



Influence of Silicon on Root Anatomy of Rice **(*Oryza sativa* L.)**

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DEDICATION

In the memory of my parents

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ABBREVIATIONS

+ Si	With silicon
– Si	Without silicon
μg	Microgram
μgcm ⁻² sec ⁻¹	Microgram per centimeter square per second
μL	Microliter
μm	Micrometer
μmolcm ⁻² sec ⁻¹	Micromole per centimeter square per second
°C	Degree centigrade
ANOVA	Analysis of variance
As	Arsenic
ATP	Adenosine triphosphate
B	Boron
bp	Base pair
Ca	Calcium
Ca(NO ₃) ₂ ·4H ₂ O	Calcium nitrate tetrahydrate
CaCl ₂	Calcium chloride
CaCl ₂ ·2H ₂ O	Calcium chloride dihydrate
CaCO ₃	Calcium carbonate
cDNA	Complementary deoxyribonucleic acid
cm	Centimeter
cm ²	Centimeter square
Cu	Copper
CuSO ₄	Copper sulfate
DNA	Deoxyribonucleic acid
Fe	Iron
FeCl ₃	Ferric chloride
FeS	Iron sulfide
g	Gram

GF-AAS	Graphite Furnace Atomic Absorption Spectrophotometer
h	Hour
H ₃ AsO ₃	Arsenious acid
H ₃ BO ₃	Boric acid
HCl	Hydrochloric acid
HF	Hydrogen fluoric acid
K	Potassium
K ₂ SiO ₃	Potassium silicate
K ₂ SO ₄	Potassium sulfate
M	Molar
m	Meter
Mg	Magnesium
mg	Milligram
mg/L	Milligram per liter
MgSO ₄	Magnesium sulfate
min	Minute
mL	Milliliter
mM	Millimolar
mm	Millimeter
Mn	Manganese
MnSO ₄	Manganese sulfate
Mo	Molybdate
mV	Millivolt
N	Nitrogen
Na ₂ MoO ₄	Sodium molybdate
NaAsO ₂	Sodium arsenite
NaCN	Sodium cyanide
NaN ₃	Sodium azide
NaNO ₂	Sodium nitrite
ng	Nanogram
NH ₄	Ammonium

$\text{NH}_4\text{H}_2\text{PO}_4$	Ammonium phosphate
NH_4HPO_4	Ammonium hydrogenphosphate
NH_4NO_3	Ammonium nitrate
NIP	Nodulin 26-like intrinsic protein
nm	Nanometer
NO_3	Nitrate
O_2	Oxygen
P	Phosphorus
PAR	Photosynthetic Active Radiation
Pht1;1	Phosphate transporter 1;1
Pht1;3	Phosphate transporter 1;3
Pht1;4	Phosphate transporter 1;4
ppm	Part per million
RNA	Ribonucleic acid
rpm	Revolutions per minute
s	Second
Si	Silicon
$\text{Si}(\text{OH})_4$	Silicic acid
$\text{SiO}_2 \cdot n\text{H}_2\text{O}$	Hydrated silica
UV	Ultra violet
v/v	Volume per volume
w/v	Weight per volume
Zn	Zinc
ZnSO_4	Zinc sulfate

ABSTRACT

Paddy rice is the most important staple food for many countries that can contain high amounts of the toxic element arsenic (As) in some conditions. Though a beneficial effect of silicon (Si) supply in rice plants have been long known, it was recently discovered that application of Si to the nutrient solution can reduce total arsenic uptake in rice. Suberized cell walls of rice roots have barrier properties against the entering of water and toxic ions.

In current study, the role of silicic acid supply on suberization in root exodermis and endodermis was investigated in rice grown in both nutrient solution and quartz sand. Microscopic examinations revealed that the silicic acid application could enhance the suberization in root exodermis and endodermis of rice grown in nutrient solution. In contrast, no effect of Si on suberization in root was observed in rice grown in quartz sand. The effect of silicic acid supply on suberization in both root exodermis and endodermis might need at least 48 h. Moreover, silicic acid application could reduce the oxidation power of rice root.

The effect of Si supply on uptake of arsenite by roots with and without lateral roots was examined. The inhibitory effect of Si on arsenite uptake and arsenic content in both root types was clearly shown. This implied that silicic acid supply affected both arsenite uptake rate and total arsenic concentration in roots due to the higher degree of suberization and lower expression of arsenite transporters in rice roots. However, no difference of arsenite uptake and arsenite content between roots with and without lateral roots. The younger zone of both roots grown with and without silicic acid supply had a higher arsenite uptake rate and contained more arsenic than the older root zone. This study demonstrated that silicic acid supply to the nutrient solution plays a role in enhancing the suberization in roots and decreasing the oxidation power as well as arsenite uptake rate of rice roots.

1. INTRODUCTION

As a global food, paddy rice has a large influence on human nutrition and improved livelihoods in all over the world. Moreover, it is the staple food for half of the world population and is almost grown in all continents of the world (Diouf, 2004). The world population is growing time by time and over next 50 years, the population of the world will increase about 50 % (Goloyugo, 2009). Rice is therefore on the frontline in the fight against world hunger and poverty. Being in tune with the idea of long-terms food security, it is inevitably necessary to boost its productivity. In recent decades, arsenic contamination of the environment becomes a life-threatening problem for millions of people via contamination of drinking water and polluted irrigation water (Quaghebeur and Rengel, 2005). Environmental arsenic pollution has been reported from many countries, with the most severe problems occurring in Asia, namely Bangladesh, China and India, which are all major Asian rice producing countries (Abedin et al., 2002).

According to food surveys, paddy rice generally contains a higher content of arsenic compared to other cereals (Tao and Bolger, 1998). The main reason for an enhanced accumulation of arsenic by flooded rice is that it was mostly grown in flooded conditions which lead to increase the bioavailability of soil arsenic (Xu et al., 2008). Thus some measures must be developed to reduce and manage the severity of this problem. Furthermore, it is essential to know how the arsenic is taken up by rice under the paddy soil condition in order to better understand the accumulation and metabolism of arsenic in the plants (Meharg and Hartley-Whitaker, 2002). This therefore calls for the need to study how arsenic enters into the root and also to investigate root anatomy of rice. However, information on rice root anatomy is relatively limited compared to the rest of the plant (Lampe, 1994). Rice grown in flooded soil condition can modify its root anatomy and silicon may play an important role in this modification of rice root system since it is known to be beneficial for enhancing plant growth by providing mechanical strength and promoting the photosynthesis of the plants (Epstein, 1994). Silicon has also been shown to improve disease resistance, water-use efficiency and induce resistance to heavy metal stress (Ma and Takahashi, 2002b; Liang et al., 2007; Gao et al., 2006; Epstein, 1999; Romero-Aranda et al., 2006; Kidd et al., 2006; Neumann and zur Neiden, 2001). Beside this, silicon application to soil is known to reduce the total arsenic uptake by rice (Guo et al., 2007; Bogdan and Schenk, 2008). Additionally, the supply of silicon can enhance the development of root exodermis in order to

improve the formation of suberin lamellae of exodermis (Nordheim, 2007). Suberin is an extracellular biopolymer and it consists of a polyaliphatic domain and a polyaromatic domain (Franke and Schreiber, 2007). In rice roots, suberization generally takes place in Casparian bands within the cell walls of endodermis and exodermis. Suberized cell walls of rice roots have barrier properties against the entering of water and ions from the soil and protect the plants from the invasion of pathogen. In addition, it acts as impervious to air as it is to water (Franke and Schreiber, 2007).

Therefore, this study aimed at investigating the effect of silicic acid application on the formation of suberin lamella of root exodermis and endodermis. It was expected that application of silicon would affect on the oxidation power of root and the uptake of arsenite due to the enhancement of suberization of root exodermis and endodermis in rice.

2. LITERATURE REVIEW

2.1 Rice root anatomy

The anatomy of rice roots is generally similar to other monocots including cereals. Rice root system is normally characterized by having shallow and compact root system and built up with a radicle (or seminal) root, mesocotyl root and nodal (or adventitious) roots (Fig. 1) (Yoshida, 1981). The distribution of root system is mostly regulated by the growth direction of nodal roots due to their bulk of rice root system (Morita and Abe, 1994 cited in Davis and Haissig, 1995). Seminal and nodal roots as well as most of lateral roots have root hairs that are $\sim 5\text{-}10\ \mu\text{m}$ in diameter and $\sim 50\text{-}200\ \mu\text{m}$ in length (Morita and Nemoto, 1995 cited in Baluska et al., 1995). The maximum depth of roots is $\sim 1\text{m}$ or deeper in upland soils and under anaerobic conditions, the roots can reach a maximum depth of $\sim 40\ \text{cm}$ (Yoshida, 1981).

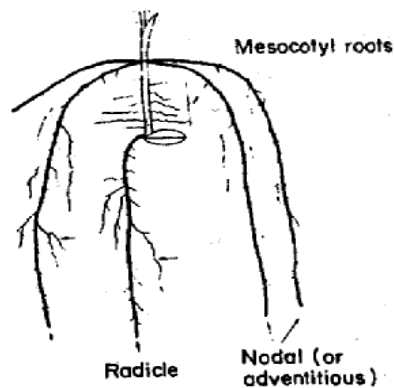


Figure 1: Three kinds of rice roots (adapted from Hoshikawa, 1975 cited in Yoshida, 1981).

