150 µl of mixture were mixed with 50 µl mixed reagent solution (2.53 µl 5N H_2SO_4; 3.9 µl 100 mM ammonium molybdate; 15.6 µl 100 mM ascorbic acid; 5.2 µl antimony potassium tartrate (1 mg/ml), freshly mix) and optical density was measured at 882 nm in Tecan after 2–4 min.

2.7.3. Sugar measurement for NAF extraction

The second aliquot was used for sugar quantification which was described in Method 2.6.2. The subcellular metabolites were calculated using the algorithm described in *Fürtauer et al.* (2016).

2.8. Molecular biological methods

2.8.1. RNA isolations and cDNA synthesis

Total RNA from Arabidopsis was isolated by NucleoSpin® RNA Plant

Kit (Machery-Nagel, Düren, Germany), whereas RNA samples from potato tubers were prepared using TRIzol reagent (Invitrogen, USA) with RNA purification column (Machery-Nagel, Düren, Germany). The RNA was finally eluted in 30 µl of RNase-free ddH2O. 1µg of isolated RNA was used for cDNA synthesis, performed using the qScript™ cDNA synthesis Kit (Quanta Biosciences, Beverly, MA, USA) according to the associated protocol. cDNA was ten times diluted with RNase-free ddH2O before using.

2.8.2. Expression analysis by qRT-PCR

Gene-specific primers **(Supplemental table 2.1)** were used for real-time quantitative PCR (qRT-PCR) in a CF X96™ real time cycler (Bio-Rad, Germany). Gene expression was quantified by interpolating the fluorescence from SYBR Green (iQ SYBR® Green; Bio-Rad Laboratories, Germany) into the template DNA. The expression of the reference gene, *AtActin2* (AT3G18780, (Gupta *et al.,* 2016) for Arabidopsis and *StEF-1α* ((LOC102577640, (Nicot *et al.*, 2005)) for the potato, were used to determine relative expression levels according to the following equation: $1000*2^{(Ct^{target\ genes} - Ct^{reference\ gene})}$.

Table 2.5 Primers used for qRT-PCR analysis

Gene ID	Gene name		Forward (5'->3')
AT4G22200	*AtAKT2*	F:	CACAGCTTCTTGTCCGTGAA
		R:	ACTCTTCGAAGTCGCCAAAA
AT3G18780	*AtActin2/8*	F:	ACGGTAACATTGTGCTCAGTGGTG
		R:	CTTGGAGATCCACATCTGCTGGA
AT4G13420	*AtHAK5*	F:	AAGAGGAACCAAATGCTGAGACA
		R:	GCCCCGATGAAGGGACAT
AT5G27150	*AtNHX1*	F:	CTACCTATTACCGCACCAGAACG
		R:	CTCAATGAACGAGTCTTGGTCC
AT1G20840	*AtTST1*	F:	TGCTTCTTCGTGATGGGTTACGGT
		R:	CCAACTAGTCCGATCGAGCT
AT4G35300	*AtTST2*	F:	CATGGATCTTTCTGGTCGAAGGAC
		R:	GATAAGACCGCGTGCACAATGC
AT1G22710	*AtSUC2*	F:	TAGCCATTGTCGTCCCTCAGATG
		R:	ACCACCGAATAGTTCGTCGAATGG
AB061263	*Stef1a*	F:	ATTGGAAACGGATATGCTCCA
		R:	TCCTTACCTGAACGCCTGTCA
X86021	*StSKT1*	F:	CTAGGAAAACCAGAAGGACC
		R:	GTTCTGAAGGGATTGTTGATATG

PGSC0003DMG400009213	*StSUT1*	F: R:	TTCCATAGCTGCTGGTGTTC TACCAGAAATGGGTCCACAA
PGSC0003DMP400056212	*StSWEET11b*	F: R:	AACCTGAAGTCATTGTGAAGG CAGTAACAACCAGTAAACCTG
PGSC0003DMT400025886	*StTST1*	F: R:	TAGCTGGCTCGACCACTTTT CGCAACTAACACAGCACCAT
PGSC0003DMT400048038	*StTST2*	F: R:	TGTTTCTCTCAGGTGCTCCA GCAGCAGCAAGTGCAATAAG

2.8.3. Expression analysis by RNA sequencing (RNASeq)

The preparation of the RNA library and the sequencing of the transcriptome was carried out by Novogene (Beijing, China). In short description: Before library preparation, the isolated RNA was first analyzed for quality (sample QC). The appropriate library was prepared according to *Arabidopsis thaliana*, and subsequently tested for its quality (Library QC). Next, a 150 bp paired-end sequencing strategy was used to sequence the samples. To remove ribosomal RNA and generate the cDNA libraries, poly(A)+ RNA enrichment and mRNA fragmentation followed by random-prime cDNA synthesis was performed. Illumina PE150 technology was used to sequence the sample and the final stage involved the bioinformatics analysis. (https://en.novogene.com/). The bioinformatics analysis was also performed by Novogene, following the program as described in **Table 2.6**. To identify specific genes responding to silicon, responding genes in silicon treated plants were compared to the plants without silicon treated plants at three time points, before drought (0 h), six hours after drought (6 h), eight days after drought (8 d). To investigate if the biological processes were highly altered, the program DESeq2 (Love *et al.*, 2014) was used. All genes with a p-value < 0.05 were included.

Table 2.6 Programs used for bioinformatics analysis of RNA-Seq.

Analyse	Software	Parameter	Funktion
Mapping	Hisat	Dta-phred33	Mapping to the reference genome
Novel gene	StringTie	Default	Assembling the transcripts
prediction	GFFCompare	Default	Comparison of the transcriptomes
Quantifikation	FeatureCounts	Default	-

Differential analysis	DESeq2	padj <= 0,05
	EdgeR	padj <= 0,005 Ilog2(FC)I >= 1

2.9. Software tools

The In preparing this thesis, a variety of editing tools were used to ensure the highest standard of writing. Among these were Grammarly, which assisted with grammar and spelling checks, and ChatGPT, which aided in refining the structure of the text. Moreover, the generation of visual content and the computation of statistical data were achieved using Prism - GraphPad. This software facilitated the precise calculation of statistical values, thereby enhancing both the validity and clarity of the research presented.

2.10. Statistics

Data from at least three independent replicates were presented as means ± SD. Analysis of variance (ANOVA) by Tukey-Kramer’s test conducting all pairwise multiple comparison procedures was performed using Prism software, version 9. A value of $p < 0.05$ was considered a significant difference, which are represented by different letters.

3. Results

Drought is a severe problem that causes decreased crop production. Therefore, enhancing plant drought tolerance is a key step in reducing yield loss. Previous reports suggest that potassium and silicon fertilization can alleviate damage from drought stress, but the underlying mechanisms are far from understood. Therefore, we aimed to investigate the physiological and molecular functions of potassium and silicon in improving plant drought tolerance. To do so, we used the model plant *Arabidopsis thaliana* and the crop plant *Solanum tuberosum* to analyze the effects of potassium and silicon on drought tolerance.

3.1. Potassium effects on drought tolerance in Arabidopsis

The major limitation for plant growth and crop production is soil water availability. Plant development depends on the vascular circulation of water, nutrients, and assimilates (Lucas *et al.*, 2013). Sucrose and potassium are the major driving forces keeping vascular circulation going. The understanding of this interaction between potassium and sucrose in vascular mass flow might be a key point in increasing plant drought tolerance. To search for potassium-dependent and sugar-related responses under drought conditions, soil drought experiments with different levels of potassium supply were established. Furthermore, a hydroponic drought system with different potassium levels supplied to the model plant Arabidopsis was created.

3.1.1. Low potassium levels impact Arabidopsis shoots growth on soil under control and drought conditions

To investigate the effects of potassium on plant drought tolerance, plants were grown in nutrition-poor soil substrate mixed with different levels of potassium fertilization (K0, 0 mg Kg^{-1} soil; K1, 27 mg Kg^{-1} soil; K2, 142 mg Kg^{-1} soil; K3, 500 mg Kg-1 soil). The water content of the soil substrates was reduced until it reached 50% of field capacity (FC) **(Method 2.2)**. Under well-watered control conditions (100% FC), there were no substantial potassium-dependent differences among the plants **(Figure 3.1 A)**. Regarding biomass, the K0 and K1 plants had about 85% of the fresh weight of K2 plants under control conditions. Interestingly, the

fresh weight of the shoots from plants grown with a high potassium supply (K3) did not significantly differ from the K2 plants. In contrast, under drought conditions, the growth of K0 plants was markedly impacted, with their fresh weight being only 0.6 times that of the plants grown with low (K1), standard (K2), and high (K3) potassium supply **(Figure 3.1 B)**. To determine the growth effects of the different potassium fertilization levels during drought stress, we calculated the relative growth index ($FW_{drought}$/ $FW_{control}$), which showed the amount of biomass reduction under drought relative to control conditions. The K2 plants exhibited a 56% reduction in biomass, while the K1 plants had only a 20% reduction. Moreover, the K3 plants did not show a significant difference compared to the K2 condition **(Figure 3.1 C)**.

To assess the recovery rate after severe drought stress, we subjected two-week-old plants to three weeks without watering, and then we allowed them to recover for two days by watering them again. We then determined the survival rate. None of the K0-treated plants survived after three weeks without watering **(Supplemental figure 1 A)**, whereas 75% of the K1- and K2-treated plants were able to survive, and only 38% of the K3-treated plants survived the severe drought treatment **(Supplemental figure 1 B)**.

Taken together, the data suggest that plants without potassium supply exhibit the lowest drought stress resistance, while those with low potassium supply did not show a worse performance in terms of biomass accumulation and drought survival compared to the K2 plants. Additionally, higher potassium supply did not improve drought tolerance.

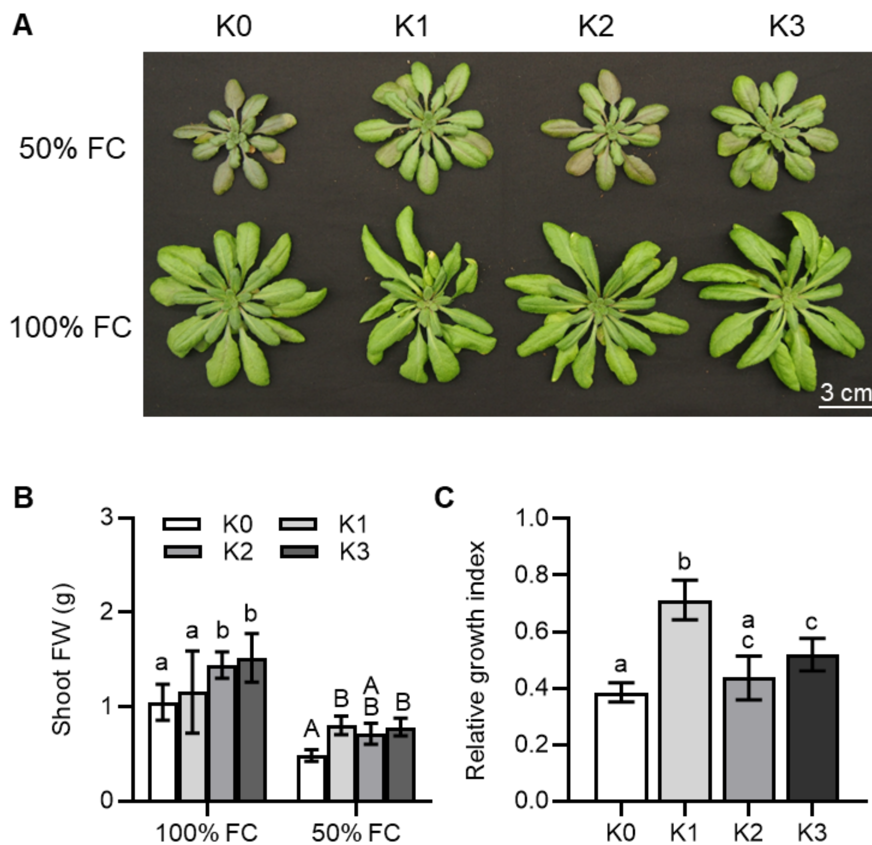


Figure 3.1 Potassium affect growth during control and drought stress conditions. Watering was ceased for three-week-old plants, which were then subjected to drought stress conditions (50% FC) for a period of three weeks. (A) Plant morphology after three weeks of cultivation under control (100% FC) or drought stress (50% FC) conditions. The plants were six-week-old at the documentation time. (B) Plant fresh weight was determined three weeks after stopping watering. (C) The relative growth index during the drought stress was calculated in comparison to the control condition. The parameter was calculated with the FW from six-week-old plants. Results are means ± SD from six or more independent biological replicates ($n \geq 6$). Different letters indicate different level of significance determined between different potassium conditions by two-way ANOVA calculation with post-hoc (Tukey's multiple comparisons test) testing to $p < 0.05$.

3.1.2. Potassium deficiency results in altered metabolite accumulation in leaves

Compatible solutes (including soluble sugars and amino acids) and inorganic ions accumulate in plant cells during drought stress (Hummel *et al.,* 2010, Takahashi *et al.,* 2020, Ilyas *et al.,* 2021). To investigate the effect of different levels of potassium fertilization on metabolite of plants, we determined the contents of sugars, starch, and potassium. Three-week-old plants grown on soil mixed with varying levels of potassium were exposed to drought stress (50% FC) for three weeks and afterwards shoots were harvested for metabolite analysis.

Under well-watered control conditions (100% FC), the total sugar levels in the respective plants were generally lower than those of plants grown under drought conditions. The accumulation of sugars in the plants with different levels of potassium was not significantly different under control conditions **(Figure 3.2 A, 100% FC)**. However, under drought stress (50% FC), the accumulation of glucose was 1.3-times higher in the shoots of K0 plants than in K1, K2, and K3 plants, while the levels of the other three sugars were not significantly different between K1, K2, and K3 plants **(Figure 3.2 A, 50% FC)**. This suggests that the K0 plants required more sugar to adjust osmotic pressure under drought stress than the K1, K2, and K3 plants. The accumulation of starch under control conditions increased in a K-dependent manner until K2, indicating that increasing potassium supply up to 142 mg per kilogram of soil led to higher starch accumulation. This K-dependent pattern was also observed in the plants under drought stress. However, a further increase of potassium supply of up to 500 mg per kilogram of soil did not result in higher starch accumulation. Additionally, like the observed sugar levels, the starch levels in the shoots during drought stress were significantly higher compared to the respective K levels under control conditions **(Figure 3.2 B)**.

Apparently, different levels of potassium fertilization alter the carbohydrate composition in plants. To confirm that the different levels of potassium also affected the potassium status in plants under both control and drought conditions, we analyzed the potassium content in the shoots. Surprisingly, the concentration of potassium in K0 plants was 1.4 times higher than in K1 plants under control conditions (100% FC). However, this pattern was not observed under drought stress (50% FC). The potassium content was not significantly different between

the different levels of potassium supply conditions under drought stress **(Figure 3.2 C)**. To confirm that the amount of potassium application affects the potassium content in the phloem, we collected the phloem exudate from plants with different levels of potassium supply. The concentration of potassium in the phloem exudate was slightly increased in a K-dependent manner and significantly higher in K3-supplied plants **(Figure 3.2 D)**.

Taken together, our results suggest that without potassium fertilization (K0), plants produce less starch in the shoots under control conditions, while the K0 plants accumulate more glucose to survive under drought stress. Increasing the level of potassium supply increases the production of starch under control conditions until the K2 level of fertilization. Further increasing potassium supply (K3) increases the potassium level in plants, but it does not increase the production of carbohydrates under control conditions.

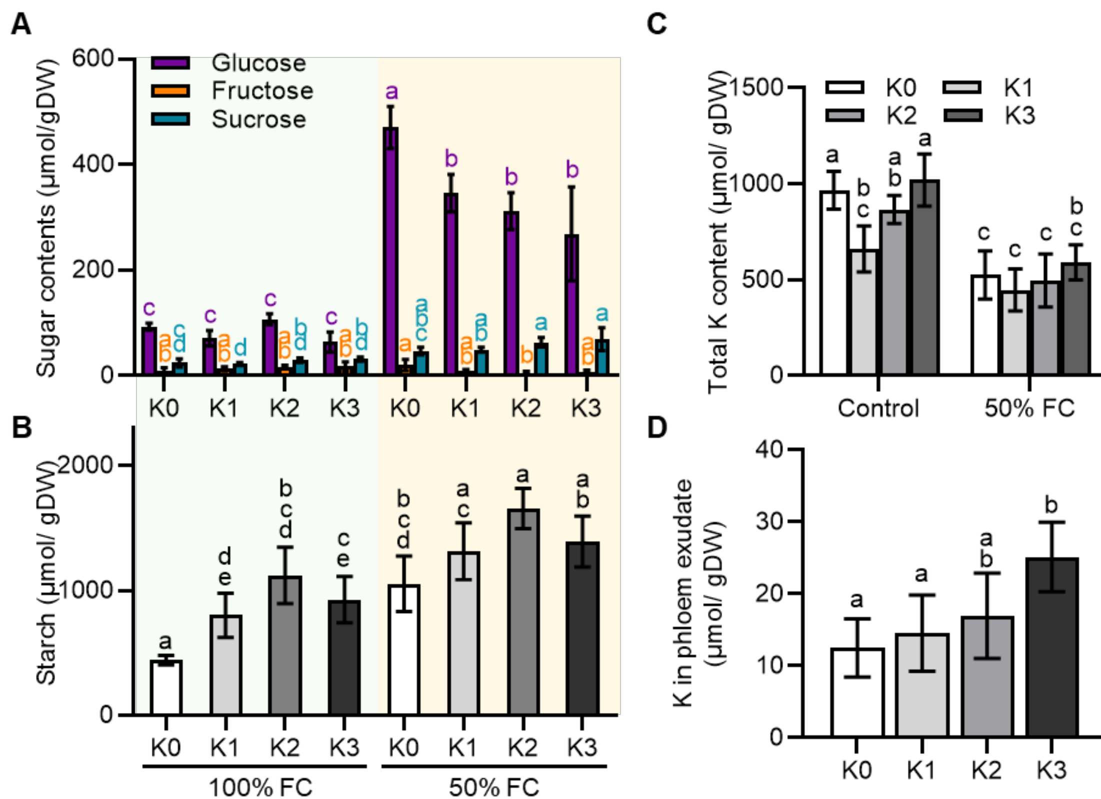


Figure 3.2. Sugar and starch accumulation of Arabidopsis rosette tissue grown under different potassium fertilization during drought conditions. The analysis was done with six-week-old plants. (A) Sugar contents and (B) starch content in shoot tissue under 100% FC (well-watered) and 50 % FC (drought) conditions. (C) Total potassium content in the shoots. (D) The level of potassium in the phloem. Results are means ± SD from four or more independent biological replicates (n ≥ 4). The letters represent the significance determined between different potassium conditions by two-way ANOVA calculation with post-hoc (Tukey's multiple comparisons test) testing to $p < 0.05$.

3.1.3. K-dependent effects in shoots and root growth in the hydroponic growth system under drought stress

To analyze K-dependent performance of plants independently and at the same be able to analyze effects on root development under drought stress, we employed a hydroponic system using a modified Hoagland solution with varying potassium levels (K0, 0 mM; K1, 0.25 mM; K2, 1.13 mM as standard condition; K3, 2.5 mM). After four weeks of growth with different K conditions, PEG8000 was applied to simulate drought stress. For the control in this analysis, the plants were continuously grown on their respective K-media.

Under control conditions, plant growth was significantly impaired in the absence of potassium fertilization (K0). However, K1, K2, and K3 treatments did not show any significant differences in plant growth **(Figure 3.3 A)**. Interestingly, different levels of potassium supply led to altered survival rates under drought stress, with only 44% of K0-plants surviving compared to 100% of K1-plants. However, higher potassium supply (K2 and K3) did not improve survival rates as effectively as the K1 treatment **(Supplemental figure 2)**. To quantify the effects of different potassium concentrations on plant development under drought stress, we measured shoot **(Figure 3.3 B-D)** and root **(Figure 3.3 E-G)** growth parameters. Under control conditions, K0-plants had significantly smaller shoot biomass than K1, K2, and K3 plants, while K1 treatment restored shoot biomass to a level comparable to that of K2-treated plants. After drought stress, shoot biomass decreased in all treatments, with K0 plants exhibiting the most significant loss and K3 plants losing more biomass than K2-treated plants **(Figure 3.3 B)**. Similarly, K0-plants had the smallest shoot dry weight, while K3 treatment did not increase plant biomass under drought stress **(Figure 3.3 C)**. The water content of shoots also followed this trend **(Figure 3.3 D)**.

For root growth, K0 treatment led to severe impairment under control conditions, with the biomass being four times lower compared to that of the K2 plants. This damage was mitigated by K1 supply, with K3-treated plants even showing a slight gain in root biomass compared to K2 plants. Under drought stress, root biomass did not change in K0, K1, and K2 treatments, but K3 plants lost more root biomass than K2-treated plants **(Figure 3.3 E)**. Similarly, K3 treatment did not increase root dry weight under drought stress compared to K2 treatment **(Figure 3.3 F)**. The

water content of roots did not differ between K0, K1, and K2 plants under drought stress, but K3 plants lost a significant amount of water **(Figure 3.3 G)**. The root-to-shoot ratio (R/S ratio) was calculated, which is known to increase in response to drought stress. Under control conditions, K0-plants had the lowest R/S ratio, while K1, K2, and K3-plants did not differ significantly. Under drought stress, all K treatments led to an increase in the R/S ratio compared to control conditions, with K3 treatment resulting in a significantly higher R/S ratio than K0 or K1 treatments **(Figure 3.3 H)**. However, the R/S ratio did not differ significantly between the K treatments after drought stress relative to their respective control conditions **(Figure 3.3 I)**. These results suggest that the growth of both shoots and roots did not differ in a K-dependent manner under control and drought conditions, except for the severely impaired growth observed under K0 treatments for both conditions relative to K2 treatment.

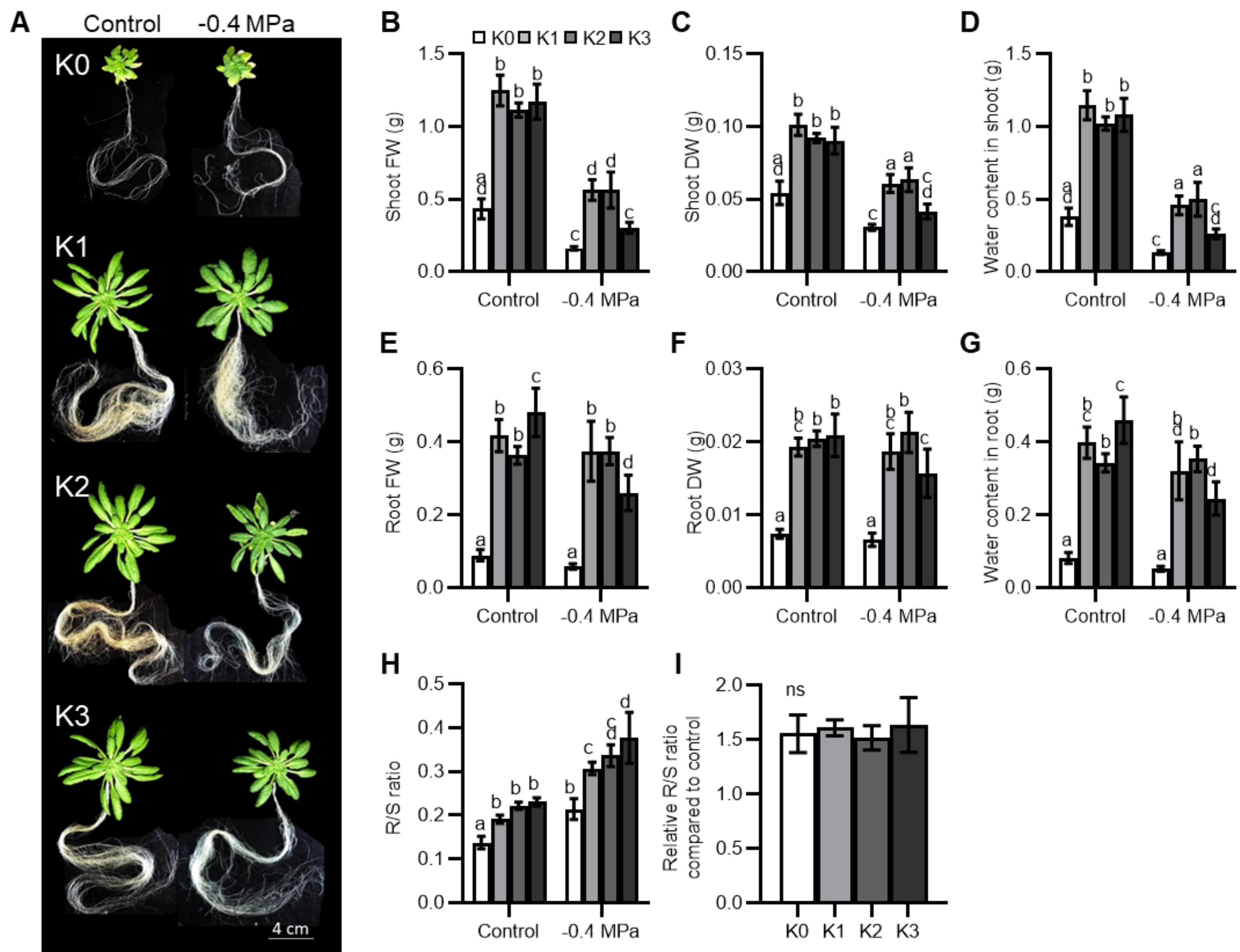


Figure 3.3. Potassium-dependent shoot and root growth effects during drought stress in a hydroponic system. The Plants were grown in the media with different levels of K (K-medias) for four weeks and then were treated with the respective K-medias as the control condition or with the K-media PEG8000 (-0.4 MPa) to simulate drought stress condition. (A) Arabidopsis plants grew under control and -0.4MPa. The photos were taken eight days after the PEG8000 treatment. Arabidopsis shoots (B) fresh weight, (C) dry weight, and (D) water content under control conditions and after PEG8000 treatment. Arabidopsis root (E) fresh weight, (F) dry weight, and (G) water content under control conditions and after PEG8000 treatment. (H) The root/shoot ratio was calculated for the dry weight. (I) Relative root/shoot ratio compared to control. All parameters were quantified eight days after the PEG8000 treatment. Results are means ± SD from five independent biological replicates (n=5). The letters represent the significance determined between different potassium conditions by two-way

ANOVA calculation with post-hoc (Tukey's multiple comparisons test) testing to $p < 0.05$.

3.1.4. Tissue-specific alterations in metabolic accumulation under different levels of potassium supply

As observed in plants grown on soil with different potassium supply, an accumulation of glucose was induced in the shoot under drought stress **(Figure 3.2)**. To confirm the levels of metabolites, such as sugars and starch, in both shoot and root tissue, the plants grown in hydroponics with modified Hoagland solution were subjected to PEG8000 (-0.4 MPa) to simulate drought stress **(Method 2.3)**. The metabolites were extracted from the plants eight days after drought treatment with PEG8000 (-0.4 MPa), while the plants grown in the same media without PEG8000 were used as the control. Under control conditions, the sugar contents in shoots were generally lower than under drought conditions. K0 plants already accumulated 4.5-times more glucose, 4.6-times more fructose, and 1.8-times more sucrose in shoots than K1, K2, and K3 plants. There was no difference between K1, K2, and K3 plants under control conditions **(Figure 3.4 A, left)**. Under drought stress (-0.4 MPa), K0 plants accumulated even more monosaccharides in shoots. Especially fructose levels were higher, around four-times higher than in K0 plants under control conditions. The accumulation of glucose in K1 and K2 plants was induced during drought stress. However, there were no differences between K1 and K2 plants. In K3 plants, not only was glucose accumulated, but also fructose and sucrose were highly accumulated in shoots; the levels were around 6.8 times for glucose, eight times for fructose, and 2.3 times for sucrose in comparison to K3 plants under control conditions. Moreover, the amplification of sugar accumulation under drought stress in K3 shoots was more substantial than in those of K1 and K2 plants **(Figure 3.4 A, right)**. Since starch accumulation depends on the metabolism of soluble sugars, we analyzed the starch content in shoots. The starch content in shoots did not significantly decrease in a K-dependent manner from K1 over K2 to K3 plants, while the starch content was reduced in shoots of plants without potassium supply (K0) under control conditions. Under drought stress, the starch content in the shoots of K0-plants was significantly lower than in K0-plants under control conditions. Compared to the respective plants under control conditions, the starch content in

shoots of K1, K2, and K3 plants was not altered by drought stress, even though the starch level in K3 plants was 25% lower than in K1 plants under drought stress **(Figure 3.4 B)**.

In root tissue, glucose in K0 plants was significantly higher than in K1 plants, and the sucrose content in K0 plants was generally higher than in other K-treatments under control conditions. Drought stress did not alter sugar contents in K0 and K2 roots, while fructose content in K1-plants was increased. In the roots of K3-plants, sucrose levels were induced by drought treatment in comparison to K3-plants under control conditions **(Figure 3.4 C)**. The starch content in the roots of K0-plants was slightly higher than in other potassium-fertilized plants under control conditions, even though this accumulation pattern in K0-plants was not observed in K0-plants under drought stress **(Figure 3.4 D)**.

Next, we analyzed the potassium levels in these plants. The concentration of potassium in the shoots of K0 plants was only around 300 µmol per gram of dry weight, while the potassium content increased with increasing amounts of potassium supply, reaching 937 µmol per gram of dry weight in K1 plants, 1145 µmol per gram of dry weight in K2 plants, and 1403 µmol per gram of dry weight in K3 plants. This increase in potassium level in the shoots in a K-dependent manner was also maintained when the plants were under drought stress **(Figure 3.4 E)**. The potassium content in the roots of K0 plants was obviously the lowest since there was no extra potassium in the K0 media. Surprisingly, K1 plants had 30% more potassium in their roots than K2 plants. Higher potassium supply (K3) did not lead to a higher potassium accumulation in the roots compared to the K2 treatment. When the plants were under drought stress, the potassium levels in the roots of K0 and K1 plants were reduced, while the K2 and K3 plants maintained the same level of potassium in the roots compared to the control conditions **(Figure 3.4 F)**. To corroborate whether the observed phenotypes were influenced by alterations in other ions as well, the concentrations of various cations and anions were examined. The levels of other ions, such as sodium (Na), were also found to be impacted by variations in potassium supply. The Na content was higher in K0 and K1 plants, most likely because the potassium in the media was replaced by sodium **(Method 2.3)**. Under drought conditions, the sodium content was 1.5-times higher in the shoots of K1 plants compared to K1 plants under the

control condition **(Supplemental Table 3.1)**. This sodium accumulation in K1 plants might as well be the effect of induced unspecific Na import by K transporters induced by low potassium conditions. These results suggest that the potassium content in the shoots increases in a K-dependent manner. Without any potassium supply (K0), sugars and starch are highly accumulating in whole plants (shoot and root tissue) under control and drought stress. High potassium supply (K3) induces higher sugar accumulation in the shoot but not roots.

Drought stress reduces sugar long-distance transport in phloem because water limitation impacts vascular mass flow (Stanfield & Bartlett, 2022). To prove whether more potassium supply also improves sugar long-distance transport in the phloem, we analyzed the sugar contents in the phloem exudate. The concentration of total sugars was significantly higher in the phloem exudate of K0 plants, which was around 2.3 times higher than in other K-treated plants, while there was no difference in phloem exudate between K1, K2, and K3 plants **(Figure 3.4 G)**. Additionally, the potassium content in the phloem of K0 plants was slightly lower, although not significantly **(Supplemental figure 3)**. This result suggests that more potassium supply does not affect the concentration of potassium in the phloem. Without potassium supply (K0), the sugar content in the phloem was increased in K0 plants, which might be due to K-deficiency leading to an obstruction of sugar translocation in plants.

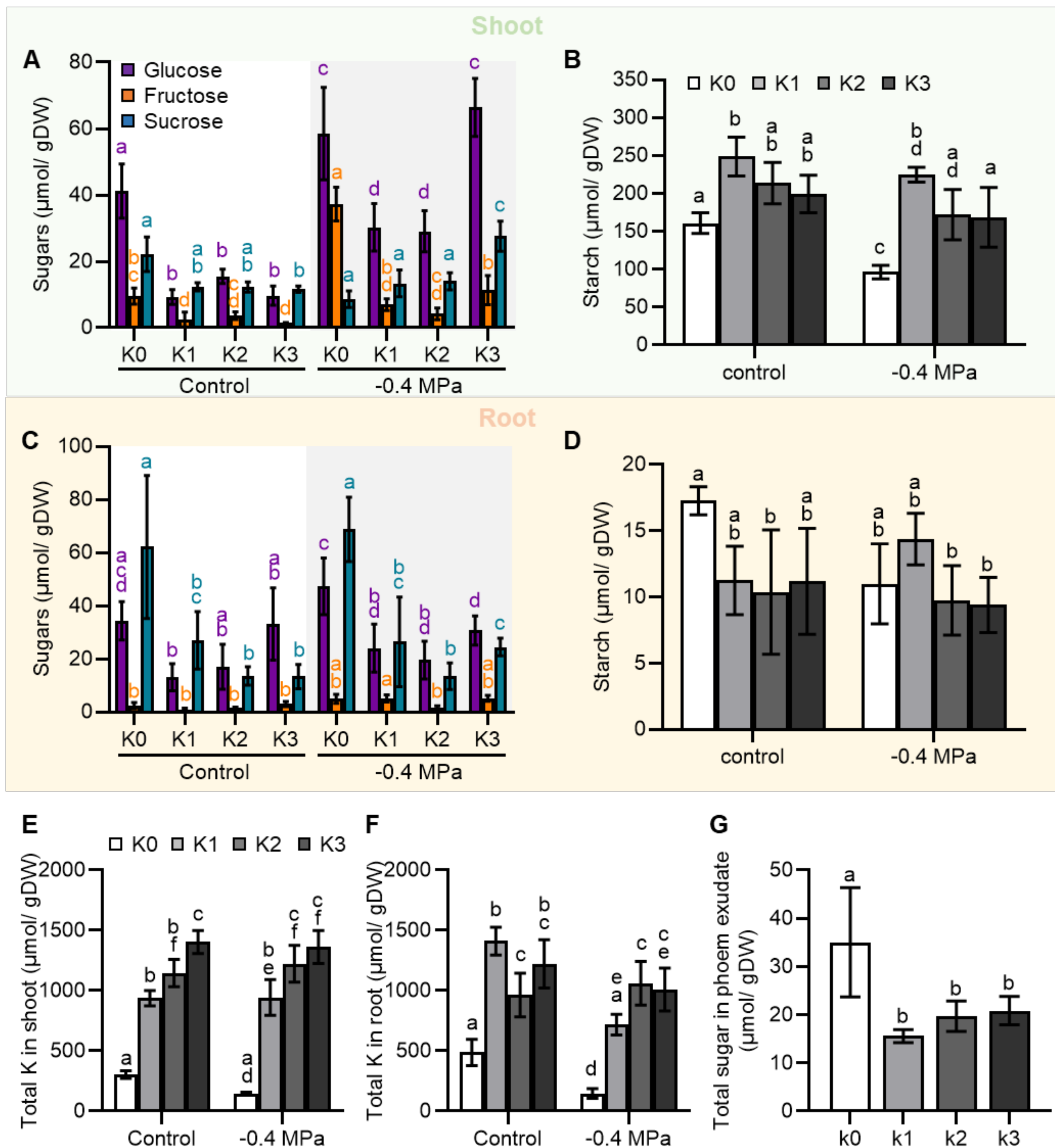


Figure 3.4. The changing of sugars, starch, and potassium in hydroponic plants during drought stress (-0.4 MPa). The metabolites were extracted eight days after the PEG8000 treatment. (A and B) Sugar and starch contents in the shoot. (C and D) Sugar and starch content in the root. (E and F) Potassium level in the shoot and root tissue. (G) Total sugar in phloem exudate. Results are means ± SD from four or more independent biological replicates ($n \geq 4$). The letters represent the significance determined between different potassium conditions by two-way ANOVA calculation with post-hoc (Tukey's multiple comparisons test) testing to $p < 0.05$.

