Introduction

Silicon (Si) is the second most abundant element after oxygen in the earth's crust and all plants contain Si in their tissues. Since there is still no evidence showing that Si is involved in the metabolism of plants, Si has not been recognized as an essential element according to the criteria of essentiality established by Arnon and Stout (1939). However, beneficial effects of Si have been observed in a wide variety of plant species. The characteristic function of Si is to help plants to overcome multiple biotic and abiotic stresses (Ma, 2004; Ma and Yamaji, 2006; Ma and Yamaji, 2008). For example, Si increases the resistance of plants to pests and pathogens. Silicon also enhances the resistance to drought and heavy-metal toxicity. Based on such important roles of Si in plants, Epstein and Bloom (2003) have proposed Si as "quasi-essential" for the growth of higher plants. In Japan, Si has been recognized as an "agronomically essential element" for rice, a typical Si-accumulating plant, because high accumulation of Si is required for optimal growth and sustainable production of rice.

Silicon is taken up by the roots in the form of silicic acid, a non-charged molecule. It is the same form to be translocated in the xylem from the roots to the shoots. In the shoot, with water loss through transpiration, silicic acid is concentrated and polymerized to silica (SiO_2) and finally deposited on the different tissues. Therefore, for the transfer of Si from the external solution (soil solution) to the different tissues, different transporters are required. Recently, great progresses have been made in identification of Si transporters involved in the uptake, translocation and distribution of Si.

Transporters Involved in Si Uptake

Radial transport of Si from the external solution into the root cells includes influx and efflux transporters.

Influx Si transporters

The first Si transporter (Lsi1) was identified from rice (Ma et al., 2006). Rice is able to accumulate Si over 10% in the shoots. By using a mutant approach, the gene *Lsi1* (Low silicon 1) was isolated by map-based cloning method. Lsi1 belongs to a Nod26-like major intrinsic protein (NIP) subfamily of aquaporin-like proteins. The predicted amino acid sequence has six transmembrane domains and two Asn-Pro-Ala (NPA) motifs, which are well conserved in typical aquaporins. By using a *Xenopus* oocyte assay system, Lsi1 shows an influx transport activity for silicic acid (Ma et al., 2006). The transport activity is not affected by low temperature (Mitani et al., 2008). Recently, it was reported that Lsi1 is also

permeable to arsenite (Ma et al., 2008).

The gene *Lsi1* is constitutively expressed in the roots, but its expression is decreased to one fourth by Si supply (Ma et al., 2006). Within a root, the expression of *Lsi1* was much lower in the root tip region between 0-10 mm than that in the basal regions (>10 mm) (Yamaji and Ma, 2007). Silicon uptake in the root tip region (0 to 10 mm) comprising both the apical meristem and the elongation zone is also much lower than that in the basal regions (>10 mm from the root tips). These observations indicate that the site of Si uptake is located in the mature regions of the roots rather than the root tips.

Investigation of rice *Lsi1* expression at different growth stages showed that the expression was transiently enhanced around the heading stage (Yamaji and Ma, 2007). A previous study showed that 67% of total Si was taken up during the reproductive stage from panicle initiation to heading in rice (Ma et al., 1989). Deficiency of Si during this stage results in a significant reduction in the grain yield, suggesting that a high Si uptake during this period is required for producing a high yield. Therefore, the increased expression of *Lsi1* during the heading stage coincides with a high Si requirement during this growth stage.

Lsi1 is localized in the main and lateral roots, but not in root hairs (Ma et al., 2006). This is consistent with the results of a previous physiological study that root hairs do not play any demonstrable role in silicon uptake, but that lateral roots contribute significantly to silicon uptake (Ma et al., 2001). Furthermore, in rice roots including seminal, lateral and crown roots, the Lsi1 protein is localized at both exodermis and endodermis, where Casparian strips exist. Interestingly, Lsi1 shows polar localization at the distal side of both the exodermis and endodermis cells (Fig. 1). Taken together, Lsi1 is responsible for the transport of silicic acid from the external solution to the root cells in rice.