Rice root is systematically composed of different types of cells, which have different functions to serve the root system. The outermost part of the root is the epidermis shown in Fig. 2. The epidermis cells are small cells and in older roots, they are tabular and often elongated into the root hairs (Clark and Harris, 1981). They have low resistance to the entry of water and minerals. Beneath the epidermis is the exodermis that is the outermost layer of the cortex. The exodermis is a special type of hypodermis which is characterized by the formation of Casparian bands in its cell walls (Peterson and Perumalla, 1990). The Casparian band of exodermis serves as a barrier function of variable resistance to the radial flow of both water and solutes (Hose et al., 2001).

The authors also reported that it is involved in regulating the internal oxygen status of the plants growing in wetland environment.

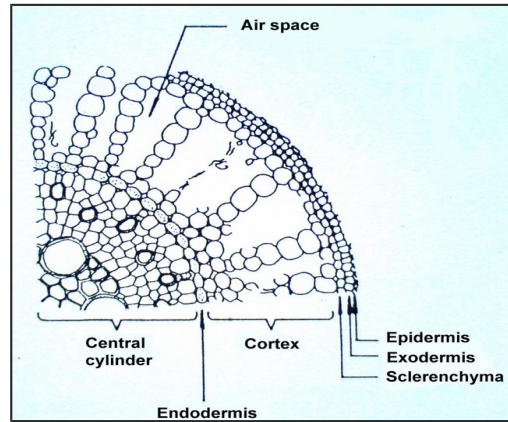


Figure 2: Cross section of a mature rice root (adapted from Hoshikawa, 1975 cited in Yoshida, 1981).

Between the exodermis and the cortex is the sclerenchyma that is composed of thick-walled lignified cells. It acts as a supporting tissue and their walls consist of lignin and cellulose (Clark et al., 1981). Under drying soil conditions, the sclerenchyma would reduce water loss from the roots and prevent collapse of cortex. In contrast, sclerenchyma might be a constraint for water absorption into the nodal root. Some rice varieties form two or three layers of sclerenchyma in the outer cortex, whereas most of other rice varieties form only one layer (Kondo et al., 2000).

Beside the sclerenchyma, the cortex is the basic part of the primary root body and occupies the greatest volume of the roots. In cortex, the cells form during the early development, die and leave gas spaces (Evans, 2003). The aerenchyma is an important system for gas exchanged under flooded conditions. The innermost layer of cortex is the endodermis which is characterized by the formation of the Casparian bands in the cell walls. The Casparian bands of endodermis also prevent the back flow of water and ions from the cortex (Zier et al., 1999). Suberin lamellae are deposited as secondary walls after the maturation of endodermal Casparian bands. However, passage cell do not develop suberin lamellae and they may decrease in number and form suberin lamellae when the root becomes older (Enstone et al., 2003). The centre of the central cylinder is formed by primary xylem toward the pericycle. The protoxylem elements are located next to pericycle, and the tips of the ridges commonly refer to the protoxylem poles (Raven et al., 1999).

A few cells from the central cylinder towards the stem are so called metaxylem that are enlarged and matured after the protoxylem (Stover, 1928).

Roots enable the plant to take up water and nutrients. Along a single root, suberization is more intense in regions at ~ 4-5 cm from the tip. Thus the entrance of water and ions might be most active between the root tip and the site of suberized region. The lateral roots might also be responsible for the uptake of ions due to their bulk of external surface area, although short fine lateral roots account for a small part of root mass (Kirk, 2003).

2.2 Arsenic uptake in rice

Because of its toxicity, arsenic is one of the considerable environmental concerns in the world. It is widely distributed from both natural and anthropogenic sources such as mining activities, wood preservation, and irrigation with arsenic (As) contaminated ground-waters. In soil-water environments, As can exist in four valency states: -3 , 0 , $+3$, and $+5$. Since adsorption is one of the reactions that controls the mobility and bioavailability of As, both arsenite ($+3$) and arsenate ($+5$) have a strong sorption affinity for iron-(hydr) oxide (Thoral et al., 2005). According to its strong binding to iron-, aluminum-, and manganese-(hydr) oxides, arsenic concentration is very low in soil solution of aerobic soils (Bogdan and Schenk, 2008). However, in anerobic conditions floodwater inhibits oxygen movement into the soil and the remaining oxygen is depleted within a short time due to the consumption of microorganisms for respiration (Trolldenier, 1988). When anaerobic conditions were achieved, dissolution of Fe and Mn (hydr) oxides occurs, causing desorption of As in soil solution (McGeehan and Naylor, 1994). In submerged soils, inorganic arsenic coexists in the soil solution and converts between the reduced inorganic species arsenite and the oxidized species arsenate (Meharg, 2004; Abedin et al., 2002). Arsenite is considered to be more mobile in soil solution because it is present as a neutral species (H_3AsO_3). In moderate reductive conditions like in paddy soils, arsenite might be the predominant form followed by arsenate and smaller amounts of methylated As species (Abedin et al., 2002). Nearly 90 % of arsenate is generally stable in the aerobic soil conditions (O'Neil, 1995 cited in Alloway, 1995). The formation of arsenate and smaller amounts of methylated As species may be enhanced by a high organic matter content resulting in a greater microbial activity (Ascar et al., 2008). Rittle and Drever (1995) stated that soluble As may decrease according to the formation of arsenic

sulfide with further decreasing redox potential. In long term, using of arsenic-rich ground water might lead to the accumulation of As in paddy soils (Dittmar et al., 2007).

Arsenate shares the same transport pathway with phosphate in higher plants (Ullrich-Eberius et al., 1989; Meharg, 1994). It may behave as a phosphate analogue and is relatively immobile like phosphate in soil. Among a number of phosphate transporters, Pht1;1 and Pht1;4 mediate the uptake of arsenate (Shin et al., 2004). Recently, Catarecha et al. (2007) found that Pht1;3 mediates arsenate uptake in the short-term and enhances the accumulation of arsenate over a longer period of growth.

Arsenite uptake is of particular importance for rice plants grown in moderate reductive conditions. Aquaporin channels can mediate arsenite influx in plant roots. According to the recent study of Ma et al. (2008), two different kinds of transporters that belong to the nodulin 26-like intrinsic protein subfamily of aquaporin (NIP) contribute arsenite transport from external medium to xylem sap. A silicon influx transporter (Lsi1) is permeable to arsenite but not to arsenate in yeast. The results of yeast expressing Lsi1 showed that silicic acid transporter can mediate the transport of arsenite in rice. Silicon efflux transporter (Lsi2) mediated not in the influx of arsenite in the roots but rather in the efflux of arsenite toward the xylem sap. In shoot and grain, Lsi2 may have more impact on the accumulation of arsenic than Lsi1. No transporter has yet been identified for entering small amounts of organic As species into plant roots (Tripathi et al., 2007).

2.3 Effect of silicon on arsenic uptake in rice

Nowadays, there is an urgent need to develop the effective strategies for limiting the detrimental impact of arsenic compounds in rice. A recent study demonstrated the inhibiting effect of indigenous silicic acid in the soil solution on As uptake by rice (Bogdan and Schenk, 2008). The authors also suggested that soil with higher available Si content may decrease the As content of rice. This can be explained by a Si induced reduced expression of arsenite transporters as recently demonstrated by Ma et al. (2008). Si application to the growing medium can also decrease the shoot and root As concentration (Guo et al., 2005; 2007). However, the detailed mechanism of Si effect on As concentration is not known so far.

2.4 Silicon uptake in rice plant

Silicon is the second most prevalent element in the Earth's crust. However, most sources of Si are insoluble and usually combined with other elements, forming oxides or silicates (Richmond and Sussman, 2003). Si concentration in soil solution typically varies from 0.1 to 0.6 mM. All plants contain Si in significant amounts and Si concentration in shoot ranges from 0.1 % to 10 % Si in dry weight (Epstein, 1994). The difference among plant species in Si accumulation is largely due to the capability of roots to take up Si (Takahashi et al., 1990; Mitani and Ma, 2005). Rice can accumulate Si to the level up to 10 % of shoot dry weight that is several times higher than that of essential macronutrients such as nitrogen, phosphorus and potassium (Savant et al., 1997; Ma et al., 2006). Rice roots take up Si in the form of uncharged silicic acid [$\text{Si}(\text{OH})_4$] (Takahashi and Hino, 1978 cited in Ma and Tami, 2003). Takahashi et al. (1990) proposed that there are three uptake modes for Si uptake in plants: active, passive and rejective uptake. Rice is a typical Si accumulating plant that shows active uptake of Si (Takahashi and Hino, 1978 cited in Ma and Tami, 2003). Si uptake in rice is inhibited by metabolic inhibitors such as NaCN, 2,4-dinitrophenol (Okuda and Takahashi, 1962 cited in Guo et al., 2005) or low temperature, particularly at low external Si concentration (Ma et al., 2002). On the other hand, Si uptake is not totally prevented by metabolic inhibitors and low temperature. Thus there is a passive Si uptake beside the active uptake (Raven, 2001 cited in Datnoff et al., 2001). According to Mitani and Ma (2005), two processes are involved in transport of Si from the external solution in the cortical cells and the release of Si from cortical cells into the xylem. This radial transport of Si in rice is mediated through specific transporters for silicic acid in the root (Ma et al., 2002).