3.1.5. Potassium treatment alters expression of sugar and potassium transporters under drought stress

The accumulation of sugars and potassium is altered in plants by drought stress **(Figure 3.4)**. The expression of genes coding for sugar and potassium transporters in both shoots and roots under control and drought conditions was compared. The gene expressions were analyzed before drought stress (zero hours) as the control and six hours after drought stress was simulated by PEG8000 treatment. The expression of genes coding for TONOPLAST SUGAR TRANSPORTERS, the major vacuolar monosaccharide transporters (Wormit *et al.*, 2006), were affected by the K supply. *AtTST1* expression was lower while *AtTST2* expression was higher in the shoot of K0-plants in comparison to other K-treatments under control conditions. There was no significant difference between K1, K2, and K3 in general. During drought stress, the expression of *AtTSTs* did not show significant differences between the different K-treatments. However, the expressions of *AtTST1* in the shoot of K1, K2, and K3 plants were reduced under drought stress in comparison to the control, respectively **(Figure 3.5 A)**. Additionally, the expression of *AtTST2* in K1, K2, and K3 plants was induced 24 hours after PEG8000 treatment **(Supplemental figure 4 A)**. The expression patterns of *AtTSTs* were different in the root. The expression of *AtTST1* was lower in plants without potassium (K0) or under low potassium levels (K1) supplied in comparison to K2 plants. *AtTST1* was strongly induced in the root of K0 and K1 plants, while it was not altered in the root of K2 and K3 plants by the drought stress treatment. The expression level of *AtTST2* in the root was not altered by different levels of potassium treatment in both control and drought conditions **(Figure 3.5 B)**. These results showed that *AtTST1* and *AtTST2* have different expression patterns under different levels of potassium fertilization under drought stress.

The SUCROSE-PROTON SYMPORTER 2 (SUC2) plays a role in sugar long-distance transport and is therefore an important key player in maintaining plant vascular flow and carbon and energy supply from source to sink tissues under both control and drought stress conditions (Stanfield & Bartlett, 2022). Therefore, we analyzed the expression of *AtSUC2*. Different levels of potassium supply did not alter the expression of *AtSUC2* in the shoot of the respective plants under control conditions. Under drought stress, the expression of *AtSUC2* was not

altered in the shoot of K0 plants compared to control condition, but *AtSUC2* expression was increased in K1, K2, and K3 plants. However, there was no significant difference between K1, K2, and K3-plants **(Figure 3.5 C)**. In root tissue, the expression of *AtSUC2* was not changed by different levels of potassium supply under control conditions. Nevertheless, the *AtSUC2* expression in K1-plants was increased but not in the other potassium-treated plants under drought stress (Figure 3.5 D).

The K^+ channel AKT2 was proposed to release potassium out of the phloem to increase the efficiency of SUC2 (Deeken *et al.,* 2002, Gajdanowicz *et al.,* 2011). Therefore, we analyzed the expression of *AKT2* in plants with different levels of K supply. Without K supply (K0), the expression of *AKT2* was already higher than in the other potassium treatments in the shoot tissue under control conditions, and this expression pattern was even more significant under drought stress. The expression of *AKT2* was not altered by the K1, K2, and K3 treatment in both control and drought conditions **(Figure 3.5 E)**. To confirm the level of potassium deficiency in the K0 condition, we measured the expression of HAK5, a HIGH-AFFINITY K TRANSPORTER, which is a marker of K deficiency stress (Chen *et al.*, 2015, Chen *et al.*, 2017). *HAK5* was strongly induced in the root tissue of K0-plants under both control and drought conditions. It did not show a significant difference in the roots of K1, K2, and K3 plants, indicating that the K1, K2, and K3 plants were not under potassium deficiency conditions **(Figure 3.5 D)**.

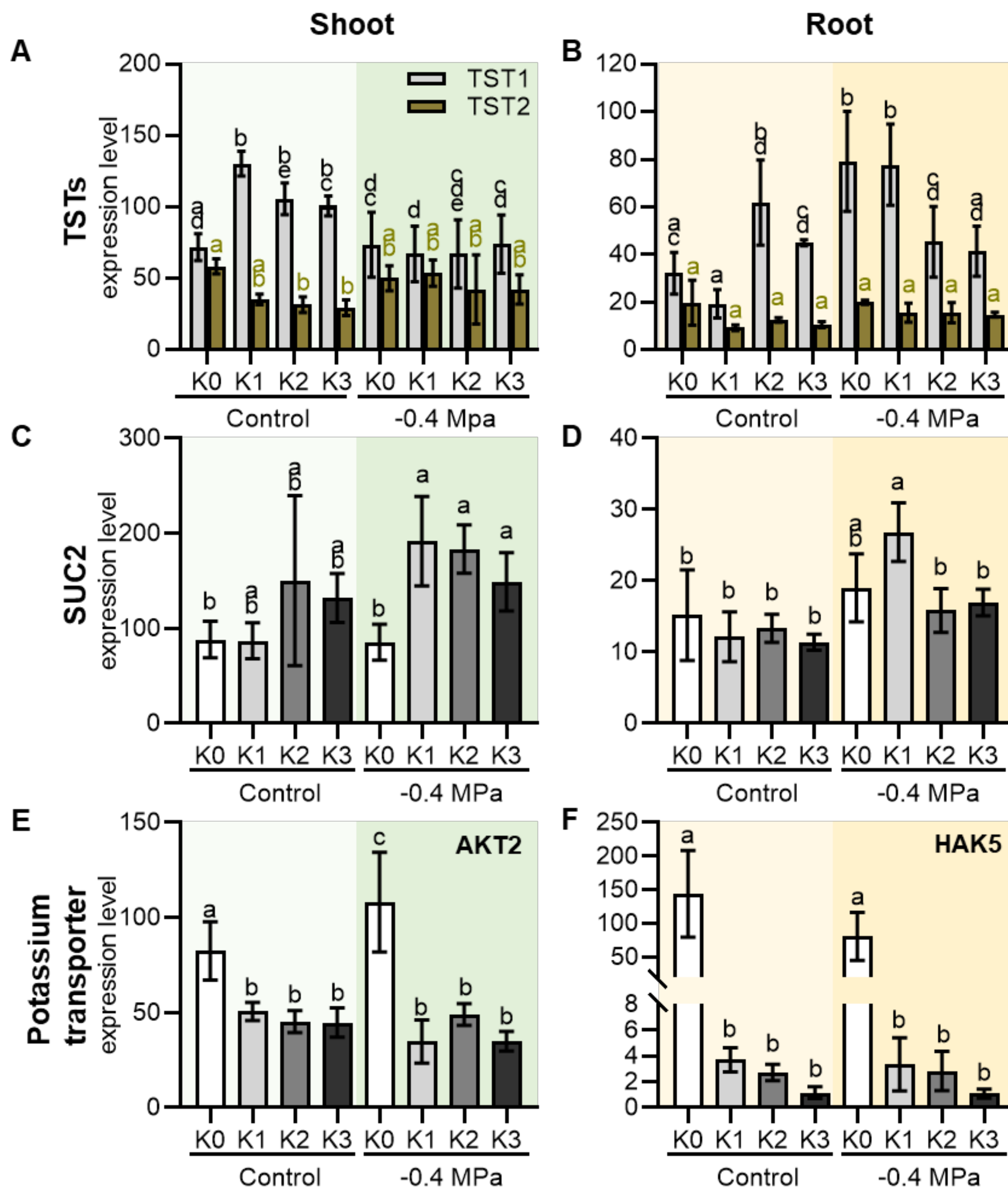


Figure 3.5. Expression of sugar and ion transporters in different tissue during drought stress with differing potassium supplies. (A and B) Expression of tonoplast sugar transporters (*TST1* and *TST2*) in shoot and root. (C and D) Expression of sucrose-proton SYMPORTER 2 (*SUC2*) in shoot and root. (E and F) Expression of potassium channel (*AKT2*) in shoot and high-affinity K transporter (*HAK5*) in roots. Total RNA was isolated from the plants before the PEG8000 treatment (control) and six hours after the PEG8000 treatment (-0.4 MPa). Relative expression level by normalizing to internal control, *AtActin2*, is shown. Results are means ± SD from four independent biological replicates (n=4). The letters represent the significance determined between different potassium conditions by two-way ANOVA calculation with post-hoc (Tukey's multiple comparisons test) testing to $p < 0.05$.

3.1.6. Potato plant growth and tuber yield under different potassium treatments

Potassium has been known to play an important role in phloem mass flow (Gajdanowicz *et al.,* 2011). In this study, we were interested in revealing the relationship between potassium fertilization and crop sink strength. Therefore, we compared the growth of "Désirée" potato plants (as a wild type in this study) with that of NTT-antisense lines. NTT-antisense lines have significantly reduced the activity of the amyloplast-located ATP/ADP translocator that supplies amyloplasts in the heterotrophic tuber tissue with ATP, thereby decreasing the fuel for tuber starch biosynthesis. Consequently, NTT-antisense lines exhibit reduced tuber starch and sink strength. This reduction in starch is accompanied by elevated soluble sugars in the tuber, which are responsible for the "ginger" phenotype of the tubers (Tjaden *et al.*, 1998). The NTT-antisense lines provide a tool to observe K-dependent sugar translocation in dependence on the plant's sink strength. Most modern tuber crops have been bred to have higher sink strength. Therefore, it will be interesting to understand whether potassium and sugar translocation efficiencies are co-regulated with sink strength. To investigate the effect of potassium (K) fertilization on potato tuber yield, growth experiments with different levels of potassium fertilization were performed. The potato tubers were planted in pots of soil with different amounts of K fertilization (K1, 27 mg Kg^{-1} soil; K2, 142 mg Kg^{-1} soil; K3, 500 mg Kg^{-1} soil, **Method 2.1.2**). Tuber yield was counted three months after the emergence of the shoot **(Method 2.1.1)**.

In general, the plants showed different growth behavior under different potassium fertilization treatments. Both WT and NTT-antisense plants grown under K1 conditions were smaller than the plants grown under K2 and K3 plants. Moreover, the K1-treated NTT-antisense lines had fewer leaves in comparison to K1-treated WT plants **(Figure 3.6 A)**. In general, the yield parameters of the NTT-antisense line were two times lower than the ones of the WT. This phenotype also has been published previously (Tjaden *et al.,* 1998). The total tuber yield of WT plants was not significantly decreased by K1 treatment in comparison to the K2-treated WT plants. However, the total tuber yield of NTT-antisense plants was reduced when supplied with the K1 fertilizer in comparison to K2-treated NTT-antisense plants. In contrast, the WT plants yielded 57 grams more of tuber when supplied with a

greater amount of potassium (K3), while the tuber yield was not significantly altered in NTT-antisense plants **(Figure 3.6 B)**. The same pattern was also found in tuber dry matter **(Figure 3.6C)**. Besides, the tuber numbers of both WT and NTT-antisense plants were not altered by different levels of potassium fertilization **(Figure 3.6 D)**.

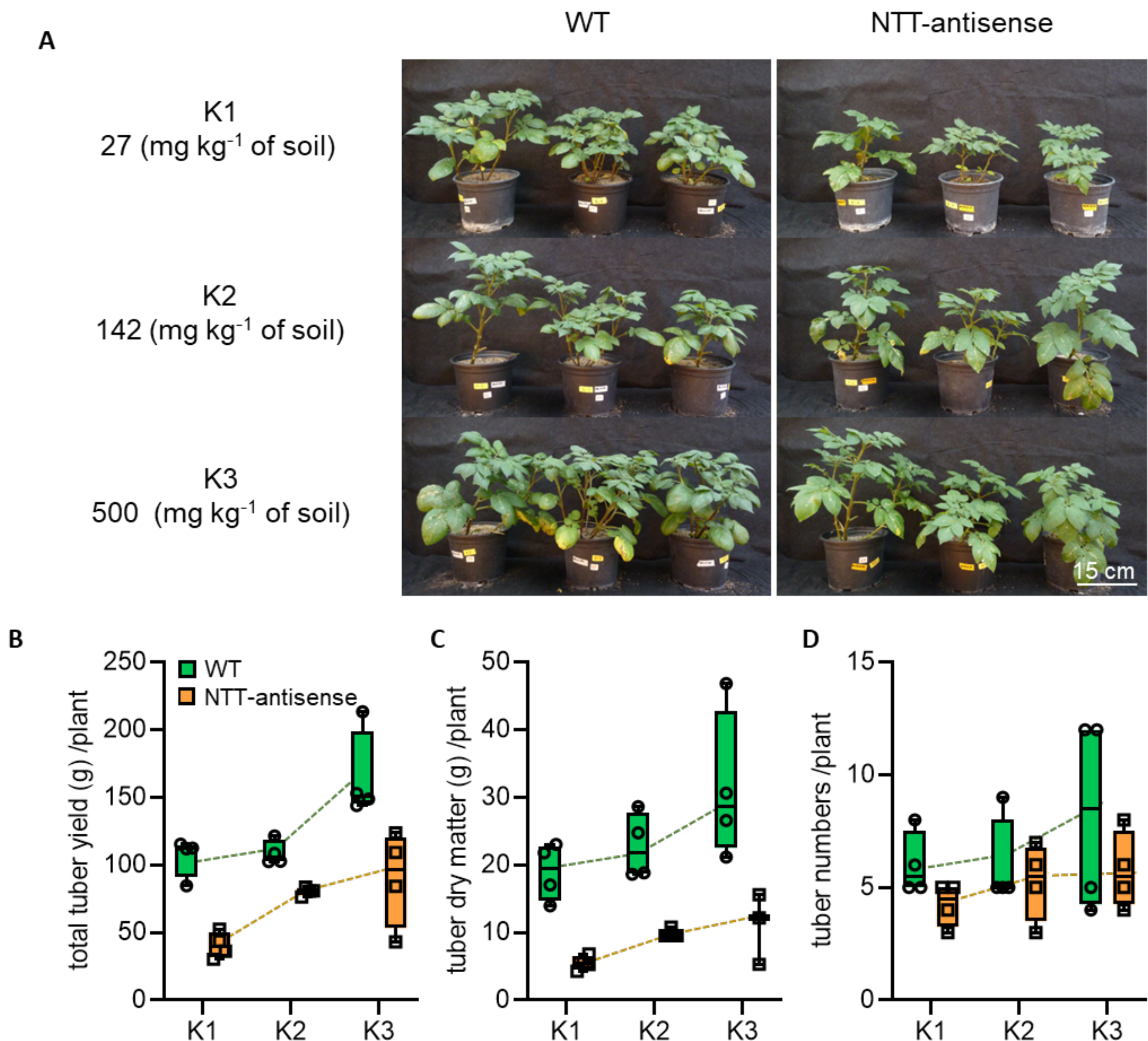


Figure 3.6. Plant phenotypes during K treatment. (A) Photos were taken 25 days after the emergence of the shoot. Bar = 15 cm. (B) Potassium fertilization effects on tuber yield, (C) biomass, and (D) tuber count. All parameters include four independent biological replicates (n = 4).

3.1.7. Potassium supply influences soluble sugars, starch, and potassium content in potato tubers

The different levels of K supply altered tuber yield. Therefore, metabolites in leaves and tubers were analyzed. Monosaccharides in leaves were not affected by K fertilization in WT plants. In NTT-antisense plants, monosaccharides were higher in K2-treated plants compared to other K-treated plants, although this difference was not statistically significant. Furthermore, accumulation of sucrose in the leaves of NTT-antisense plants was not affected by potassium fertilization **(Figure 3.7 A)**. Potassium fertilization did not alter leaf starch content in both WT and NTT-antisense plants **(Figure 3.7 B)**. Investigating the different levels of potassium supply during plant growth might alter the potassium content in leaves of both WT and NTT-antisense plants. Therefore, potassium content in the leaves was also analyzed. Different potassium fertilization did not alter potassium content in leaves of both WT and NTT-antisense plants **(Figure 3.7 C)**.

The monosaccharide contents were generally higher than the sucrose content in the potato tubers of both WT and NTT-antisense plants. The glucose and fructose contents in NTT-antisense plants were 2.5 times and two times higher, respectively, than in WT plants in all potassium treatments. The sucrose content in both WT and NTT-antisense plants was not different **(Figure 3.7 D)**. The starch yield in tubers of WT plants was generally higher than in NTT-antisense plants. The starch yield in WT showed a K-dependent increasing pattern ($p = 0.05$), meaning that supplying more potassium increases the starch production in tubers. This K-dependent increasing pattern was more pronounced in NTT-antisense plants **(Figure 3.7 E)**. The accumulation of K in tubers was significantly increased in both WT and NTT-antisense plants with K3 treatments. Furthermore, the potassium content in NTT-antisense tubers was generally lower in comparison to that in the WT **(Figure 3.7 F)**. These results suggested that the effects of potassium in NTT-antisense plants were limited.

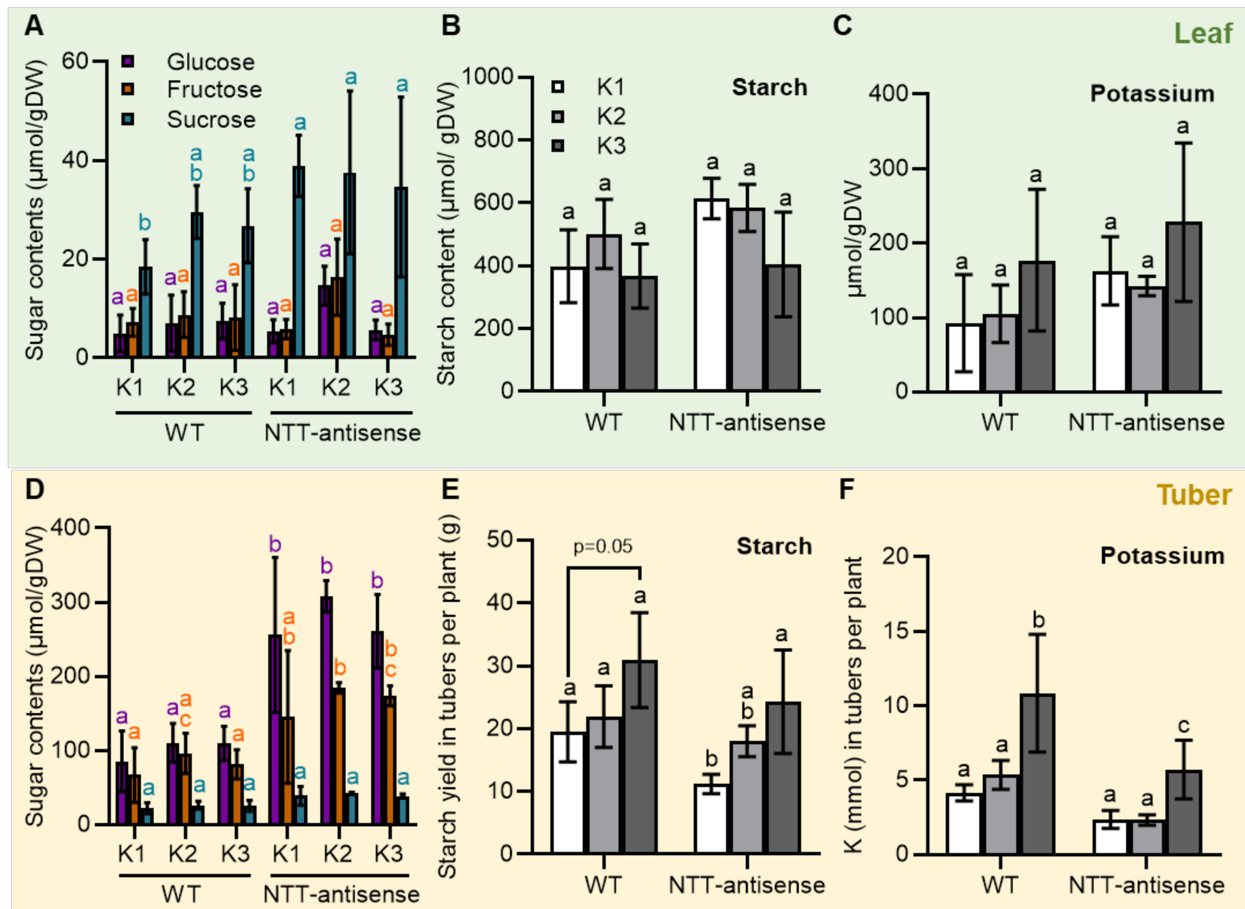


Figure 3.7. Potassium affects sugars and starch in the potato. (A) Sugar, (B) starch, and (C) potassium contents in leaves. (D) Sugar contents in tubers. (E) Starch yield per plant. (F) Accumulation of potassium in tubers (per plant). Potato leaf samples were harvested 30 days after the emergence of the shoot. Potato tuber samples were harvested three months after the emergence of the shoot. Each sample includes three leaves or tubers. Results are means ± SD from four independent biological replicates (n = 4). The letters represent the significance determined between different potassium conditions by two-way ANOVA calculation with post-hoc (Tukey's multiple comparisons test) testing to $p < 0.05$.

3.1.8. Expression of sugar transporters and potassium channels

To understand the effect of sugar transport during potassium fertilization, the transcript level of genes coding for sugar transporters and potassium channels in leaves and tubers in both WT- and NTT-antisense plants grown under the different potassium regimes were measured. Sugar transporters, *St*SUT1, the phloem-associated sucrose loaders (Kühn *et al.*, 2003), *St*TST1 and *St*TST2, the putative tonoplast localized *At*TST homolog, and the potassium channel *St*SKT1 (Zimmermann *et al.*, 1998), which is related to members of the AKT family of potassium channels previously identified in *Arabidopsis thaliana* and potato, were included into these analyses.

The expression of *StSUT1* in leaves of WT plants did not show significant differences in the various potassium treatments, although it was slightly lower under the K3 treatment. The expression of *StSUT1* in NTT-antisense plants was in general higher than in the WT plants, especially in K2 NTT-antisense plants, which was two times higher than in the WT plants **(Figure 3.8 A)**. The expression of *StSUT1* in tubers was no different in both WT and NTT-antisense plants under different potassium treatments **(Figure 3.8 B)**. In Arabidopsis, *TSTs* expression greatly depends on cytosolic monosaccharide concentration, and *AtTST1* levels are upregulated by high glucose levels (Wingenter *et al.*, 2010). In general, the expression of *StTST1* was higher than that of *StTST2* in both organs **(Figure 3.8 C-F)**. *StTST1* responded clearly to potassium fertilization in leaves of NTT-antisense plants so that the expression was increased when potassium supplied was decreased. Moreover, the expression of *StTST1* in the leaves of K1 NTT-antisense plants was higher than in WT plants **(Figure 3.8 C)**. This pattern was not found in potato tubers **(Figure 3.8 D)**. Simultaneously, the expression of *StTST2* in the leaves of K1 NTT-antisense plants was twelve times higher than in K1 WT plants and the transcript level of *StTST2* was decreased when more potassium was supplied **(Figure 3.8 E)**. Furthermore, *StTST2* was decreased in potato tuber of K1-NTT-antisense, although the overall expression level in tubers was low **(Figure 3.8 F)**. This result suggested that low potassium might induce sugar accumulation in leaves of the NTT-antisense line since a higher expression of *StTSTs*.

AKT2 plays a role in maintaining the K^+ gradient for phloem (re)loading processes (Gajdanowicz *et al.,* 2011). To clarify the different amounts of K supply altered the phloem transport, the expression of *StSKT1*, which is an orthologue to *At*AKT2, was analyzed. In leaves, the expression of *SKT1* was decreased in both WT and NTT-antisense plants when the plants grew under higher potassium fertilization (K3, **Figure 3.8 G**). In contrast, *StSKT1* was higher in tubers of both WT and NTT-antisense plants with K3 treatment **(Figure 3.8 H)**. This result suggests that the expression of *StSKT1* was reduced by higher potassium supplied since the level of potassium in the phloem of K3-plants (WT and NTT-antisense) might be sufficient to improve the phloem mass flow that a higher expression of *StSKT1* is unnecessary.

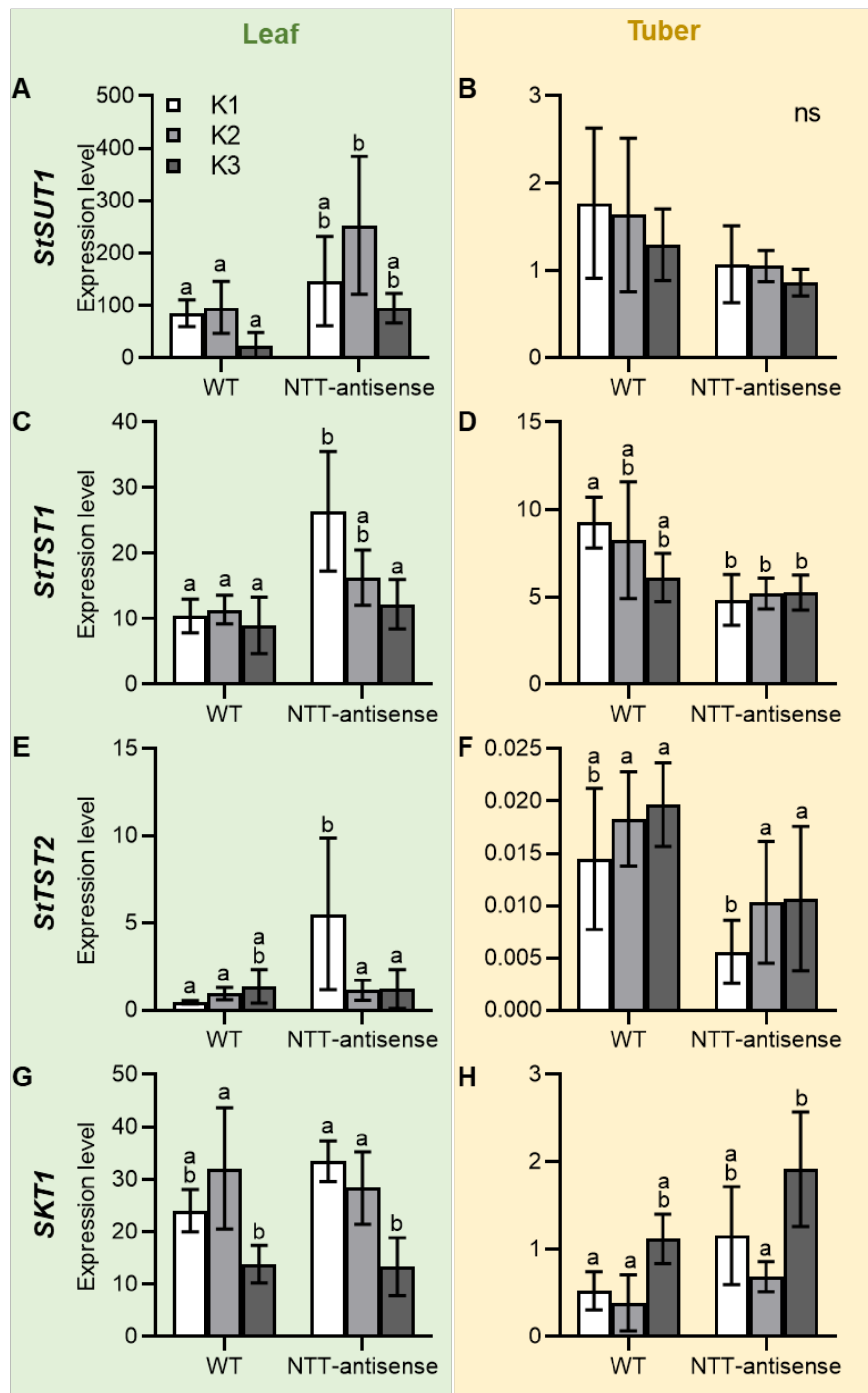


Figure 3.8. Genes expression in potato organs under different potassium fertilization. (A, B) *St*SUT1; (C, D) *St*TST1; (E, F) *St*TST2; (G, H) SKT1. Leaf samples were harvested 30 days after the emergence of the shoot. Potato tuber samples were harvested three months after the emergence of the shoot. Total RNA was isolated from various organs, and cDNA was used for qRT-PCR with gene-specific primers. Relative expression level by normalizing to internal control, *StEF-1a*, is shown. Results are means ± SD from four independent biological replicates (n = 4).

3.2. The role of silicon during drought stress

Silicon is the second most abundant element in Earth's crust (Takahashi *et al.,* 1990). It has been shown that silicon supply is not only helpful for plant growth but also a beneficial fertilizer to improve biotic and abiotic stress tolerance. However, the underlying mechanisms are still unknown. Here, the effects of silicon on plant growth and the transcriptional changes during drought stress were analyzed.

3.2.1. Silicon promotes plant resistance to drought

To investigate the role of silicon in plants during drought stress, we established a hydroponic drought system using a modified Hoagland solution with two millimolar of monosilicic acid. Plants were first grown in the media with or without Si for four weeks and then PEG8000 was applied to simulate drought stress (-0.4MPa). Plants grew slightly better with silicon treatment already before the drought stress. After the drought treatment started by the addition of PEG8000, the Si-dependent growth effect was even more pronounced and significant. The Si-treated plants performed better under drought stress in comparison to plants without Si supplied **(Figure 3.9 A)** and around 20% of plants of the non-Si-treated plants died during the drought stress **(Figure 3.9 B)**. To quantify the effects of Si on plant growth under drought stress, leaf phenotypes, and shoot and root biomass were measured eight days after PEG8000-induced drought stress. In average, plants without Si supply developed 0.7 dead leaves, 3.3 wilted leaves, and 1.4 yellow leaves per plant while Si-treated plants developed 0.2 dead leaves, 0.4 wilted leaves, and 1.3 yellow leaves per plant. Apparently, Si treatment reduced the number of wilted, and dead leaves, although it did not mitigate leaf yellowing **(Figure 3.9 C)**. The effect of Si is not only in reducing the drought damage in leaves but also in increasing plant biomass. Si treatment not only increased 47% of fresh weight (FW) but also increased 48% of dry weight (DW) and 55% of water content in comparison to non-Si-treated plants under control conditions **(Figure 3.9 D-F, left)**. This positive effect was also found for plants that were put under drought stress. The FW and DW of Si-treated plants were 50% higher than of plants without Si supplied (non-Si-treated plants; **Figure 3.9 D and E, right**). Nevertheless, the water content in the shoot tissue did not show a significant difference between the non-Si and Si-treated plants during drought stress (-0.4

MPa**; Figure 3.9 F**). The Si-dependent growth effects were also found in roots under control conditions. The fresh weight, dry weight, and water content of root in the Si-treated plants were in general 1.6 times higher than of the non-Si-treated plants under the control condition **(Figure 3.9 G-I, left)**. However, no growth effects of Si on roots were found during drought stress **(Figure 3.9 G-I, right)**.

The root-to-shoot ratio (R/S ratio) has long been known to be enhanced in plants under drought stress (Harries, 2015). By the calculation of the R/S ratio, the non-Si-treated plants revealed an increase of 1.3 times the R/S ratio under control conditions, which was the same in the Si-treated plants during the drought stress **(Figure 3.9 J)**. To confirm that those growth and drought phenotypes were due to an accumulation of Si within the plant, the Si content in the shoots was analyzed. The Arabidopsis plants accumulated a small amount of Si which was around three µmol per one gram of dry weight in shoot tissue without any Si fertilization, while the plants accumulated around nine micromoles of Si per one gram of dry weight in the shoot when supplying Si. The Si content in the shoot was not altered during drought **(Figure 3.9 K)**. Nevertheless, when the Si fertilizer was given, the Si was absorbed and then accumulated in the shoot which altered the plant growth.

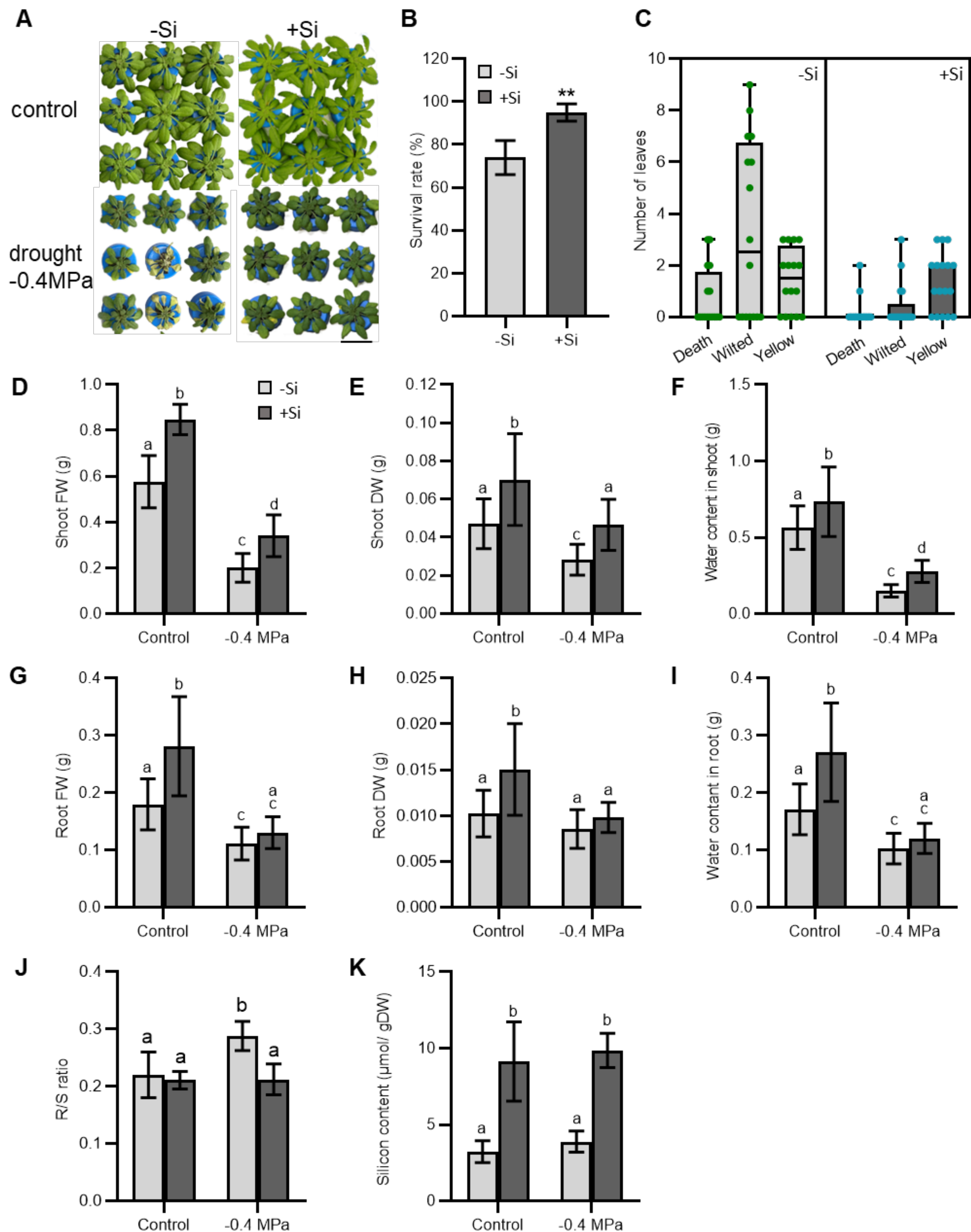


Figure 3.9. Silicon treatment mitigates drought stress symptoms. Four-week-old plants were treated with PEG8000 (-0.4 Mpa) to simulate drought stress. The same age of plants grown in the media without PEG8000 was used as the control. All parameters were quantified eight days after the treatment. (A) The morphology of the plants grown in hydroponic media with or without Si under control and drought (-0.4 MPa) conditions. Bar = 3 cm. (B) Plant survival rate was determined at eight days after

PEG8000 treatment (-0.4 MPa). The result means ± SD from four times technical repeats. (C) Leaf phenotype from surviving plants. (n=16). Shoot (D) fresh weight and (E) dry weight under control, and drought stress (-0.4 Mpa). (F) Relative water content in the shoot. Root (G) fresh weight and (H) dry weight under control, and drought stress (-0.4 Mpa). (I) Relative water content in the root. (J) The root/shoot ratio (R/S ratio) was calculated by the plant's dry weight. (K) Silicon content in the shoot (n = 5). All parameters were analyzed from surviving plants. (D-J) are means ± SD from ten or more independent biological replicates (n > 10). The letters represent the significance determined between different potassium conditions by two-way ANOVA calculation with post-hoc (Tukey's multiple comparisons test) testing to $p < 0.05$.