Following identification of rice Lsi1, Si influx transporters have also been identified in maize (ZmLsi1) and barley (HvLsi1) (Chiba et al., 2009; Mitani et al., 2009). At amino acid level ZmLsi1 and HvLsi1 show 82% and 82% identity with OsLsi1, respectively. Both HvLsi1 and ZmLsi1 also show Si influx transport activity like OsLsi1, but their cell-type specificities of localization and expression patterns are different from OsLsi1. HvLsi1 and ZmLsi1 are localized at the epidermal, hypodermal and cortical cells (Chiba et al., 2009; Mitani et al., 2009). Furthermore, the expression levels of both *HvLsi1* and *ZmLsi1* are unaffected by Si. These differences may be related to the root structures and Si uptake ability among different species.

Efflux transporters of silicon

The efflux transporter gene (*Lsi2*) of Si was also cloned using a novel rice mutant (*lsi2*) defective in Si uptake (Ma et al., 2007). This gene *Lsi2* is predicted to encode a membrane protein with 11 transmembrane domains, belonging to a putative anion transporter without any similarity with the silicon influx transporter Lsi1. In *Xenopus laevis* oocytes, Lsi2 does

not show influx transport activity for silicic acid but shows efflux transport activity (Ma et al., 2007). These results suggest that in contrast to the influx transporter Lsi1, Lsi2 is a silicon efflux transporter, which is capable of transporting silicon out of the cells. Interestingly, the efflux of Si was inhibited by a low temperature treatment and by three protonophores; 2,4-dinitrophenol (DNP), carbonylcyanide 3-chlorophenylhydrazone (CCCP) and carbonylcyanide *p*-(trifluoromethoxy)penylhydrazone (FCCP). Furthermore, the efflux activity of Lsi2 is increased at lower external pH values (Ma et al., 2007). All these results suggest that transport of silicon by Lsi2 is an energy-dependent active process, which is driven by the proton gradient.

Lsi2 is mainly expressed in the roots as is *Lsi1*. The mRNA accumulation was constitutive but it decreased to one fourth by continuous silicon supply (Ma et al., 2007). Furthermore, there is little accumulation of *Lsi2* transcripts in the root tip (0-10 mm), but much accumulation in the mature parts of the roots. These expression patterns are similar to those of *Lsi1* (Ma et al., 2006, Yamaji and Ma, 2007), suggesting that the expression of *Lsi1* and *Lsi2* may be regulated in a similar manner. Comparison of the promoter region revealed common domains in the *Lsi1* and *Lsi2* (Ma et al., 2007).

Like Lsi1, Lsi2 is also localized at the exodermis and the endodermis cells of the roots. However, in contrast to Lsi1 localized on the distal side, Lsi2 was localized on the proximal side of the exodermis and the endodermis cells. Si efflux transporters in other plant species have not been identified.