The gene of silicic acid transporter *Lsi1* is mapped to chromosome 2 of rice and contains five exons and four introns. The complementary DNA of this gene is 1409 bp long and the deduced protein comprises 298 amino acids. The gene encodes with a membrane protein which has similarities with major intrinsic protein (NIP) subfamily of aquaporin-like proteins (Ma et al., 2006). Staining with an anti-*Lsi1* polyclonal antibody showed localization of *Lsi1* on the plasma membrane of the distal sides of both exodermis and endodermis cells, where Casparian bands exist (Ma and Yamaji, 2006). The authors also found that the expression of *Lsi1* is much lower in the root tip region between 0 and 1 cm than that in the basal regions (>1 cm from the root tip). This observation indicated that *Lsi1* plays an importance role in Si uptake, mainly in the mature

regions of root rather than the root tips. In contrast to influx transporter Lsi1, Lsi2 (low silicon 2) is a Si efflux transporter which is capable to transport silicic acid out of cells (Ma et al., 2007). It could be mapped to the distal region of chromosome 3. The gene consists of two exons and one intron, the open reading frame for Lsi2 is 1416 bp long, and the deduced protein includes 427 amino acid residues. The protein is predicted to have 11 transmembrane domains (Ma et al, 2007; Ma and Yamaji, 2008). It is localized on the proximal side of root exodermis and endodermis (Ma et al, 2007). Like Lsi1, its accumulation of transcripts was low in the root tip (0-1 cm) compared to the mature parts of roots. Ma and Yamaji (2008) mentioned that the transport of silicic acid by Lsi2 is an energy dependent active process that is driven by the proton gradient.

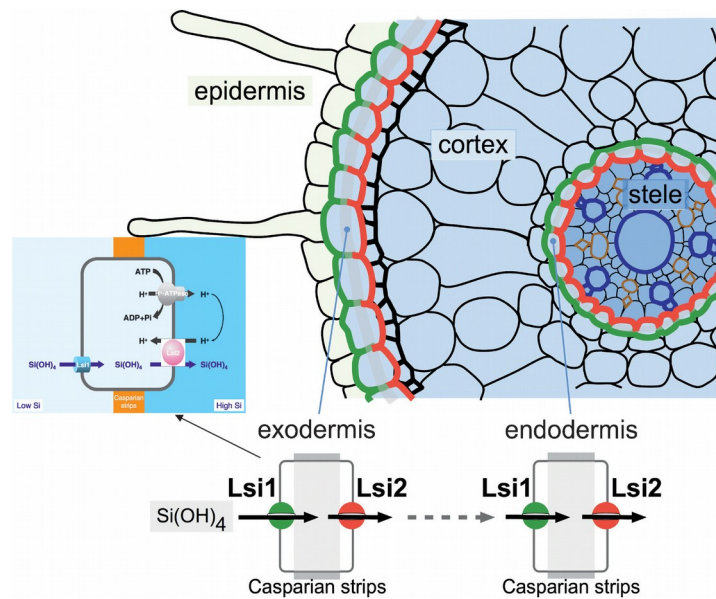


Figure 3: A schematic presentation of Si transport system in rice (adapted from Ma and Yamaji, 2008).

Silicic acid passes passively from external solution in root exodermal cells by Lsi1 and is then released actively by Lsi2 into the apoplast of a spoke like structure across the aerenchyma (Fig. 3). After that silicic acid moves in the apoplast and enters endodermis cells by Lsi1 and is released to the stele by Lsi2. Recently, Yamaji et al. (2008) observed that Lsi6 (low silicon 6) is also a Si transporter which is involved in distribution of Si in the shoot. Lsi6 belongs to the nodulin 26-like intrinsic membrane protein (NIP) subfamily of aquaporin-like proteins. In contrast to Lsi1 and Lsi2, Lsi6 is expressed in the leaf sheath and leaf blade as well as in the root

tip. It is present in the xylem parenchyma cells of the leaf sheath and leaf blades. Furthermore, Lsi6 shows polar localization at the side facing towards the vessels. Interestingly, it is more expressed in the immature region (0-1 cm) of root tip. This expression pattern is quite different from Lsi1 and Lsi2 that are more expressed in the mature region of the root. This different expression pattern indicated that it plays a role different from Lsi1 in Si transport. Although Lsi6 contributes as a transporter to Si uptake, its contribution to the whole root uptake is small (Yamaji et al., 2008).

It is generally agreed that silicic acid taken up by the root is translocated to the shoot through the transpiration stream (Ma et al., 2002). Because of transpiration, silicic acid is gradually concentrated and polymerized to colloidal and finally to silica gel [$\text{SiO}_2 \cdot n\text{H}_2\text{O}$] (Mitani et al., 2005). Si from the xylem is deposited in leaf blades and leaf sheaths. In addition to leaf blades and leaf sheath, silicified cells are also found in the epidermis and vascular tissues of the stem, and hull (Ma and Yamaji, 2006). Si deposition can be seen in the endodermis and exodermis of the root (Gong et al., 2003). In rice root, the endodermis serves as the major organ for storing of Si and silica is predominantly deposited on the inner tangential and radial faces in endodermal cells of the root. The amount of Si deposition in roots is several times lower than in the leaves (Lux et al., 1999). In the leaves, Si is mainly deposited beneath the cuticle and the epidermis where Si deposition acts as a physical barrier (Ma and Yamaji, 2006).

2.5 Effect of silicon on rice plant

Si has still not been listed among the essential elements in plants due to lack of direct evidence that Si is part of the molecule of an essential plant constitute (Epstein, 1994; 1999). Nevertheless, Si has long been counted as an important nutrient for rice growth (Savant et al., 1997). It enhances the mechanical strength of rice plants to get and maintain an erect habit conducive to light interception (Epstein, 1994). Si also reduces the transpirational water loss by deposition of Si in epidermal cells under cuticle (Ma and Takahashi, 2002b). It has beneficial effects under physical stress such as lodging, drought, radiation, high temperature and freezing (Ma et al., 2008) and also stimulates the translocation of photoassimilates to the panicles. As it well known, the effect of Si on growth of rice is markedly pronounced at the reproductive stage of the plants (Ma et al., 1989). Consequently Si nutrition increases the yield due to correlation with higher Si

content in flag leaves (Epstein, 1994). The application of Si may alleviate iron and manganese toxicity in plants since uptake of iron and manganese is decreased (Okuda and Takahashi, 1959 cited in Lewin and Reimann, 1969). Similar results were found by Epstein (1994) and Nwugo and Huerta (2008) in case of aluminum and cadmium toxicities. It can also alleviate other mineral stresses such as salinity stress and phosphorus deficiency (Matoh et al., 1986; Ma and Takahashi, 2002a). It is widely accepted that Si provides resistance of plants to diseases caused by both fungi and bacteria such as rice blast, powdery mildew, sheath blight, ring spot, rust, leaf spot and gray leaf spot. It controls insect pests for instance stem borer, brown plant hopper, rice green plant hopper and non-insect pests like leaf spider and mites. These protective effects might be related to a cuticle Si double layer that makes it difficult to penetrate and chew plant materials by insects (Ma and Yamaji, 2008). One recent finding indicates that silicic acid application had lead to better development of root exodermis (Nordheim, 2007).

2.6 Oxidation power of rice plant

Waterlogged soils are usually anaerobic and have a low redox potential (Ponnamperuma, 1984 cited in Colmer, 2003a). Most of vascular plant cannot withstand in anaerobiosis resulting from extended flooding or waterlogging (Tiner, 1999). However, rice and other wetland plants can grow in flooded conditions (Armstrong and Armstrong, 1991). When a soil is submerged, oxygen supply to the roots through the soil is impossible due to the interruption of the gaseous exchange between soil and air (Trolldenier, 1988). The trapped oxygen is consumed by plant roots and microbial respiration (Reddy et al., 1980).

Rice roots obtain their oxygen through a system of air-filled intercellular spaces (aerenchyma) by which oxygen moves from foliar parts down to the root (Trolldenier, 1988). Aerenchyma is the term given to plant tissues containing internal, longitudinal and continuous gas spaces from shoots to roots formed by separation or breakdown of specific cells (Ito et al., 1999). It is formed as an integral part of ordinary root development (Kawai et al., 1998). It also promotes the long distance oxygen transport to the roots and facilitates the counter flow of volatile compounds which include ethanol, carbon dioxide and methane in the anaerobic soil and plant tissues. The formation of aerenchyma causes high porosity in primary and adventitious roots (Justin and Armstrong, 1987). Exodermis of root forms a barrier to radial oxygen loss (ROL) in rice (Clark

and Harris, 1981). It can be hypothesized that a physical barrier to ROL may have drawbacks for some root functions in wetland plants and this feature may impede water and nutrient uptake (Armstrong, 1979). Some oxygen diffuses into the soil and oxygen diffusion from roots also contributes to rhizosphere oxidation (Ando et al., 1983). Oxygen diffusion from roots acts as an important part of oxidizing power of rice roots. The oxidizing power of rice roots protects against excessive ferrous iron or hydrogen sulfide that may reach phytotoxic concentrations in waterlogged soils (van Breemen and Moormann, 1978; Tadano and Yoshida, 1978). It may influence the productivity of rice (Armstrong, 1967; Joshi et al., 1979). The oxidizing power of rice roots may be comprised of two different processes:

- Release of molecular oxygen into the rhizosphere and
- Enzymatic oxidation on root surface (Ando et al., 1983).