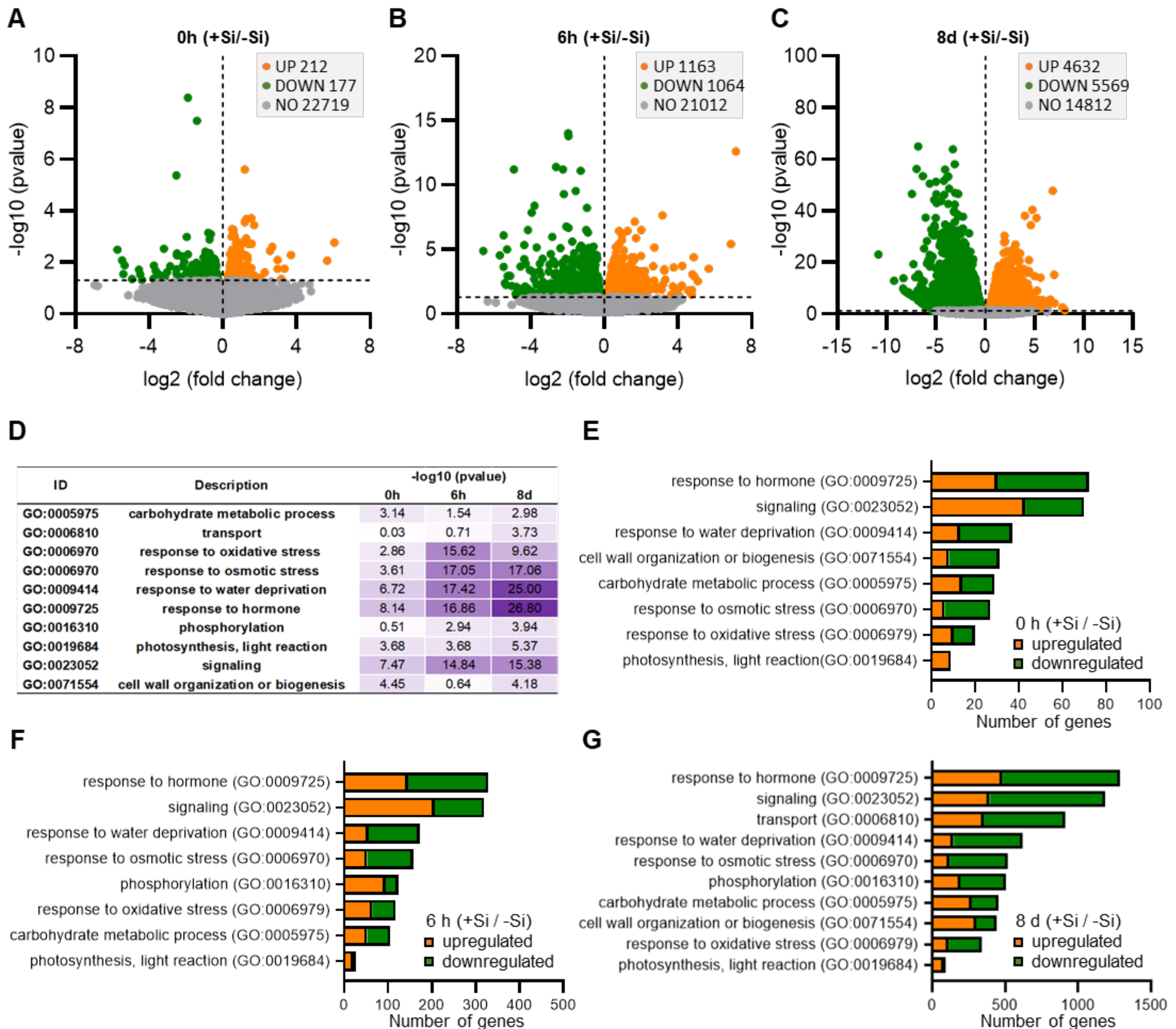
3.2.2. Transcriptome analysis showed Si-dependent differences between early and long-term responses to drought

To further understand the role of Si in drought tolerance, the RNA-sequencing of shoots from plants grown hydroponically with or without monosilicic acid and treated with PEG8000 to simulate drought stress for either six hours (6h) or eight days (8d) was performed. The differentially expressed genes (DEGs) between Si and without Si-supplied plants with a p-value < 0.05 were identified at the two different drought stress time points and in addition before the onset of the drought stress (control, 0h). At 0h, 389 DEGs were identified, which were significantly altered in response to Si. Of these, 212 genes were upregulated and 177 were downregulated **(Figure 3.10 A)**. At 6h after the onset of the drought stress, this number markedly increased to 2,227 DEGs in total, from which 1,163 genes were upregulated, and 1,064 genes were downregulated **(Figure 3.10 B)**. After 8d of drought exposure, the total number of Si-dependent DEGs increased to 10,201, from which 4,632 genes were upregulated, and 5,569 genes were downregulated **(Figure 3.10 C)**.

To reveal the biological processes implicated by Si-responsive genes, the gene ontology (GO) enrichment analysis with all DEGs significantly altered (p-value < 0.05 according to DESeq2) in Si-treated plants compared to non-Si-treated plants grown under control, and two different drought time points were performed. At all three time points GO terms associated with carbohydrate metabolic process (GO: 0005975), response to oxidative stress (GO:0006970), response to osmotic stress (GO:0006970), response to water deprivation (GO:0009414), response to hormone (GO:0009725), photosynthesis, light reaction (GO:0019684) were significantly regulated. GO terms associated with phosphorylation (GO:0016310) and transport (GO:0006810) were altered specifically after drought. Interestingly, the genes associated with cell wall organization or biogenesis were altered by silicon only under control and long-term drought stress **(Figure 3.10 D)**.

The different time points of drought stress application influenced the number of DEGs within the different GO terms **(Figure 3.10 E-F)**. The duration of the drought period increased the number of genes in all GO term groups. Under the control condition (0h), 72 and 70 of 389 responded genes were associated with hormones and signaling, respectively. Si upregulated about 61% of signaling-associated

genes already before drought stress. This upregulation could also be found in early drought stress response (6h, **Figure 3.10 E and F**). About seven and ten percent of silicon-regulated genes were involved in water deprivation or osmotic stress, respectively before drought stress. The silicon-regulated genes involved in carbohydrate metabolic processes and photosynthesis under the control condition were also found. Interestingly, all the genes related to photosynthesis were upregulated when silicon was supplied **(Figure 3.10 E)**. Furthermore, Si increased the expression of genes associated with phosphorylation, where 76% of genes associated with phosphorylation genes were upregulated **(Figure 3.10 F)**, suggesting stimulation of signaling pathways and post-translational regulation of protein functions during early drought stress response. By prolonging drought stress (8d), 60 and 68% of genes associated with carbohydrate metabolic process and cell wall organization, respectively, were stronger upregulated. Interestingly, at this late time point transcripts associated with the GO term “transport” were significantly regulated with Si, which was not observed at the time points before **(Figure 3.10 G)**. The later observations strongly suggest that Si fertilization adjusts primary metabolism but also the distribution of metabolites to mitigate the damage from long-term drought stress.



ID	Description	-log10 (pvalue) 0h	6h	8d
GO:0005975	carbohydrate metabolic process	3.14	1.54	2.98
GO:0006810	transport	0.03	0.71	3.73
GO:0006970	response to oxidative stress	2.86	15.62	9.62
GO:0006970	response to osmotic stress	3.61	17.05	17.06
GO:0009414	response to water deprivation	6.72	17.42	25.00
GO:0009725	response to hormone	8.14	16.86	26.80
GO:0016310	phosphorylation	0.51	2.94	3.94
GO:0019684	photosynthesis, light reaction	3.68	3.68	5.37
GO:0023052	signaling	7.47	14.84	15.38
GO:0071554	cell wall organization or biogenesis	4.45	0.64	4.18

Figure 3.10. Transcriptional changes were highlighted by comparing Si-supplied plants to non-Si-supplied plants before drought treatment (0h), at 6 hours (6h), and 8 days (8d) after drought treatment. (A-C) Volcano plot analysis of three-time points by setting p-value < 0.05, |log2 fold change| > 0. (D) Go term analysis of Si-responding genes. Numbers mean the significance of altered biological processes. (E-G) Analysis of differentially expressed genes of selected biological processes in shoots at 0h, 6h, and 8d after PEG8000 supply, simulating drought stress. Bars mean the number of responded genes up or downregulated in the respective biological processes.

3.2.3. Si effects on photosynthesis and carbohydrate metabolism

By the RNA seq analysis, photosynthesis and carbohydrate metabolism were observed to be altered by Si application. This suggests that Si application may enhance the ability of plants to maintain photosynthetic activity under stress conditions, by increasing the expression of genes involved in this process. Therefore, the photosynthetic performance of Si-treated plants and non-Si-treated plants was analyzed by PAM measurement. The analysis revealed that the ETR (electron transport rate) was at maximum at 98 µmol m^{-2} s^{-1} in the plant without Si supplied, while the ETR was increased to 109 µmol m^{-2} s^{-1} when the plants were supplied with Si under the control condition (**Supplemental figure 5 A)**. The quantum yield in photosystem II (Y(II)) was also higher in Si-treated plants under control conditions (**Supplemental figure 5 B)**, suggesting that Si treatment upregulated the genes corresponding to photosynthesis further increasing photosynthesis under control conditions, although Si treatment did not increase the ETR and Y(II) under drought stress. Moreover, the Si application reduced the NPQ level under control conditions **(Supplemental figure 5 C).**

The transcriptional analysis revealed that genes associated with the carbohydrate metabolic process were regulated in Si-treated plants under both control and drought stress **(Figure 3.11 D-G)**. Therefore, metabolites were isolated from the plants eight days after PEG8000 treatment (as drought stress) to confirm the levels of sugars and starch in both shoots and roots. Under control conditions, non-Si-treated plants accumulated an average of 6 µmol g^{-1} dry weight of glucose in the shoot. With Si treatment, plants accumulated 16 µmol g^{-1} dry weight of glucose in the shoot. The fructose content was also three times higher in the shoot of Si-treated plants, but the sucrose content was not altered by Si treatment under control condition **(Figure 3.11 A, left)**. Under drought stress, the sugars were highly induced in the shoot of Si- or non-Si-treated plants, and the glucose content was 1.9 times higher in Si-treated plants compared to non-Si-treated plants. Although the fructose and sucrose contents were also increased in shoot, there was no significant difference between Si-treated and non-Si-treated plants **(Figure 3.11 A, right)**. Starch accumulation in the shoot was not altered by Si treatment under control conditions. However, starch accumulation in shoot was increased in Si-plants under drought stress, which was 2.7-times higher compared

to the control plants **(Figure 3.11 B)**. The accumulation of the amino acid proline is indicative of the stress level in many plants (Hanson *et al.,* 1977; Yan *et al.,* 2021). Under control conditions, Si treatment did not lead to a change in the proline level in the shoot tissue. Drought stress induced a higher accumulation of proline in shoots, but it was 50% reduced in shoots of Si-treated plants compared to non-Si-treated plants. Other amino acids, such as branched-chain amino acids (BCAAs; leucine, isoleucine, and valine), which have been reported to be highly accumulated in Arabidopsis during drought stress (Shim *et al.*, 2022), were also found to be highly induced in non-Si-treated plants under drought stress **(Supplemental table 3.2)**, suggesting that Si supply mitigates the level of drought stress in the shoot. It has been reported that Si application promotes relocation of potassium (K) from roots to shoots in barley (Beier *et al.*, 2022). Therefore, we measured the K content in both Si- and non-Si-treated plants. The K content in the shoot did not show a significant change under control conditions, although it was decreased in Si-treated plants under drought stress **(Figure 3.11 D)**. Other ions, such as nitrate, were also decreased under drought stress. However, Si-treated plants contained more nitrate in the shoot, which was around 210 µmol gDW^{-1} more than plants without Si treatment **(Supplemental table 3.3)**. These results suggest that Si treatment mitigated the drought stress level by increasing sugar contents or reducing the reduction of nitrate in the shoot.

In the root tissue, sugar contents were not altered by Si treatment under control conditions. All sugar contents were induced in the root of both Si- and non-Si-treated plants by drought stress. Glucose content was 40% higher in Si-treated plants than in non-Si-treated plants under drought stress **(Figure 3.11 E)**. In contrast, the starch accumulation in the root of Si-treated plants was significantly lower than in non-Si-treated plants under control conditions. Under drought stress, the starch content in non-Si-treated plants was reduced by 90% compared to plants under control conditions, while the starch content in Si-treated plants was only reduced by 46% **(Figure 3.11 F)**. The proline content was also induced in the root by drought stress, but there was no difference between Si and non-Si-treated plants **(Figure 3.11 G)**. The potassium content was decreased in roots by drought stress, but there was no difference between Si and non-Si plants **(Figure 3.11 H)**. Interestingly, the nitrate contents in roots showed the same effects as in the shoot tissue. The nitrate content was 2.5 times higher in Si-treated plants compared to

non-Si-treated plants under drought stress **(Supplemental table 3.3)**. These results suggest that the drought stress level in the root was not mitigated by Si treatment, although the sugar content in the root was induced and the nitrate content was higher in plants with Si treatment. This might be explained by the fact that Si-treated plants had better growth in shoot tissue but not in root tissue under drought stress, since the Si treatment reduced the drought stress level only in the shoot.

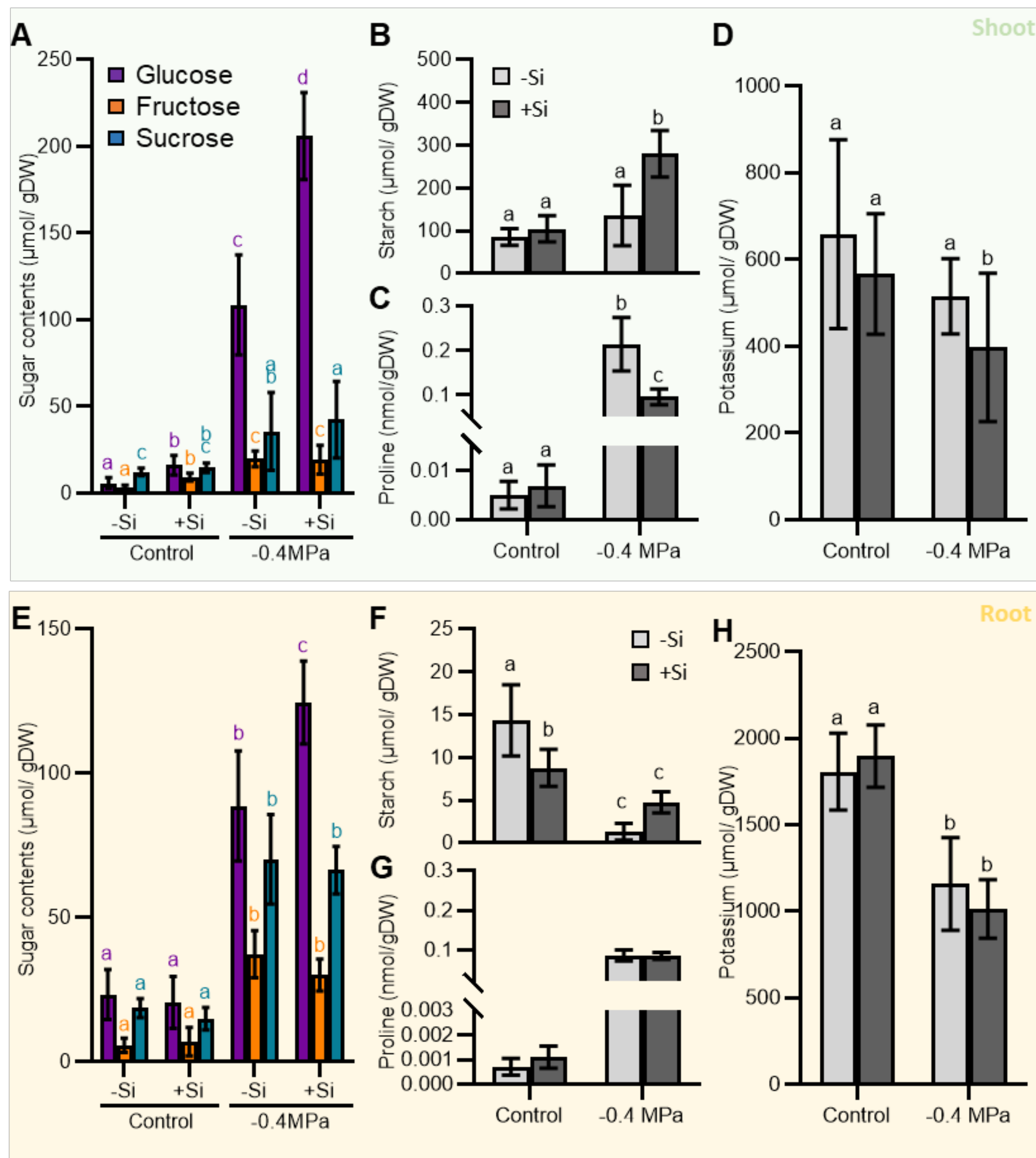


Figure 3.11. Influence of silicon on the metabolism under drought stress. Four-week-old plants were treated with PEG8000 (-0.4 Mpa) to simulate drought stress. The same age of plants grown in the media without PEG8000 was used as the control. The metabolisms were extracted eight days after the treatment and then analyzed. The same age of plants grown in the media without PEG8000 was used as the control. (A) Sugar, (B) starch, (C) proline, and (D) potassium contents in shoots. (E) Sugar, (F) starch, (G) proline, and (H) potassium contents in roots. Results are mean± SD from nine or more independent biological repeats (n > 9).

3.2.4. Influence of silicon on subcellular sugar distribution

Si treatment induced sugar accumulation in the shoots. However, the subcellular localization of sugars is a key factor in altering the carbohydrate metabolic process since high cytosolic sugar led negative feedback on photosynthesis (DYSON *et al.*, 2015, Weise *et al.*, 2019). To analyze the subcellular localization of metabolites in both control and drought stress conditions, we isolated sugars from the plastid, cytosol, and vacuole using non aqueous fractionation (NAF) (**Method 2.7)**. Glucose was equally accumulating in the cells of both Si- and non-Si-treated plants. However, 50% of glucose was accumulated in the vacuole when silicon was supplied. This pattern was also observed in plants under drought stress **(Figure 3.12 A)**. Fructose was distributed equally in the cells of both Si- and non-Si-treated plants under control conditions. Under drought stress, the fructose content in the plastid was decreased and distributed to the cytosol in non-Si-treated plants, while the fructose was distributed to the vacuole in Si-treated plants **(Figure 3.12 B)**. About 40% of sucrose was found in the plastid in both Si- and non-Si-treated plants under control conditions. The sucrose content was increased in the plastid but decreased in the vacuole in non-Si-treated plants during drought. This phenomenon was not observed in Si-treated plants. Furthermore, irrespective of the presence of control or drought stress conditions, or whether Si treatment was administered, the sucrose content in the cytosol remained unchanged **(Figure 3.12 C)**. The subcellular localization of ions was also measured. Drought stress induced ion re-localization to the vacuole, although there were no differences between Si- and non-Si-treated plants **(Supplemental figure 6)**. This result suggests that silicon altered the subcellular localization of sugars, which might be the reason for drought stress mitigation by Si supplementation.

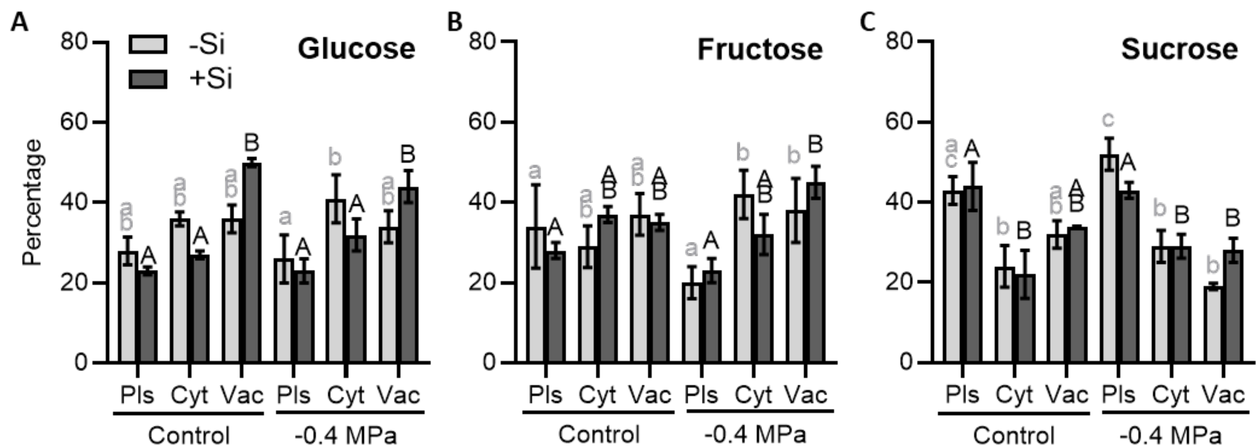


Figure 3.12. Subcellular localization of metabolites. Percentage of (A) glucose, (B) fructose, and (C) sucrose in plant cell compartments from shoot tissue. Four-week-old plants were treated with PEG8000 (-0.4 Mpa) to simulate drought stress. The same age of plants grown in the media without PEG8000 was used as the control. The shoot tissue was harvested eight days after the treatment and then analyzed. Results are means ± SD from three or more independent biological replicates (n ≥ 3).

3.2.5. Si-regulated sugar transporters and enzymes involved in primary sugar metabolism under drought stress

By analyzing the plant metabolism, we found that Si may mitigate the effects of drought stress by altering the subcellular distribution of sugars. This can be due to alterations in the expression of different subcellular sugar transport proteins or general changes in primary sugar metabolism in the plant cell Therefore, the expression of sugar transporters and enzymes involved in primary carbohydrate metabolism in the chloroplast, vacuole, and plasma membrane that responded to drought stress and compared their expression between non-Si and Si-treated plants was first analyzed. Regarding chloroplast transport proteins, compared to plants under control conditions (0h), only non-Si-treated plants strongly induced *AtGPT2*, the gene encoding a glucose 6-phosphate/phosphate translocator that is upregulated by high cytosolic sugar levels (DYSON et al., 2015), at eight days after drought stress (8d) **(Figure 3.13 A, chloroplast)**, indicating that non-Si-

treated plants accumulated a high level of cytosolic sugars. This result was consistent with the results of subcellular localization of metabolites, which showed that glucose and fructose were higher in the cytosol of non-Si-treated plants under drought stress **(Figure 3.12)**. The expression of *AtpGlcT* was downregulated by drought stress (6h and 8d) in non-Si-treated plants, while the level of *AtpGlcT* was not altered by drought stress in Si-treated plants **(Figure 3.13 A, chloroplast)**, suggesting that Si treatment mitigated the drought stress reduction of glucose export from the chloroplast.

To further investigate the regulation of sugar relocation in plant cells, we analyzed the expression of vacuolar sugar transporters. The expression levels of most of the *Early Responsive to Dehydration6-Likes (ERDLs)* members (*AtESL1, AtERD6, AtERDL6, AtERDL4*), which are vacuole sugar exporters, were upregulated in non-Si-treated plants at 8d compared to the plants at 0h. However, the drought stress-induced upregulation of *AtERDLs* in Si-treated plants was not as significant as in non-Si-treated plants, suggesting that Si might reduce the export of sugars from the vacuole to the cytosol (**Figure 3.13 A, vacuole)**. In contrast, the expression of the major vacuolar sugar importer, *AtTST1*, was 2.3 times higher in Si-treated plants compared to non-Si-treated plants at 8d of drought stress. However, the expression of *AtTST2* in Si-treated plants was 0.59 times lower than in non-Si-treated plants at 8d under drought **(Figure 3.13 A, vacuole)**. Recently, the CBL-CIPK6 complex was identified in cotton to phosphorylate TST2, thus regulating sugar homeostasis (Deng *et al.*, 2020). Therefore, we investigated the expression of *AtCIPK6*. Under control conditions, the transcript level of *AtCIPK6* was not altered by Si treatment. However, *AtCIPK6* in Si-treated plants was around 1.8 times higher than in non-Si-treated plants under long period drought stress (8d), although *AtCIPK6* was three times higher in both Si- and non-Si-treated plants at six hours after drought stress (6h) **(Supplemental figure 7)**. This suggests that Si treatment might increase the phosphorylation of AtTST2 by inducing the expression of *AtCIPK6*, thus directing towards higher sugar accumulation in the vacuole. Besides, *AtSWEET17*, a vacuole fructose transporter important for drought tolerance (Valifard *et al.*, 2021), was upregulated in Si-treated plants at 8d **(Figure 3.13 A, vacuole)**. The data suggest that drought stress induces the accumulation of sugars in the cytosol, which may then induce negative feedback to reduce glucose exportation from the chloroplast. Si

treatment alleviated these drought syndromes by altering sugar localization into the vacuole. This sugar re-localization might improve osmotic balance and maintain sugar metabolism processes in plants under drought stress.

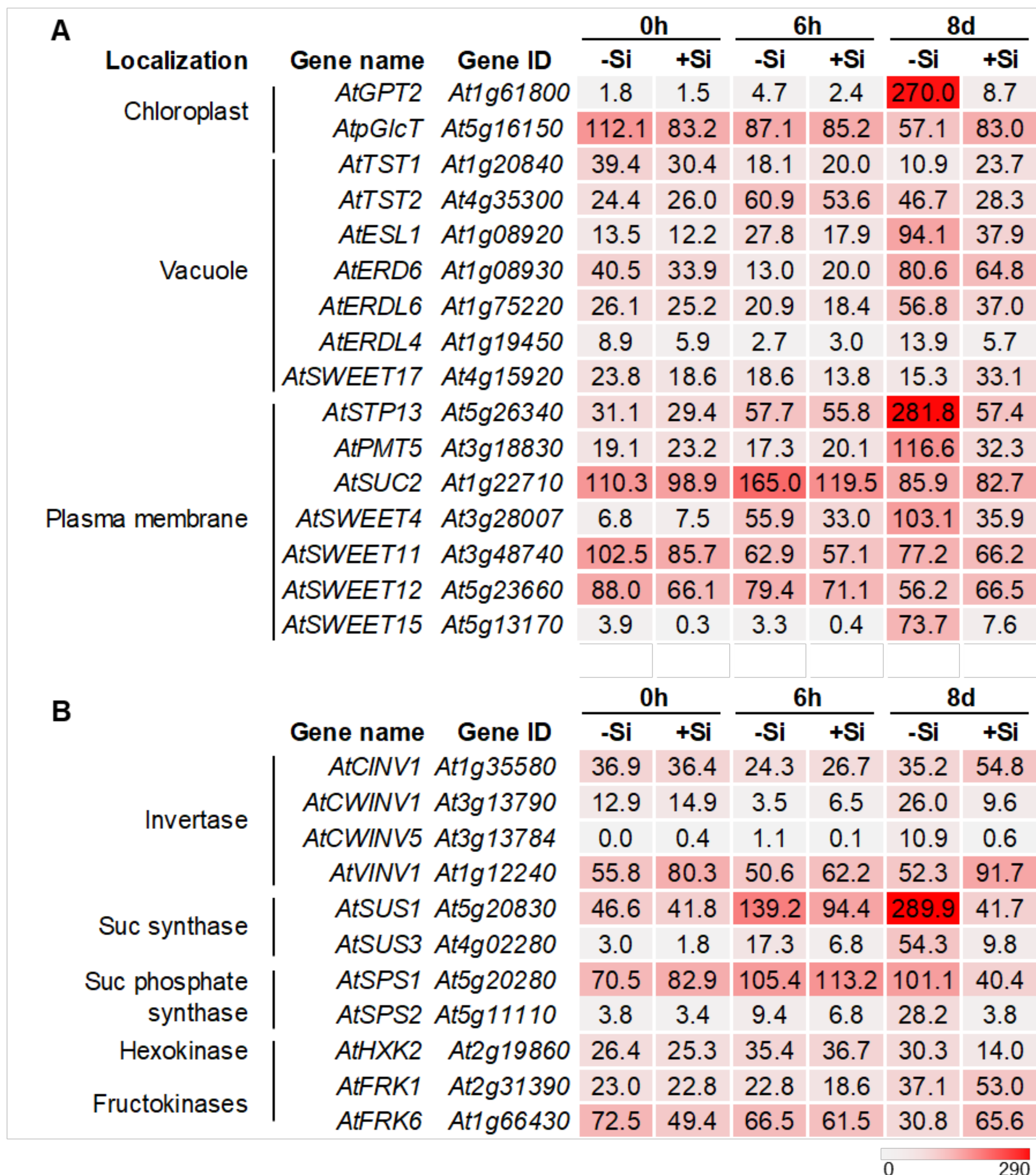
The source-to-sink sucrose transport capacity can also be limited by drought stress. Therefore, the sugar transporters involved in phloem loading were analyzed. *AtSUC2*, the plasma membrane sucrose-proton symporter, was already 0.7 times lower in Si-treated plants compared to non-Si-treated plants at 0h of drought stress. The expression of *AtSUC2* was 1.5 times higher in both Si- and non-Si-treated plants at 6h of drought compared to their respective plants at 0h of treatment, although *AtSUC2* in non-Si-treated plants was still higher than in Si-treated plants under drought stress **(Figure 3.13 A, plasma membrane)**. *AtSWEET11* and *AtSWEET12*, key players in sucrose efflux from phloem parenchyma cells involved in the phloem loading process (Chen *et al.,* 2012), were slightly (0.8 times) lower expressed in Si-treated plants compared to non-Si-treated plants at 0h of treatment. A reduction in the expression of *AtSWEET11* was observed in both Si- and non-Si-treated plants due to drought. However, the expression of *AtSWEET12* was decreased only in non-Si-treated plants at 8d of drought stress **(Figure 3.13 A, plasma membrane)**, meaning Si treatments mitigated the reduction of *AtSWEET12*. In addition, the expression pattern of other plasma membrane sugar transporters was altered by Si treatment under drought stress. The expression of *AtSTP13*, a major contributor to monosaccharide uptake from the apoplast (Yamada *et al.,* 2011), was dramatically increased in non-Si-treated plants but not in Si-treated plants at 8d compared to their respective plants under control conditions. The same expression pattern was found for *AtPMT5*, encoding a low-specificity H^+-symporter that mediates the energy-dependent uptake of hexoses and inositol across the plasma membrane **(Figure 3.13 A, plasma membrane)**. Moreover, *AtSWEET4*, which plays a role in transporting sugars into the endosperm (Liu *et al.,* 2016), and *AtSWEET15*, known to be induced by abiotic stress (Seo *et al.,* 2011), were highly upregulated in non-Si-treated plants at 8d, suggesting that Si treatment alleviated the severity of drought stress in the plants **(Figure 3.13 A, plasma membrane)**. This data suggests that sugars might be accumulated in the apoplast since drought stress reduces phloem loading, which might induce sugar re-uptake from the apoplast. Si treatment might mitigate the reduction of phloem loading when the plants are

under drought stress conditions so that the induction of sugar re-uptake from the apoplast is not exhibited.

Not only was the expression of sugar transporters altered, but also the expression of genes coding for enzymes involved in primary sugar metabolism was regulated. At 0h, the expression of *cytosol invertase 1* (*AtCINV1*) did not show a difference between Si-treated and non-Si-treated plants, while *AtCINV1* was 1.5 times increased by a longer drought stress treatment (8d) in Si-treated plants but not in non-Si-treated plants compared to respective plants under control conditions **(Figure 3.13 B, invertase)**. The expression of *Cell Wall Invertase 1* (*AtCWINV1*) was first decreased in both Si- and non-Si-treated plants at 6h. Then, the transcription level was increased in non-Si-treated plants but decreased in Si-treated plants at 8d compared to respective plants at 0h **(Figure 3.13 B, invertase)**. The expression of *AtCWINV5* was also highly induced only in non-Si-treated plants at 8d compared to plants at 0h, although the expression level of it was extremely low in both Si- and non-Si-treated plants **(Figure 3.13 B, invertase)**. On the other hand, the expression of *vacuolar invertase 1* (*VINV1*) in Si-treated plants was already 1.4 times higher than in non-Si-treated plants at 0h, while this difference was more significant at 8d **(Figure 3.13 B, invertase)**, suggesting that Si increases sucrose hydrolysis to yield glucose and fructose in the cytosol and vacuole, promoting plant growth and enhancing drought tolerance.

Sucrose synthesis (SUS) and sucrose phosphate synthesis (SPS) are also key enzymes involved in sucrose hydrolysis and synthesis (Ruan, 2014). Therefore, the transcript levels of *AtSUSs* and *AtSPSs* were analyzed. *AtSUS1* and *AtSUS3* were three and five times higher, respectively, in non-Si-treated plants at 6h, and these expression patterns were more significant at 8d. However, these high inductions of *AtSUSs* by drought stress were not observed in Si-treated plants **(Figure 3.13 B, Suc synthase)**, indicating that Si treatment reduced the induction of sucrose decomposition to fructose and UDP-glucose. On the other hand, *AtSPS1* and *AtSPS2*, which synthesize sucrose in the cytosol, were slightly increased in non-Si-treated plants but two times lower in Si-treated plants at 8d compared to respective plants at 0h **(Figure 3.13 B, Suc phosphate synthesis)**. This suggests that Si treatment might reduce sucrose synthesis to keep more monosaccharides in plant cells. The hexose-phosphorylating enzymes (HXK and

FRK) are also involved in sugar metabolism processes and signaling in different compartments of plant cells (Granot *et al.,* 2013). The expression of *AtHXK2* was not altered by Si treatment under control and earlier drought stress. When extending the drought period to 8d, the expression of *AtHXK2* in Si-treated plants was two times lower than in non-Si-treated plants **(Figure 3.13 B, hexokinase)**. The expression of *AtFRK1* was also upregulated in Si-treated plants at 8d after drought, which was 2.4 times higher compared to the plants under control. Interestingly, the expression of *AtFRK6* showed a different pattern, which was downregulated two times in non-Si-treated plants at eight days after drought stress compared to the non-Si-treated plants under control **(Figure 3.13 B, fructokinase)**. The regulation of these sugar transporters and enzymes in the primary carbohydrate metabolism suggests that Si treatment altered the sugar relocation and sugar metabolism processes. Thus, the plants showed improved growth under both control and drought conditions when supplied with silicon.



