2 - Silicon: transcellular and apoplastic absorption and transport in the xylem

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2.1 Introduction

Before the absorption of chemical elements by the roots, there is ion-root contact, either by the ion movement in the rhizosphere soil solution (diffusion or mass flow), or by the root growth itself that encounters the ion (root interception) (Prado, 2021). To the best of our knowledge, the predominant form of contact of silicon (Si) with roots has not yet been determined. By either of these processes, and upon the contact with the roots, an ion may immediately enter the symplast by crossing the plasma membrane of an epidermal cell, or it may penetrate the apoplast and diffuse between the epidermal cells through the cell walls. From the apoplast of the cortical parenchyma, the ion can either be transported across the plasma membrane of a cortical cell, thus entering the symplast, or diffuse radially into the endoderm via the apoplast. The apoplast forms a continuous phase, from the root surface through the cortical parenchyma. However, in all cases, the ions must enter the symplast before entering the stelae, due to the presence of the striae of Caspary, a lignified or suberized layer that forms rings around endoderm cells and blocks the entry of water and solutes into the endoderm of the stele via apoplast (Assmann, 2017).

Si is absorbed by the roots from the soil solution or nutrient solution in monosilicic acid (H_4SiO_4) form, a neutrally charged molecule. The maximum solubility of H_4SiO_4 in solution is about 2 mmol L^{-1} and most soils usually contain H_4SiO_4 in solution between 0.1 - 0.6 mmol L^{-1} (Ma e Takahashi, 2002). After its absorption it is transported to the shoot by the xylem in the same chemical form (Bauer et al., 2011; Takahashi e Hino, 1979).

In general, there are four main areas of research on Si in plants: its absorption (or its absence), its classification as beneficial for the plants, its application as a fertilizer, and the mechanisms by which it attenuates biotic and abiotic stresses. Among these, one of the most intriguing properties of Si is its differential absorption by plants. Under similar conditions, plant species have different abilities to accumulate Si. As the benefits of Si are generally linked to the amount absorbed, some plant species benefit more from Si fertilization compared to others (Coskun et al., 2019).

Plants are classified according to the Si content in the shoot into three categories. Non-accumulators such as tomato accumulate less than 5 g kg^{-1}, while intermediate ones such as cucumbers, show contents between 5 and 10 g

kg^{-1} and accumulators, for example, rice accumulate morethan 10 g kg^{-1} of this element (Ma e Takahashi, 2002). The difference in Si accumulation between species has been attributed to differences in the Si absorption capacity by the roots (Ma e Yamaji, 2006). Thus, three modes of Si uptake were proposed: active, passive and rejection (Mitani-Ueno e Ma, 2021).

2.2 Active uptake of Si

This absorption system is normally represented by Si accumulating plants, such as rice, barley, maize and wheat. Here, transporters are necessary for the movement of Si from the soil solution to the plant organs. In an experiment with wheat plants grown in a nutrient solution with 0.02 mmol L^{-1} of Si, the concentration of this element in the xylem exudate has reached values as 400 times higher than those of the initial nutrient solution (8 mmol L^{-1}) in just 10 minutes. This reveals active mechanisms that absorb Si from the nutrient solution or soil solution and transport it to the xylem (CASEY et al., 2004).

H_4SiO_4 transporters were firstly described in rice. In this species, two transporters, Lsi1 and Lsi2 (Low silicon 1 and 2, so named due to the low Si content observed in the respective mutants with loss of function of these transporters) are expressed mainly in the mature zone of the roots and not in the root hairs, performing the Si transport from the soil solution to the xylem. These conveyors are of two types i.e. channel type and transport type (Ma e Yamaji, 2015).

Lsi1 is a bidirectional channel-type Si transporter that performs passive transport of Si by concentration difference (Mitani-Ueno e Ma, 2021). According to Coskun et al., (2021) and articles cited by them, homologous to rice Lsi1 were characterized in other plants as maize (*Zea mays*), barley (*Hordeum vulgare*), pumpkin (*Curcurbita moschata*), wheat (*Triticum aestivum*), horsetail (*Equisetum arvense*), soybean (*Glycine max*), poplar (*Populus trichocarpa*), cucumber (*Cucumis sativus*), tobacco (*Nicotiana sylvestris*), date palm (*Phoenix dactylifera*), grape (*Vitis vinifera*) and tomato (*Solanum lycopersicum*).