Armstrong (1967) reported that along the root axis, the maximum rate of oxygen diffusion is found within about 1 cm distance behind the root tip. Moreover, enzymatic oxidation of roots also contributes the basic mechanism for higher oxidation activity of roots (Armstrong, 1967). Mitsui (1965) cited in Yoshida, 1981 suggested that rice roots might have a glycolic acid pathway where glycolic acid is oxidized to carbon dioxide and water. ATP liberated during this oxidation is transferred to hydrogen peroxide and then this peroxide is decomposed into molecular oxygen and water.

2.7 Suberization

Generally, suberin forms internal barriers in plants to separate the plant body from the surrounding environment or appeared to form internal barriers in plants (Schreiber et al., 2005). Deposition of suberin in anticlinal, including Casparian bands, and tangential cell walls of root exodermis act as a hydrophobic barrier that contributes to the plant's overall resistance under unfavorable growth conditions, such as low oxygen levels or high salinity. Moreover, a suberized exodermis seems to prevent loss of water and stored solutes into the rhizosphere during drought periods (Hose et al., 2001).

Suberin is a complex biopolymer closely attached to the inner primary cell walls (Schreiber et al., 1999) and represents up to 50 % of the chemical composition of suberized cells (Pereira, 1988). The biopolymer suberin is composed of aliphatic domain and aromatic domain in plant cells.

These two domains are chemically linked and distinct from each other (Franke and Schreiber, 2007; Bernards et al., 2004). The aliphatic domains are attached to the phenolic domain via ester bonds (Bernards and Lewis, 1992; Kolattukudy, 1980; 1984), whereas the aromatic domains are attached to the cell wall carbohydrates (Yan and Stark, 2000). Recently, glycerol has been identified as one of the important structural elements in the suberin monomers, which is supposed to cross-link the aliphatic and aromatic domains during suberin polymerization. (Moire et al., 1999; Graça and Pereira, 2000). The aliphatic domains are synthesized via the fatty acid biosynthetic pathway, catalyzed by fatty acid elongases in the root cells (Schreiber et al., 2000). Hydroxylation is typically catalyzed by cytochrome P450-dependent enzymes, converting ω -hydroxyacids to either 1, ω -dicarboxylic acids or alcohols (Le Bouquin et al., 2001). The assembly of the aromatic monomers proceeds via the general phenylpropanoid pathway (Kolattukudy, 2001). The polymerization step of aromatic monomers has been involved a peroxidase reaction (Bernards et al., 2004). The detailed biosynthesis and deposition of suberin still remain enigmatic.

3. OBJECTIVES AND HYPOTHESES

3.1 Objectives

The objectives of this study are:

- To determine the influence of silicon on formation of suberin of root exodermis and endodermis
- To understand the interaction between silicon supply and oxidation power of root
- To understand the influence of suberization and lignification on arsenic - flux into roots

3.2 Hypotheses

- The application of silicon will enhance the suberization of root exodermis and endodermis.
- The application of silicon will influence on the oxidation power of root.
- The application of silicon will affect the uptake of arsenite.

4. MATERIALS AND METHODS

4.1 Plant cultivation

Rice seeds of the variety *Selenio* were germinated with tap water for 5 days in the growth chamber (14 h light, 10 h dark at 220 $\mu\text{molm}^{-2}\text{s}^{-1}$ PAR; 25°C during light; 20°C during dark period; 70 % relative atmospheric humidity). The seedlings were then rolled in filter paper and placed into a plastic beaker that was filled with tap water for about one third. After 7 days, the uniform seedlings were transplanted in nutrient solution (Tab. 1) or quartz sand.

Table 1: Composition of nutrient solution.

Nutrient	Salt	Nutrient concentration (ppm)
N	NH ₄ NO ₃ Ca(NO ₃) ₂ x 4 H ₂ O NH ₄ H ₂ PO ₄	40 (60% NO ₃ ; 40% NH ₄)
P	NH ₄ H ₂ PO ₄	46.46
K	K ₂ SO ₄	139.21
Ca	Ca(NO ₃) ₂ x 4 H ₂ O CaCl ₂ x 2 H ₂ O	20
Mg	MgSO ₄	19.4
B	H ₃ BO ₃	0.08
Cu	CuSO ₄	0.51
Mo	Na ₂ MoO ₄	0.004
Zn	ZnSO ₄	0.052
Mn	MnSO ₄	0.549
Fe	Sequestrene (6 %)	2

For control, no Si was added. In all other treatments 50 mg/L Si was applied to nutrient solution in form of K₂SiO₃. Additional K₂SO₄ was applied to the treatments of without Si supply for comparable potassium concentration. When rice was grown in nutrient solution alone, nutrient solution was renewed every 4 days. The pH was measured every day and adjusted to 5.5 with 10 % sulfuric acid or 0.1 M potassium hydroxide. Fig. 4 shows schematic drawing of rice grown in quartz sand. In this case, 250 mg/L sugar was added to nutrient solution. The size of pot was 10 cm in diameter and 30 cm in length. The redox potential of the quartz sand was monitored

everyday by means of a Pt-electrode that was permanently immersed in the quartz sand. For the first 2 days grown in quartz sand, the redox potential of both Si treatments was about 200 mV and after that the redox potential was reduced by about - 200 mV in both Si treatments. Quartz sand was covered by a 3-4 cm layer of nutrient solution. The rice seedlings were harvested 30 days later.

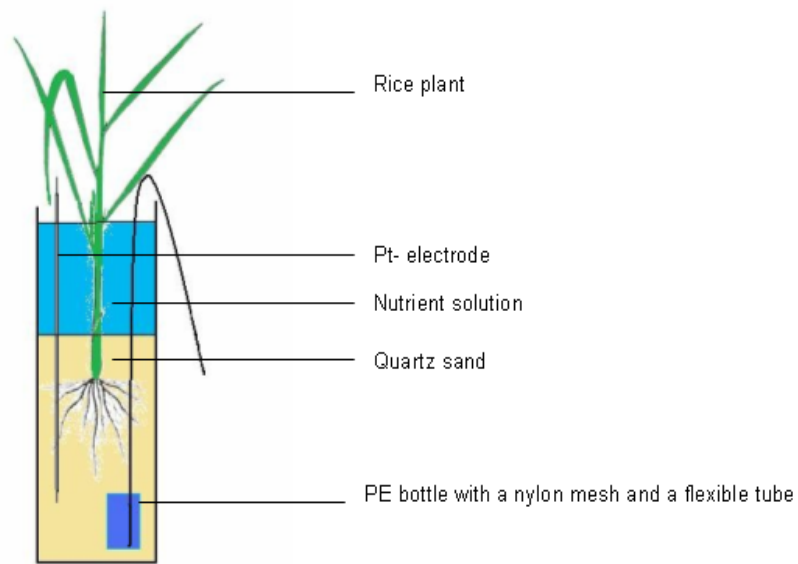


Figure 4: A schematic drawing of the quartz sand cultivation system.

4.2 Analytical procedures

4.2.1 Root morphology and anatomy

4.2.1.1 Root growth rate

The determination of root length was carried out in both with and without silicic acid supply (Fig. 5). After growing in the nutrient solution, 1 cm from the tip of single nodal root per plant was carefully marked with a water proof marker. Root lengths of nodal roots were measured in 24 h, 48 h and 72 h, respectively. The results showed that average root length of both + and – Si treatments was more or less increased at the same rate (1 cm/24 h).

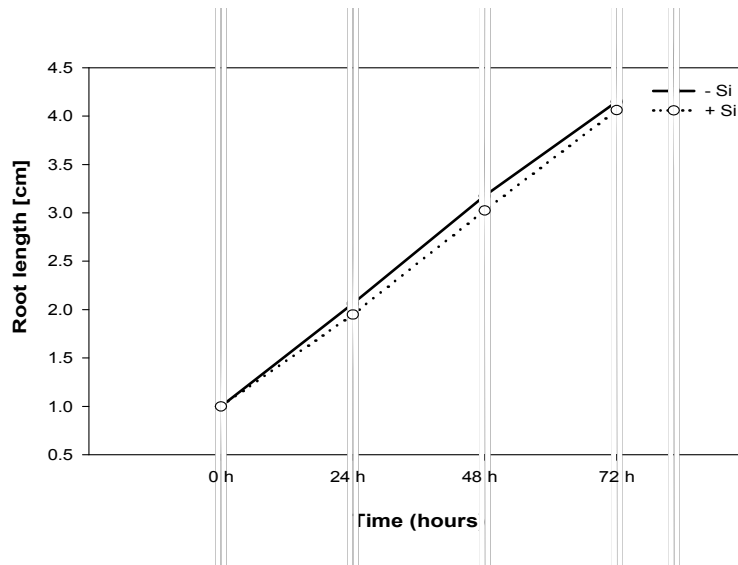


Figure 5: Average root lengths of + and – Si treatments measured in 24 h, 48 h and 72 h, respectively.

4.2.1.2 Suberin staining

To examine the formation of suberization in root exodermis and endodermis, samples of fresh nodal roots without lateral roots were taken 1-2 cm, 4-5 cm, 8-9 cm and 12-13 cm distance from the root tip using a hand-held sharp blade. The root cross sections were stained with aniline blue fluorescent according to Brundrett et al. (1988). Freehand sections were transferred into holder chambers and stained in 0.1 % (w/v) berberine hemi-sulphate in distilled water for 1 h. Thereafter, sections were rinsed by passing holders through several changes of distilled water. Root cross sections were then stained with 0.5 % (w/v) aniline blue in distilled water for 30 min. After that holders were transferred into 0.1 % (w/v) FeCl_3 in 50 % (v/v) glycerine (prepared by adding glycerine to filtered aqueous FeCl_3). After several minutes in this solution, root sections were transferred to slides and mounted in the same solution. Root cross sections were examined using Zeiss Axiophoto microscope with UV illumination (UV-G 365). They were photographed by means of a camera (AxioCam) and picture recording software (Axiovision Ac Version 4). For determination of suberin stages in both root exodermis and endodermis, 4 plants per replicate were used. In every plant, 1 root per plant was used and cut into four distances. For every root distance, 4 cuts per each section were taken. Each Si treatment was replicated for four times. The suberization stages were counting 10 cells per each root cross section under a microscope.