A

Localization	Gene name	Gene ID	0h -Si	0h +Si	6h -Si	6h +Si	8d -Si	8d +Si
Chloroplast	*AtGPT2*	*At1g61800*	1.8	1.5	4.7	2.4	270.0	8.7
	AtpGlcT	*At5g16150*	112.1	83.2	87.1	85.2	57.1	83.0
Vacuole	*AtTST1*	*At1g20840*	39.4	30.4	18.1	20.0	10.9	23.7
	AtTST2	*At4g35300*	24.4	26.0	60.9	53.6	46.7	28.3
	AtESL1	*At1g08920*	13.5	12.2	27.8	17.9	94.1	37.9
	AtERD6	*At1g08930*	40.5	33.9	13.0	20.0	80.6	64.8
	AtERDL6	*At1g75220*	26.1	25.2	20.9	18.4	56.8	37.0
	AtERDL4	*At1g19450*	8.9	5.9	2.7	3.0	13.9	5.7
	AtSWEET17	*At4g15920*	23.8	18.6	18.6	13.8	15.3	33.1
Plasma membrane	*AtSTP13*	*At5g26340*	31.1	29.4	57.7	55.8	281.8	57.4
	AtPMT5	*At3g18830*	19.1	23.2	17.3	20.1	116.6	32.3
	AtSUC2	*At1g22710*	110.3	98.9	165.0	119.5	85.9	82.7
	AtSWEET4	*At3g28007*	6.8	7.5	55.9	33.0	103.1	35.9
	AtSWEET11	*At3g48740*	102.5	85.7	62.9	57.1	77.2	66.2
	AtSWEET12	*At5g23660*	88.0	66.1	79.4	71.1	56.2	66.5
	AtSWEET15	*At5g13170*	3.9	0.3	3.3	0.4	73.7	7.6

B

	Gene name	Gene ID	0h -Si	0h +Si	6h -Si	6h +Si	8d -Si	8d +Si
Invertase	*AtCINV1*	*At1g35580*	36.9	36.4	24.3	26.7	35.2	54.8
	AtCWINV1	*At3g13790*	12.9	14.9	3.5	6.5	26.0	9.6
	AtCWINV5	*At3g13784*	0.0	0.4	1.1	0.1	10.9	0.6
	AtVINV1	*At1g12240*	55.8	80.3	50.6	62.2	52.3	91.7
Suc synthase	*AtSUS1*	*At5g20830*	46.6	41.8	139.2	94.4	289.9	41.7
	AtSUS3	*At4g02280*	3.0	1.8	17.3	6.8	54.3	9.8
Suc phosphate synthase	*AtSPS1*	*At5g20280*	70.5	82.9	105.4	113.2	101.1	40.4
	AtSPS2	*At5g11110*	3.8	3.4	9.4	6.8	28.2	3.8
Hexokinase	*AtHXK2*	*At2g19860*	26.4	25.3	35.4	36.7	30.3	14.0
Fructokinases	*AtFRK1*	*At2g31390*	23.0	22.8	22.8	18.6	37.1	53.0
	AtFRK6	*At1g66430*	72.5	49.4	66.5	61.5	30.8	65.6

Figure 3.13. Expression of sugar transporters and enzymes involved in primary sugar metabolism. Heatmap showing FPKM transcriptional levels of significantly differentially expressed genes associate with (A) sugar transporters and (B) enzymes involved in the primary carbohydrate metabolism process. 0h, before drought stress; 6h, six hours after drought stress; 8d, eight days after drought stress. For each sample, three independent biological replicates (n ≥ 3) were included.

3.2.6. Silicon increases tuber fresh weight at control but not drought conditions

The effect of silicon on crop plants greatly depends on the species and their ability to accumulate silicon in tissues and cells. Grass species, especially rice, heavily rely on sufficient silicon nutrition for proper growth and during generative development. However, the role of silicon for dicotyledonous species that produce tubers and tuberous roots has so far been neglected. This study provides the first data on the effects of silicon on tuber yield, sugar, and starch metabolism in the "Désirée" variety of potato plants, which exhibit reduced tuber sink strength. To study the effects of silicon on potato tuber formation during drought stress, WT potato plants (Désirée) were grown in soil mixed with or without silicon fertilizer for four weeks. Then, the water content was decreased to 30% to induce drought stress **(Method 2.2)**. The shoot dry weight, tuber yield, and tuber numbers of Si-treated plants under control and drought conditions were measured seven weeks after reducing the water supply **(Figure 3.14)**. The WT potato plants generally developed more tubers under drought stress conditions than under control conditions. However, the size of the tubers was larger under control conditions compared to plants under drought stress. Si treatment did not cause a significant difference under both control and drought conditions **(Figure 3.14 A)**. From the previous results in Arabidopsis, where Si supply improved the growth of Arabidopsis, the fresh weight of shoots and tubers were measured. The shoot fresh weight was not significantly altered by the Si treatment in potato plants **(Figure 3.14 B)**. However, Si-treated plants produced heavier tubers compared to non-Si-treated plants under control and drought conditions, although the total yield per plant did not show a significant difference **(Figure 3.14 C)**. Subsequently, the stages of tuberization were analyzed. For this analysis, the tubers were categorized by size in accordance with the method presented in the publication by Weeda and colleagues (Weeda *et al.*, 2009). Tubers smaller than 0.6 g were grouped into developing tubers, from 0.6 g to 10 g of tubers were defined as young tubers, and tubers exceeding 10 g were mature tubers. At the harvest time point, Si-treated plants had produced a lower number of developing and young tubers and a higher number of mature tubers under control conditions but not under drought conditions, although the total tuber numbers were not significantly altered by the Si treatment under control and drought conditions **(Figure 3.14 D)**. These

data suggested that Si fertilization might promote tuber development and accelerate tuber maturation in potato plants under well-watered conditions but not under drought stress.

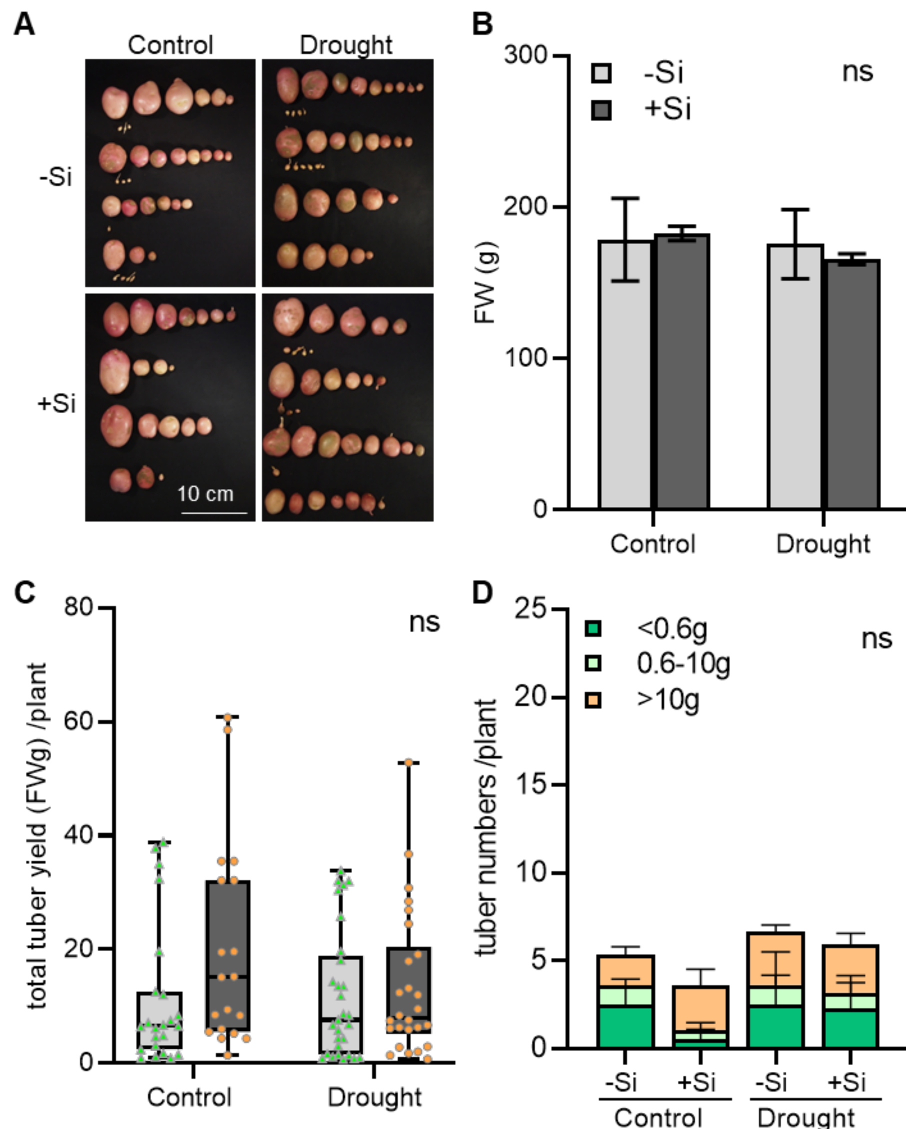


Figure 3.14. Potato tuber yields under control and drought conditions with or without silicon treatment. (A) Tuber formation, Bar = 10 cm. (B) Shoot fresh weight. (C) Effects of silicon treatment on total tuber yield per plant during drought. (D) Numbers of Tubers in different stages. Désirée was used as WT in this analysis. Samples were harvested at 68 days after drought stress treatment. Results are means ± SD from four independent biological replicates (n = 4).

3.2.7. The effects of silicon treatment on metabolites altering during drought stress in potato plants

Accumulation of the amino acid proline is indicative of the stress level in many plants and tissues, including potato (Liu *et al.*, 2019, Yan *et al.*, 2021). Therefore, the stress level in the potato plants was analyzed by measuring the level of proline in both leaves and tubers. The tubers were grown in soil mixed with or without silicon fertilizer for four weeks, and then the water content was decreased to 30% to induce drought stress for 47 days **(Method 2.2)**. Under control conditions, the silicon treatment did not lead to significant changes in the proline levels in potato leaves. Under drought stress conditions, the proline content was strongly increased in non-Si-treated plants, while the proline content was not altered in Si-treated plants **(Figure 3.15 A)**. Drought-induced proline accumulation was also found in the potato tubers, although the level of proline accumulation was not as high as in leaves. The Si treatment did not alter the proline accumulation in the potato tubers **(Figure 3.15 B)**. To confirm if Si application mitigates the level of drought stress in potato plants, Si content in potato leaves and tubers was analyzed. Under control conditions, the concentration of Si in the leaves of Si-treated plants was 1.3 times higher than in non-Si-treated plants **(Figure 3.15 C)**. The same level of Si content in leaves was also found in the plants under drought stress **(Figure 3.15 C)**, meaning drought stress did not affect Si accumulation in leaves. Si application did not alter the level of Si in tubers in both Si-treated and non-Si-treated plants under both control and drought conditions **(Figure 3.15 D)**. This result suggested that the Si application did not alter the Si content in the tubers and did not mitigate the drought stress level in potato tubers. Nevertheless, Si application did mitigate the drought stress in the shoot of potato plants.

Based on our results on the effects of silicon on starch and sugar accumulation in Arabidopsis, we analyzed the respective contents in potato plants. There was no difference in starch accumulation in potato leaves and tubers with or without Si treatment under both control and drought conditions **(Figure 3.15 E and F)**. The glucose content in the leaves of plants under drought stress was higher than that in the plants under control conditions. The fructose content in the potato leaves was slightly increased by drought stress, although this pattern was not statistically significant in comparison to the plants under control conditions. There was no

difference in the level of sucrose in the plants under both control and drought conditions **(Figure 3.15 G)**. Nevertheless, the total sugar contents in the leaves were generally higher in the plants under drought stress compared to the plants under control conditions, even though Si application did not alter the accumulation of sugars in the potato leaves under both control and drought conditions. The total sugar contents in the potato tubers were slightly lower in Si-treated plants under both control and drought conditions compared to non-Si-treated WT plants, although the observations were not significant. Moreover, the drought stress did not alter the accumulation of sugars in the potato tubers **(Figure 3.15 H)**.

As Si application promoted potassium (K) relocation from root to shoot in barley (Beier *et al.,* 2022), this effect might be also observed in potato plants. Therefore, the K content in the phloem exudate, leaves, and tubers of potato plants was verified. Under control conditions, the Si application did not alter the K content in the phloem. The K content was decreased in non-Si-treated plants, while the K content did not show the difference in Si-treated plants under drought stress **(Supplemental figure 11 A)**. Moreover, the Si treatment did not alter the potassium content in the potato leaves and tubers under both control and drought stress conditions **(Supplemental figure 11 B-C)**. These data showed that the effects of Si on drought tolerance in potatoes were tissue dependent. However, the mechanism is still unclear.

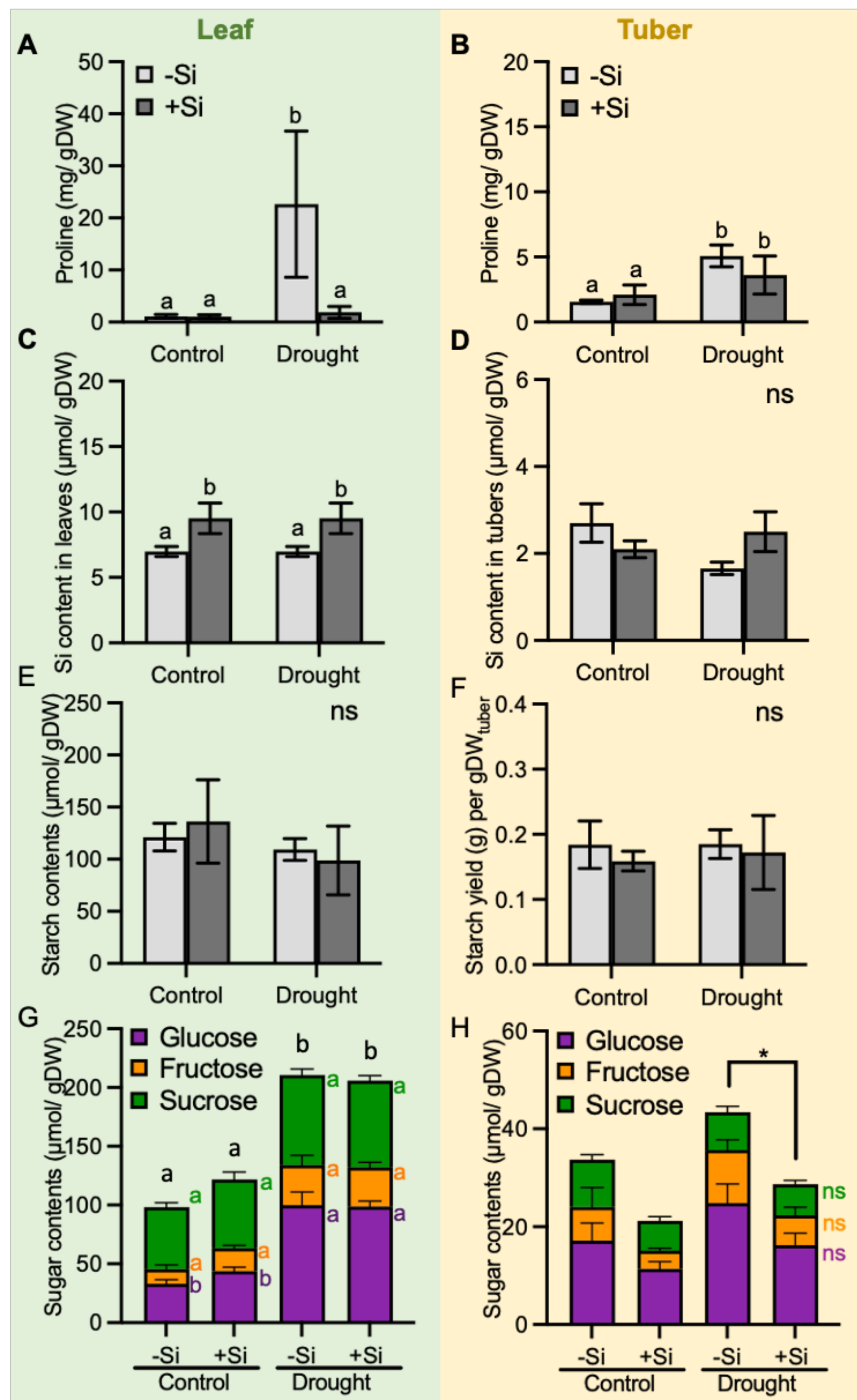


Figure 3.15. Silicon-dependent effects on metabolisms in potato plants during drought stress. The level of proline in (A) leaves and (B) tubers. Silicon content in (C) leaves and (D) tubers. Starch content in (E) leaves and (F) tubers. Sugar content in (G) leaves and (H) tubers. Samples were harvested seven weeks after stopping the water supply. Results are means ± SD from four independent biological replicates (n = 4). The statistics for (G) and (H) were separated by the levels of glucose (purple), fructose (orange), sucrose (green), and total sugar levels (black).

3.2.8. Silicon induces the expression of sugar transporters under drought in leaves of WT but not NTT-antisense potato plants

Since sugar accumulations were affected by drought and/or Si treatments, we analyzed the expression of genes encoding plasma membrane and vacuolar membrane-localized sugar transporters in leaves and tubers, as previously introduced in result 3.3.4. These included *St*SUT1 and *St*SWEET11, which are sugar transporters for phloem loading (Kühn *et al.,* 2003, Abelenda *et al.*, 2019), and *St*TST1 and *St*TST2, the homologs of Arabidopsis tonoplast-localized sugar transporters (*At*TSTs). The gene encoding *StSUT1* was not affected by Si supply in potato plant leaves under control conditions. Under drought stress, the expression of *StSUT1* was two times higher in the leaves of Si-treated plants compared to the plants under control conditions, while this induction was not found in the leaves of non-Si-treated plants **(Figure 3.16 A)**. In tubers, there were no differences in the expression of StSUT1 under both Si and drought treatments **(Figure 3.16 B)**. *StSWEET11* expression was not different under control conditions, but it was slightly higher in the leaves of Si-treated plants under drought stress, although the result was not statistically significant **(Figure 3.16 C)**. Si application did not alter the expression of *StSWEET11* in tubers under both control and drought conditions **(Figure 3.16 D)**. Nevertheless, Si application stimulated the expression of StSUT1 in potato leaves under drought stress, suggesting that sugar loading into the phloem might be enhanced in the shoot of potato plants under drought stress due to Si application.

The expression of *StTSTs* is indicative of the re-localization of sugar in plant cells during drought stress. Therefore, we analyzed the expression of respective family members in potato plants to investigate the Si-dependent sugar re-localization. The expression of *StTST1* in potato leaves did not change significantly due to Si supply under control conditions. However, StTST1 expression was two times higher in the leaves of Si-treated plants compared to non-Si-treated plants under drought stress **(Figure 3.16 E)**. The same induction pattern of *StTST1* under drought stress was observed in the tubers of Si-treated plants **(Figure 3.16 F)**. The expression of *StTST2* did not show significant differences in both Si-treated and non-Si-treated plants under control and drought stress conditions in both leaves and tubers **(Figure 3.16 E and F)**. These results suggest that Si may

mitigate drought stress by increasing sugar storage in the vacuole through the upregulation of *StTST1* expression in the leaves of potato plants.

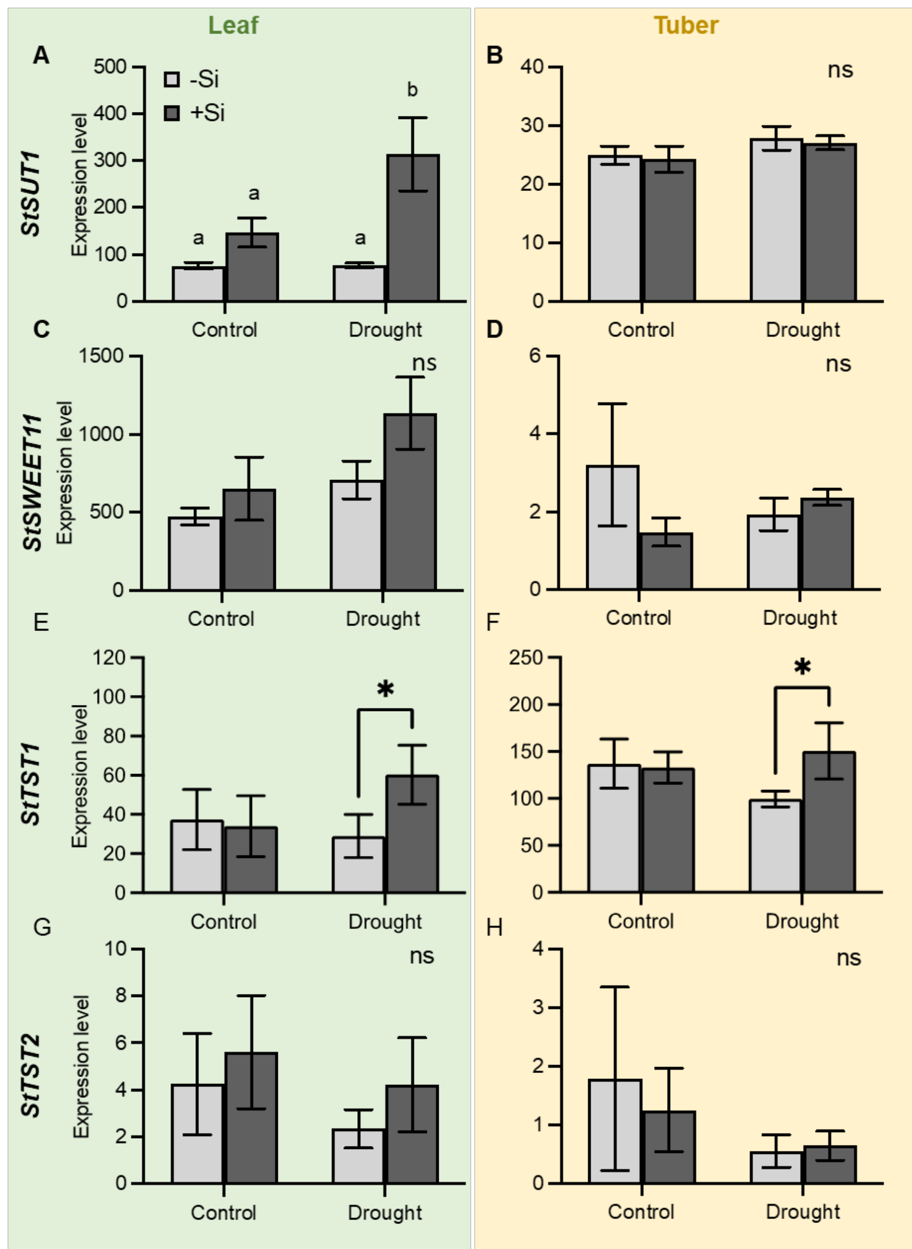


Figure 3.16. The expression of sugar transporters in different tissues of potato plants with Si supply under control and drought stress. The expression of *StSUT1* in (A) leaves and (B) tubers; the expression of *StSWEET11* in (C) leaves and (D) tubers; the expression of *StTST1* in (E) leaves and (F) tubers; the expression of *StTST2* in (G) in leaves and (H) tubers. The samples were harvested seven weeks after stopping the water supply. Total RNA was isolated from various organs, and cDNA was used for qRT-PCR with gene-specific primers. Relative expression level by normalizing to internal control, *StEF-1a*, is shown. Results are means ± SD from four independent biological replicates (n = 4).

4. Discussion

Proper nutrient supply is helpful for plants that deal with abiotic stress. This study aimed to investigate how nutrition fertilization exhibits improved drought tolerance such as better growth, improved phloem long-distance transport, and reduced yield loss during drought stress. This project began with two plant nutrients. I first studied how potassium maintains the phloem mass flow under drought stress. The second part of this project was to investigate whether Si improves drought tolerance and what where the underlying mechanisms. In this study, the mechanisms of these two nutrients in drought stress tolerance were investigated herein with *Arabidopsis thaliana* and further verified in the potato.

4.1. Potassium effects on sugar loading under drought stress

Potassium (K) has been known to be essential for plant growth, osmotic potential adjustment, and phloem transportation (Marschner, 2011). In this work, two approaches were employed to identify physiological, biochemical, and molecular effects of K in the plant's response to drought. First, a soil-substrate based cultivation system using a low nutrient-containing substrate (Pommerrenig *et al.*, 2018, Ho *et al.,* 2020) was used to adjust K levels in a soil condition. The solute ion content measurement in the soil showed that the K^+ level increased when more K was supplied **(Supplemental figure 12 A)**. This approach reflected K usage and K effects in a situation mirroring natural growth conditions but lacked the possibility to access and analyze root tissue. Therefore, a second approach using a hydroponic cultivation system, in which drought stress was mimicked by the application of the osmotic PEG8000. The latter approach neglected root-soil interacting factors but allowed for fast and uncomplicated separation of roots. In contrast to growth on soil substrate, the hydroponic cultivation allowed for more precise adjustment of the nutrient composition because the zero-soil substrate contained – although at low levels – still basic levels of different nutrients (Method), which might also vary between individual batches and shipments. Although it is hard to compare these two approaches since the background of K in media is different, the drought recovery rate showed a similar pattern **(Supplement figure**

1 and 2). Thus, we connected the results of K-dependent effects on drought tolerance from these two approaches.

4.1.1. Severe potassium deficiency can lead to a slowing down of phloem mass transport, further impacting drought tolerance

Drought stress can cause plants to accumulate sugars to improve water retention by increasing solute potential, according to research (Hoekstra *et al.,* 2001, Taiz *et al.,* 2015, Takahashi *et al.,* 2020). However, potassium deficiency under drought stress can reduce the ability to maintain osmotic potential, causing plants to accumulate more sugar to generate turgor and lower osmotic potential. This was observed in a study comparing plants with different levels of potassium (K1, K2, K3, and K0) under drought stress, where plants without K fertilization (K0) had a 1.3-times increase in glucose compared to other plants **(Figure 3.2 A)**. In a similar hydroponic system (HS), K0-HS plants had high monosaccharides accumulation in the shoot under control and drought stress (-0.4 MPa) condition **(Figure 3.4 A)**. The vacuolar K^+ pool plays a crucial role in generating turgor and driving cell expansion by lowering osmotic potential. However, potassium deficiency can affect the K^+ content of the phloem, which is critical for the translocation of K^+ and sugars to different parts of the plant. K0 plants had similar levels of K^+ as K2 and K3 plants under control conditions **(Figure 3.2 C)**, but the K^+ content in the phloem of K0 plants was significantly lower (1.4 times) than in K3 plants **(Figure 3.2 D)**. Previous research has shown that potassium deficiency reduces sugar export from the leaflet of oil palm (*Elaeis guineensis*), supporting this result (Cui *et al.*, 2020). Additionally, the higher accumulation of sugars in the phloem of K0-HS plants might be due to slower sugar movement through the phloem **(Figure 3.4 G)**. Under control conditions, the expression of the tonoplast sugar transporter (*TST1*), which imports sugars from the cytosol into the vacuole, was already 1.5 times lower in K0-HS plants in comparison to in other plants **(Figure 3.5 A)**, potentially leading to sugar accumulation in the cytosol or apoplast of the shoot. The vacuole is the largest organelle that plays a role in cell turgor under standard conditions and osmotic stress (Sampaio *et al.*, 2022). The lower amount of sugar in the vacuole of K0-HS plants in comparison to the higher K supplies reduced their tolerance to drought stress, resulting in low survival rates under drought stress, regardless of whether the soil field capacity was reduced,

or drought stress was simulated by adding PEG8000 **(Supplemental figures 1 and 2)**. A further investigation is needed to confirm this hypothesis through subcellular metabolism localization analysis.

Moreover, loss of the AKT2/3 potassium channel (AKT2) reduces sugar loading into the phloem due to the lower phloem potential (Deeken *et al.,* 2002). It might be also the resume that the sugar accumulated in the plants of K0-HS plant under the control condition. (Figure 3.4 A). However, the expression of AKT2 in shoot of K0-HS plants was 1.6 times higher than other plants under the control condition. Drought stress even induced the expression of AKT2 more than under control condition in shoot of K0-HS plants **(Figure 3.5E)**. It can be explained that K deficiency might stimulate the expression of AKT2 to help the plants maintain the phloem potential for transport.

4.1.2. The negative impact of high potassium fertilization on Arabidopsis under drought stress

Despite numerous reports on the role of potassium (K) in phloem sugar loading, a detailed investigation into the mechanisms by which external K fertilization might promote long-distance sugar transport in plants has yet to be conducted. Our investigation into the effect of external K fertilization on Arabidopsis plants showed that increasing K3 fertilization (500 mg Kg^{-1} soil) did not result in an increase in biomass or sugar and starch contents compared to standard K fertilization **(Figure 3.1 and 3.2)**. While we did observe a slight increase in the K content in leaves and phloem of K3 plants under well-watered conditions **(Figure 3.2)**, it appears that external K fertilization in soil culture system may not be an effective strategy for improving the growth and sugar transport of Arabidopsis plants. Similarly, the results in hydroponic culture system showed that K3 supply did not significantly alter the sugar and starch contents in shoots, although there was a slight increase in glucose content in the root **(Figure 3.4)**. The expression of *SUC2* and *AKT2* genes, which are involved in sucrose reloading in the phloem transport system, remained unaltered in K3-HS plants compared to K2-HS plants **(Figure 3.5)**. While transporter activity may be regulated by phosphorylation (Ma *et al.*, 2020, Held *et al.*, 2011, Ma *et al.*, 2019a), we observed that the total sugar and K content in the phloem did not change upon increasing K supply in the hydroponic media

(Figure 3.4 G and supplemental figure 3) indicating that increasing K fertilization may not be an efficient strategy to improve long-distance sugar transport. However, it is important to note that Arabidopsis is not an ideal model for studying sugar long-distance transport as it lacks a strong sink. Therefore, further studies on the interaction between K fertilization and sugar long-distance transport under drought stress should be conducted in different plant species. These studies may provide a more comprehensive understanding of the mechanisms underlying sugar transport and K fertilization in plants, which could lead to the development of more effective strategies for improving crop productivity under stressful conditions.

While high potassium fertilization has been suggested to promote better yield and enhance plants' drought tolerance (Lu *et al.*, 2001, Jin *et al.*, 2007, Zahoor *et al.*, 2017), our experiments with Arabidopsis showed that supplying more K (K3, 500 mg) did not improve growth under drought stress. The recovery rate of plants treated with K3 was 50% lower than that of K2-treated plants (under standard K conditions) in terms of recovering from drought stress **(Supplemental figure 1)**. Similarly, in a hydroponic growth system (HS) with PEG8000 treatment to simulate drought stress, higher K supply did not improve drought tolerance **(Figure 3.3 B and C)**. In fact, shoot and root growth were reduced in K3-HS plants under drought stress compared to K2-HS plants (standard condition) **(Figure 3.3 B and E)**. Although Zhao *et al.* (2020) suggested that excess K is toxic for Arabidopsis, our experimental settings in both soil and hydroponic solutions did not exceed the toxic threshold of 105 mM external KCl. Nevertheless, our results demonstrated that K3-HS plants lost 80% of their shoot fresh weight when exposed to drought stress induced by the addition of PEG8000 **(Figure 3.3 A)**. One possible explanation is that while the amount of K used in our experiments was not toxic under control conditions, it might become toxic when the plants are under drought stress. Drought stress can lead to salinity stress, as salts become concentrated in the soil solution, resulting in combined drought and salinity stress (Stavi *et al.*, 2021). Additionally, Zhao *et al.* (2020) showed that KCl suppresses or even blocks sucrose degradation, and we observed a similar effect in K3-HS plants under drought stress **(Figure 3.4 A)** that the accumulation of sucrose was around two times higher than in K0, K1, and K2-HS plants. This may be due the higher KCl in K3-HS plants inhibited the sucrose hydrolyzation under drought stress and that

sucrose might have repressed the photosynthesis via a feedback inhibition, resulting in reduction of shoot and root growth in comparison to the other plants under drought stress **(Figure 3.3 B-G)**. A further analysis of high K reducing sugar metabolism under drought stress is needed to fully understand the complex interactions between nutrient availability, drought stress, and plant growth.

The other possible explanation is that higher K^+ might cause the inhibition of calcium (Ca) and magnesium (Mg) uptake and translocation. The salt stress-induced reduction of Ca^{2+} concentration has been reported in Arabidopsis, sugar beet, maize, and sorghum (Bernstein *et al.*, 1993, FORTMEIER & SCHUBERT, 1995, Wakeel *et al.*, 2011). In this study, drought stress reduced the Ca^{2+} and Mg^{2+} contents in shoot in all K-HS plants in comparison to them in control condition although the different K supply did not cause the alteration in the Ca^{2+} and Mg^{2+} contents in the shoots **(Supplemental table 3.1)**. While this could be a contributing factor to the reduction of drought tolerance in K3-HS plants, it may not be the major reason in this study.

Overall, our results suggest that the K3-HS plants' reduced drought tolerance is likely due to ion toxicity resulting from the high KCl concentration. This finding provides new insights into the effects of KCl on plant growth and development under drought stress, which may have implications for crop management and agriculture.

4.1.3. Low amount of sodium may be a benefit for plant under drought stress

Potassium is an essential nutrient for plant growth and has been used in crop fields for over a century. Its deficiency can lead to stunted growth, yield reductions, and increased susceptibility to diseases and pests. K plays a critical role in plant stress response (Cakmak, 2005, Wang *et al.*, 2013). In the setting of K-dependent hydroponic culture, the lower K fertilization (K1-HS) did not result in decreased shoot and root growth compared to standard K conditions (K2-HS) under drought stress. In fact, it increased the recovery rate from drought compared to the K2-HS plants **(Supplemental figure 2)**, where the only difference was the amount of K and Na in the hydroponic culture solution **(Table 2.3)**.

The ion contents analysis showed that the Na^+ in shoot of K1-HS plants was always 3.6 times higher than K2-HS plants under both control and drought stress. Interestingly, the K1-HS root accumulated four times of Na^+ in comparison to its shoot **(Supplemental table 3.1)**. In principle, sodium (Na) is not essential for either growth and development or for reproduction. Moderate and high levels of salt are detrimental to the majority of plants which are classified as glycophytes. At low concentrations, sodium (Na^+) can be a useful nutrient for plants, especially in low K conditions. A survey of published studies reveals that Na^+ is reported as a beneficial micronutrient nutrient (≤1 mM) for certain plant species, such as barley, maize, and tomato, contributing to an increase in the plants' biomass (Brownell, 1968, Ohta *et al.*, 1988, Woolley, 1957). This is because Na^+ and K^+ are chemically and structurally similar. An example of benefits is the accumulation of Na in the vacuole, where it provides turgor and thus can positively affect plant growth. Additionally, a study on Beet (Beta vulgaris L.) reported that leaf water content increased when the plant was supplied with 16 millequivalents per litre of sodium during drought stress (Subbarao *et al.*, 1999, LAWLOR & MILFORD, 1973). The expression of *AtNHX1*, a major Na-H-exchanger on tonoplast membrane, was two times higher in the shoot and root of K1-HS under drought stress in comparison to them under control conditions **(Supplemental figure 4 B and C)**, suggesting that the Arabidopsis plants might maintain the cell turgor by Na^+ and use the limited K^+ to keep the metabolism for plants growth. Thus, we assume the low amount of Na fertilization has positive effects on maintaining turgor in Arabidopsis during drought stress. Further research could be conducted to investigate the specific mechanisms by which Na^+ helps maintain turgor and positively affects plant growth, especially in low K conditions. Additionally, further studies could be conducted to explore the potential of Na fertilization as an alternative or supplement to K^+ fertilization in agriculture, especially in areas where K^+ is scarce or expensive.