Lsi1 is a member of the Nodulin26-like intrinsic protein III (NIP-III) subgroup of Major Intrinsic Proteins (MIPs; also known as aquaporins) that carry out the passive transport of water and/or small uncharged solutes such as H_4SiO_4 (Ma e Yamaji, 2015). Thus, at the molecular level, aquaporins from plants belonging to the NIP-III subgroup with a GSGR (glycine-serine-glycine-arginine) selectivity filter and two NPA (asparagine-proline-alanine) domains separated by 108 amino acids are permeable to H_4SiO_4 (Coskun et al., 2019).

Lsi2 is an energy-dependent, antiport-type H_4SiO_4 efflux transporter. H_4SiO_4 crosses the plasma membrane along with H^+ but in opposite directions. However, unlike Lsi1, the relationship between structure and function of Lsi2 for now remains unknown (Coskun et al., 2021).

In rice, roots have a distinct anatomy, characterized by two Caspary streaks, one in the exoderm and the other in the endoderm, and also formation of aerenchyma (spaces without cells) in certain regions of the cortex. Therefore,

H_4SiO_4 from the soil solution or nutrient solution first crosses the epidermis via the apoplast and then is transported into the exodermis by Lsi1 located polarly on the distal side (farther from the root center), followed by efflux to the cortex by Lsi2 located polarly on the proximal side (closer to the root center) of the membrane of the same cell. These transporters are therefore located on opposite sides of the same cell. H_4SiO_4 flows via the apoplast to the endoderm, being transported in and out by Lsi1 and Lsi2, respectively, towards the xylem (Figure 2.1 A) (Mitani-Ueno e Ma, 2021).

In addition to the polar location of Lsi1 and Lsi2 in rice roots, the presence of the exoderm with the Caspary stria contributes to increase the accumulation of Si. It prevents its reflux, as it has been shown by the concentration of Si between the exoderm and the endoderm which duplicated due to the presence of Caspary's striae in the exodermis, in relation to its removal (Sakurai et al., 2015).

The expression of Si influx and efflux transporters is higher during the day than at night. This is because during the day, both the rate of transpiration and the flow of sap from the xylem is higher and the Si absorbed in the root is then efficiently transported to the upper tissues. Contrarily, during the night, the transpiration rate is lower as the xylem sap flow and therefore Si is not transported efficiently (Sakurai et al., 2017). According to the same authors, by analogy, when a conveyor belt is moving quickly, a lot of luggage can be carried, but carrying a lot of luggage on a slow conveyor is not a good strategy.

Recently, an H_4SiO_4 efflux transporter, located in the pericycle cells (layer of cells after the endoderm) of rice roots was identified and names Lsi3 (Figure 2.1 A). After one day of increasing the concentration of Si from 0.2 to 1.0 mmol L^{-1}, the expression of Lsi3 was decreased. Furthermore, Lsi3 expression was much higher in the mature root region (> 10 mm from the root tip) than in the root tip region. In the vegetative stage, OsLsi3 was mainly expressed in the roots and in the reproductive stage. Lsi3 was also not only expressed in the roots, but also expressed in the nodes (Huang et al., 2022). The same authors identified a significant negative correlation between Si accumulation in shoots and Lsi3 expression, indicating that Si accumulation in shoots suppressed Lsi3 expression in roots. The location of OsLsi3 in the pericycle was responsible for 30% of the total Si loading to the xylem at low Si concentrations but did not affect the absorption at high Si concentrations.

In maize roots, the expression of the ZmLsi1 transporter depends on the type of root. In the seminal (seed) roots, ZmLsi1 was located only in the epidermis and hypodermis cells (corresponding to the rice exoderm). In the lateral seminal roots, ZmLsi1 was expressed from the epidermis to the endodermis. In the crown roots, the localization of ZmLsi1 was observed in some of the epidermal and hypodermic cells. In all root types, ZmLsi1 showed polar location on the distal side of cells (N. Mitani et al., 2009). To check the morphology of maize roots, see Hochholdinger (2009).