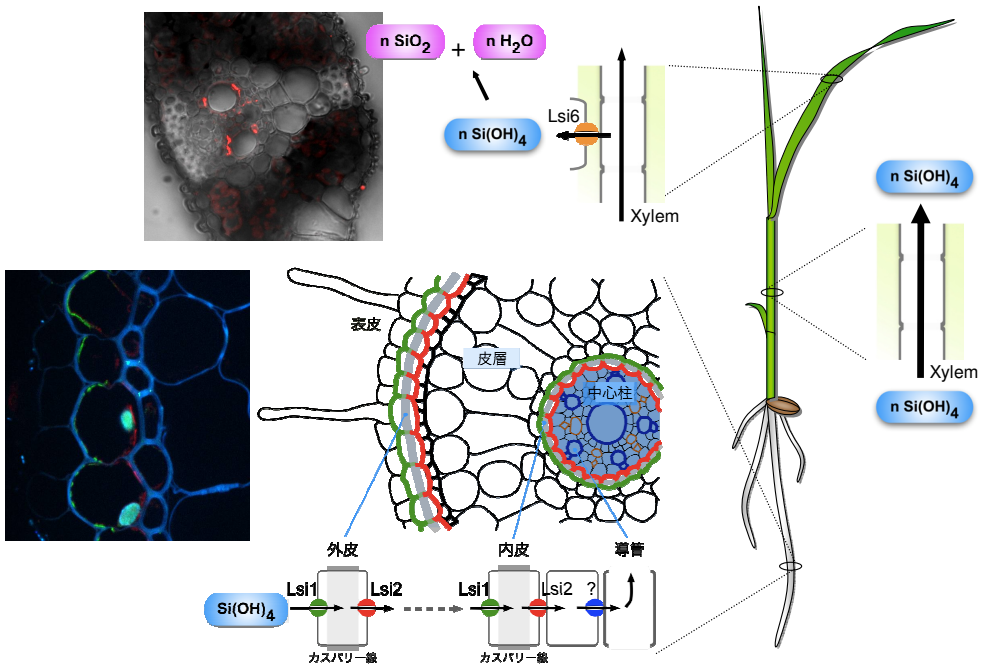

Figure 1. Silicon transporters involved in the uptake and distribution of Si in rice.

An efficient transport system for Si in rice by coupling of Lsi1 and Lsi2

As described above, both Si transporters (Lsi1 and Lsi2) are localized on the exodermis and endodermis, where Casparian strips exist (Fig. 1). Casparian strips prevent solutes to pass freely from the external solution to the stele. In fact, silicon is highly deposited on the exodermis and endodermis. Both Lsi1 and Lsi2 are required for transcellular transport of silicon to the stele. Moreover, rice roots are characterized by the formation of aerenchyma, which is accompanied by the destruction of almost all cortex cells except the exodermis and the endodermis. Therefore, Si transported into the exodermis cells by Lsi1 is released by Lsi2 into the apoplast of a spoke-like structure across the aerenchyma. Silicon is then transported into the endodermis cells by Lsi1 and released into the stele by Lsi2 (Fig.1). Coupling of Lsi1 and Lsi2 in the same cell in the Casparian strips is required for efficient transport of Si across the cells into the stele.

A recent study showed that genotypic differences in the Si accumulation in rice can result from the difference in the expression of Si transporter genes in roots (Ma et al., 2007). The expression of both Lsi1 and Lsi2 was lower in a variety with low Si uptake capacity than in a variety with high Si uptake capacity. However, there is no difference in the cellular localization of these two transporters between low and high Si uptake varieties. Therefore, high expression of both Lsi1 and Lsi2 is required to enhance Si uptake.

Transporters Involved in Translocation and Distribution of Si

Silicon transported by Lsi1 and Lsi2 into the stele is then translocated to the shoot by transpirational flow through the xylem. More than 90% of Si taken up by the roots is translocated to the shoots (Ma and Takahashi, 2002). Silicon is present in the xylem at high concentrations and in the form of monosilicic acid (Casey et al., 2003; Mitani et al., 2005). Recently, a transporter (Lsi6), which is responsible for the export of silicic acid from the xylem and for the subsequent distribution of Si, was identified in rice.

Lsi6 is a homolog of Lsi1. It also shows transport activity for silicic acid in the oocyte assay. However, different from Lsi1 and Lsi2, Lsi6 is also expressed in the leaf sheath and leaf blades in addition to the root tips (Yamaji et al., 2008). Lsi6 is localized in the adaxial side of the xylem parenchyma cells in the leaf sheath and leaf blades (Fig. 1). Knockout of Lsi6 does not affect the uptake of Si by the roots, but affects the silica deposition pattern in the leaf blades and sheaths. The density of silicified dumbbell-shape and motor cells in the knockout line is decreased compared with wild-type rice. The abaxial epidermis cells is observed to be silicified in the mutant, but infrequently in the wild-type rice (Yamaji et al., 2008). Furthermore, knockout of Lsi6 results in an increased excretion of Si in the guttation fluid. These results suggest that knockout of *Lsi6* resulted in alteration of the Si pathway to

the specific cells. Therefore, cell-type specific silicification depends on the symplastic pathway of Si delivered by Lsi6 rather than apoplastic pathway. A similar transporter ZmLsi6 has also been identified in maize (Mitani et al., 2009).

The level of Si accumulation in the shoots greatly differ among plant species, ranging from 0.1% to 10% of dry weight (Hodson et al., 2005; Ma and Takahashi, 2002). However, the molecular mechanisms for these differences are still unknown. Identification of more Si transporters in different plant species in future, will help to better understand the mechanisms of Si uptake, translocation and distribution in plants.

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