4.2.2 Determination of oxidizing power of rice roots

4.2.2.1 Visualization of oxidation power

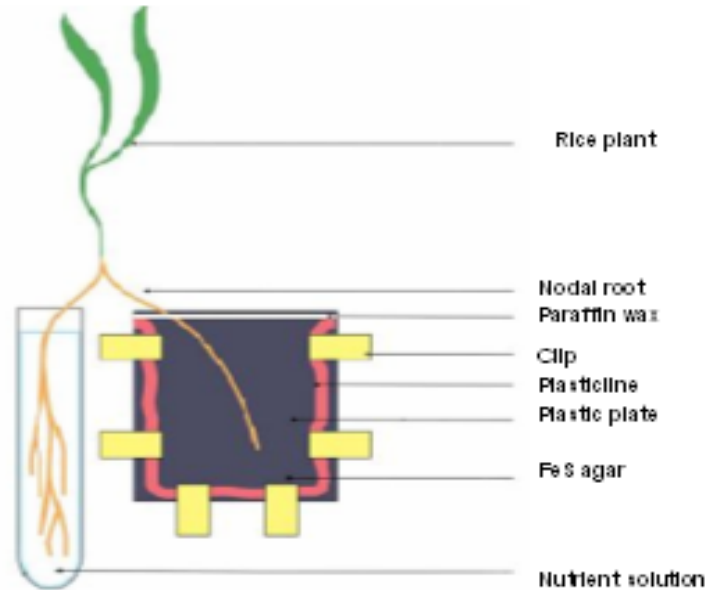


Figure 6: A schematic drawing of the experimental set up for visualization of oxidation power of rice root.

To visualize the oxidation power of rice roots, a single nodal root was embedded in semisolid (Trolldenier, 1988). The FeS agar was prepared as follows:

- The medium was prepared by adding 0.8 % agar to nutrient solution without iron and then it was solubilized by heating.
- Thereafter the solution was cooled to 40°C in a water bath for 15 min and further 50 mL of nutrient solution supplemented with 0.14 g of ferrous sulfide and 0.032 g of sodium sulfide was added. Thereafter, it developed a black ferrous sulfide precipitation. The medium was buffer with 0.05 g of CaCO₃ per 100 mL and adjusted to pH 6.0.

A single nodal root per plant was placed on the plastic plate (5 cm x 14 cm) and covered with another plastic plate which was sealed by the plastic line and clipped together (Fig. 6). The rest of the roots were in nutrient solution. The media was filled into the box and the top of the plates was sealed with paraffin wax (melting temperature of 40°C). After that the plates were wrapped with black foil for protecting the light. The oxidation status of root was seen one day after embedding

the root in the agar medium. In this study, both Si treatments were replicated for five times. Photos were taken after each 24 h and 48 h later after filled with medium. Near the root became reddish brown while the rest of area remained black in color after embedding the agar for 24 h. Photo of an oxidized status of the root was taken after each 24 h and 48 h later after filled with medium.

4.2.2.2 Quantification of oxidation power

Nodal root samples of 0.2 g was taken from each 1-5 cm and 8-12 cm distances from the root tip and immersed in 10 mL of 20 ppm alpha-naphthylamine (α -NA) test solution at 25°C. The plastic test tubes (1 cm x 6 cm) were closed by a stopper and wrapped with black foil. Then they were incubated 0.5 h, 1 h, 1.5 h and 2 h, respectively. After incubation, 2 mL of the α -NA solution was pipetted in 10 mL of 0.1 % sulfanilic acid (in 3 % acetic acid) and 1 mL of 1000 ppm NaNO₂. The absorbance of the colored solution was determined by a photometer at 530 nm. The oxidation power of root was calculated from the decrease of α -NA concentration during incubation.

4.2.3 Molecular Biology Methods

4.2.3.1 Isolation of RNA

Nodal roots with and without laterals of + and – Si treatments were harvested at 0-2 cm and 4-6 cm from the root tip and frozen in liquid nitrogen. Total RNA was extracted by using the TRIsure reagent from the Bioline using the following protocol. Homogenize root samples were added to 1 mL of TRIZOL® Reagent per 100 mg of root samples and it was incubated for 5 min at room temperature. After that 0.2 mL of chloroform per 1 mL of was added and incubated for 3 min at room temperature. It was centrifuged at a speed of 12000 rpm for 15 min at 8°C. Then supernatant was transferred into a new Eppendorf tube and 0.5 mL of isopropyl alcohol was added. The homogenous sample was incubated for 10 min at 4°C and centrifuged at 12000 rpm for 15 min at 4°C. The supernatant was removed and the pellet was washed with 75 % ethanol. It was vortexed and centrifuged at 7500 rpm for 5 min at 4°C. The RNA pellet was left to dry after removing the supernatant. Then the RNA pellet was dissolved in 20 μ L of RNase-free water by

passing the solution a few times through a pipette tip. RNA concentration was measured using a spectrophotometer.

4.2.3.2 RT-PCR (Real Time-Polymerase Chain Reaction)

For Real Time-PCR experiment, total RNA from non-pooled samples was used. 1 µg PAR of total RNA was taken for first-strand cDNA synthesis using the Revert Aid™ H Minus Kit (Fermentas) and random hexamer primer (Fermentas) following the manufacturer's instructions. In Real Time-PCR experiments, 50 ng cDNA were used as template in a 25 µl reaction-mix with SYBR-Green (Invitrogen) for quantitative analysis. As endogenous control, *eukaryotic elongation factor 1-alpha (eEF 1-α)* was used due to its stable expression in rice (Jain, M. et al. 2006; Jain, M. 2009). Real Time-PCR runs were performed in a Stepone™ Real Time cyler (Applied Biosystems), with primer sets as follows: 5'-CCAGCAACAACCTCGAGAACA-3' and 5'-TCATGAACACCAGCAGGAAC-3' for *Lsi1*; 5'-AACATGGTCATGCTGCTCTG-3' and 5'-GGGTGCTTGGTCGTTATGTT-3' for *Lsi2*; 5'-TCAAGTTTGCTGAGCTGGTG-3' and 5'-AAAACGACCAAGAGGAGGGT-3' for *eEF 1-α*.

4.2.4 Arsenite uptake along the root

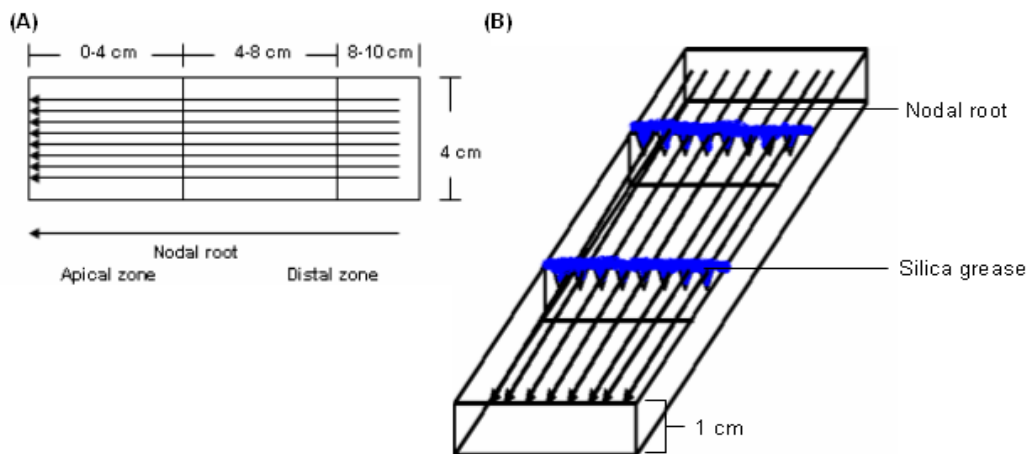


Figure 7: Schematic drawings of a micro rhizotrone.

The uptake of arsenite along nodal roots with and without lateral roots was determined by compartmented rhizotrons. These micro-rhizotrons were made of 2 mm thick. Perspex plates and

consisted of 3 compartments (4 cm x 4 cm x 1 cm; 4 cm x 4 cm x 1 cm and 4 cm x 2 cm x 1 cm; respectively) as shown in Fig. 7. These plates were fixed with liquid glue for plastics (Revell GmbH & Co. KG; Bünde; Germany). Nodal roots (10 cm long) of rice grown in the nutrient solution with and without Si were excised and washed in the nutrient solution. In each rhizotron, 8 roots were placed in the compartment contained a solution with 1 mM CaCl₂ and 2 μM H₃BO₃ at pH 5.5. The compartments were sealed with silicon grease to avoid arsenite contamination between the chambers (Fig. 7 B). Arsenite was applied in the form of (NaAsO₂) in the treated compartment to give a concentration of 0.3 mg/L which was shown to be not yet toxic (Hofmann, personal communication). After treated with arsenite for 6 h, 3.8 mm (0-3.8 cm, 4.1-7.9 cm and 8.1-10 cm) were cut out of each zone to avoid contamination by silicon grease. Fresh weight of roots was determined according to Schenk and Barber (1979). Root length and the surface area of roots were determined by means of photo-analysis software (WinRHIZO, Canada, Regent Instruments Inc.; www.regentinstruments.com) based on the line intersect method of Tennant (1975). Treatments were replicated for four times. The arsenite content of nutrient solution in each compartment and of root zones were determined.