In barley (*Hordeum vulgare*) seminal roots, HvLsi1 is localized in epidermal cells and all cortical cells. In the lateral roots, HvLsi1 is expressed in the hypodermic cells. Furthermore, HvLsi1 is more expressed in the mature region of the root than in its tip. This transporter has a polar location on the distal side of the epidermal and cortical cells of the seminal and lateral roots. (Chiba et al., 2009). However, HvLsi6 was highly expressed in the root tips compared to the mature root region. This transporter was located polarly in the epidermis and cortex of the root tip region (Yamaji et al., 2012). HvLsi1 is therefore responsible for Si absorption in the mature root region, while HvLsi6 is involved in Si absorption at root tips. On the other hand, Lsi2 from barley and maize is present only in the endoderm of the basal region of the roots and does not have a polar distribution. This means it is expressed throughout the plasma membrane of the endoderm cell (Namiki Mitani et al., 2009).

In this context, the proposed model for the uptake of H_4SiO_4 via roots in barley and maize indicates that this element is taken up by HvLsi1 or ZmLsi1, respectively, which is located polarly in epidermal, hypodermic and cortical cells. Unlike rice roots, barley and maize generally lack aerenchyma in cortical cells. Thus, H_4SiO_4 is transported to the endodermis by the symplast pathway. The transfer of H_4SiO_4 from the endoderm to the stele is performed by HvLsi2 or ZmLsi2 (Figure 2.1 B). This absorption system also results in high Si accumulation in the shoot but is not as efficient as the system present in rice (Mitani-Ueno e Ma, 2021).

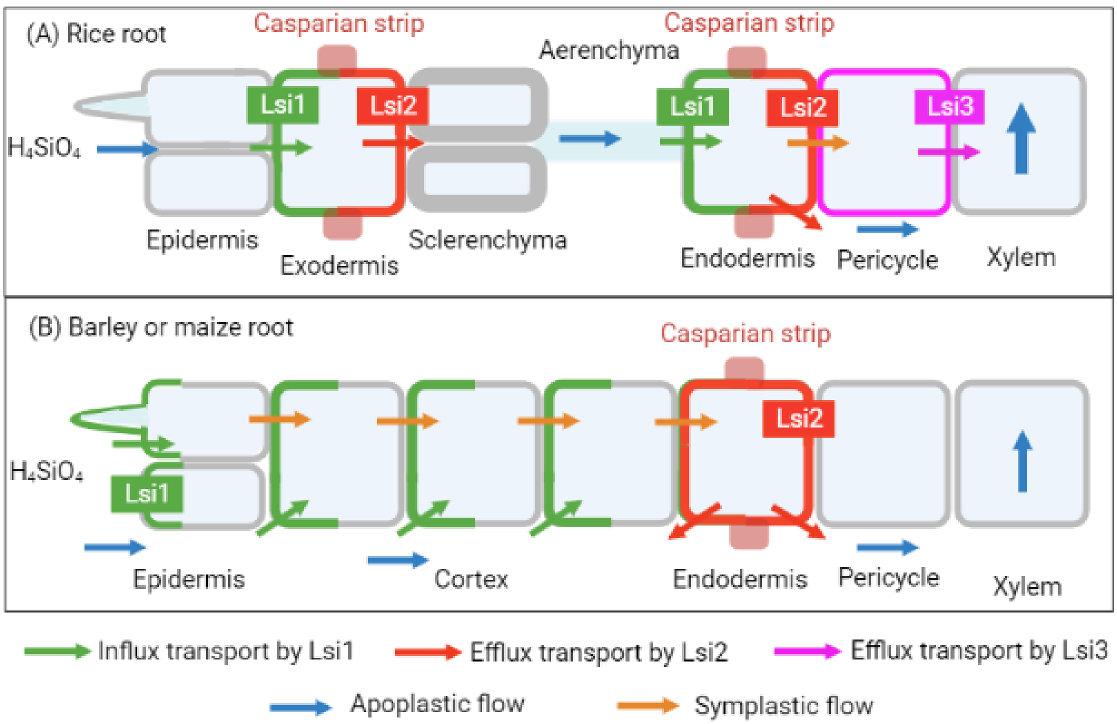

Figure 2.1 - Schematic presentation of Si uptake system in different plant species. (A) Active Si uptake system in rice. Si uptake is cooperatively mediated by Lsi1 and Lsi2, which are polarly localized at the distal and proximal side, respectively, of both exodermis and endodermis. In addition, loading of Si to the xylem is

facilitated by Lsi3, localized to the pericycle. (B) Active Si uptake system in other Si-accumulating species. Si uptake in these species such as barley is also cooperatively mediated by Lsi1 and Lsi2, but localized at different cell layers. Redrawn from Mitani-Ueno e Ma (2021). Created with BioRender.com