4.1.4. Potassium fertilization increases starch accumulation potato tubers

Arabidopsis is not ideal for studying sink strength due to its weak carbohydrate storage organs. To investigate the hypothesis that increased potassium (K) fertilization promotes long-distance sugar transport to increase yield under

drought stress, we turned to potato plants. According to the U.S. Department of Agriculture's National Nutrient Database, the Désirée potato variety typically contains 16-18% starch (https://fdc.nal.usda.gov/fdc-app.html#/food-details/168470/nutrients). In our K fertilization experiment, WT (Désirée) produced around 100 grams of fresh potato containing 20g of starch under standard K (K2) conditions, indicating that our K fertilization system was well-established.

We also investigated the NTT-antisense potato line, which produces less starch due to a reduction in the activity of the amyloplast-located ATP/ADP translocator (Tjaden *et al.,* 1998), resulting in higher sugar accumulation. Our experiment showed that while the starch content of NTT-antisense was not significantly lower than WT under K2 conditions, the monosaccharide accumulations were 2.5 to three times higher in NTT-antisense tubers than in WT plants **(Figure 3.7)**. However, the tuber yield of NTT-antisense was lower than WT plants, indicating that NTT-antisense plants exhibited lower sink strength **(Figure 3.6)**.

Biomass production in plants depends on leaf photosynthetic characteristics and sucrose-to-starch conversion in storage organs (Hu *et al.*, 2016, Du *et al.*, 2020a). We found that increasing K application enhances starch accumulation and K content in tubers **(Figure 3.7)**. Interestingly, in NTT-antisense plants, the leaf assimilation rate (AR) and stomatal conductance (SC) both decreased with an increase in K supply while CO_2 content in leaves did not show a significant change, unlike in WT plants **(Supplemental figure 10)**. Previous studies in sweet potato and cotton have shown that low AR and SC with increased CO_2 content can be due to non-stomatal factors such as low chlorophyll content, an unbalanced chlorophyll a to chlorophyll b ratio, negative chlorophyll fluorescence parameters, and decreased carboxylation efficiency (Hu *et al.,* 2016, Gao *et al.*, 2021). Therefore, it is possible that the high level of K reduces the assimilation rate in NTT-antisense plants, caused by non-stomatal factors. The reduced sink strength may cause the potato plants to alter their C metabolism. Finally, we found that K3 NTT-antisense plants with lower assimilation rates did not exhibit a reduction in starch accumulation, although tuber yield was not significantly increased compared to WT plants under K3 conditions.

In conclusion, our findings suggest that increased K fertilization may promote long-distance sugar transport in potatoes, leading to higher yields and starch content even in low sink strength varieties.

4.1.5. Potassium fertilization in altering sugar and K^+ loading in potato plants

Sugar transporters are essential components of the plant's vascular system, enabling the long-distance transport of photosynthetically produced sugars from source to sink organs.

Potato sucrose transporter StSUT1 localizes to sieve elements in potato tuber phloem and influences tuber physiology and development (Kühn *et al.,* 2003). With the increased K fertilization, the expression of *StSUT1* was slightly decreased in leaves of WT plants, though it is not statistical difference **(Figure 3.8)**, suggesting that K may not have a direct effect on sugar transporter expression in potato plants. Rather, sugar transporters may be regulated through phosphorylation, as is the case with the apple sucrose transporter MdSUT2.2 which is a phosphorylation target for protein kinase MdCIPK22 in response to drought (Ma *et al.,* 2019a). Further research is needed to fully understand the mechanisms underlying the regulation of sugar transporter expression in potato plants and the potential role of K fertilization in this process.

In addition to sugar transporters, K^+ channels also play a crucial role in sugar long-distance transport in plants. In Arabidopsis, AKT2 is known to regulate sugar loading into the phloem. In potato plants, SKT1 is the inward-rectifying K^+ channel that is the highest homology to Arabidopsis AKT1 (Schroeder *et al.*, 1994, Zimmermann *et al.,* 1998). However, the physiology function of about SKT1 is still unclear. With the increased K fertilization, the expression of *SKT1* was decrease in leaves and increased in tubers. This expression pattern was found in both WT and NTT-antisense plants **(Figure 3.8)**, suggest that SKT1 may have different function in leaf tissue and tuber. We hypothesize that SKT1 may be capable of converting into a non-rectifying channel, similar to AKT2, which would enable it to mediate both K^+ uptake and release (Gajdanowicz *et al.,* 2011). This suggests that SKT1 may help facilitate sugar loading from the phloem into the tuber by releasing K^+ from the phloem to the apoplast in tuber, creating an electrochemical gradient.

This process could help to overcome energy limitations and enable the efficient loading of sugars into sink cells (i.g., tuber).

Sugar translocation in plants is a complex process that involves the interplay of various transporters. It is not only the long-distance transport of sugars from source to sink, but also the subcellular localization of sugars that plays a crucial role in plant growth and yield production. Tonoplast sugar transporters (TSTs) have also been implicated in sugar transport and are known as the major vacuolar monosaccharide importers in Arabidopsis (Wormit *et al.,* 2006). Furthermore, the study in melon suggested that the expression of CmTST1 in roots was induced by a relatively high level of sucrose, glucose, and fructose (Lu *et al.*, 2020). Interestingly, our study revealed that lower K fertilization (K1) induced the expression of tonoplast sugar transporters in the leaves of NTT-antisense potato plants **(Figure 3.8)**. These findings suggest that higher sugar accumulations in K1 NTT-antisense plants resulted in the induction of *StTSTs* to facilitate the import of sugars into the vacuole. Additionally, these expression patterns of *TSTs* were not found in WT plants, and the sucrose levels in WT plants were generally lower than in NTT-antisense plants. This hints that the NTT-antisense plant with low sink strength reduced sugar transport from source to sink. While the sugar levels in NTT-sense plants were not significantly altered by K fertilization, starch accumulation was slightly decreased when more K was supplied **(Figure 3.8)**. This result suggests that more K supply improves sugar transport to the sink, even in low sink strength potato plants.

Furthermore, the flow of K in the phloem creates an electrochemical gradient for maintaining sugar loading (Gajdanowicz *et al.,* 2011), and it is possible that a similar effect occurs in the sugar imported from the cytosol to the vacuole. Under low K conditions, plants may require more TSTs to import sugars, suggesting a possible role of TSTs in regulating sugar transport under varying K conditions. Although little is known about potato TSTs, our results in this study hint that low K may reduce sugar long-distance transport and induce the expression of *StTSTs*, highlighting the potential importance of K in regulating sugar transport in potato plants.

4.2. The function of silicon in drought tolerance

4.2.1. *Arabidopsis thaliana* is a silicon excluder species

Plant species have different abilities to accumulate silicon (Si). Active accumulators, accumulate around 1.5% to 10% Si content in shoots, e.g., rice, wheat, maize, and sorghum. The passive accumulators are plants with a shoot Si content of 0.5–1.5%, e.g., cucumber, bitter gourd, and melon. The plants with a Si content of less than 0.2% are classified as Si excluders, which was associated with most dicots, such as tomato, potato, canola, and lentil (Takahashi *et al.,* 1990). The Si content analysis showed that the Si-treated Arabidopsis accumulated around ten µmol, which was around 0.03% of Si per one gram of dry weight **(Figure 3.9 K)**. This result indicated that *Arabidopsis thaliana* belongs to Si excluders. Also, this property makes Arabidopsis an ideal species to study the mechanisms underlying silicon uptake and transport in plants. Furthermore, studies have shown that silicon (Si) can mitigate the negative effects of drought stress in Si excluder plant species such as tomatoes and canola (Shi *et al.,* 2016, Habibi, 2014). In contrast, wheat landraces are high Si accumulators, but no significant differences in growth or stress tolerance were observed under water stress conditions (Crusciol *et al.,* 2009). This mentions the possibility of Arabidopsis as a model to study the mechanism of Si under drought stress in plants.

4.2.2. Silicon mitigates drought stress effects in Arabidopsis leaf tissue but not in root tissue

In the present study, the application of silicon (Si) under drought stress conditions led to a reduction in the number of dead and wilted leaves, and an increase in biomass and water content in the leaves **(Figure 3.9 C-F)**. However, the Si-dependent manner of root growth in Arabidopsis was observed only under control conditions and not under drought stress conditions **(Figure 3.9 G-H)**. Si has been reported that can increase root growth in sorghum and rice, particularly under drought stress conditions (Ming *et al.,* 2012, Fleck *et al.*, 2011), while the lack of effects on the root/shoot ratio in tomato (Shi *et al.,* 2016), suggesting Si have different impacts on all plants under water limitation conditions.

Proline is an amino acid that is known to accumulate in plants under stress conditions, including drought stress. It has been suggested that proline could be used as a biochemical marker for screening drought-tolerant crop cultivars, as cultivars that accumulate higher levels of proline are more sensitive to drought. This has been observed in common beans, potato, and maize, among other crops (Blum & Ebercon, 1976, Ibarracaballero *et al.,* 1988, Crusciol *et al.,* 2009, Arteaga *et al.,* 2020). The observation in this study showed a lower proline accumulation in the shoot of Si-treated plants in comparison to non-Si-treated plants under drought stress **(Figure 3.11 C)**. This suggests that Si application could be a strategy for improving plant growth or further productivity under drought-stress conditions. However, the Si application did not alter the level of proline in root tissue when the plants were under drought stress **(Figure 3.11 G)**. It is possible that the mechanisms of drought tolerance differ between root and shoot tissues. Therefore, Si application may have a tissue-specific effect on drought tolerance.

Overall, these results suggest that Si application has a beneficial effect on the growth of Arabidopsis plants, but this beneficial effect varies depending on the plant species and tissue type.

4.2.3. Silicon application increased the accumulation of sugars in leaf tissue via altering the primary carbohydrate metabolism processes

It is generally acknowledged that the accumulation of soluble sugars in response to osmotic stress, such as salt and drought stress, serves as a mechanism for plants to increase their solute potential (Ψs), which in turn improves the ability of cells to retain water (Hoekstra *et al.,* 2001, Taiz *et al.,* 2015, Takahashi *et al.,* 2020). Si has been reported that increases K content in maize under drought stress (Kaya *et al.,* 2006). However, in our study, Si supply did not alter the potassium content in both leaf and root tissue **(Figure 3.11 D and H)**, suggesting the potassium content was not the primary cause of Si-dependent mitigation of drought stress. On the other hand, the sugar content analysis showed that the application of silicon (Si) appears to increase the accumulation of soluble sugars in the shoot of plants under both control and drought stress conditions **(Figure 3.11 A)**. This suggests that Si application may enhance the ability of plants to cope with osmotic stress by increasing their capacity to accumulate osmolytes such as soluble sugars. One

possible explanation for the observed effect of Si on soluble sugar accumulation is the following. (1) Si application increases the photosynthesis and transpiration rate (Verma *et al.*, 2020, Chen *et al.*, 2016), (2) Si alters the sugar metabolism processes, (3) Si regulates the starch remobilization to produce solute sugars.

Genes associated with photosynthesis were found to be upregulated in Si-treated plants both before and after drought stress **(Figure 3.10 E-G)**. The fact that the Si treatment had a positive effect on electron transport rate (ETR) and quantum yield in photosystem II (Y(II)) only under non-stressful conditions, as opposed to under drought stress conditions. **(Supplemental figure 5 A)**. The genes associated with photosynthesis, such as chlorophyll a-b binding proteins (LHCB) and photosystem II reaction center protein I (PsbI), have been reported to function in balancing the relative excitation of photosystem I and photosystem II. However, less information is available about the phenotype that results from overexpression of these genes, particularly with regard to alterations in ETR and photosynthesis (Lu, 2016). The photosynthetic system is a complex network that involves the regulation of electron transport, phosphorylation, oxidation-reduction, and other processes. Therefore, further study is needed to investigate the interaction between the photosynthetic system and Si. Although Si application did not always lead to a corresponding increase in photosynthetic performance, we hypothesized that Si increases sugar accumulation under drought stress may through other regulating processes, such as sugar metabolism or starch remobilization.

Sugar metabolism, which includes sucrose hydrolysis and synthesis, plays a crucial regulatory role in plant growth. High level of sucrose in plant cells can enhance plants resistance to drought stress (Hoekstra *et al.,* 2001, Taiz *et al.,* 2015, Takahashi *et al.,* 2020). However, the result in our analysis showed that Si application increased the level of glucose but not sucrose in leaf tissue in comparison to the plants without Si apply under drought stress **(Figure 3.11 A)**. Additionally, it was observed that Si application had an impact on enzymes such as invertases (INVs), sucrose synthases (SUSs), and sucrose phosphate synthases (SPSs) under drought stress conditions **(Figure 3.13 B)**, suggesting a connection between Si treatment and sucrose metabolism.

SUSs are another enzyme involved in sucrose hydrolysis which catalyzes the reversible cleavage of sucrose to fructose and UDP/ADP-glucose. In non-Si-

treated plants, the expression of sucrose synthases (SUSs) is induced by drought stress, while the expression of sucrose phosphate synthases (SPSs) is upregulated. However, Si application mitigates this induction of both SUSs and SPSs during drought stress **(Figure 3.13 B)**. Our findings indicate that Si has a differential impact on sucrose metabolism in plants under drought stress, and the expression of *INVs* provides evidence for this conclusion. The expression of both *VINV1* and *CINV1*, with a 1.7-fold higher expression observed in Si-treated plants compared to plants without Si application eight days after drought stress **(Figure 3.13 B)**.

The importance of vacuole invertase (VINV) in plant resilience to adverse environmental conditions has been highlighted. Overexpression of VINV, such as in cucumber (CsVI2), enhances its activity and drought tolerance by providing energy and contributing to the osmotic potential of the cell (Chen *et al.*, 2021). Additionally, Cytosolic invertases (CINV1 or At-A/N-InvG) down-regulate oxidative stress defense gene expression, which suggests that they produce glucose as a substrate for mitochondria-associated hexokinase and contribute to mitochondrial re active oxygen species homeostasis (Xiang *et al.*, 2011). Taken together with the observed glucose accumulation in leaves **(Figure 3.11 A)**, it is suggested that Si can enhance drought tolerance in Arabidopsis by altering sucrose metabolism. This may occur through two mechanisms: (1) increasing the expression of *VINV* to accumulate more hexoses, such as glucose, in the vacuole, which can help maintain the osmotic potential of the cell during extended periods of drought stress, and (2) enhancing energy metabolism and oxidative stress response by increasing the expression of cytosolic invertase (CINV) and potentially interacting with mitochondrial reactive oxygen species homeostasis **(Figure 4.1)**. However, further studies are needed to confirm this interaction between Si and mitochondrial reactive oxygen species homeostasis.

Starch remobilization is another way to increase the soluble sugars available for providing energy and osmoprotectants in cells under drought stress (Krasensky & Jonak, 2012). However, it is in agreement with several earlier studies that the C balance (i.e. the amount of C left available in the rosette after the requirements for growth and respiration are fulfilled) was strongly increased by water deficit. (Kim *et al.*, 2000, Bogeat-Triboulot *et al.*, 2006). Over-sugar accumulation in leaf tissue

causes negative feedback on photosynthesis. In our case, the observed the higher starch accumulation in Si-treated plants during drought stress suggest that Si application increases starch accumulation **(Figure 3.11 B)** for maintaining the carbon (C) balancing in leaf tissue. Another observation in this study, the expression of *GPT2*, also provides evidence for this conclusion. GPT2 is a glucose 6-phosphate/phosphate translocator with a role in cytosolic sugar level maintenance which regulating and stabilizing photosynthetic electron transport and carbon metabolism (DYSON et al., 2015, Weise et al., 2019).The expression of GPT2 is strongly induced by drought stress in the plants, but only in the plants without Si application **(Figure 3.13 A)** indicating a high level of sugar in cytosol. Si application maintains the cellular C balancing in plant cells during drought stress.

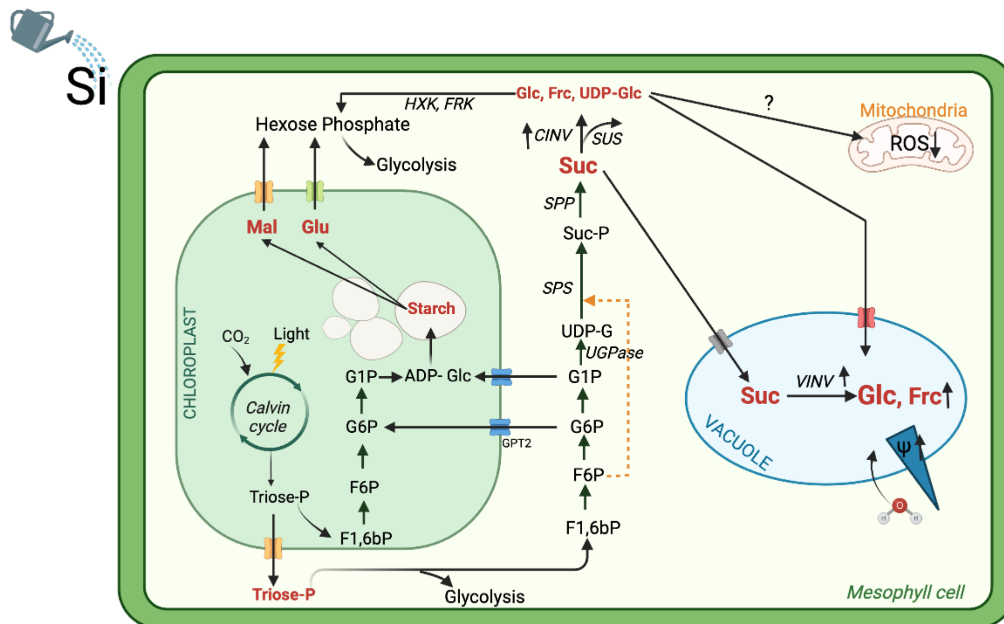


Figure 4.1. Hypotheses of the function of silicon in sugar metabolism under drought stress. In times of drought stress, plants must adjust their metabolic processes to cope with the lack of water. One adaptation is the hydrolysis of sucrose into glucose and fructose, which is facilitated by the enzyme cytosolic invertase (CINV). These monosaccharides are then utilized in one of two ways: either as an energy source to enhance energy metabolism and oxidative stress response in the mitochondria, or they are transported into the vacuole. The sugars stored in the vacuole contribute to the osmotic potential of the cell, which is critical for maintaining water balance and cell turgor pressure under drought stress conditions. This osmotic regulation helps prevent water loss and damage to cellular structures, ultimately improving the plant's ability to withstand the stress.

4.2.4. Silicon increases drought tolerance through increasing sugar accumulation in vacuole

Sugars occur in different subcellular compartments, which is of crucial importance for the sugar metabolism and plant development (Tiessen & Padilla-Chacon, 2013). The vacuole is the largest organelle, which plays a role in cell turgor under standard conditions but also osmotic stress (Sampaio *et al.,* 2022). As mentioned earlier, the induction of *GPT2* expression in response to drought stress in plants without Si application may be associated with changes in vacuolar glucose and fructose levels. Our subcellular metabolic analysis revealed that Si application resulted in a 10% increase in vacuolar glucose levels compared to plants without Si treatment, both under control and drought stress conditions **(Figure 3.12 A)**. Furthermore, Si treatment specifically led to an increase in vacuolar fructose levels from 35% to 45% specifically under drought stress conditions. These findings suggest that Si application may have a modulatory effect on the accumulation of glucose and fructose in the vacuole, indicating a potential role in regulating subcellular sugar localization and metabolism in response to drought stress.

The findings of Si application modulating vacuolar accumulation of glucose and fructose, as mentioned earlier, may be related to the function of sugar transporters, as intracellular sugar distribution relies on the activity of various sugar transporters. These sugar transporters may regulate sugar accumulation by either increasing sugar import into the vacuole or decreasing sugar export from the vacuole. Our results showed that Si application had an impact on the expression of various tonoplast sugar transporters under drought stress conditions **(Figure 3.13 A, vacuole)**. The expression of sugar transporters, such as *AtTST1* and *AtSWEET17*, which facilitate the import of sugar from cytosol to the vacuole (Wormit *et al.,* 2006, Guo *et al.*, 2013), was higher in Si-treated plants compared to those without silicon application. AtSWEET17, known as a fructose transporter, has been reported to play a role in drought tolerance as mutants with knocked-out *AtSWEET17* show impaired drought tolerance (Guo *et al.,* 2013; Valifard *et al.,* 2021). These findings strongly suggest that Si enhances sugar accumulation in the vacuole under drought stress.

In a study by Slawinski *et al*. (2021), it was suggested that *AtTST2* functions in sugar accumulation for osmotic adjustment during the early stage of drought stress,

while *AtTST1* is downregulated to facilitate the remobilization of sugars from the vacuole during prolonged drought periods. Our results showed a similar expression pattern, with *AtTST1* being downregulated at eight days after drought stress, while *AtTST2* was upregulated six hours after drought stress **(Figure 3.13 A, vacuole)**. However, Si application reduced the level of downregulation of *AtTST1*. This can be explained that Si helps maintaining sugar accumulation in the vacuole by keeping the expression of *AtTST1*. Furthermore, TST2 has been reported to be phosphorylated by CIPK6 (CBL-interacting protein kinases 6), and overexpression of GhCIPK6 in Arabidopsis led to higher sugar accumulation (Deng *et al.,* 2020). Gene expression results showed that Si application maintained a higher level of *AtCIPK6* expression, while this pattern did not occur in plants without Si treatment at eight days after drought stress **(Supplemental Figure 7)**, indicating that Si may regulate sugar homeostasis by phosphorylating the activity of TST2 to enhance sugar accumulation in vacuole under drought stress. CBL-interacting protein kinases (CIPKs) participate in a signaling pathway that responds to stress by briefly elevating calcium levels, which are detected by a complex formed by CBL calcium-binding proteins and CIPKs. However, the application of Si did not result in any significant difference in the calcium (Ca) content of leaves **(Supplemental table 3.3)** or its subcellular localization under drought stress (**Supplemental figure 6 C**). It is difficult to definitively conclude that Si did not have an effect on signaling transduction via calcium level alteration, as any changes in Ca content may have occurred in the early stages of drought stress and may not have been detectable in our study. Therefore, we hypothesize that Si may also have a direct role in signaling transduction, independent of its effect on calcium levels. Further studies are needed to elucidate the potential signaling pathways affected by Si under stress conditions. Future research could explore the potential interactions between Si and other signaling pathways, and how these interactions may contribute to enhanced drought tolerance. Nevertheless, these findings suggest that Si application promotes the import of sugars into the vacuole, potentially enhancing drought stress tolerance.

On the other hand, vacuole sugar accumulation is also associated with the regulation of sugar export from vacuole. For example, AtERDL6 (AtESL1.02) is a proton coupled vacuolar glucose exporter showing downregulation in cold to keep sugar in vacuole (Klemens *et al.*, 2014). Vacuolar sugar accumulation is a critical

metabolic process in response to drought or cold stress, as it contributes to the development of abiotic stress tolerance. The transporters involved in sugar efflux from vacuole, such as *AtERDL6, AtERD6*, and *AtESL1*, were induced in the non-Si-treated plants under drought stress. Si application mitigated the drought-dependent induction **(Figure 3.13 A, vacuole)**, indicating Si helps sugar accumulation in the vacuole. However, AtERDL6 (AtESL1.02) is a proton coupled vacuolar glucose exporter showing downregulation in cold to keep sugar in vacuole (Klemens *et al.,* 2014). However, Slawinski *et al*. (2021) suggested that the upregulation of *AtERDL6* and under severe water deficit conditions might be necessary for the remobilization of sugars from mature wilted leaves and their reallocation to young sink organs. It could be explained that Si application may help maintain a certain level of subcellular sugar metabolism, potentially reducing the need for excessive sugar export from vacuole to support continued growth under drought stress conditions, resulting more sugar accumulated in vacuole for osmotic adjustment. This idea is also support by the higher expression of *AtGPT2*, associated with increased cytosolic sugar levels (Weise *et al.,* 2019), observed in the non-Si-treated plants, indicating that drought stress impacts cytosolic sugar metabolism and leads to higher sugar accumulation in the cytosol. This, in turn, may trigger a negative feedback loop that downregulates AtpGlcT, a plastid glucose transporter, reducing glucose export from the plastid to the cytosol (Cho *et al*., 2011). Si application, on the other hand, may maintain sugar metabolism by enhancing cytosolic invertase (CINV), resulting in improved growth **(Figure 4.1)**. Overall, this suggests a plausible mechanism involving Si application, sugar metabolism, and osmotic adjustment in vacuole under drought stress conditions **(Figure 4.2)**.

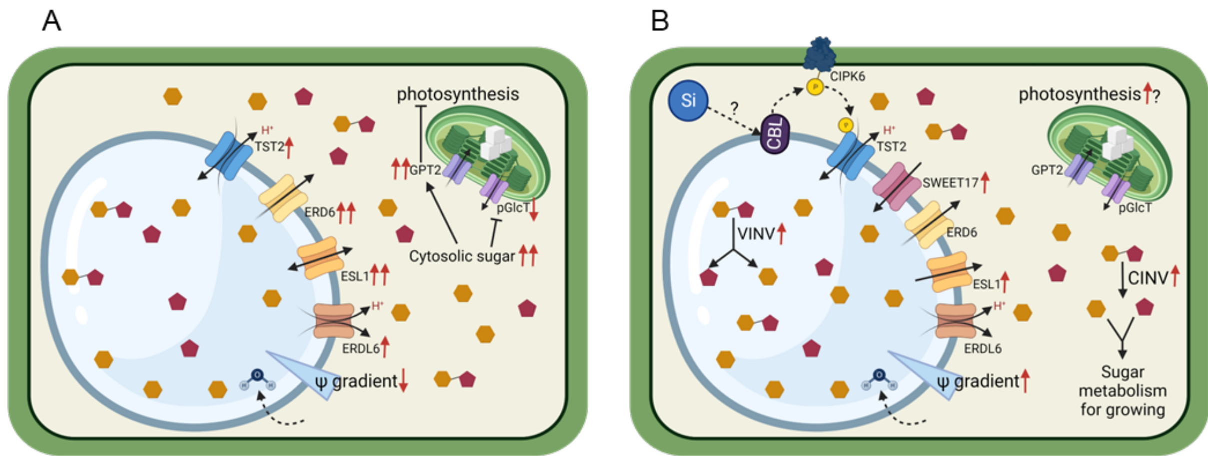


Figure 4.3 Overview of the regulation of vacuole sugar transporters under drought stress. (A) Under drought stress conditions without Si supplementation, the responses of sugar transporters were induced. Drought stress led to the efflux of sugar from vacuoles, resulting in the accumulation of sugar in the cytosol after eight days of stress. The high cytosolic sugar levels induced the expression of AtGPT2, which in turn in reduced photosynthesis and sugar export from plastids to the cytosol. (B) Si application may mitigate the efflux of sugars from the vacuole to the cytosol, allowing for more sugar to be retained in the vacuole. This can result in an increased water gradient, facilitating water movement into the vacuole.

4.2.5. Silicon increases sugar long distance transport via increase the hydraulic conductivity in vascular tissue

Sugar transport from source to sink organs (e.g. roots), supplying carbon as an energy source to meet the energy demand of growth and stress-responsive adaptive mechanisms, is crucial for plants to survive under drought stress (Durand *et al.,* 2016; Kaur *et al.,* 2021). Arabidopsis sucrose transporters SWEET11, SWEET12, and SUC2 are known to play key roles in phloem loading and have been reported to maintain sugar transportation under drought stress. Knockout mutants of these transporters have been shown to exhibit a drought-sensitive phenotype (Gong *et al.,* 2015; Chen *et al.,* 2022). However, in our study, the expression of these sugar transporters (*AtSWEET11*, *AtSWEET12*, and *AtSUC2*) involved in phloem loading did not show significant changes in response to Si application under drought stress **(Figure 3.13 A, plasma membrane)**. The regulation of sugar transporters by protein phosphorylation has been suggested, with studies showing that SWEET11 and SWEET12 are phosphorylated by SnRK2 to enhance root growth under drought stress (Chen *et al.,* 2022). However, the expression of SnRK2 was not altered by either drought or Si treatments in our study **(Supplemental figure 8)**. While our findings suggest that Si may enhance drought tolerance by promoting the import of sugars into the vacuole, it is important to note that the protein interactions between SWEET transporters and SnRK2 with Si treatment under drought stress have not been analyzed in our study, leaving the possibility of SnRK2 phosphorylation under drought stress unexplored. Despite the challenge of explaining how Si application improves sugar phloem loading under drought stress, our results showed that glucose content in the root was 40% higher in Si-treated plants compared to non-Si-treated plants under drought stress **(Figure 3.11 E)**. Additionally, Si application mitigated the degradation of starch in the roots **(Figure 3.11 F)**, indicating that sugar long-distance transport in Si-treated plants may be more efficient than in plants without Si supply.

Some of the transported sucrose is cleaved in the vascular tissues to support vascular development and functioning. Fructose kinases (FRKs) have been reported to contribute to vascular tissues development. Inhibited FRK in tomatoes resulted in decreased hydraulic conductivity and sugar transportation, indicating

impaired vascular circulation (Damari-Weissler *et al.*, 2009, Stein *et al.*, 2017, Stein *et al.*, 2016). Maintaining vascular circulation is crucial for plants to survive under drought stress. Our gene expression analysis revealed that the expression of *AtFRK1* was significantly increased by 2.3 times in Si-treated plants compared to the control group, eight days after drought treatment. However, there was no significant change observed in the expression of AtFRK1 in plants without Si treatment. Additionally, the expression of AtFRK6 was reduced by 57% in plants without Si application, eight days after drought stress **(Figure 3.13 B)**. This suggests that Si application may contribute to better vascular tissue development, which could help maintain vascular circulation under drought stress and potentially enhance drought tolerance.

Overall, while the regulation of sugar transporters by Si and protein phosphorylation requires further investigation, our results suggest that Si-treated plants may exhibit more efficient sugar long-distance transport. Additionally, Si application may contribute to better vascular tissue development which might be a mechanism of Si-mediated drought stress tolerance. Further research is warranted to fully understand the mechanisms underlying Si-mediated enhancement of vascular tissue development and improving sugar transport.

4.2.6. Silicon alleviates drought stress in leaves, but not in tubers

According to Takahashi et al. (1990), potatoes are classified as Si excluders. However, some studies have reported that silicon (Si) can mitigate the negative effects of drought stress in Si excluder plant species such as tomatoes and canola (Habibi, 2014, Shi *et al.,* 2016). Previous studies in tomatoes have suggested that Si application can increase the average number of fruits per plant by 23% (Lu *et al.*, 2016). Our study in Arabidopsis also demonstrated that Si supply improves plant growth under control and drought stress conditions **(Discussion 4.2.2)**. Therefore, we established a Si supply system in potato plants and performed drought stress experiments **(Method 2.1.2)**. Si-treated plants accumulated around ten µmol of Si per gram of dry weight in leaves (0.02%), which is consistent with the findings of Takahashi et al. (1990). We also observed that Si treatment slightly promoted potato plants to produce bigger tubers under well-watered conditions **(Figure 3.14 D)**, although Si supply did not cause a significant difference in shoot

growth under both control and drought stress conditions **(Figure 3.14 B)**. These results suggest the possibility of Si application in potato to increase potato tuber yield. However, the results of the metabolic analysis showed that the application of Si did not cause a difference in starch content, and sugar contents in the tuber were slightly decreased **(Figure 3.15)**. Sugars not only serve as an energy source but also play a crucial role in cell wall restructuring. Si has been reported that regulates the activities of enzymes involved in carbohydrate metabolism and affects the lignification of cell walls, consequently regulating assimilate synthesis and transport efficiency (Ming *et al.,* 2012, Gong *et al.,* 2005, Kaya *et al.,* 2006, Sonobe *et al.,* 2010). It is possible that Si did not have a direct effect on promoting carbohydrate accumulation in potato tubers, but instead affected the cell wall structure. Further research is needed to fully understand the mechanisms by which Si influences tuber development and yield.

Under drought stress conditions supplying Si under drought stress conditions mitigated the accumulation of proline in leaves. However, we observed that Si did not have a significant effect on proline accumulation in potato tubers **(Figure 3.15)**. This phenomenon is consistent with what has been observed in Arabidopsis **(Figure 3.11 G)**. Additionally, the report indicates that silicon supplementation in tomatoes did not significantly affect the root-to-shoot ratio nor alter the proline levels in the roots (Shi *et al.,* 2016). This suggests that Si may have a similar effect on Si excluder plant species that Si is more effective in shoot tissue in Si excluder plant species.

The observation of the mitigation of drought stress levels in leaves with Si treatment is promising, yet our study found no significant changes in the sugar and starch contents in Si-treated leaves under drought stress **(Figure 3.15)**. While the result in Arabidopsis suggests that Si supply may increase drought tolerance by altering sugar metabolism processes **(Discussion 4.2.3)**, it remains unclear whether this effect is universal across plant species. Hence, we cannot assume that Si directly affects carbohydrate accumulation in leaves under drought stress conditions in potato. Further studies are needed to investigate the potential effects of Si on other aspects of plant physiology, such as cell wall structure, hydraulic conductance, or ROS levels, in order to fully understand the mechanisms underlying the observed mitigation of drought stress levels in Si-treated leaves.

4.2.7. Silicon may regulate subcellular localization in drought-stressed potato leaves

Our study found that Si supply altered the expression of vacuole sugar transporters under drought stress in potato plants. Specifically, the expression of *tonoplast sugar transporter* (*StTST1*) in potato was found to be two times higher in Si-treated plants under drought stress compared to non-Si-treated plants **(Figure 3.16 E)**. This suggests that Si may regulate sugar storage in the vacuole for osmotic adjustment, ultimately leading to increased drought tolerance. Furthermore, the vacuole is the largest organelle in plant cells and plays a critical role in maintaining cell turgor under standard and osmotic stress conditions (Sampaio *et al.,* 2022). Interestingly, while Si supply did not alter the sugar and starch contents in leaves, sugar translocation may still play a key role in mitigating drought stress in leaves. The result in Arabidopsis suggests that Si supply increases drought tolerance by increasing sugar accumulation in the vacuole **(Discussion 4.2.4)**, which may ultimately help maintain cell turgor and reduce the negative impacts of drought stress on plant growth and yield.

Additionally, the expression of *StSUT1* was four times higher in Si-treated plant under drought stress in comparison to non-Si-treated plants **(Figure 3.16 F)**. This is consistent with previous studies that have shown that drought stress can lead to changes in the expression of *SUT* genes in various plant species. In apoplasmic loaders such as Arabidopsis, soybean, barley, rice, wheat, and maize, the SUT responsible for phloem loading is up-regulated under drought stress, but not in tomato or potato (Xu *et al.,* 2017).