The arsenite uptake rate of root (I ; μgcm⁻²s⁻¹) was calculated as follow:

$$I = \frac{C_1 + C_2}{SA} \times \frac{1}{t}$$

where C_1 = As content of the whole root length (μg)

C_2 = As content of nutrient solution in the compartments that were not supplied with arsenite (μg)

SA = Surface area of root that was exposed to arsenite (cm²)

t = Time of arsenite exposure (s)

4.2.5 Chemical Analysis

4.2.5.1 Determination of silicon content in rice roots and shoots

After harvesting the plants were divided into roots and shoots and dried at 60°C for 3 days. After milling, 10 mg of dry matter were digested with 1.5 ml of a mixture of 1 M HCl and 2.3 M HF

according to Novozamsky et al. (1984). The samples were put on a shaker overnight and diluted to 1:10 (100 μ L of sample, 900 μ L of millipore-purified water). After that, 250 μ L of boric acid (3.2 %) was added to 50 μ L of the sample and shake overnight. Then, 50 μ L of samples were added with 250 μ L of tartaric acid and 250 μ L of ascorbic acid. Si concentration of roots and shoots was measured by the colorimetric molybdenum blue method at 811 nm with a spectrophotometer (van der Vorm, 1987).

4.2.5.2 Arsenic determination in plant matter and nutrient solution

Root zones were dried at 60°C to reduce moisture content for 1 day. After that they were digested over night in 500 μ L double distilled ultra pure nitric acid under continuous shaking at room temperature. Total arsenic contents in roots were determined by GF-AAS (Unicam 939 QZ; Analytical Technologies Inc; Cambridge; UK) at a wavelength of 193.7 nm and ashing phase of 20 s at 400°C and an atomization phase of 3 s at 2100°C. Total As in the nutrient solution of each compartment was also measured by GF-AAS.

4.3 Statistical Analysis

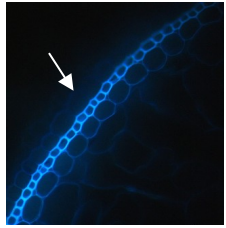
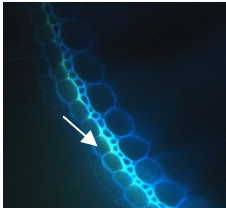
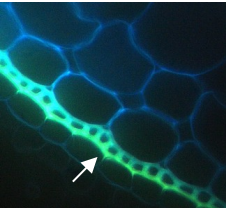
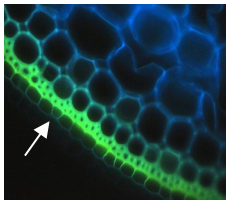
Treatments were replicated four times in a Completely Randomized Design (CRD). Results were analyzed by using SAS Version 9.2 (SAS Institute INC, Cary, U.S.). One or two factorial ANOVA was employed in order to analyze the effect of each treatment and interaction among the treatments. Mean comparisons were carried out according to the Tukey test at 5 % significance level ($p < 0.05$).

5. RESULTS

5.1 Effect of silicic acid supply on suberin layer formation in rice root

For each cross section suberin formation was evaluated each of 10 cell walls and classified in four stages as detailed in Tab. 2.

Table 2: Stages of suberin layer formation in root cross sections of rice.

Stage	Colour of suberin layer	Casparian Bands	Cross sections stained with berberine aniline blue
I	Blue, only autofluorescence	Suberin layer formation (0 % of total length of the anticlinal wall of cell)	
II	Dim green-yellow	Suberin layer formation (25 % total length of the anticlinal wall of cell)	
III	Bright yellow-green	Suberin layer formation (50 % of total length of the anticlinal wall of cell)	
IV	Intense yellow	Suberin layer formation (100 % of total length of the anticlinal wall of cell)	

5.1.1 Influence of silicic acid supply on suberin layer formation in root of rice grown in nutrient solution

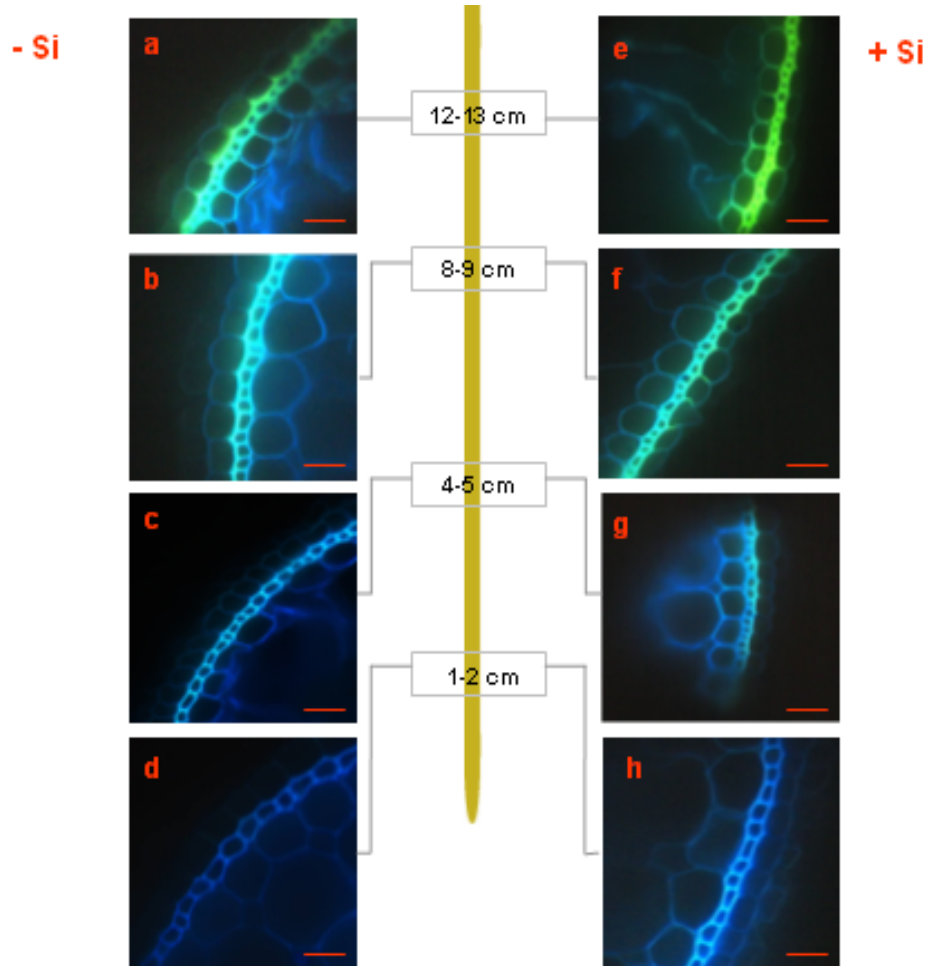
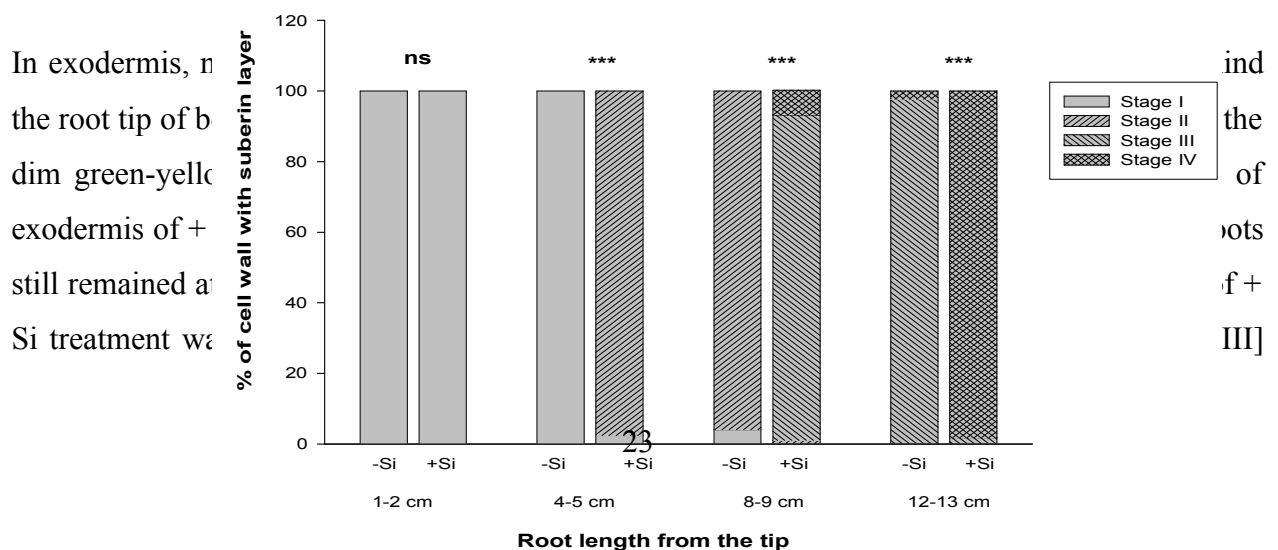


Figure 8: Overview of development of suberization of root exodermis of rice grown in nutrient solution with and without silicic acid supply. Root cross sections were stained with Berberine.