2.3 Passive uptake of Si

This absorption system is more common in intermediate Si accumulator plants, such as cucumber (CsLsi1 and CsLsi2) and pumpkin (CmLsi1 and CmLsi2). Both influx transporters, CsLsi1 and CmLsi1, are expressed in almost all root cells, around the entire plasma membrane, except for CsLsi1 which shows polar location in the endoderm. On the other hand, Lsi2 of both species are expressed only in endoderm cells without showing polar location (Figure 2.2 A). Thus, the lack of polar location and the existence of the two transporters in general in the same cells result in low efficiency for Si absorption (Mitani-Ueno e Ma, 2021).

2.4 Rejection uptake of Si

The uptake of rejection occurs in plants that do not accumulate Si, such as tomato. However, as in rice, tomato has SlLsi1, a functional Si transporter. SlLsi1 was expressed at the tips and basal regions of the roots, in the plasma membrane of the cells but without polar distribution, i.e. without distribution on one side of the cell's plasma membrane but across the entire membrane. However, the Si efflux transporter, SlLsi2, is not expressed in tomato, which is attributed to the low accumulation of Si in this plant species. Based on this knowledge, the proposed model for the absorption of Si in tomato indicates that this element can be absorbed through the epidermis and cortex via apoplast, symplast and SlLsi1 but due to the lack of the efflux transporter, SlLsi2 functional in the endodermis in the roots, Si in endodermal cells is not actively exported to the stele, resulting in low accumulation in the shoot (Figure 2.2 B). Furthermore, SlLsi1 shows bidirectional transport and part of the Si absorbed in the root cells can be released to the apoplast if there is a concentration gradient between the cytosol and the external solution. SlLsi1 showed 53-66% similarity with other Lsi1 from rice, cucumber and squash (Sun et al., 2020).