Under water stress, *SUT* transcript abundance can be upregulated (Ibraheem *et al.*, 2011b, Xu et al., 2017), or the transporters can be stabilized to prevent their breakdown from the plasma membrane (Ma *et al.*, 2019b), which would increase loading and strengthen the gradient for water uptake from the xylem (Stanfield & Bartlett, 2022). Our results suggest that Si treatment may regulate the expression of *StSUT1* under drought stress in potato, potentially contributing to the maintenance of water uptake and transport under these conditions. However, further studies are needed to fully understand the role of SUTs in drought stress response and the potential mechanisms by which Si treatment may affect their expression and function.

4.3. Conclusion

This dissertation highlights the crucial role of nutrition, particularly potassium (K), in plant development and drought tolerance, with a focus on its impact on sugar metabolism and transportation under drought stress conditions. The results suggest that optimal K supplementation is essential for efficient utilization of sugars as an energy source for growth and development, as well as for maintaining plant growth in adverse conditions. The study found that K-deficient plants had stunted growth and inefficient sugar metabolism, while excessive K supplementation inhibited sucrose metabolism and reduced drought tolerance. These findings suggest that the amount of K supplementation is critical, and the right balance needs to be achieved for optimal plant growth and development. Interestingly, low levels of sodium (Na) under low K conditions were found to enhance plant drought tolerance. This is because Na can accumulate in the vacuole to control water potential, which in turn reduces the negative effects of drought stress. This finding provides insights into the role of Na in plant drought tolerance and its potential use in improving crop productivity in water-limited regions.

Additionally, this study highlights the potential benefits of silicon (Si) application in enhancing plant resilience to drought stress. Si was found to increase photosynthesis rates, promote sugar accumulation in the vacuole, and alter sugar metabolism processes, potentially through the induction of invertase and fructose kinase expression. The study also suggests that Si may regulate the expression of vacuole sugar transporters and SUT genes in potato plants under drought stress, leading to increased sugar accumulation in the vacuole and enhanced phloem loading for water uptake. While Si did not significantly alter the sugar and starch contents in leaves, the regulation of sugar translocation and phloem loading may play a crucial role in reducing the effects of drought stress in plants. The study highlights the need for further research to fully understand the mechanisms underlying the effects of Si on plant physiology and to explore its potential as a tool for improving plant drought tolerance.

Overall, this study provides valuable insights into the potential benefits of optimal K supplementation and Si application in improving plant resilience to drought stress, which could have significant implications for agriculture and food security in water-limited regions. The findings also suggest that the right balance of K

supplementation, in combination with other essential nutrients, can enhance plant growth and development, and further research in this area is needed.

4.4. Outlook

Potassium has long been recognized as a beneficial element for plant growth and is widely used in agriculture. The study suggests that K fertilization can enhance tuber yield in different sink strengths of potato. However, the mechanism behind K's role in improving sugar reloading in phloem and sugar long-distance transportation still requires further investigation. Additionally, the study found that K deficiency under drought stress can affect the K content of the phloem, which may impact the plant's ability to maintain water balance and carry out essential physiological processes. Further research is needed to understand the relationship between different K levels and sugar translocation in plants.

Low levels of Na under low K conditions may benefit plants by improving their drought tolerance. Na can accumulate in the vacuole to control water potential. Future research could investigate this relationship further to determine how Na accumulation affects plant drought tolerance under low K conditions. The study found that excess K supplementation does not improve drought tolerance and may inhibit sucrose metabolism. Further research could explore how excess K affects sucrose metabolism and the potential mechanisms behind this relationship.

Silicon has been identified as a potential fertilizer for improving drought stress tolerance. The study's findings provide insights into the mechanisms underlying the beneficial effects of silicon application on drought tolerance in Arabidopsis. However, further studies are needed to fully elucidate the potential signaling pathways affected by Si under stress conditions. This could involve exploring the interactions between Si and other signaling pathways, and how these interactions may contribute to enhanced drought tolerance. Additionally, investigating the effect of Si on the expression and phosphorylation of sugar transporters and other key proteins involved in sugar metabolism and signaling transduction could provide a more comprehensive understanding of the molecular mechanisms underlying Si-induced drought tolerance.

The study also suggests that Si supply may have potential benefits for improving drought tolerance in potato plants. However, more research is needed to fully understand the mechanisms underlying Si-mediated drought tolerance in potato, particularly with regards to the role of sugar metabolism and transporters. Investigating the effects of Si on other aspects of plant physiology, such as cell wall structure and ROS levels, may provide further insight into its potential as a drought mitigation strategy. These findings could have important implications for agriculture, as improving drought tolerance in crops can help to mitigate the negative effects of climate change on food production.

5. Reference

Abelenda, J. A., Bergonzi, S., Oortwijn, M., Sonnewald, S., Du, M. R., Visser, R. G. F., *et al.* (2019) Source-Sink Regulation Is Mediated by Interaction of an FT Homolog with a SWEET Protein in Potato. *Current Biology,* 29, 1178-+.

Adrees, M., Ali, S., Rizwan, M., Zia-ur-Rehman, M., Ibrahim, M., Abbas, F., *et al.* (2015) Mechanisms of silicon-mediated alleviation of heavy metal toxicity in plants: a review. *Ecotoxicology and Environmental Safety,* 119, 186-197.

Agarie, S., Uchida, H., Agata, W., Kubota, F. and Kaufman, P. B. (1998) Effects of Silicon on Transpiration and Leaf Conductance in Rice Plants (Oryza sativa L.). *Plant Production Science,* 1, 89-95.

Albrecht, V., Weinl, S., Blazevic, D., D'Angelo, C., Batistic, O., Kolukisaoglu, U., *et al.* (2003) The calcium sensor CBL1 integrates plant responses to abiotic stresses. *Plant Journal,* 36, 457-470.

Andersen, M. N., Jensen, C. R. and Losch, R. (1992) The Interaction Effects of Potassium and Drought in Field-Grown Barley .1. Yield, Water-Use Efficiency and Growth. *Acta Agriculturae Scandinavica Section B-Soil and Plant Science,* 42, 34-44.

Arteaga, S., Yabor, L., Diez, M. J., Prohens, J., Boscaiu, M. and Vicente, O. (2020) The Use of Proline in Screening for Tolerance to Drought and Salinity in Common Bean (Phaseolus vulgaris L.) Genotypes. *Agronomy-Basel,* 10.

Asai, T., Tena, G., Plotnikova, J., Willmann, M. R., Chiu, W. L., Gomez-Gomez, L., *et al.* (2002) MAP kinase signalling cascade in Arabidopsis innate immunity. *Nature,* 415, 977-983.

Beier, S., Marella, N. C., Yvin, J.-C., Hosseini, S. A. and von Wirén, N. (2022) Silicon mitigates potassium deficiency by enhanced remobilization and modulated potassium transporter regulation. *Environmental and Experimental Botany,* 198, 104849.

Benabdellah, K., Ruiz-Lozano, J. M. and Aroca, R. (2009) Hydrogen peroxide effects on root hydraulic properties and plasma membrane aquaporin regulation in Phaseolus vulgaris. *Plant Molecular Biology,* 70, 647-661.

Bernstein, N., Silk, W. K. and Läuchli, A. (1993) Growth and development of sorghum leaves under conditions of NaCl stress. *Planta,* 191, 433-439.

Billings, S. A. and Phillips, N. (2011) Forest Biogeochemistry and Drought. In: *Forest Hydrology and Biogeochemistry: Synthesis of Past Research and Future Directions.* (Levia, D. F., Carlyle-Moses, D. and Tanaka, T., eds.). Dordrecht: Springer Netherlands, pp. 581-597.

Blum, A. and Ebercon, A. (1976) Genotypic responses in Sorghum to drought stress. III. Free Proline accumulation and drought resistance 1. *Crop science,* 16, 428-431.

Bogeat-Triboulot, M.-B. a., Brosché, M., Renaut, J., Jouve, L., Le Thiec, D., Fayyaz, P., *et al.* (2006) Gradual Soil Water Depletion Results in Reversible Changes of Gene Expression, Protein Profiles, Ecophysiology, and Growth Performance in Populus euphratica, a Poplar Growing in Arid Regions. *Plant Physiology,* 143, 876-892.

Bokor, B., Bokorova, S., Ondos, S., Svubova, R., Lukacova, Z., Hyblova, M., *et al.* (2015) Ionome and expression level of Si transporter genes (Lsi1, Lsi2, and Lsi6) affected by Zn and Si interaction in maize. *Environmental Science and Pollution Research,* 22, 6800-6811.

Bray, E. A. (2004) Genes commonly regulated by water-deficit stress in Arabidopsis thaliana. *Journal of Experimental Botany,* 55, 2331-2341.

Brownell, P. F. (1968) Sodium as an essential micronutrient element for some higher plants. *Plant and Soil,* 28, 161-164.

Cakmak, I. (2005) The role of potassium in alleviating detrimental effects of abiotic stresses in plants. *Journal of Plant Nutrition and Soil Science,* 168, 521-530.

Castiglioni, P., Warner, D., Bensen, R. J., Anstrom, D. C., Harrison, J., Stoecker, M., *et al.* (2008) Bacterial RNA chaperones confer abiotic stress tolerance in plants and improved grain yield in maize under water-limited conditions. *Plant Physiology,* 147, 446-455.

Chen, D., Cao, B., Wang, S., Liu, P., Deng, X., Yin, L., *et al.* (2016) Silicon moderated the K deficiency by improving the plant-water status in sorghum. *Scientific Reports,* 6, 22882.

Chen, G., Hu, Q. D., Luo, L., Yang, T. Y., Zhang, S., Hu, Y. B., *et al.* (2015) Rice potassium transporter OsHAK1 is essential for maintaining potassium-mediated growth and functions in salt tolerance over low and high potassium concentration ranges. *Plant Cell and Environment,* 38, 2747-2765.

Chen, G., Liu, C., Gao, Z., Zhang, Y., Jiang, H., Zhu, L., *et al.* (2017) OsHAK1, a High-Affinity Potassium Transporter, Positively Regulates Responses to Drought Stress in Rice. *Frontiers in Plant Science,* 8.

Chen, L.-Q., Qu, X.-Q., Hou, B.-H., Sosso, D., Osorio, S., Fernie, A. R., *et al.* (2012) Sucrose efflux mediated by SWEET proteins as a key step for phloem transport. *Science,* 335, 207-211.

Chen, L., Zheng, F., Feng, Z., Li, Y., Ma, M., Wang, G., *et al.* (2021) A Vacuolar Invertase CsVI2 Regulates Sucrose Metabolism and Increases Drought Tolerance in Cucumis sativus L. *Int J Mol Sci,* 23.

Chen, Q., Hu, T., Li, X., Song, C.-P., Zhu, J.-K., Chen, L., *et al.* (2022) Phosphorylation of SWEET sucrose transporters regulates plant root:shoot ratio under drought. *Nature Plants,* 8, 68-77.

Chen, W., Yao, X., Cai, K. and Chen, J. (2011) Silicon Alleviates Drought Stress of Rice Plants by Improving Plant Water Status, Photosynthesis and Mineral Nutrient Absorption. *Biological Trace Element Research,* 142, 67-76.

Cheong, Y. H., Kim, K. N., Pandey, G. K., Gupta, R., Grant, J. J. and Luan, S. (2003) CBL1, a calcium sensor that differentially regulates salt, drought, and cold responses in Arabidopsis. *Plant Cell,* 15, 1833-1845.

Cho, M.-H., Lim, H., Shin, D. H., Jeon, J.-S., Bhoo, S. H., Park, Y.-I., *et al.* (2011) Role of the plastidic glucose translocator in the export of starch degradation products from the chloroplasts in Arabidopsis thaliana. *New Phytologist,* 190, 101-112.

Cramer, M. D., Hawkins, H.-J. and Verboom, G. A. (2009) The importance of nutritional regulation of plant water flux. *Oecologia,* 161, 15-24.

Crusciol, C. A. C., Pulz, A. L., Lemos, L. B., Soratto, R. P. and Lima, G. P. P. (2009) Effects of Silicon and Drought Stress on Tuber Yield and Leaf Biochemical Characteristics in Potato. *Crop science,* 49, 949-954.

Cui, J., Lamade, E. and Tcherkez, G. (2020) Potassium deficiency reconfigures sugar export and induces catecholamine accumulation in oil palm leaves. *Plant Science,* 300, 110628.

D'Autreaux, B. and Toledano, M. B. (2007) ROS as signalling molecules: mechanisms that generate specificity in ROS homeostasis. *Nature Reviews Molecular Cell Biology,* 8, 813-824.

Damari-Weissler, H., Rachamilevitch, S., Aloni, R., German, M. A., Cohen, S., Zwieniecki, M. A., *et al.* (2009) LeFRK2 is required for phloem and xylem differentiation and the transport of both sugar and water. *Planta,* 230, 795-805.

Deeken, R., Geiger, D., Fromm, J., Koroleva, O., Ache, P., Langenfeld-Heyser, R., *et al.* (2002) Loss of the AKT2/3 potassium channel affects sugar loading into the phloem of Arabidopsis. *Planta,* 216, 334-344.

Deng, J., Yang, X., Sun, W., Miao, Y., He, L. and Zhang, X. (2020) The Calcium Sensor CBL2 and Its Interacting Kinase CIPK6 Are Involved in Plant Sugar Homeostasis via Interacting with Tonoplast Sugar Transporter TST21. *Plant Physiology,* 183, 236-249.

Deshmukh, R. K., Vivancos, J., Guerin, V., Sonah, H., Labbe, C., Belzile, F., *et al.* (2013) Identification and functional characterization of silicon transporters in soybean using comparative genomics of major intrinsic proteins in Arabidopsis and rice. *Plant Molecular Biology,* 83, 303-315.

Dien, D. C., Mochizuki, T. and Yamakawa, T. (2019) Effect of various drought stresses and subsequent recovery on proline, total soluble sugar and starch metabolisms in Rice (Oryza sativa L.) varieties. *Plant Production Science,* 22, 530-545.

Du, X., Xinyue, Z., Xi, M. and Kong, L. (2020a) Split application enhances sweetpotato starch production by regulating the conversion of sucrose to starch under reduced nitrogen supply. *Plant Physiology and Biochemistry,* 151.

Du, Y., Zhao, Q., Chen, L., Yao, X., Zhang, H., Wu, J.*, et al.* (2020b) Effect of Drought Stress during Soybean R2–R6 Growth Stages on Sucrose Metabolism in Leaf and Seed. *International Journal of Molecular Sciences,* 21, 618.

Dubouzet, J. G., Sakuma, Y., Ito, Y., Kasuga, M., Dubouzet, E. G., Miura, S.*, et al.* (2003) OsDREB genes in rice, Oryza sativa L., encode transcription activators that function in drought-, high-salt- and cold-responsive gene expression. *Plant Journal,* 33, 751-763.

Durand, M., Porcheron, B., Hennion, N., Maurousset, L., Lemoine, R. and Pourtau, N. (2016) Water deficit enhances C export to the roots in Arabidopsis thaliana plants with contribution of sucrose transporters in both shoot and roots. *Plant Physiology,* 170, 1460-1479.

DYSON, B. C., ALLWOOD, J. W., FEIL, R., XU, Y., MILLER, M., BOWSHER, C. G.*, et al.* (2015) Acclimation of metabolism to light in Arabidopsis thaliana: the glucose 6-phosphate/phosphate translocator GPT2 directs metabolic acclimation. *Plant, Cell & Environment,* 38, 1404-1417.

Elizamar Ciríaco da, S., Manoel Bandeira de, A., André Dias de Azevedo, N. and Carlos Dias da Silva, J. (2013) Drought and Its Consequences to Plants – From Individual to Ecosystem. In: *Responses of Organisms to Water Stress.* (Sener, A., ed.). Rijeka: IntechOpen, pp. Ch. 2.

Epstein, E. (1994) The Anomaly of Silicon in Plant Biology. *Proceedings of the National Academy of Sciences of the United States of America,* 91, 11-17.

Farooq, M. A., Ali, S., Hameed, A., Ishaque, W., Mahmood, K. and Iqbal, Z. (2013) Alleviation of cadmium toxicity by silicon is related to elevated photosynthesis, antioxidant enzymes; suppressed cadmium uptake and oxidative stress in cotton. *Ecotoxicology and Environmental Safety,* 96, 242-249.

Fischer, B. B., Hideg, É. and Krieger-Liszkay, A. (2013) Production, detection, and signaling of singlet oxygen in photosynthetic organisms. *Antioxid Redox Signal,* 18, 2145-2162.

Fleck, A. T., Nye, T., Repenning, C., Stahl, F., Zahn, M. and Schenk, M. K. (2011) Silicon enhances suberization and lignification in roots of rice (Oryza sativa). *Journal of Experimental Botany,* 62, 2001-2011.

FORTMEIER, R. and SCHUBERT, S. (1995) Salt tolerance of maize (Zea mays L.): the role of sodium exclusion. *Plant, Cell & Environment,* 18, 1041-1047.

Fujita, Y., Fujita, M., Satoh, R., Maruyama, K., Parvez, M. M., Seki, M.*, et al.* (2005) AREB1 is a transcription activator of novel ABRE-dependent ABA signaling that enhances drought stress tolerance in Arabidopsis. *Plant Cell,* 17, 3470-3488.

Fujita, Y., Yoshida, T. and Yamaguchi-Shinozaki, K. (2013) Pivotal role of the AREB/ABF-SnRK2 pathway in ABRE-mediated transcription in response to osmotic stress in plants. *Physiologia Plantarum,* 147, 15-27.

Fürtauer, L., Weckwerth, W. and Nägele, T. (2016) A Benchtop Fractionation Procedure for Subcellular Analysis of the Plant Metabolome. *Frontiers in Plant Science,* 7.

Gajdanowicz, P., Michard, E., Sandmann, M., Rocha, M., Corrêa, L. G. G., Ramírez-Aguilar, S. J.*, et al.* (2011) Potassium (K⁺) gradients serve as a mobile energy source in plant vascular tissues. *Proceedings of the National Academy of Sciences,* 108, 864-869.

Gao, X., Zou, C., Wang, L. and Zhang, F. (2005) Silicon improves water use efficiency in maize plants. *Journal of Plant Nutrition,* 27, 1457-1470.

Gao, X. P., Zou, C. Q., Wang, L. J. and Zhang, F. S. (2006) Silicon decreases transpiration rate and conductance from stomata of maize plants. *Journal of Plant Nutrition,* 29, 1637-1647.

Gao, Y., Tang, Z., Xia, H., Sheng, M., Liu, M., Pan, S.*, et al.* (2021) Potassium Fertilization Stimulates Sucrose-to-Starch Conversion and Root Formation in Sweet Potato (Ipomoea batatas (L.) Lam.). *Int J Mol Sci,* 22.

Gaymard, F., Pilot, G., Lacombe, B., Bouchez, D., Bruneau, D., Boucherez, J.*, et al.* (1998) Identification and Disruption of a Plant Shaker-like Outward Channel Involved in K+ Release into the Xylem Sap. *Cell,* 94, 647-655.
Gong, H., Zhu, X., Chen, K., Wang, S. and Zhang, C. (2005) Silicon alleviates oxidative damage of wheat plants in pots under drought. *Plant Science,* 169, 313-321.
Guerriero, G., Stokes, I., Valle, N., Hausman, J.-F. and Exley, C. (2020) Visualising silicon in plants: histochemistry, silica sculptures and elemental imaging. *Cells,* 9, 1066.
Gunes, A., Pilbeam, D. J., Inal, A. and Coban, S. (2008) Influence of silicon on sunflower cultivars under drought stress, I: Growth, antioxidant mechanisms, and lipid peroxidation. *Communications in Soil Science and Plant Analysis,* 39, 1885-1903.
Guo, W.-J., Nagy, R., Chen, H.-Y., Pfrunder, S., Yu, Y.-C., Santelia, D.*, et al.* (2013) SWEET17, a Facilitative Transporter, Mediates Fructose Transport across the Tonoplast of Arabidopsis Roots and Leaves *Plant Physiology,* 164, 777-789.
Gupta, A., Dixit, S. K. and Senthil-Kumar, M. (2016) Drought Stress Predominantly Endures Arabidopsis thaliana to Pseudomonas syringae Infection. *Frontiers in Plant Science,* 7.
Habibi, G. (2014) Silicon supplementation improves drought tolerance in canola plants. *Russian Journal of Plant Physiology,* 61, 784-791.
Hattori, T., Sonobe, K., Inanaga, S., An, P. and Morita, S. (2008) Effects of Silicon on Photosynthesis of Young Cucumber Seedlings Under Osmotic Stress. *Journal of Plant Nutrition,* 31, 1046-1058.
Helaly, M. N., El-Hoseiny, H., El-Sheery, N. I., Rastogi, A. and Kalaji, H. M. (2017) Regulation and physiological role of silicon in alleviating drought stress of mango. *Plant Physiology and Biochemistry,* 118, 31-44.
Held, K., Pascaud, F., Eckert, C., Gajdanowicz, P., Hashimoto, K., Corratgé-Faillie, C.*, et al.* (2011) Calcium-dependent modulation and plasma membrane targeting of the AKT2 potassium channel by the CBL4/CIPK6 calcium sensor/protein kinase complex. *Cell Res,* 21, 1116-1130.
Ho, L.-H., Rode, R., Siegel, M., Reinhardt, F., Neuhaus, H. E., Yvin, J.-C.*, et al.* (2020) Potassium application boosts photosynthesis and sorbitol biosynthesis and accelerates cold acclimation of common plantain (Plantago major L.). *Plants,* 9, 1259.
Hoekstra, F. A., Golovina, E. A. and Buitink, J. (2001) Mechanisms of plant desiccation tolerance. *Trends in Plant Science,* 6, 431-438.
Hosseini, S. A., Maillard, A., Hajirezaei, M. R., Ali, N., Schwarzenberg, A., Jamois, F.*, et al.* (2017) Induction of Barley Silicon Transporter HvLsi1 and HvLsi2, increased silicon concentration in the shoot and regulated Starch and ABA Homeostasis under Osmotic stress and Concomitant Potassium Deficiency. *Frontiers in Plant Science,* 8.
Hsiao, T. C. and Xu, L. K. (2000) Sensitivity of growth of roots versus leaves to water stress: biophysical analysis and relation to water transport. *Journal of Experimental Botany,* 51, 1595-1616.
Hu, W., Huang, C., Deng, X. M., Zhou, S. Y., Chen, L. H., Li, Y.*, et al.* (2013) TaASR1, a transcription factor gene in wheat, confers drought stress tolerance in transgenic tobacco. *Plant Cell and Environment,* 36, 1449-1464.
Hu, W., Jiang, N., Yang, J., Meng, Y., Wang, Y., Chen, B.*, et al.* (2016) Potassium (K) supply affects K accumulation and photosynthetic physiology in two cotton (Gossypium hirsutum L.) cultivars with different K sensitivities. *Field Crops Research,* 196, 51-63.
Huber, D., Römheld, V. and Weinmann, M. (2012) Chapter 10 - Relationship between Nutrition, Plant Diseases and Pests. In: *Marschner's Mineral Nutrition of Higher Plants (Third Edition).* (Marschner, P., ed.). San Diego: Academic Press, pp. 283-298.
Hummel, I., Pantin, F., Sulpice, R., Piques, M., Rolland, G., Dauzat, M.*, et al.* (2010) Arabidopsis plants acclimate to water deficit at low cost through changes of carbon usage: an integrated perspective using growth, metabolite, enzyme, and gene expression analysis. *Plant Physiology,* 154, 357-372.
Hussain, S. S., Kayani, M. A. and Amjad, M. (2011) Transcription factors as tools to engineer enhanced drought stress tolerance in plants. *Biotechnol Prog,* 27, 297-306.

Ibarracaballero, J., Villanuevaverduzco, C., Molinagalan, J. and Sanchezdejimenez, E. (1988) Proline Accumulation as a Symptom of Drought Stress in Maize - a Tissue Differentiation Requirement. *Journal of Experimental Botany,* 39, 889-897.

Ibraheem, O., Dealtry, G., Roux, S. and Bradley, G. (2011a) The effect of drought and salinity on the expressional levels of sucrose transporters in rice ('Oryza sativa'nipponbare) cultivar plants. *Plant OMICS,* 4, 68-74.

Ibraheem, O., Dealtry, G., Roux, S. and Bradley, G. (2011b) The Effect of Drought and Salinity on the Expressional Levels of Sucrose Transporters in Rice ('Oryza sativa'Nipponbare) Cultivar Plants. *Plant OMICS,* 4.

Ilyas, M., Nisar, M., Khan, N., Hazrat, A., Khan, A. H., Hayat, K.*, et al.* (2021) Drought tolerance strategies in plants: a mechanistic approach. *Journal of Plant Growth Regulation,* 40, 926-944.

Ings, J., Mur, L. A., Robson, P. R. and Bosch, M. (2013) Physiological and growth responses to water deficit in the bioenergy crop Miscanthus x giganteus. *Front Plant Sci,* 4, 468.

Iraki, N. M., Bressan, R. A., Hasegawa, P. and Carpita, N. C. (1989) Alteration of the physical and chemical structure of the primary cell wall of growth-limited plant cells adapted to osmotic stress. *Plant Physiology,* 91, 39-47.

Javot, H. and Maurel, C. (2002) The role of aquaporins in root water uptake. *Annals of Botany,* 90, 301-313.

Jin, H., Zhang, L., Li, B., Han, M. and Liu, X. (2007) Effect of potassium on the leaf nutrition and quality of Red Fuji apple. *Acta Agric. Bor Occid. Sin,* 16, 100-104.

Kaiser, W. M. (1982) Correlation between changes in photosynthetic activity and changes in total protoplast volume in leaf tissue from hygro-, meso-and xerophytes under osmotic stress. *Planta,* 154, 538-545.

Kaldenhoff, R., RIBAS-CARBO, M., Sans, J. F., Lovisolo, C., Heckwolf, M. and Uehlein, N. (2008) Aquaporins and plant water balance. *Plant, Cell & Environment,* 31, 658-666.

Kaur, H., Manna, M., Thakur, T., Gautam, V. and Salvi, P. (2021) Imperative role of sugar signaling and transport during drought stress responses in plants. *Physiologia Plantarum,* 171, 833-848.

Kaya, C., Tuna, L. and Higgs, D. (2006) Effect of Silicon on Plant Growth and Mineral Nutrition of Maize Grown Under Water-Stress Conditions. *Journal of Plant Nutrition,* 29, 1469-1480.

Keller, I., Müdsam, C., Rodrigues, C. M., Kischka, D., Zierer, W., Sonnewald, U.*, et al.* (2021) Cold-Triggered Induction of ROS- and Raffinose Metabolism in Freezing-Sensitive Taproot Tissue of Sugar Beet. *Frontiers in Plant Science,* 12.

Kim, J. Y., Mahé, A., Brangeon, J. and Prioul, J. L. (2000) A maize vacuolar invertase, IVR2, is induced by water stress. Organ/tissue specificity and diurnal modulation of expression. *Plant Physiology,* 124, 71-84.

Klemens, P. A. W., Patzke, K., Trentmann, O., Poschet, G., Büttner, M., Schulz, A.*, et al.* (2014) Overexpression of a proton-coupled vacuolar glucose exporter impairs freezing tolerance and seed germination. *New Phytologist,* 202, 188-197.

Krasensky, J. and Jonak, C. (2012) Drought, salt, and temperature stress-induced metabolic rearrangements and regulatory networks. *Journal of Experimental Botany,* 63, 1593-1608.

Kruse, E., Uehlein, N. and Kaldenhoff, R. (2006) The aquaporins. *Genome biology, 7*, 1-6.

Kuchenbuch, R., Claassen, N. and Jungk, A. (1986) Potassium availability in relation to soil moisture. *Plant and Soil,* 95, 221-231.

Kühn, C., Hajirezaei, M. R., Fernie, A. R., Roessner-Tunali, U., Czechowski, T., Hirner, B.*, et al.* (2003) The sucrose transporter StSUT1 localizes to sieve elements in potato tuber phloem and influences tuber physiology and development. *Plant Physiology,* 131, 102-113.

Kumar, S., Sachdeva, S., Bhat, K. V. and Vats, S. (2018) Plant Responses to Drought Stress: Physiological, Biochemical and Molecular Basis. In: *Biotic and Abiotic Stress Tolerance in Plants.* (Vats, S., ed.). Singapore: Springer Singapore, pp. 1-25.

Kurepa, J. and Smalle, J. A. (2022) Auxin/Cytokinin Antagonistic Control of the Shoot/Root Growth Ratio and Its Relevance for Adaptation to Drought and Nutrient Deficiency Stresses. *Int J Mol Sci,* 23.

LAWLOR, D. W. and MILFORD, G. F. J. (1973) The Effect of Sodium on Growth of Water-stressed Sugar beet. *Annals of Botany,* 37, 597-604.

Lebaudy, A., Very, A. A. and Sentenac, H. (2007) K+ channel activity in plants: Genes, regulations and functions. *Febs Letters,* 581, 2357-2366.

Lemoine, R., La Camera, S., Atanassova, R., Dédaldéchamp, F., Allario, T., Pourtau, N.*, et al.* (2013) Source-to-sink transport of sugar and regulation by environmental factors. *Frontiers in Plant Science,* 4.

Liang, Y. C., Si, J. and Romheld, V. (2005) Silicon uptake and transport is an active process in Cucumis sativus. *New Phytologist,* 167, 797-804.

Liu, P., Yin, L., Deng, X., Wang, S., Tanaka, K. and Zhang, S. (2014a) Aquaporin-mediated increase in root hydraulic conductance is involved in silicon-induced improved root water uptake under osmotic stress in Sorghum bicolor L. *Journal of Experimental Botany,* 65, 4747-4756.

Liu, P., Yin, L. N., Wang, S. W., Zhang, M. J., Deng, X. P., Zhang, S. Q.*, et al.* (2015) Enhanced root hydraulic conductance by aquaporin regulation accounts for silicon alleviated salt-induced osmotic stress in Sorghum bicolor L. *Environmental and Experimental Botany,* 111, 42-51.

Liu, W. W., Tai, H. H., Li, S. S., Gao, W., Zhao, M., Xie, C. X.*, et al.* (2014b) bHLH122 is important for drought and osmotic stress resistance in Arabidopsis and in the repression of ABA catabolism. *New Phytologist,* 201, 1192-1204.

Liu, Y., Wang, L., Li, Y., Li, X. and Zhang, J. (2019) Proline metabolism-related gene expression in four potato genotypes in response to drought stress. *Biologia plantarum,* 63, 757-764.

Love, M. I., Huber, W. and Anders, S. (2014) Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome biology,* 15, 550.

Lu, B., Wen, S., Zhu, P., Cao, H., Zhou, Y., Bie, Z.*, et al.* (2020) Overexpression of Melon Tonoplast Sugar Transporter CmTST1 Improved Root Growth under High Sugar Content. *Int J Mol Sci,* 21.

Lu, J., Chen, F., Wan, Y., Liu, D., Yu, C., Wang, Y.*, et al.* (2001) Effect of application of potassium on the yield and quality of navel orange. *J. Fruit Sci,* 18, 272-275.

Lu, M. M., Silva, D. M. D., Engelbert, P., Alvin, F. and Milagros, P. (2016) Growth and Yield of Tomato Applied with Silicon Supplements with Varying Material Structures.

Lu, Y. (2016) Identification and Roles of Photosystem II Assembly, Stability, and Repair Factors in Arabidopsis. *Front Plant Sci,* 7, 168.

Lucas, W. J., Groover, A., Lichtenberger, R., Furuta, K., Yadav, S.-R., Helariutta, Y.*, et al.* (2013) The Plant Vascular System: Evolution, Development and FunctionsF. *Journal of Integrative Plant Biology,* 55, 294-388.

Lux, A., Luxova, M., Hattori, T., Inanaga, S. and Sugimoto, Y. (2002) Silicification in sorghum (Sorghum bicolor) cultivars with different drought tolerance. *Physiologia Plantarum,* 115, 87-92.

Ma, J. F. (2004) Role of silicon in enhancing the resistance of plants to biotic and abiotic stresses. *Soil science and plant nutrition,* 50, 11-18.

Ma, J. F., Tamai, K., Yamaji, N., Mitani, N., Konishi, S., Katsuhara, M.*, et al.* (2006) A silicon transporter in rice. *Nature,* 440, 688-691.

Ma, J. F. and Yamaji, N. (2006) Silicon uptake and accumulation in higher plants. *Trends in Plant Science,* 11, 392-397.

Ma, J. F., Yamaji, N., Mitani, N., Tamai, K., Konishi, S., Fujiwara, T.*, et al.* (2007) An efflux transporter of silicon in rice. *Nature,* 448, 209-212.

Ma, Q. J., Sun, M. H., Kang, H., Lu, J., You, C. X. and Hao, Y. J. (2019a) A CIPK protein kinase targets sucrose transporter MdSUT2.2 at Ser(254) for phosphorylation to enhance salt tolerance. *Plant Cell and Environment,* 42, 918-930.

Ma, Q. J., Sun, M. H., Lu, J., Kang, H., You, C. X. and Hao, Y. J. (2019b) An apple sucrose transporter MdSUT2. 2 is a phosphorylation target for protein kinase MdCIPK22 in response to drought. *Plant Biotechnology Journal,* 17, 625-637.

Ma, X., Li, Q. H., Yu, Y. N., Qiao, Y. M., Haq, S. U. and Gong, Z. H. (2020) The CBL-CIPK Pathway in Plant Response to Stress Signals. *Int J Mol Sci,* 21.

Mahouachi, J., Socorro, A. R. and Talon, M. (2006) Responses of papaya seedlings (Carica papaya L.) to water stress and re-hydration: Growth, photosynthesis and mineral nutrient imbalance. *Plant and Soil,* 281, 137-146.

Mandlik, R., Thakral, V., Raturi, G., Shinde, S., Nikolic, M., Tripathi, D. K.*, et al.* (2020) Significance of silicon uptake, transport, and deposition in plants. *Journal of Experimental Botany,* 71, 6703-6718.

Marschner, H. (2011) *Marschner's mineral nutrition of higher plants*. Academic press.

Martínez, J.-P., Silva, H., Ledent, J.-F. and Pinto, M. (2007) Effect of drought stress on the osmotic adjustment, cell wall elasticity and cell volume of six cultivars of common beans (Phaseolus vulgaris L.). *European journal of agronomy,* 26, 30-38.

Michel, B. E. (1983) Evaluation of the Water Potentials of Solutions of Polyethylene Glycol 8000 Both in the Absence and Presence of Other Solutes. *Plant Physiology,* 72, 66-70.

Ming, D., Pei, Z., Naeem, M., Gong, H. and Zhou, W. (2012) Silicon alleviates PEG-induced water-deficit stress in upland rice seedlings by enhancing osmotic adjustment. *Journal of Agronomy and Crop Science,* 198, 14-26.

Mitani-Ueno, N., Yamaji, N. and Ma, J. F. (2011) Silicon efflux transporters isolated from two pumpkin cultivars contrasting in Si uptake. *Plant Signaling & Behavior,* 6, 991-994.

Mitani, N., Chiba, Y., Yamaji, N. and Ma, J. F. (2009) Identification and Characterization of Maize and Barley Lsi2-Like Silicon Efflux Transporters Reveals a Distinct Silicon Uptake System from That in Rice. *Plant Cell,* 21, 2133-2142.

Mitani, N., Yamaji, N., Ago, Y., Iwasaki, K. and Ma, J. F. (2011) Isolation and functional characterization of an influx silicon transporter in two pumpkin cultivars contrasting in silicon accumulation. *Plant Journal,* 66, 231-240.

Munné-Bosch, S. and Alegre, L. (2004) Die and let live: leaf senescence contributes to plant survival under drought stress. *Funct Plant Biol,* 31, 203-216.