(Fig. 8 f), whereas in the – Si treatment there was only Stage II in root exodermis (Fig. 8 b). The intense yellow of 100 % fully developed suberin lamellae [Stage IV] occurred in the root exodermis of + Si treatment at 12- 13 cm distance from the root tip (Fig. 8 e). However, at the same distance formation of suberin layer in root exodermis of – Si treatment was only reached to Stage III (Fig. 8 a).

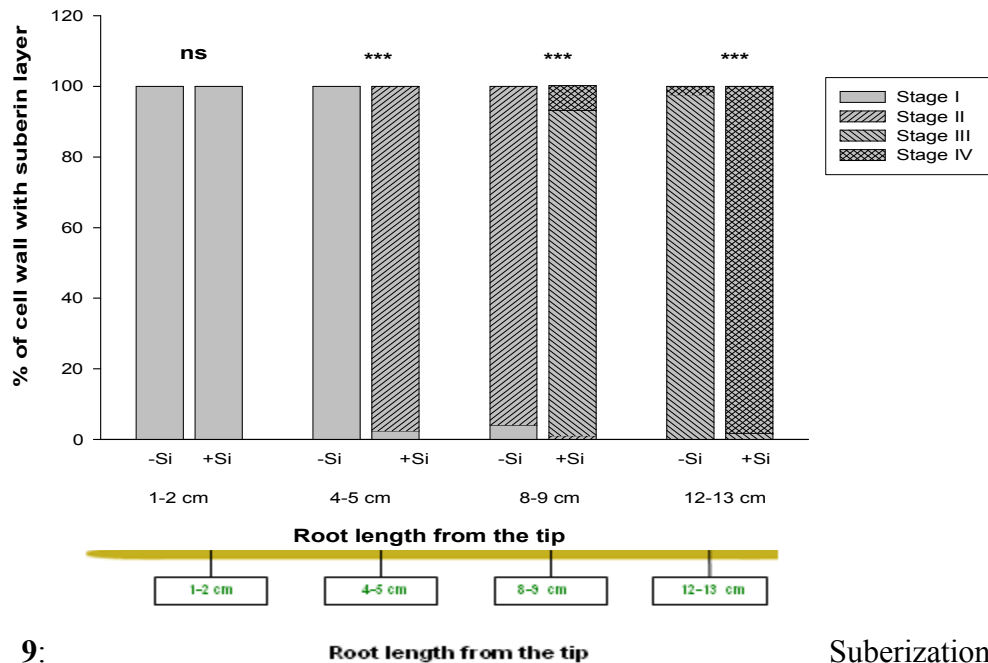


Figure 9: Suberization of exodermis along the root of rice grown in nutrient solution as affected by silicic acid application.

The percentage of suberization stages in exodermis along the root was given in Fig. 9. In 1-2 cm distance from the root tip all cell walls of both treatments was not suberized. In contrast, in the following rest sections suberization of + Si treatment was always one stage further developed. Since suberin is not only deposited in cell walls of root exodermis but also in root endodermis, the effect of Si supply on suberization stages was also examined in root endodermis (Fig 10 and 11). The formation of suberin lamellae in root endodermis of + Si treatment reached to Stage IV already at 1-2 cm distance from the root tip (Fig. 10 h), whereas that of – Si treatment remained at Stage I (Fig. 10 d). In all other root sections, cell walls of endodermis were fully suberized independent of the Si supply.

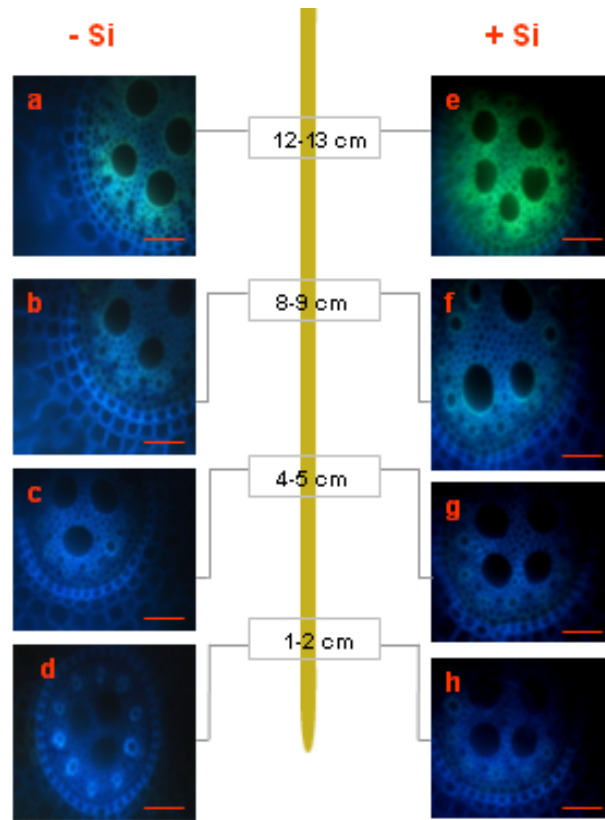


Figure 10: Overview of development of suberization of root endodermis of rice grown in nutrient solution with and without silicic acid supply. Root cross sections were stained with Berberine-aniline Blue fluorescent [bar = 20 μ m].

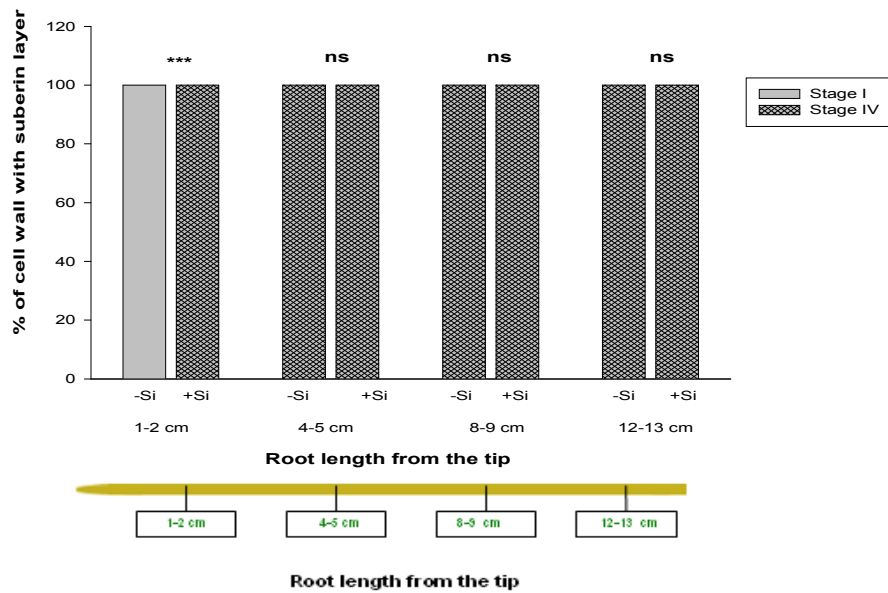


Figure 11: Influence of silicic acid supply on suberization of endodermis along the root of rice grown in nutrient solution.

5.1.2 Influence of silicic acid supply on suberin layer formation in root of rice grown in quartz sand

In this study, the concentration of Si in nutrient solution was measured by the colorimetric molybdenum blue method at 811 nm with a spectrophotometer (Van der Vorm, 1987). For + Si treatment, Si concentration was more or less 50 mg/L Si at all times of measurement (Fig. 12). Si concentration in nutrient solution without Si supply was about 1 mg/L Si until two weeks after growing in quartz sand. After that, Si concentration in nutrient solution of – Si treatment was increased to about 8.5 mg/L Si.

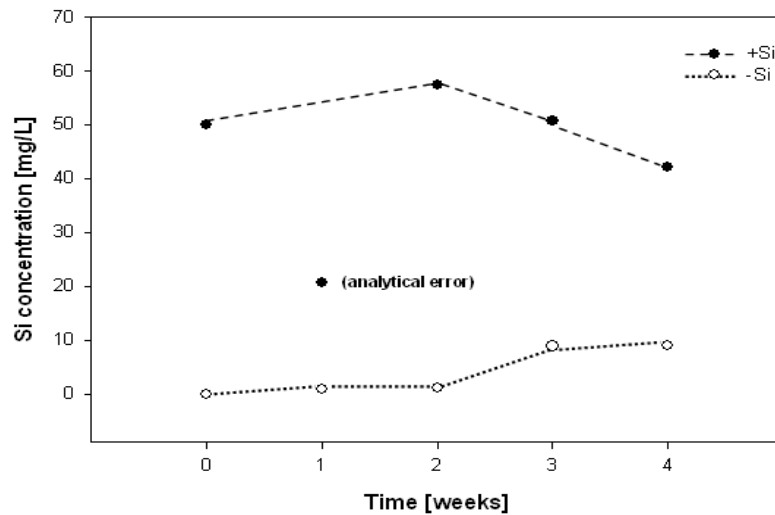


Figure 12: Silicon concentration in nutrient solution of + and - Si treatments.

Fig. 13 and 14 show the development of suberization in root exodermis of both + and – Si treatments of rice grown in quartz sand. Formation of suberin layers in root exodermis of both + and – Si treatments were reached Stage IV at 1-2 cm distance from the root tip (Fig. 13 d and h) in both regardless of the Si concentration in the applied nutrient solution. Similarly, suberization of endodermis was not different for Si treatments (Fig. 15 and 16).