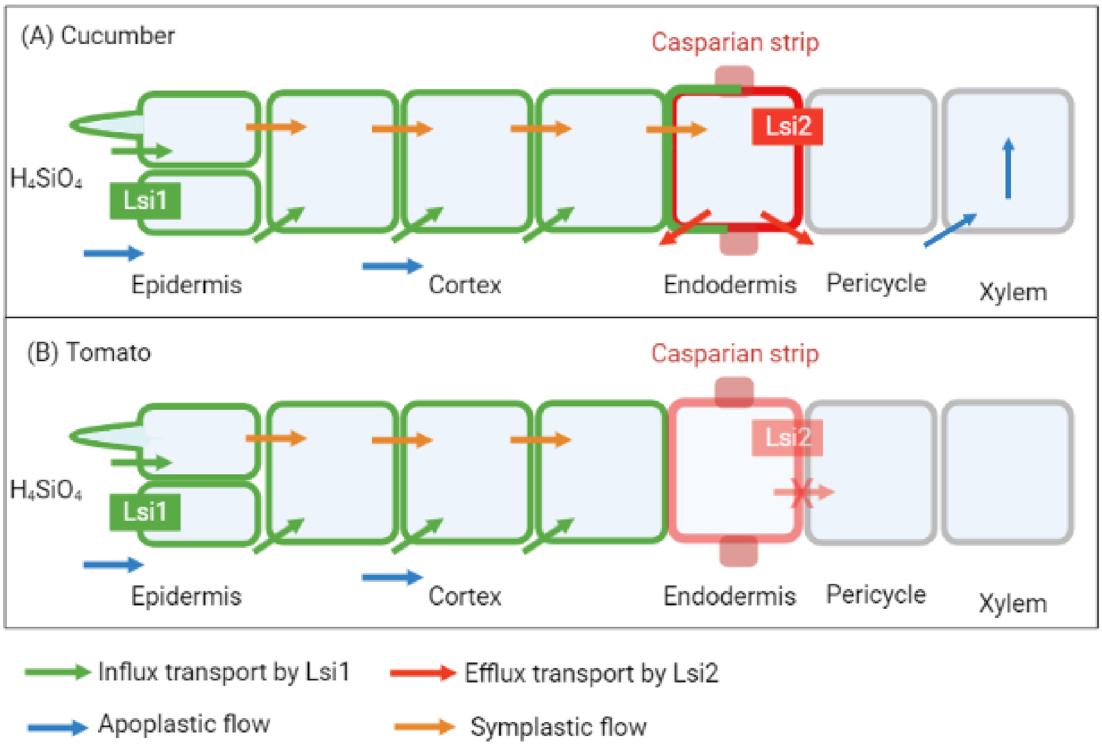

Figure 2.2 - Schematic presentation of Si uptake system in different plant species. (A) Passive uptake system. Si uptake in these plant species such as cucumber, is mediated by CsLsi1 expressed at all cell layers without polarity, and CsLsi2 at the endodermis without polarity. (B) Rejective uptake system. This system is employed by low Si-accumulator such as tomato, which has functional Lsi1, but lacks Lsi2. Redrawn from Mitani-Ueno e Ma (2021). Created with BioRender.com

2.5 Si transport in the xylem

Xylem is a vascular plant tissue responsible for the transport of water and chemical elements from the roots to the other plant parts. Xylem cells are therefore set to function as water-conducting of tracheary elements. Each plant has its own transporters to guarantee the right movement of ions from inside to the outside of the cells through the xylem, and Si is no exception. Uptake and transport of Si in plants occur in radial manner, from the cortical cells of roots to xylem vessels (Gaur et al., 2020).

More than 90% of the Si taken up by the roots is transferred into the shoot, and then it gets distributed within the plant depending on the transpiration rate of the several organs (Ma, 2010). Additionally, Si accumulator plants such as rice, have a Si efflux transporter (Lsi6) which is responsible for the release of silicic acid from the xylem and its subsequent distribution into the leaf sheath and midrib (Haynes, 2017; Kaur e Greger, 2019).

Lsi6 is polarly localized at the adaxial side of the xylem parenchyma cells in the leaf sheaths and leaf blades. Suppression of Lsi6 affects silica deposition pattern in the leaf blades and sheaths. As so, other Si-accumulator crops such as barley have also shown this type of transporter to accumulate higher concentrations in leaves and grains. During the reproductive stage, Lsi6 is also expressed in node 1 below the panicles and is involved in the transfer of Si to vascular bundles connected to the panicles (Mitani-Ueno e Ma, 2021).

At the reproductive stage, most Si will be distributed into the grains and mainly accumulated in the husk. This distribution is mediated by three Si transporters; Lsi6, Lsi2, and Lsi3 in rice (Figure 2.3), which are localized at different cell layers of node I. OsLsi6 at the xylem transfer cells of the enlarged vascular bundles is responsible for the unloading of Si from the xylem, while OsLsi2 and its homolog OsLsi3 localized at the bundle sheath and parenchyma bridge cells are responsible for further transfer of Si to the diffuse vascular bundles (Mitani-Ueno e Ma, 2021).