Nicot, N., Hausman, J. F., Hoffmann, L. and Evers, D. (2005) Housekeeping gene selection for real-time RT-PCR normalization in potato during biotic and abiotic stress. *Journal of Experimental Botany,* 56, 2907-2914.

Ohta, D., Matsui, J., Matoh, T. and Takahashi, E. (1988) Sodium Requirement of Monocotyledonous C4 Plants for Growth and Nitrate Reductase Activity. *Plant and Cell Physiology,* 29, 1429-1432.

Oosterhuis, D. M., Loka, D. A., Kawakami, E. M. and Pettigrew, W. T. (2014) The Physiology of Potassium in Crop Production. *Advances in Agronomy, Vol 126,* 126, 203-233.

Pilot, G., Lacombe, B., Gaymard, F., Cherel, I., Boucherez, J., Thibaud, J. B.*, et al.* (2001) Guard cell inward K+ channel activity in Arabidopsis involves expression of the twin channel subunits KAT1 and KAT2. *Journal of Biological Chemistry,* 276, 3215-3221.

Pirasteh-Anosheh, H., Saed-Moucheshi, A., Pakniyat, H. and Pessarakli, M. (2016) Stomatal responses to drought stress. In: *Water Stress and Crop Plants.* pp. 24-40.

Piro, G., Leucci, M. R., Waldron, K. and Dalessandro, G. (2003) Exposure to water stress causes changes in the biosynthesis of cell wall polysaccharides in roots of wheat cultivars varying in drought tolerance. *Plant Science,* 165, 559-569.

Pommerrenig, B., Ludewig, F., Cvetkovic, J., Trentmann, O., Klemens, P. A. W. and Neuhaus, H. E. (2018) In Concert: Orchestrated Changes in Carbohydrate Homeostasis Are Critical for Plant Abiotic Stress Tolerance. *Plant and Cell Physiology,* 59, 1290-1299.

Razem, F. A. and Bernards, M. A. (2002) Hydrogen peroxide is required for poly(phenolic) domain formation during wound-induced suberization. *Journal of Agricultural and Food Chemistry,* 50, 1009-1015.

Riesmeier, J. W., Willmitzer, L. and Frommer, W. B. (1992) Isolation and characterization of a sucrose carrier cDNA from spinach by functional expression in yeast. *The EMBO Journal,* 11, 4705-4713.

Ritchie, R., xa, J and Bunthawin, S. (2010) The Use of Pulse Amplitude Modulation (PAM) Fluorometry to Measure Photosynthesis in a CAM Orchid, Dendrobium spp. (D. cv. Viravuth Pink). *International Journal of Plant Sciences,* 171, 575-585.

Roitsch, T. and González, M.-C. (2004) Function and regulation of plant invertases: sweet sensations. *Trends in Plant Science,* 9, 606-613.

Ruan, Y.-L. (2014) Sucrose metabolism: gateway to diverse carbon use and sugar signaling. *Annu Rev Plant Biol,* 65, 33-67.

Sakuma, Y., Liu, Q., Dubouzet, J. G., Abe, H., Shinozaki, K. and Yamaguchi-Shinozaki, K. (2002) DNA-binding specificity of the ERF/AP2 domain of Arabidopsis DREBs, transcription factors involved in dehydration- and cold-inducible gene expression. *Biochemical and Biophysical Research Communications,* 290, 998-1009.

Sakuma, Y., Maruyama, K., Osakabe, Y., Qin, F., Seki, M., Shinozaki, K.*, et al.* (2006a) Functional analysis of an Arabidopsis transcription factor, DREB2A, involved in drought-responsive gene expression. *Plant Cell,* 18, 1292-1309.

Sakuma, Y., Maruyama, K., Qin, F., Osakabe, Y., Shinozaki, K. and Yamaguchi-Shinozaki, K. (2006b) Dual function of an Arabidopsis transcription factor DREB2A in water-stress-responsive and heat-stress-responsive gene expression. *Proceedings of the National Academy of Sciences of the United States of America,* 103, 18822-18827.

Sampaio, M., Neves, J., Cardoso, T., Pissarra, J., Pereira, S. and Pereira, C. (2022) Coping with Abiotic Stress in Plants—An Endomembrane Trafficking Perspective. *Plants,* 11, 338.

Sato, A., Sato, Y., Fukao, Y., Fujiwara, M., Umezawa, T., Shinozaki, K.*, et al.* (2009) Threonine at position 306 of the KAT1 potassium channel is essential for channel activity and is a target site for ABA-activated SnRK2/OST1/SnRK2.6 protein kinase. *Biochemical Journal,* 424, 439-448.

Savant, N., Snyder, G. and Datnoff, L. (1996) Silicon management and sustainable rice production. *Advances in agronomy,* 58, 151-199.

Savant, N. K., Korndorfer, G. H., Datnoff, L. E. and Snyder, G. H. (1999) Silicon nutrition and sugarcane production: A review. *Journal of Plant Nutrition,* 22, 1853-1903.

Savvas, D., Gizas, G., Karras, G., Lydakis-Simantiris, N., Salahas, G., Papadimitriou, M.*, et al.* (2007) Interactions between silicon and NaCl-salinity in a soilless culture of roses in greenhouse. *European Journal of Horticultural Science,* 72, 73-79.

Schroeder, J. I., Ward, J. M. and Gassmann, W. (1994) Perspectives on the Physiology and Structure of Inward-Rectifying K+ Channels in Higher Plants: Biophysical Implications for K+ Uptake. *Annual Review of Biophysics and Biomolecular Structure,* 23, 441-471.

Sergeeva, L. I., Keurentjes, J. J., Bentsink, L., Vonk, J., van der Plas, L. H., Koornneef, M.*, et al.* (2006) Vacuolar invertase regulates elongation of Arabidopsis thaliana roots as revealed by QTL and mutant analysis. *Proceedings of the National Academy of Sciences,* 103, 2994-2999.

Shen, X. F., Zhou, Y. Y., Duan, L. S., Li, Z. H., Eneji, A. E. and Li, J. M. (2010) Silicon effects on photosynthesis and antioxidant parameters of soybean seedlings under drought and ultraviolet-B radiation. *Journal of Plant Physiology,* 167, 1248-1252.

Shi, Y., Zhang, Y., Han, W., Feng, R., Hu, Y., Guo, J.*, et al.* (2016) Silicon enhances water stress tolerance by improving root hydraulic conductance in Solanum lycopersicum L. *Frontiers in Plant Science,* 7, 196.

Shim, J. S., Jeong, H. I., Bang, S. W., Jung, S. E., Kim, G., Kim, Y. S., *et al.* (2022) DROUGHT-INDUCED BRANCHED-CHAIN AMINO ACID AMINOTRANSFERASE enhances drought tolerance in rice. *Plant Physiology*.

Soares Pereira, T., Klynger, A., Lobato, S., Kean, D., Tan, Y., Costa, D., *et al.* (2013) Positive interference of silicon on water relations, nitrogen metabolism, and osmotic adjustment in two pepper (Capsicum annuum) cultivars under water deficit. *Australian Journal of Crop Science,* 58, 481-486.

Sonobe, K., Hattori, T., An, P., Tsuji, W., Eneji, A. E., Kobayashi, S., *et al.* (2010) Effect of silicon application on sorghum root responses to water stress. *Journal of Plant Nutrition,* 34, 71-82.

Stadler, R. and Sauer, N. (1996) The Arabidopsis thaliana AtSUC2 Gene is Specifically Expressed in Companion Cells. *Botanica Acta,* 109, 299-306.

Stanfield, R. C. and Bartlett, M. K. (2022) Coordination Between Phloem Loading and Structure Maintains Carbon Transport Under Drought. *Frontiers in Plant Science,* 13.

Stavi, I., Thevs, N. and Priori, S. (2021) Soil Salinity and Sodicity in Drylands: A Review of Causes, Effects, Monitoring, and Restoration Measures. *Frontiers in Environmental Science,* 9.

Stein, O., Avin-Wittenberg, T., Krahnert, I., Zemach, H., Bogol, V., Daron, O., *et al.* (2017) Arabidopsis fructokinases are important for seed oil accumulation and vascular development. *Frontiers in Plant Science,* 7, 2047.

Stein, O., Damari-Weissler, H., Secchi, F., Rachmilevitch, S., German, M. A., Yeselson, Y., *et al.* (2016) The tomato plastidic fructokinase SlFRK3 plays a role in xylem development. *New Phytologist,* 209, 1484-1495.

Stitt, M., Lilley, R. M., Gerhardt, R. and Heldt, H. W. (1989) [32] Metabolite levels in specific cells and subcellular compartments of plant leaves. In: *Methods in Enzymology.* Academic Press, pp. 518-552.

Subbarao, G. V., Wheeler, R. M., Stutte, G. W. and Levine, L. H. (1999) How far can sodium substitute for potassium in red beet? *Journal of Plant Nutrition,* 22, 1745-1761.

Sweet, W. J., Morrison, J. C., Labavitch, J. M. and Matthews, M. A. (1990) Altered synthesis and composition of cell wall of grape (Vitis vinifera L.) leaves during expansion and growth-inhibiting water deficits. *Plant and Cell Physiology,* 31, 407-414.

Taiz, L., Zeiger, E., Møller, I. M. and Murphy, A. (2015) *Plant physiology and development*. Sinauer Associates Incorporated.

Takahashi, E., Ma, J. and Miyake, Y. (1990) The possibility of silicon as an essential element for higher plants. *Comments on Agricultural and Food Chemistry,* 2, 99-102.

Takahashi, F., Kuromori, T., Urano, K., Yamaguchi-Shinozaki, K. and Shinozaki, K. (2020) Drought Stress Responses and Resistance in Plants: From Cellular Responses to Long-Distance Intercellular Communication. *Frontiers in Plant Science,* 11.

Takahashi, S. and Murata, N. (2008) How do environmental stresses accelerate photoinhibition? *Trends in Plant Science,* 13, 178-182.

Tanguilig, V. C., Yambao, E. B., O' Toole, J. C. and De Datta, S. K. (1987) Water stress effects on leaf elongation, leaf water potential, transpiration, and nutrient uptake of rice, maize, and soybean. *Plant and Soil,* 103, 155-168.

Tjaden, J., Möhlmann, T., Kampfenkel, K. and Neuhaus, G. H. a. E. (1998) Altered plastidic ATP/ADP-transporter activity influences potato (Solanum tuberosum L.) tuber morphology, yield and composition of tuber starch. *The Plant Journal,* 16, 531-540.

Valifard, M., Le Hir, R., Müller, J., Scheuring, D., Neuhaus, H. E. and Pommerrenig, B. (2021) Vacuolar fructose transporter SWEET17 is critical for root development and drought tolerance. *Plant Physiology,* 187, 2716-2730.

Vass, I., Cser, K. and Cheregi, O. (2007) Molecular mechanisms of light stress of photosynthesis. *Annals of the New York Academy of Sciences,* 1113, 114-122.

Verbruggen, N. and Hermans, C. (2008) Proline accumulation in plants: a review. *Amino acids,* 35, 753-759.
Verma, K. K., Song, X.-P., Zeng, Y., Li, D.-M., Guo, D.-J., Rajput, V. D., *et al.* (2020) Characteristics of Leaf Stomata and Their Relationship with Photosynthesis in Saccharum officinarum Under Drought and Silicon Application. *ACS Omega,* 5, 24145-24153.
Wakeel, A., Farooq, M., Qadir, M. and Schubert, S. (2011) Potassium Substitution by Sodium in Plants. *Critical Reviews in Plant Sciences,* 30, 401-413.
Wang, M., Ding, L., Gao, L. M., Li, Y. R., Shen, Q. R. and Guo, S. W. (2016) The Interactions of Aquaporins and Mineral Nutrients in Higher Plants. *International Journal of Molecular Sciences,* 17.
Wang, M., Zheng, Q., Shen, Q. and Guo, S. (2013) The critical role of potassium in plant stress response. *Int J Mol Sci,* 14, 7370-7390.
Wang, S. M., Wan, C. G., Wang, Y. R., Chen, H., Zhou, Z. Y., Fu, H., *et al.* (2004) The characteristics of Na+, K+ and free proline distribution in several drought-resistant plants of the Alxa Desert, China. *Journal of Arid Environments,* 56, 525-539.
Waraich, E. A., Ahmad, R. and Ashraf, M. Y. (2011) Role of Mineral Nutrition in Alleviation of Drought Stress in Plants. *Australian Journal of Crop Science,* 5, 764-777.
Weeda, S., Kumar, G. and Knowles, N. (2009) Developmentally linked changes in proteases and protease inhibitors suggest a role for potato multicystatin in regulating protein content of potato tubers. *Planta,* 230, 73-84.
Weise, S. E., Liu, T., Childs, K. L., Preiser, A. L., Katulski, H. M., Perrin-Porzondek, C., *et al.* (2019) Transcriptional Regulation of the Glucose-6-Phosphate/Phosphate Translocator 2 Is Related to Carbon Exchange Across the Chloroplast Envelope. *Front Plant Sci,* 10, 827.
Wingenter, K., Schulz, A., Wormit, A., Wic, S., Trentmann, O., Hoermiller, I. I., *et al.* (2010) Increased Activity of the Vacuolar Monosaccharide Transporter TMT1 Alters Cellular Sugar Partitioning, Sugar Signaling, and Seed Yield in Arabidopsis. *Plant Physiology,* 154, 665-677.
Woolley, J. T. (1957) Sodium and Silicon as Nutrients for the Tomato Plant. *Plant Physiology,* 32, 317-321.
Wormit, A., Trentmann, O., Feifer, I., Lohr, C., Tjaden, J., Meyer, S., *et al.* (2006) Molecular Identification and Physiological Characterization of a Novel Monosaccharide Transporter from Arabidopsis Involved in Vacuolar Sugar Transport. *The Plant Cell,* 18, 3476-3490.
Xiang, L., Le Roy, K., Bolouri-Moghaddam, M. R., Vanhaecke, M., Lammens, W., Rolland, F., *et al.* (2011) Exploring the neutral invertase-oxidative stress defence connection in Arabidopsis thaliana. *Journal of Experimental Botany,* 62, 3849-3862.
Xu, Q., Chen, S., Yunjuan, R., Chen, S. and Liesche, J. (2017) Regulation of Sucrose Transporters and Phloem Loading in Response to Environmental Cues. *Plant Physiology,* 176, 930-945.
Xu, W., Cui, K., Xu, A., Nie, L., Huang, J. and Peng, S. (2015) Drought stress condition increases root to shoot ratio via alteration of carbohydrate partitioning and enzymatic activity in rice seedlings. *Acta physiologiae plantarum,* 37, 1-11.
Yan, L., Li, S., Riaz, M. and Jiang, C. (2021) Proline metabolism and biosynthesis behave differently in response to boron-deficiency and toxicity in Brassica napus. *Plant Physiology and Biochemistry,* 167, 529-540.
Yin, L. N., Wang, S. W., Liu, P., Wang, W. H., Cao, D., Deng, X. P., *et al.* (2014) Silicon-mediated changes in polyamine and 1-aminocyclopropane-1-carboxylic acid are involved in silicon-induced drought resistance in Sorghum bicolor L. *Plant Physiology and Biochemistry,* 80, 268-277.
Yoshida, S., Ohnishi, Y. and Kitagishi, K. (1962) Chemical forms, mobility and deposition of silicon in rice plant. *Soil science and plant nutrition,* 8, 15-21.
Zahoor, R., Dong, H., Abid, M., Zhao, W., Wang, Y. and Zhou, Z. (2017) Potassium fertilizer improves drought stress alleviation potential in cotton by enhancing

photosynthesis and carbohydrate metabolism. *Environmental and Experimental Botany,* 137, 73-83.

Zhang, H., Sun, X. and Dai, M. (2022) Improving crop drought resistance with plant growth regulators and rhizobacteria: Mechanisms, applications, and perspectives. *Plant Commun,* 3, 100228.

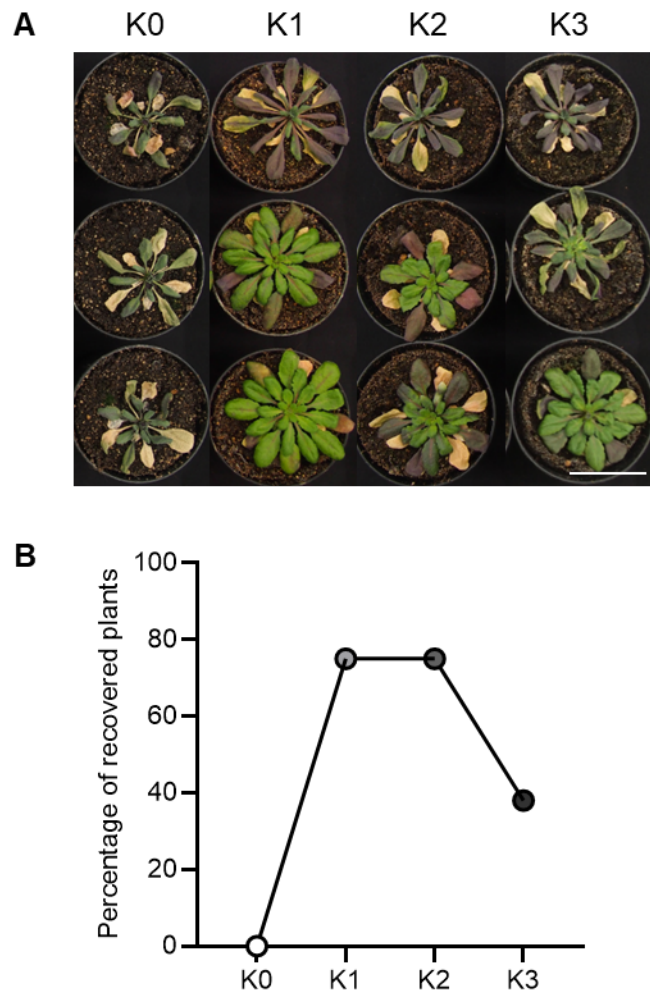
Zhao, W., Faust, F. and Schubert, S. (2020) Potassium is a potential toxicant for Arabidopsis thaliana under saline conditions. *Journal of Plant Nutrition and Soil Science,* 183, 455-467.

Zheng, M., Meng, Y., Yang, C., Zhou, Z., Wang, Y. and Chen, B. (2014) Protein expression changes during cotton fiber elongation in response to drought stress and recovery. *Proteomics,* 14, 1776-1795.

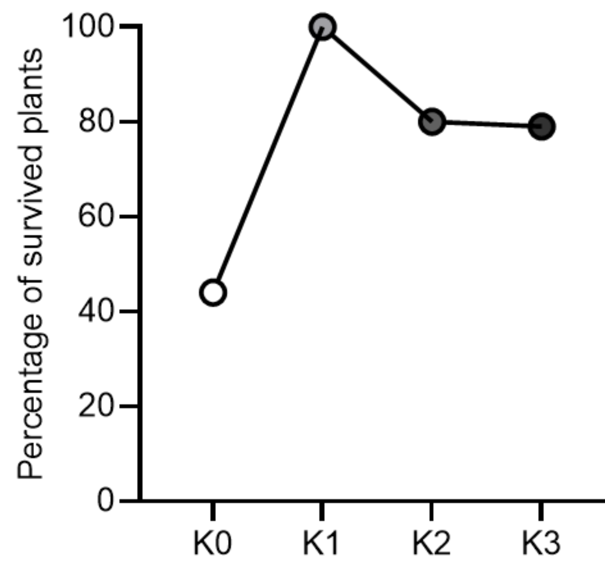
Zhu, Y. and Gong, H. (2014) Beneficial effects of silicon on salt and drought tolerance in plants. *Agronomy for sustainable development,* 34, 455-472.

Zimmermann, S., Talke, I., Ehrhardt, T., Nast, G. and Müller-Röber, B. (1998) Characterization of SKT1, an Inwardly Rectifying Potassium Channel from Potato, by Heterologous Expression in Insect Cells. *Plant Physiology,* 116, 879-890.

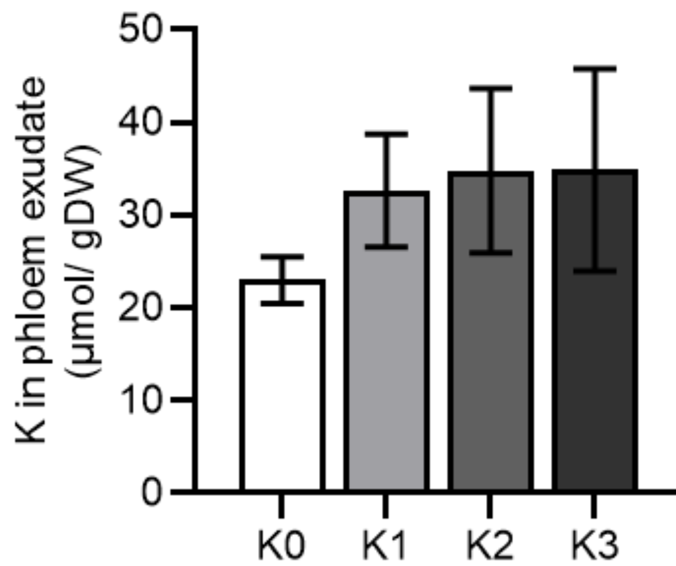
6. Supplementary figures and tables



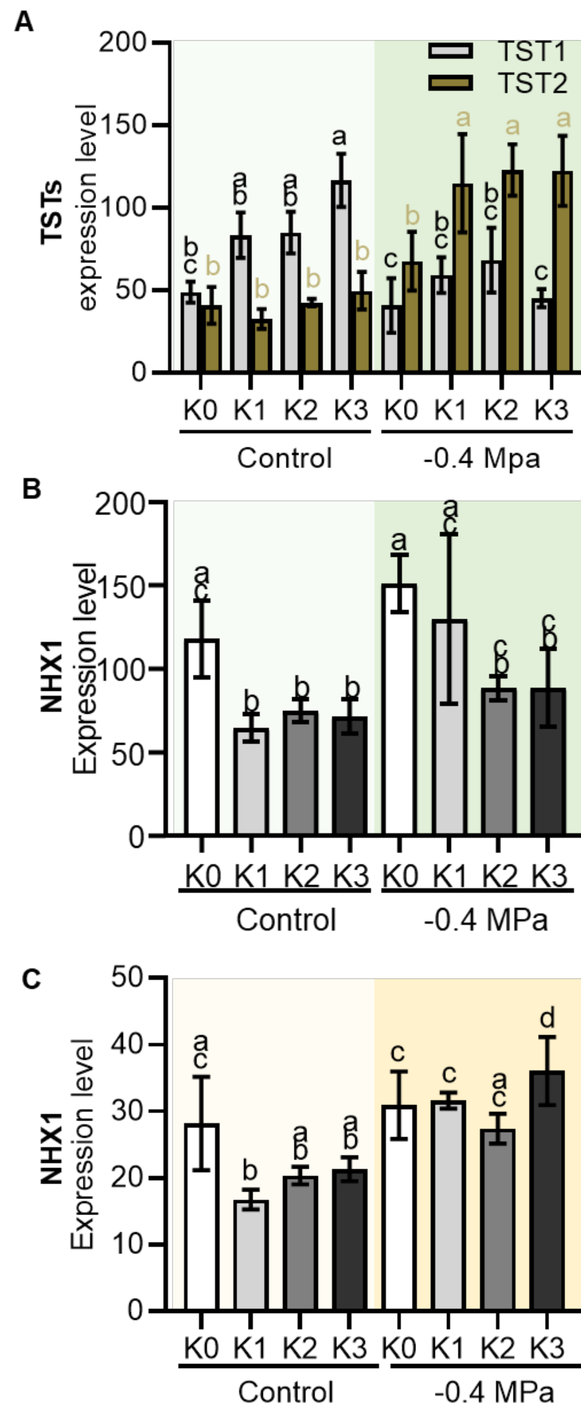
Supplemental figure 1. The survival rate of Arabidopsis plants on soil with different levels of potassium during drought stress. (A) The phenotype of plants after serious drought stress. Three-week-old plants were stopped watering for three weeks and then recovered by re-watering. The photo was taken two days after recovering. Bar = 3 cm. (B) Percentage of survived plants after serious drought stress. The Survival rate was documented two days after recovering.



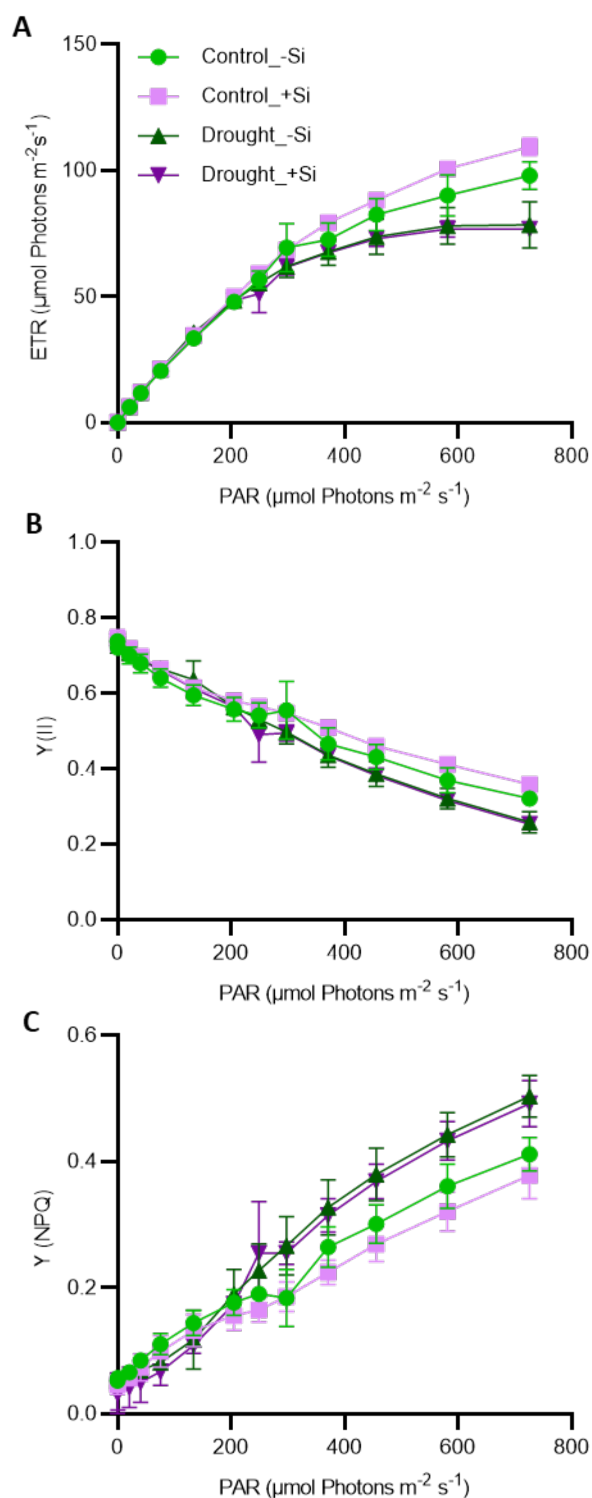
Supplemental figure 2. The survival rate of Arabidopsis in hydroponic culture. Four-week-old plants were treated with respectively K-medias as control or with PEG8000 (-0.4MPa). The survived plants were counted eight days after PEG8000 treatment.



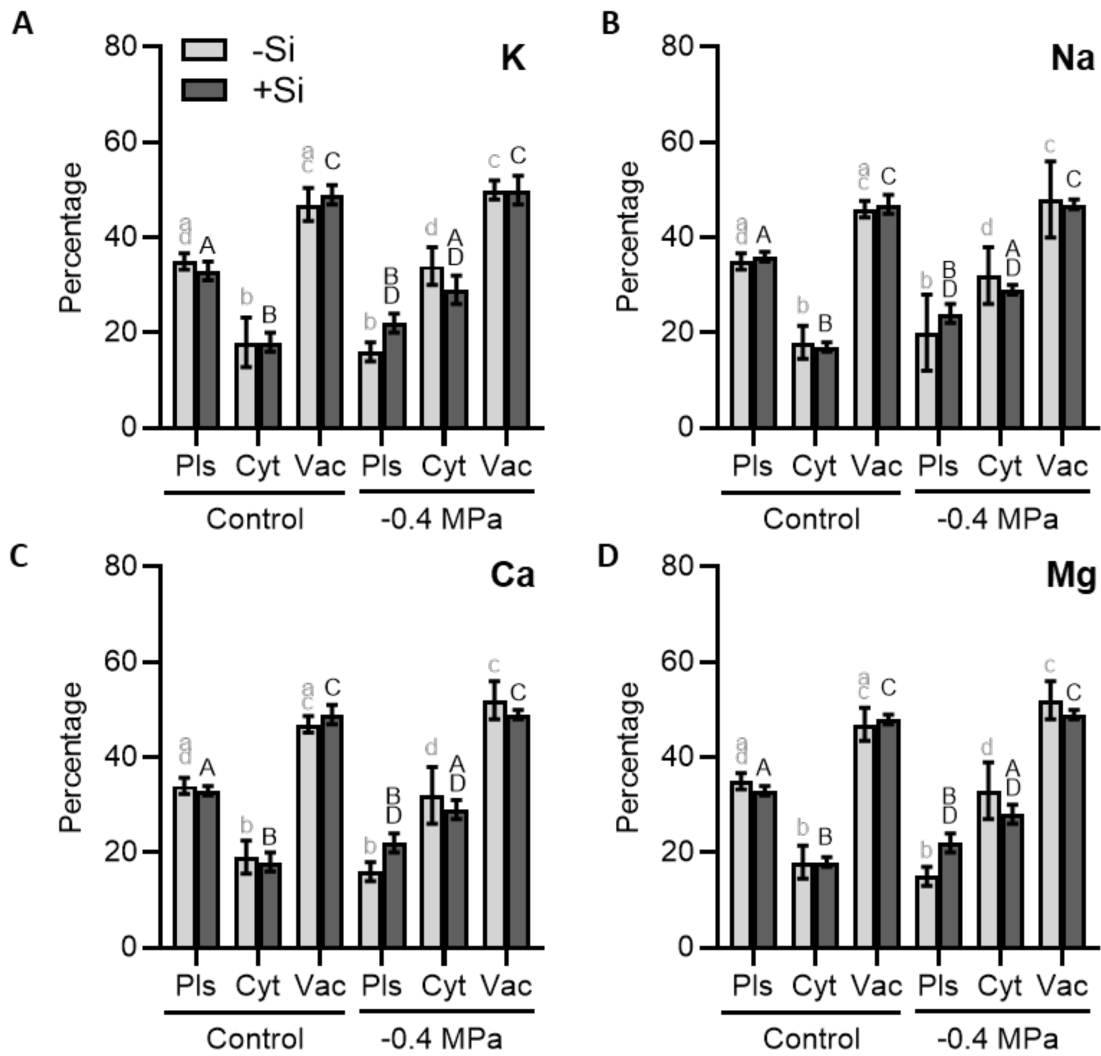
Supplemental figure 3. Potassium content in phloem exudate. The metabolites were extracted eight days after PEG8000 treatment. Results are means ± SD from four or more independent biological replicates.



Supplemental figure 4. The transcript level analysis. (A)The expression of TSTs 24 hours after PEG8000 treatment. (B) The expression of NHX1 in shoot and (C) root. The transcript level of NHX1 was measured six hours after PEG8000 treatment. Results are means ± SD from four independent biological replicates.



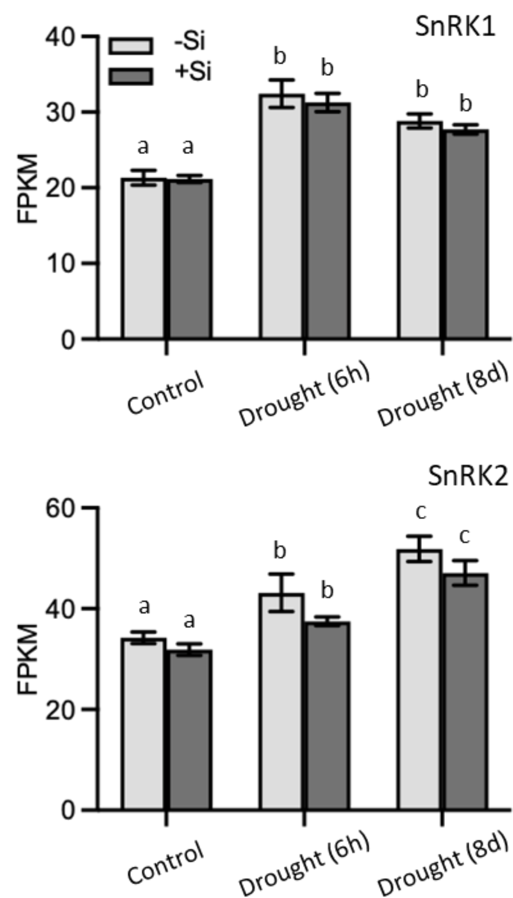
Supplemental figure 5. Photosynthetic performance of PSII in the plants with or without silicon treatment under control and drought. (A) ETR, electron transport rate. (B) Y(II), quantum yield in photosystem II; (C) Y(NPQ), quantum yield of regulated non-photochemical energy loss in PSII. Results are means ± SD from five independent biological replicates.



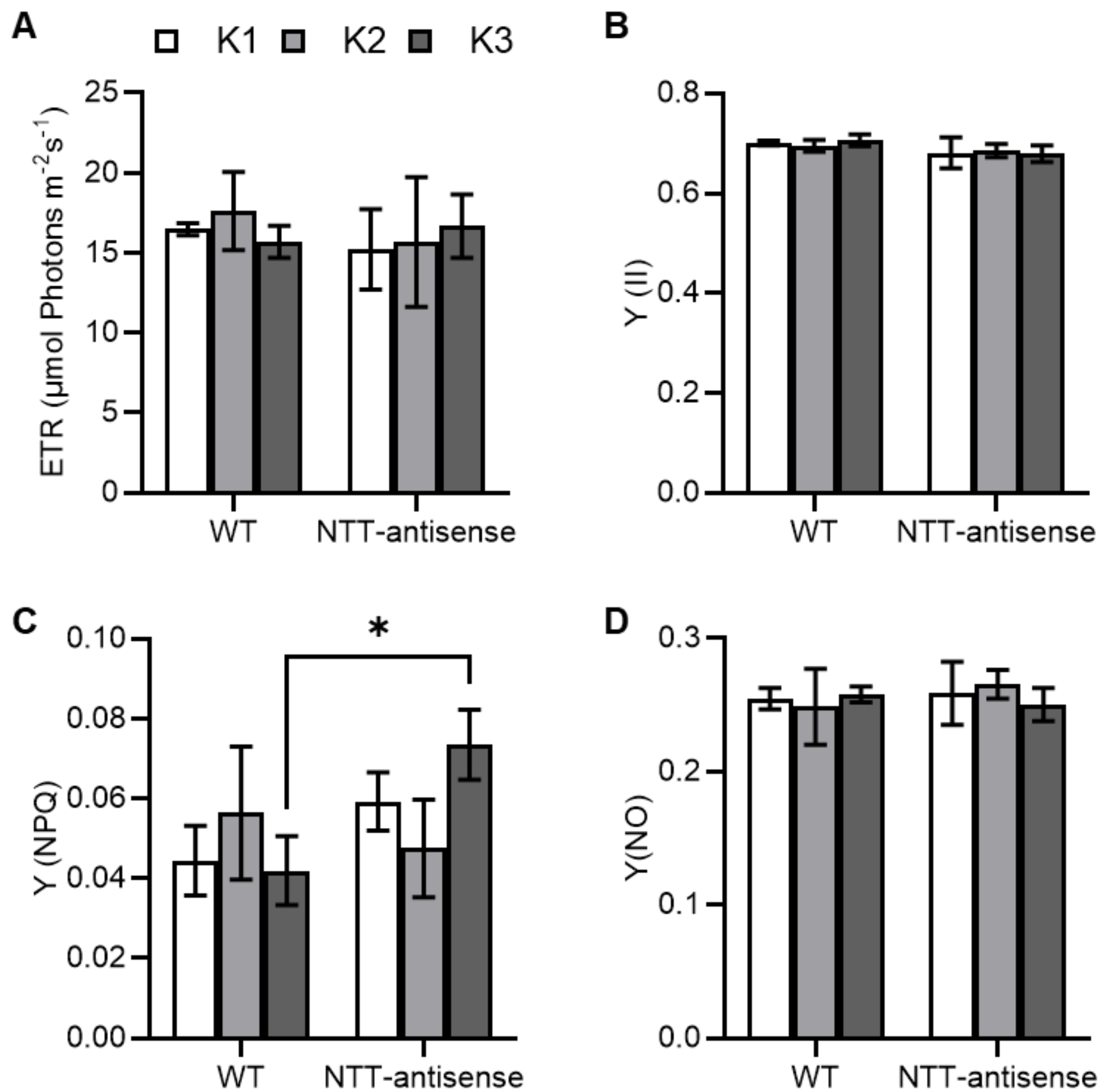
Supplemental figure 6. Subcellular localization of metabolisms analysis. Percentage of (A) potassium, (B) sodium, (C) calcium, and (D) Magnesium in plant cell components from shoot tissue. Results are means ± SD from three or more independent biological replicates.

		0h		6h		8d	
		-Si	+Si	-Si	+Si	-Si	+Si
AtCIPK1	*At3g17510*	7.10	8.33	12.27	11.57	7.10	30.51
AtCIPK2	*At5g07070*	2.05	2.08	2.39	2.84	2.05	3.48
AtCIPK3	*At2g26980*	19.73	16.97	11.03	10.81	19.73	27.54
AtCIPK4	*At4g14580*	2.12	2.00	2.09	1.30	2.12	3.35
AtCIPK5	*At5g10930*	8.61	4.96	19.97	14.39	8.61	8.26
AtCIPK6	***At4g30960***	**48.84**	**54.40**	**145.96**	**140.81**	**48.84**	**99.37**
AtCIPK7	*At3g23000*	49.86	38.58	99.61	78.84	49.86	46.69
AtCIPK8	*At4g24400*	14.02	12.46	21.35	17.77	14.02	24.42
AtCIPK9	*At1g01140*	37.75	37.78	29.12	31.98	37.75	27.78
AtCIPK10	*At5g58380*	5.64	8.85	11.33	11.94	5.64	14.90
AtCIPK11	*At2g30360*	0.00	0.00	0.00	0.00	0.00	0.00
AtCIPK12	*At4g18700*	19.82	22.58	27.41	25.95	19.82	65.39
AtCIPK13	*At2g34180*	0.95	0.66	0.94	0.54	0.95	0.25
AtCIPK14	*At5g01820*	13.71	11.12	27.19	20.06	13.71	27.49
AtCIPK15	*At5g01810*	4.15	6.20	11.49	11.38	4.15	12.99
AtCIPK16	*At2g25090*	0.00	0.03	0.13	0.02	0.00	0.21
AtCIPK17	*At1g48260*	0.96	0.79	0.74	0.30	0.96	1.41
AtCIPK18	*At1g29230*	0.37	0.44	1.24	0.97	0.37	1.77
AtCIPK19	*At5g45810*	0.03	0.04	0.11	0.07	0.03	1.40
AtCIPK20	*At5g45820*	1.82	0.76	0.76	0.57	1.82	0.43
AtCIPK21	*At5g57630*	31.17	32.79	34.69	32.05	31.17	74.95
AtCIPK22	*At2g38490*	0.57	0.27	0.77	0.37	0.57	1.25
AtCIPK23	*At1g30270*	10.94	9.85	13.43	12.76	10.94	22.82
AtCIPK24	*At5g35410*	2.19	3.17	3.64	3.58	2.19	4.16
AtCIPK25	*At5g25110*	2.36	1.27	12.84	7.20	2.36	12.51

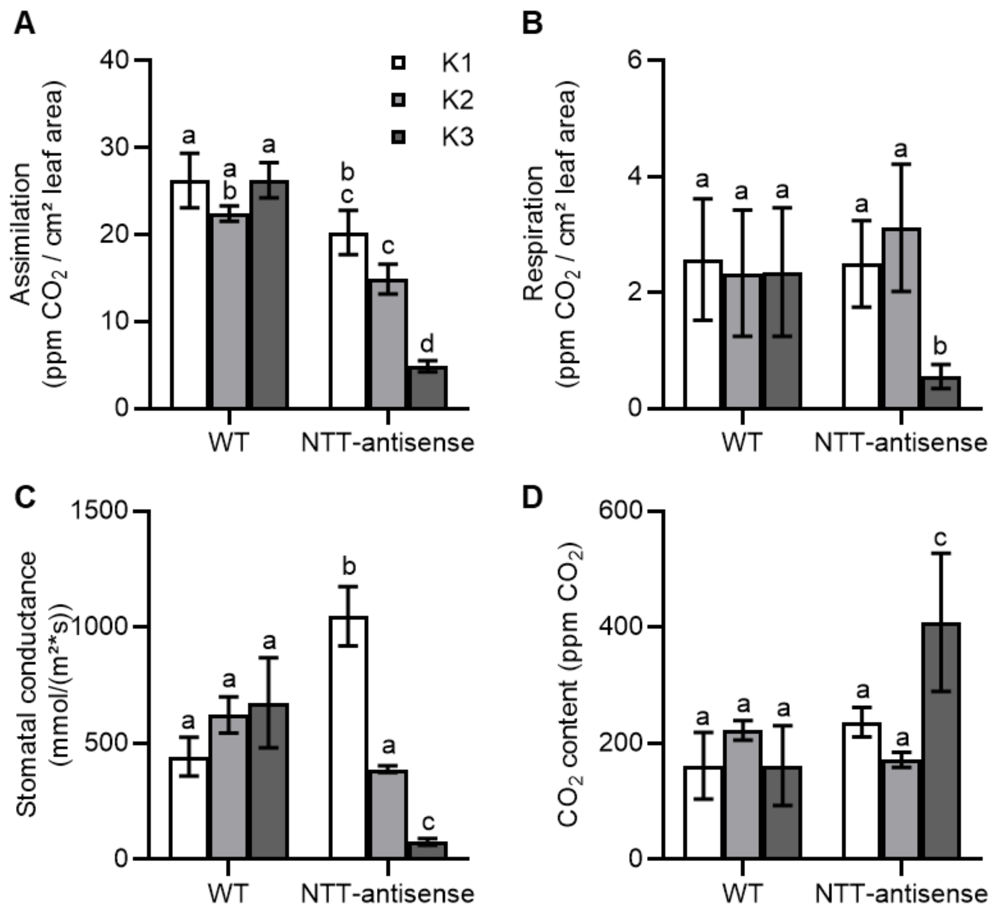
Supplemental figure 7. The transcript level of CIPKs in shoot under control and drought stress conditions. 0h: before drought stress; 6h: six hours after drought stress; 8d: eight days after drought stress.



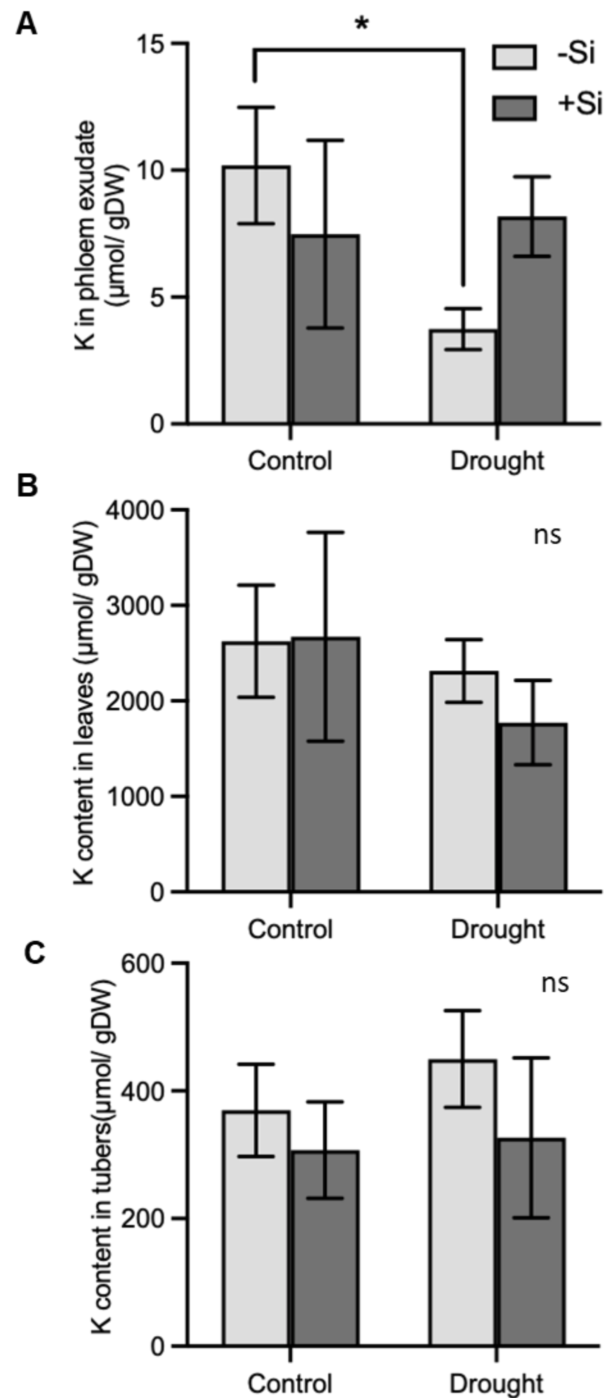
Supplemental figure 8. The transcript level of SnRK1 and SnRK2 in shoot under control and drought stress conditions. 0h: before drought stress; 6h: six hours after drought stress; 8d: eight days after drought stress. Results are means ± SD from three independent biological replicates. FPKM (Fragments Per Kilobase Million)



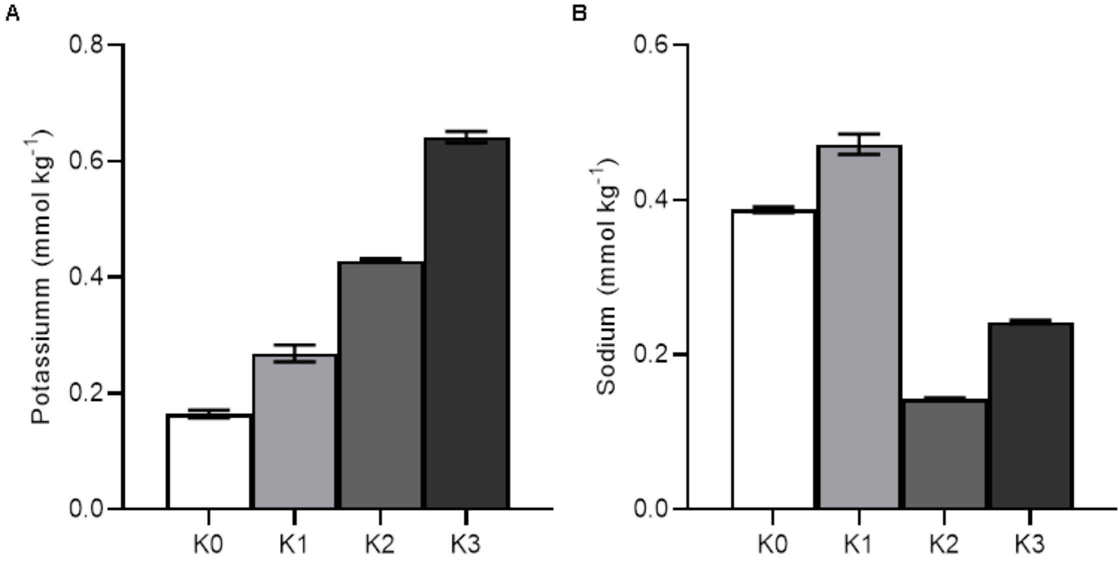
Supplemental figure 9. Photosynthetic performance of PSII in the potato plants with different levels of K supplication. (A) ETR, electron transport rate. (B) Y(II), quantum yield in photosystem II; (C) Y(NPQ), quantum yield of regulated non-photochemical energy loss in PSII; (D) Y(NO), quantum yield of nonregulated non-photochemical energy loss in PSII. Results are means ± SD from three independent biological replicates.



Supplemental figure 10. **Effect of K treatment on CO_2 assimilation.** (A) Assimilation rate, (B) respiration rate, (C) stomatal conductance, and (D) CO_2 content in the leaf of WT- and NTT-antisense plants with different levels of potassium treatment. Measurements were performed 35 days after the emergence of the shoot which was within the reproductive stage. Results are means ± SD from four independent biological replicates (n = 4).



Supplemental figure 11. The potassium content in WT and NTT-antisense plants under control and drought stress conditions. K content in (A) phloem exudate, (B) leaves, and (C) tubers.



Supplemental figure 12. The ion contents in the soil with different levels of potassium. (A) Potassium content in the soil. (B) Sodium content in the soil. The results are means ± SD from three replicates.

Supplemental table 3.1. The ion contents in Arabidopsis shoots and roots under both control and drought stress conditions.

	Shoot							
	K0		K1		K2		K3	
	Control	**-0.4 MPa**	**Control**	**-0.4 MPa**	**Control**	**-0.4 MPa**	**Control**	**-0.4 MPa**
Sodium	160 ± 17.5^{a}	144 ± 14.7^{a}	98.4 ± 2.22^{b}	145 ± 13^{a}	27.2 ± 3.69^{c}	40.3 ± 3.19^{c}	23.3 ± 0.971^{c}	41.1 ± 2.82^{c}
Ammonium	59.2 ± 7.85^{b}	96.6 ± 5.4^{a}	22.9 ± 0.442^{c}	29.7 ± 3.66^{c}	19.3 ± 1.25^{c}	25.6 ± 2.01^{c}	17.7 ± 0.907^{c}	20.8 ± 1.58^{c}
Calcium	224 ± 13.2^{cde}	84 ± 5.7^{f}	386 ± 12.7^{a}	298 ± 37.6^{bc}	255 ± 12.6^{bd}	204 ± 10.8^{de}	309 ± 10.9^{ab}	165 ± 15.4^{e}
Magnesium	246 ± 25.3^{a}	140 ± 7.61^{c}	272 ± 3.55^{a}	255 ± 17.4^{a}	223 ± 6.95^{ab}	216 ± 11.9^{ab}	228 ± 7.13^{ab}	183 ± 11.8^{bc}
Nitrate	1230 ± 111^{c}	790 ± 109^{c}	2710 ± 337^{a}	2250 ± 275^{ab}	2610 ± 126^{a}	2430 ± 129^{a}	2810 ± 190^{a}	1490 ± 192^{bc}
Phosphate	3 ± 0.468^{ab}	0.56 ± 0.0743^{c}	4.24 ± 0.361^{a}	3.37 ± 0.139^{ab}	4.2 ± 0.586^{a}	3.76 ± 0.237^{ab}	3.61 ± 0.535^{ab}	1.9 ± 0.629^{bc}
Sulfate	33.3 ± 4.5^{a}	22.7 ± 0.97^{b}	16.2 ± 0.636^{bc}	18.1 ± 1^{bc}	16.1 ± 1.89^{bc}	18 ± 1.04^{bc}	12.9 ± 1.42^{c}	14.4 ± 2.33^{bc}
Chloride	39.8 ± 9.82^{bc}	77.4 ± 5.41^{ab}	12.7 ± 1.57^{c}	50.5 ± 5.86^{bc}	18.6 ± 1.6^{c}	50.5 ± 3.46^{bc}	104 ± 5.99^{a}	115 ± 21.1^{a}
	Root							
	K0		K1		K2		K3	
	Control	**-0.4 MPa**	**Control**	**-0.4 MPa**	**Control**	**-0.4 MPa**	**Control**	**-0.4 MPa**
Sodium	194 ± 24.2^{b}	274 ± 47.8^{b}	397 ± 34.7^{a}	23.4 ± 1.72^{c}	26.5 ± 0.731^{c}	27.3 ± 2.24^{c}	33 ± 2.98^{c}	29.2 ± 2.82^{c}
Ammonium	51.4 ± 4.17^{a}	17 ± 2.71^{b}	22.2 ± 1.94^{b}	4.1 ± 0.376^{d}	15.9 ± 5.39^{bc}	3.03 ± 0.643^{d}	4.93 ± 0.444^{cd}	3.52 ± 0.337^{d}
Calcium	25.6 ± 4.4^{ab}	1.45 ± 0.483^{c}	5.22 ± 3.39^{bc}	11.6 ± 4.15^{bc}	4.39 ± 1.31^{c}	13.1 ± 3.09^{ac}	16.2 ± 6.71^{ac}	31.2 ± 4.91^{a}
Magnesium	68.4 ± 10.2^{a}	8.26 ± 0.759^{c}	11.3 ± 1.87^{c}	49.6 ± 11.5^{ac}	18.5 ± 11.3^{bc}	45.7 ± 12.2^{ac}	65.8 ± 15.4^{ab}	34.7 ± 2.38^{ac}
Nitrate	68.4 ± 10.2^{a}	8.26 ± 0.759^{c}	11.3 ± 1.87^{c}	49.6 ± 11.5^{ac}	18.5 ± 11.3^{bc}	45.7 ± 12.2^{ac}	65.8 ± 15.4^{ab}	34.7 ± 2.38^{ac}
Phosphate	7.93 ± 1.59^{b}	1.34 ± 0.189^{b}	24.6 ± 4.65^{a}	2.64 ± 1.33^{b}	8.26 ± 4.28^{b}	3.68 ± 1.62^{b}	2.9 ± 0.461^{b}	3.2 ± 1.04^{b}
Sulfate	161 ± 113	9.65 ± 1.04	35 ± 8.45	158 ± 53	135 ± 38.2	172 ± 57	182 ± 27	215 ± 32.9
Chloride	37.3 ± 3.21	84.2 ± 8.8	12.7 ± 1.52	43.5 ± 12.6	50.9 ± 21.3	18.5 ± 6.1	86.6 ± 20.2	68.1 ± 42.4

Values are means ± SEM, n = 5 per treatment group. $^{a-d}$Means in a row without a common superscript letter differ ($P < 0.05$) as analyzed by two-way ANOVA and the TUKEY test.

.

Supplemental table 3.2 Amino acid content in Arabidopsis shoots and roots under both control and drought stress conditions.

	Shoot			
	Control		**-0.4 MPa**	
	-Si *(n = 9)* mg/ gDW	**+Si** *(n = 9)* mg/ gDW	**-Si** *(n = 5)* mg/ gDW	**+Si** *(n = 9)* mg/ gDW
Aspartate	0.560±0.038^{ab}	0.574±0.051^{a}	0.365±0.039^{b}	0.404±0.047^{a}
Glutamate	1.528±0.101^{a}	1.477±0.178^{a}	0.785±0.073^{b}	1.037±0.147^{ab}
Serine	2.085±0.137	2.194±0.319	1.977±0.081	2.806±0.171
Glycine	0.061±0.006^{c}	0.065±0.012c	0.317±0.033^{a}	0.133±0.009^{b}
Glutamine	2.642±0.134	3.023±0.354	2.219±0.160	2.809±0.214
Histidine	0.026±0.003^{b}	0.029±0.005^{b}	0.117±0.044^{a}	0.042±0.005^{b}
Threonine	0.919±0.044^{b}	0.983±0.098^{b}	0.996±0.113^{b}	1.402±0.072^{a}
Alanine	0.414±0.028^{c}	0.567±0.063^{bc}	1.150±0.115^{a}	0.734±0.038^{b}
Arginine	0.093±0.0123	0.101±0.028	0.132±0.030	0.110±0.015
Ammonium	0.337±0.026	0.359±0.027	0.299±0.032	0.285±0.020
Proline	0.581±0.106^{c}	0.798±0.161^{c}	24.718±3.096^{a}	11.028±0.684^{b}
Cystine	NA	NA	NA	NA
Tyrosine	0.031±0.007^{c}	0.050±0.008^{bc}	0.129±0.016^{a}	0.086±0.011^{ab}
Valine	**0.135±0.007^{c}**	**0.190±0.025^{c}**	**0.661±0.099^{a}**	**0.349±0.031^{b}**
Methionine	0.004±0.0005	0.005±0.0008	0.004±0.001	0.005±0.0009
Isoleucine	**0.042±0.0028^{b}**	**0.066±0.007^{b}**	**0.367±0.049^{a}**	**0.101±0.010^{b}**
Leucine	**0.040±0.003^{c}**	**0.077±0.010^{c}**	**0.428±0.024^{a}**	**0.168±0.017^{b}**
Lysine	0.062±0.009^{c}	0.102±0.011^{c}	0.267±0.015^{a}	0.172±0.017^{b}
Phenylalanine	0.065±0.003^{b}	0.080±0.008^{b}	0.355±0.057^{a}	0.110±0.011^{b}
	Root			
	Control		**-0.4 MPa**	
	-Si *(n = 9)* mg/ gDW	**+Si** *(n = 9)* mg/ gDW	**-Si** *(n = 5)* mg/ gDW	**+Si** *(n = 9)* mg/ gDW
Aspartate	2.002±0.219^{A}	1.849±0.131^{A}	0.792±0.109^{B}	0.544±0.039^{B}
Glutamate	2.780±0.328^{A}	2.547±0.108^{A}	1.497±0.417^{B}	1.255±0.053^{B}
Serine	2.074±0.194	2.139±0.149	2.714±0.295	2.249±0.180
Glycine	0.061±0.013^{C}	0.054±0.003^{C}	0.234±0.032^{B}	0.325±0.029^{C}
Glutamine	2.639±0.219^{B}	2.828±0.248^{AB}	4.029±0.585^{B}	2.444±0.251^{A}
Histidine	0.023±0.005	0.033±0.006	0.053±0.017	0.032±0.007
Threonine	0.743±0.0612	0.776±0.032	0.993±0.150	0.938±0.043
Alanine	0.377±0.036^{C}	0.385±0.025^{C}	2.389±0.129^{B}	3.604±0.337^{A}
Arginine	0.042±0.016	0.038±0.004	0.021±0.008	0.032±0.007
Ammonium	0.440±0.073	0.309±0.016	0.347±0.027	0.323±0.021
Proline	0.097±0.018^{B}	0.127±0.017^{B}	9.908±0.72^{A}	9.799±0.342^{A}
Cystine	0.026±0.008	0.058±0.015	0.035±0.005	0.034±0.007
Tyrosine	0.036±0.005^{C}	0.038±0.004^{BC}	0.073±0.014^{A}	0.062±0.005^{AB}
Valine	**0.170±0.015^{B}**	**0.175±0.011^{B}**	**0.299±0.041^{A}**	**0.217±0.014^{B}**
Methionine	0.030±0.004^{A}	0.022±0.002^{AC}	0.010±0.001^{BC}	0.010±0.002^{B}
Isoleucine	**0.080±0.017**	**0.069±0.005**	**0.118±0.021**	**0.082±0.009**
Leucine	**0.104±0.012^{B}**	**0.098±0.007^{B}**	**0.227±0.039^{A}**	**0.143±0.011^{B}**
Lysine	0.116±0.013^{B}	0.110±0.008^{B}	0.253±0.043^{A}	0.159±0.012^{B}
Phenylalanine	0.074±0.010^{A}	0.056±0.004^{AB}	0.040±0.005^{B}	0.041±0.004^{B}

Values are means ± SEM. $^{a\text{-}c}$Means in a row without a common superscript letter differ ($P < 0.05$) as analyzed by two-way ANOVA and the TUKEY test.

Supplemental table 3.3 Ions contents in Arabidopsis shoots and roots under both control and drought stress conditions.

Values are means ± SEM. $^{a\text{-}c}$Means in a row without a common superscript letter

	Shoot				Root			
	control		-0.4 Mpa		control		-0.4 Mpa	
	-Si (*n* = 18) µmol/gDW	+Si (*n* = 17) µmol/gDW	-Si (*n* = 17) µmol/gDW	+Si (*n* = 18) µmol/gDW	-Si (*n* = 10) µmol/gDW	+Si (*n* = 10) µmol/gDW	-Si (*n* = 9) µmol/gDW	+Si (*n* = 10) µmol/gDW
Sodium	109 ± 5.5	85.4 ± 7.42	96.9 ± 6.15	91.5 ± 8.58	90.9 ± 5.85	76.3 ± 3.41	77 ± 8.01	93.6 ± 4.65
Ammonium	28.8 ± 2.09	27.7 ± 2.59	22.6 ± 2.17	23.1 ± 2.11	34.6 ± 1.5^{B}	27.7 ± 1.69^{C}	38.5 ± 2.38^{AB}	43.1 ± 1.49^{A}
Calcium	434 ± 35.7^{a}	376 ± 16.6^{a}	160 ± 26.2^{b}	144 ± 19.1^{b}	23.2 ± 16.2	3.72 ± 0.587	25.5 ± 18.4	3.31 ± 0.861
Magnesium	261 ± 17.8^{a}	229 ± 9.19^{a}	158 ± 10.9^{b}	148 ± 15^{b}	6.98 ± 0.722^{B}	7 ± 0.582^{B}	12.7 ± 1.81^{A}	11.4 ± 1.42^{AB}
Nitrate	1200 ± 27^{a}	1300 ± 20^{a}	630 ± 95^{b}	840 ± 66^{b}	980 ± 68^{A}	1000 ± 47^{A}	190 ± 53^{C}	470 ± 48^{B}
Phosphate	3.4 ± 0.17^{a}	3.1 ± 0.19^{ab}	1.2 ± 0.18^{c}	2.4 ± 0.27^{b}	38 ± 4.2^{A}	34 ± 1.6^{AB}	22 ± 3^{BC}	15 ± 1.4^{C}
Sulfate	4.4 ± 0.12^{c}	5.5 ± 0.48^{bc}	20 ± 1.7^{a}	7.5 ± 0.86^{b}	25 ± 4.2	24 ± 1.7	31 ± 1.4	30 ± 2.8

differ ($P < 0.05$) as analyzed by two-way ANOVA and the TUKEY test.

7. Glossary

%	Percent
°C	Degrees Celsius
µl	Microliter
g	Gram
ml	Milliliter
mM	Millimolar
Ψ	Water Potential
6h	Six Hours
8d	Eigh Days
ABA	Abscisic Acid
AKT2	Potassium Channel AKT2/3
At	Arabidopsis Thaliana
CBL	Calcineurin B-Like Protein
CIPK	CBL-Interacting Protein Kinase
CO_2	Carbon Dioxide
CWE	Cell Wall Elasticity
$_{dd}H_2O$	Double-Distilled Water
DEGs	Differentially Expressed Genes
DW	Dry Weight
ERDL	Early Response To Dehydration Six-Like
ESL1	Early Response To Dehydration Six-Like 1
EtOH	Ethanol
ETR	Electron Transport Rate
F6P	Fructose-6-Phosphate
FC	Soil Field Capacity
FRK	Fructokinase
FW	Fresh Weight
G6P	Glucose-6-Phosphate
Glu	Glucose
GO	Gene Ontology
H^+	Hydrogen Ion
H2O2	Hydrogen Peroxide
HXK	Hexokinase
i.e.	Id Est
INV	Invertase
K	Potassium
MPa	Megapascal
$NADP^+$	Nicotinamide Adenine Dinucleotide Phosphate
NADPH	Nicotinamide Adenine Dinucleotide Phosphate Hydrogen
ns	Not Statistically Significant
NTT	ATP/ADP Translocator
O_2	Oxygen Gas

PCs	Parenchyma Cells
PEG	Polyethylene Glycols
PPTCs	Phloem Parenchyma Transfer Cells
qRT-PCR	Real-Time Quantitative PCR
RNASeq	RNA Sequencing
ROS	Reactive Oxygen Species
RWC	Relative Water Content
SD	Standard Deviation
Si	Silicon
SPS	Sucrose Phosphate Synthase
St	Solanum Tuberosum
SUC	Sucrose Carrier
Suc	Sucrose
SUS	Sucrose Synthase
SUT	Sucrose Transporter
SWEET	Sugar Will Eventually Be Exported Transporter
TST	Tonoplast Sugar Transporters
UDP	Uridine Diphosphate
UGPase	UDP-Glucose Pyrophosphorylase
Y(II)	Yield Of Photosynthesis
Y(NO)	Yield Of Non-Regulated Non-Photochemical Energy Loss
Y(NPQ)	Yield Of Regulated Non-Photochemical Energy Loss

Acknowledgment

Firstly, I wish to convey my profound appreciation to Prof. Dr. H Ekkehard Neuhaus. Having him as my Ph.D. supervisor has truly been an honor. His outstanding supervision and insightful guidance have been invaluable. Furthermore, my sincere thanks go to Prof. Dr. Matthias Hahn for his assessment of my dissertation, and to Prof. Dr. Johannes Herrmann, whose friendly and proactive approach as the chair of my doctoral committee was greatly beneficial.

I owe a special debt of gratitude to Dr. Benjamin Pommerreing, whose extraordinary support, particularly during the thesis writing process, was indispensable. This entire dissertation is the product of his insightful suggestions, creative ideas, and thorough analysis. I commend his assistance in unravelling the complexities of the Silicon project, and his readiness to lend a listening ear when required.

I wish to express my appreciation to Dr. Seyed Abdollah HOSSEINI and the Roullier Group from France. They provided this invaluable opportunity and generously supported the project.

My gratitude also goes to Prof. Dr. Thomas Nägele for dispatching his PhD student, Anastasia Kitashova, who lent her support for the NAF analysis.

How can I not mention my former Supervisor, Prof. Woei-Jiun Guo. she guidance and encouragement during my master's degree were the driving force behind my decision to go to Germany for my PhD degree.

I extend heartfelt thanks to my lab colleagues: Kathi, Leo, and Isabel, whose invaluable suggestions for the project, thesis writing, and emotional support were instrumental. Their patience and encouragement bolstered my confidence. A special mention for Kathi, my stalwart support during challenging times. Additionally, Marzieh deserves my thanks for her precious time, motivation, and counsel, extending beyond the realm of research into my personal life.

My deepest appreciation goes to Christa, whose assistance with official documentation from my initial arrival was invaluable. As a non-German speaker, her support was genuinely heartening. My gratitude extends to Torsten, Ruth, Verena, Maike, Regina, and Ute, particularly our Technicians (Ruth, Verena, Maike, and Regina), whose humble and helpful attitudes, combined with their assistance with experimental

analysis, made a significant difference. Thanks are also due to Crissi, Annalisa, Vanessa, Gloria for their lab support and cultural insights. I'd also like to acknowledge Kuni, my former officemate. Despite our brief time together, her assistance and job-search advice were beneficial.

I am also grateful to friends Alina, Timo, Yu-Wei, Yi-Le, Chi-En, Yi-Hsuan, and Damian, who brought joy and lightness to my taxing Ph.D. journey.

Lastly, an immense and special thanks to my family: my parents, whose unparalleled upbringing instilled in me an unwavering belief in education and the confidence to believe that everything is possible, coupled with their unconditional love. My brothers and sister have been a constant source of comfort during periods of stress and depression, their unwavering love propelling me forward.

Above all, I want to express gratitude towards myself for persevering through such challenging times. I owe a significant acknowledgment to myself. Li-Hsuan, you've done an exceptional job.

Curriculum Vitae

Personal data

Name	Li-Hsuan Ho
Birthday	01.07.1992
Place of birth	Taichung city, Taiwan
Nationality	Taiwanese

Working Experience

2022-date	**Postdoctoral researcher**
	Cell Biology and Plant Biochemistry, University of Regensburg
2017-2018	**Research assistant**
	Bioscience and Biotechnology, NCKU

Education

2018-date	**PhD student, Plant Physiology**
	Department of Biology
	Technische Universität Kaiserslautern
2014-2017	**Master of Science, Tropical Plant Sciences**
	Department of Institute of Tropical Plant Sciences and Microbiology
	National Cheng Kung University (NCKU)
2012-2014	**Bachelor of Science, Medical Laboratory Science and Biotechnology**
	Department of Medical Laboratory Science and Biotechnology
	Central Taiwan University of Science and Technology (CTUST)
2008-2012	**Associate of Science, Medical Laboratory Science and Biotechnology**
	Department of Medical Laboratory Science and Biotechnology
	Central Taiwan University of Science and Technology (CTUST)

Publication

Ko, H.-Y., Tseng, H.-W., Ho, L.-H., Wang, L., Chang, T.-F., Lin, A., Ruan, Y.-L., Neuhaus, H. E., & Guo, W.-J. (2022). Hexose translocation mediated by Sl SWEET5b is required for pollen maturation in Solanum lycopersicum. Plant Physiology, 189(1), 344-359.

Ko, H.-Y., Ho, L.-H., Neuhaus, H. E., & Guo, W.-J. (2021). Transporter SlSWEET15 unloads sucrose from phloem and seed coat for fruit and seed development in tomato. Plant Physiology, 187(4), 2230-2245.

Ho, L. H., Lee, Y. I., Hsieh, S. Y., Lin, I. S., Wu, Y. C., Ko, H. Y., Klemens, P. A., Neuhaus, H. E., Chen, Y. M., & Huang, T. P. (2021). Ge SUT4 mediates sucrose import at the symbiotic interface for carbon allocation of heterotrophic Gastrodia elata (Orchidaceae). Plant, Cell & Environment, 44(1), 20-33.

Ho, L.-H., Rode, R., Siegel, M., Reinhardt, F., Neuhaus, H. E., Yvin, J.-C., Pluchon, S., Hosseini, S. A., & Pommerrenig, B. (2020). Potassium application boosts photosynthesis and sorbitol biosynthesis and accelerates cold acclimation of common plantain (Plantago major L.). Plants, 9(10), 1259.

Ho, L.-H., Klemens, P. A., Neuhaus, H. E., Ko, H.-Y., Hsieh, S.-Y., & Guo, W.-J. (2019). Sl SWEET1a is involved in glucose import to young leaves in tomato plants. Journal of Experimental Botany, 70(12), 3241-3254.

Chen, H. Y., Huh, J. H., Yu, Y. C., Ho, L. H., Chen, L. Q., Tholl, D., Frommer, W. B., & Guo, W. J. (2015). The Arabidopsis vacuolar sugar transporter SWEET 2 limits carbon sequestration from roots and restricts Pythium infection. The Plant Journal, 83(6), 1046-1058.

Statutory Declaration

Ich erkläre wahrheitsgemäß, dass ich die eingereichte Dissertation selbst angefertigt und alle für die Arbeit benutzten Hilfsmittel und Hilfestellungen in der Arbeit angegeben habe. Die Dissertation oder Teile hiervon habe ich bisher nicht als Prüfungsarbeit für eine staatliche oder andere wissenschaftliche Prüfung eingereicht. Ebenso habe ich die gleiche oder eine andere Abhandlung nicht bei einem anderen Fachbereich oder einer anderen Universität als Dissertation eingereicht.

English

I truthfully declare that I have independently prepared the submitted dissertation and have disclosed all the aids and assistance used in the work. I have not previously submitted the dissertation or parts thereof as an examination paper for a state or other academic examination. Similarly, I have not submitted the same or a different treatise as a dissertation to another department or university

Kaiserslautern, June 12, 2023

(Li-Hsuan Ho)