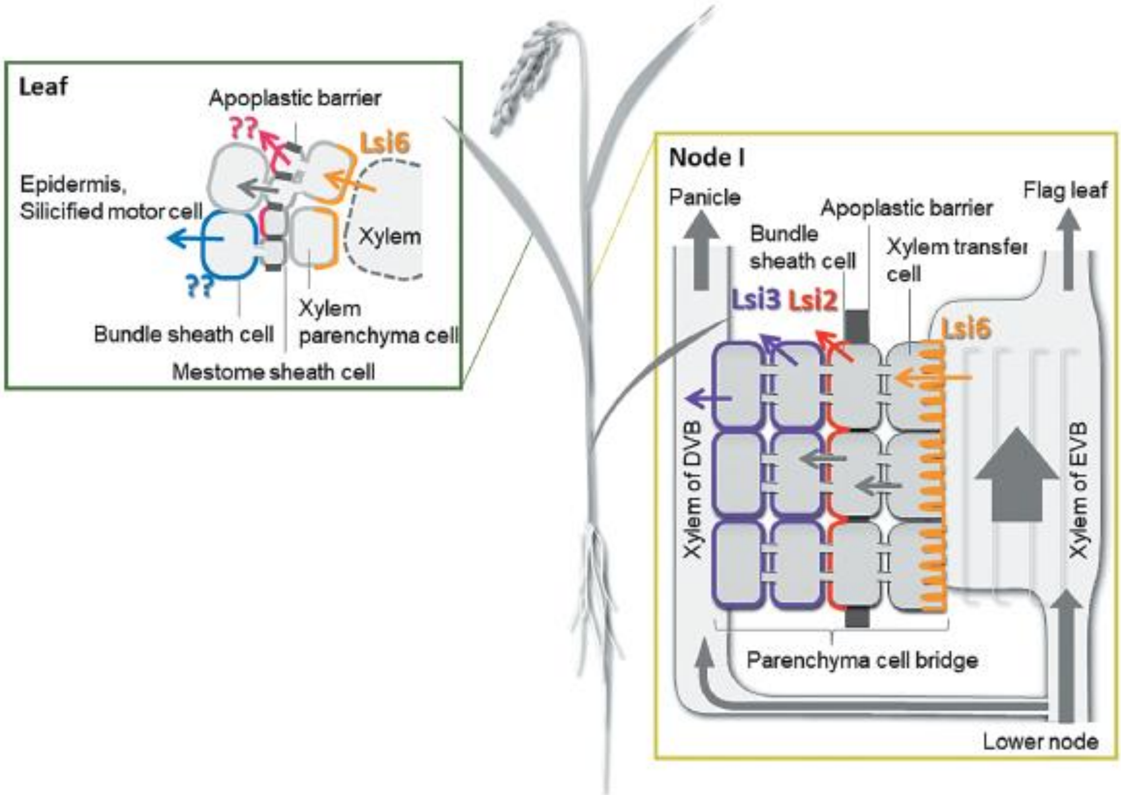

Figure 2.3 - Schematic presentation of Si distribution in shoot of rice. In leaves, Si in the xylem sap is unloaded by Lsi6, but transporters for further deposition at specific cells are unidentified. In nodes, Si in the xylem of enlarged vascular bundle is first unloaded by Lsi6 localized at the xylem transfer cells, followed by releasing Si toward diffuse vascular bundles by OsLsi2 polarly localized at the bundle sheath cell layer and OsLsi3 localized in the parenchyma tissues between enlarged vascular bundles and diffuse vascular bundles. Arrows with different colors indicate transport processes mediated by different transporters and symplastic flow of Si (Mitani-Ueno e Ma, 2021).

Once transported, Si reaches other parts of the plants it gets deposited. This deposition occurs in plant tissues where transpiration rate is higher; Si precipitation is a chemical reaction named condensation. Because transpiration is the main driver for Si accumulation and deposition in plant cells, the growth stage duration of the plant is an important factor that determines that older cells will accumulate more Si than the younger ones (Iler, 1979). When the concentration if silicic acid is higher than 100 - 200 mg kg^{-1}, monomers will form dimers and then oligomers with stable nuclei that eventually grow to form particles (Greenberg, 1959). When these particles grow up to 1 - 3 nm they become to carry a surface negative change, with enables interaction with the surrounding environment such as the cell walls (Haynes, 2017).

Deposition of silica can occur in a myriad of shapes and sizes. Often, silica precipitation in plants originates phytoliths, but that is not always the case. Phytoliths are amorphous microscopic opal structures produced in and between the cells of plants (Neethirajan et al., 2009). They are found in cells from the leaf epidermis and the covering of seeds and fruits, the epidermis of bracts which surround and protect grass seeds, and in the subepidermal tissue of orchid and palm leaves. Interestingly, at the reproductive stage, most Si will be found in cells from grains, especially accumulated in the husk (Mitani-Ueno e Ma, 2021).

Phytoliths range in size from 10 to 30 μm and are occasionally over 1000 μm in diameter. Colours can be as different as transparent or brown depending on the carbon coating extension. Phytoliths shapes and sizes differ greatly as they assume the shapes and sizes of their host cells (Rashid et al., 2019).

Silicified cells are of two types: 1) the silica cell and ii) silica body or silica motor cell. While silica cells are located on vascular bundles, showing a dumbbell-shape, silica bodies are in bulliform cells of rice leaves. Below concentration of 5%SiO_2, only silica cells are formed. Above this threshold, silica bodies start to form, increasing with Si shoot concentration (Datnoff et al., 2001). In conclusion, among crop plants, Si concentrations in tops generally increase in the order legumes<fruit crops<vegetable crops<grasses<grain crops (Haynes, 2017).

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