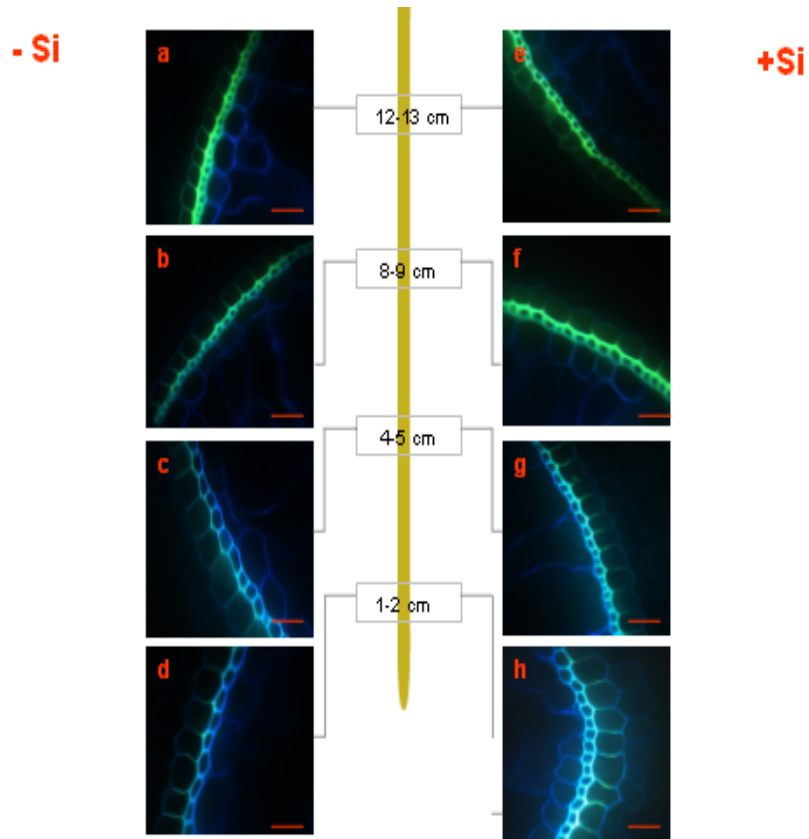


Figure 13: Overview of development of suberization of root exodermis of rice grown in quartz sand with and without silicic acid supply. Root cross sections were stained with Berberine-aniline Blue fluorescent [bar = 20 μm].

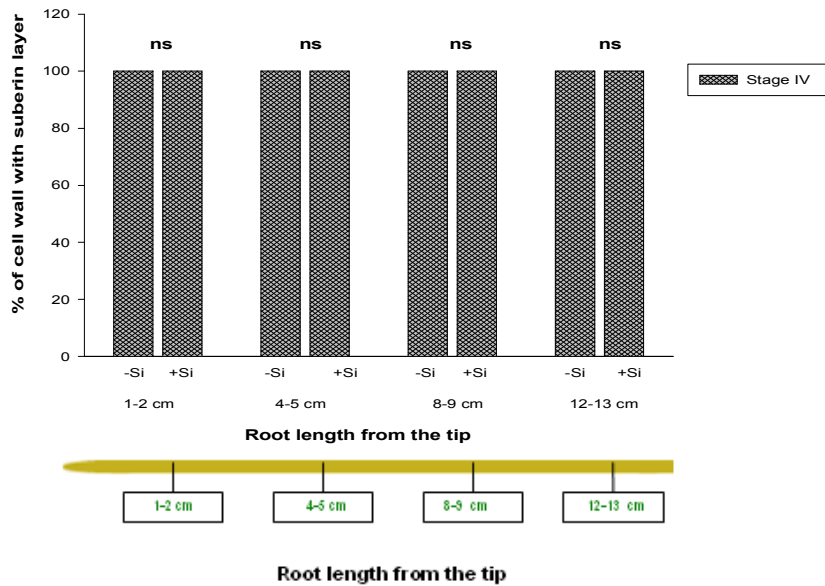


Figure 14: Influence of silicic acid supply on suberization of exodermis along the root of rice grown in quartz sand.

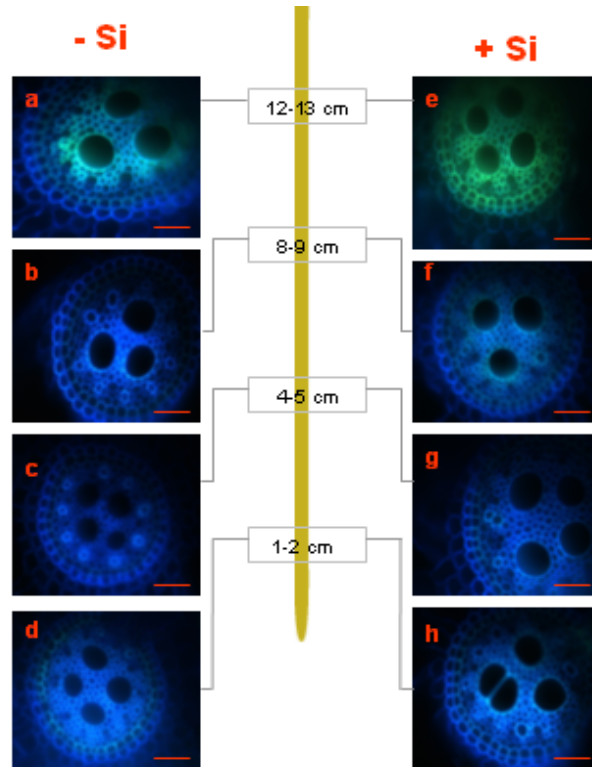


Figure 15: Overview of development of suberization of root exodermis of rice grown in quartz sand with and without silicic acid supply. Root cross sections were stained with Berberine-aniline Blue fluorescent [bar = 20 μm].

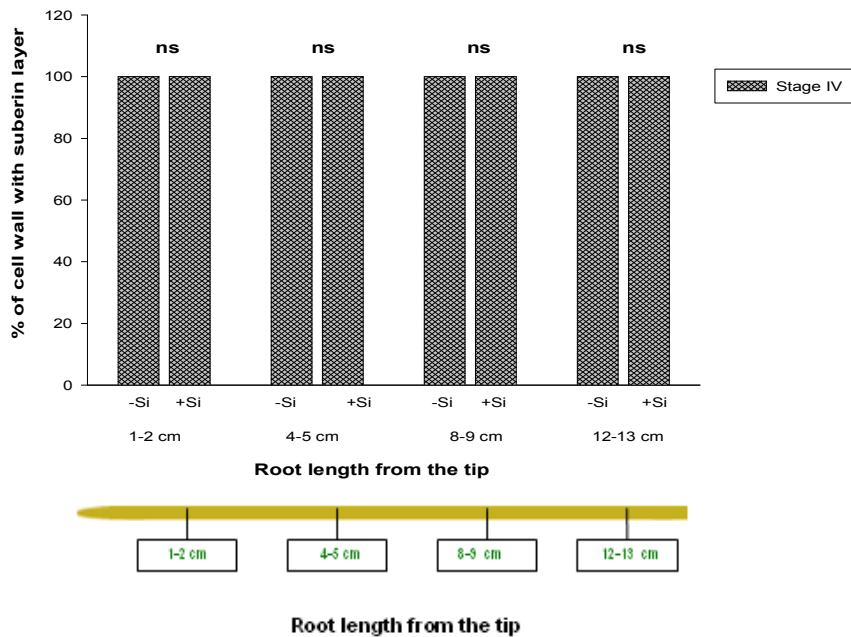


Figure 16: Influence of silicic acid supply on suberization of endodermis along the root of rice grown in quartz sand.

5.1.3 Influence of changing silicic acid supply on suberin layer formation in root of rice grown in nutrient solution

In order to determine the effect of changing Si supply on suberin layer formation in rice root, four Si treatments were carried out as follow:

- (1) -/- Si - no Si was added to nutrient solution
- (2) -/+ Si - Si was not applied for 28 days and after that Si was supplied to nutrient solution
- (3) +/+ Si - Si was continuously supplied to nutrient solution and
- (4) +/-Si - Si was applied for 28 days and thereafter no Si was added to nutrient solution.

In this case, nodal roots were cut into three zones (0-2 cm, 4-6 cm and 8-10 cm distance from the root tip). By means of microscopic examination, the percentage of suberization stages in root exodermis and endodermis of rice was carried out.

The results confirm that suberization of exodermis was enhanced by Si application in the section 4-6 cm and 8-10 cm distance from the root tip (Fig. 17 B and C), whereas no difference of suberization was observed in the section 0-2 cm (Fig. 17 A). Thus in this part the changing of Si supply did not influence on suberization. In the section 4-6 cm and 8-10 cm, removed of Si (+/-) delayed suberization at 48 h and 72 h changing with Si supply, whereas within the same periods addition of Si (-/+) enhanced suberization. However, 24 h after changing Si supply no effect was visible.

Fig. 18 confirms that suberization of the endodermis was enhanced by Si supply in the 0-2 cm section, whereas in the older root parts all treatments were fully suberized. Thus change of Si supply affected suberization only in the section 0-2 cm. The removed of Si (+/-) delayed suberization after 48 h and 72 h, whereas silicic acid supply enhanced suberization within this period. After 24 h the change of Si supply was not yet effective. In summary, the change of Si supply affected suberization of exodermis and endodermis in the same pattern.

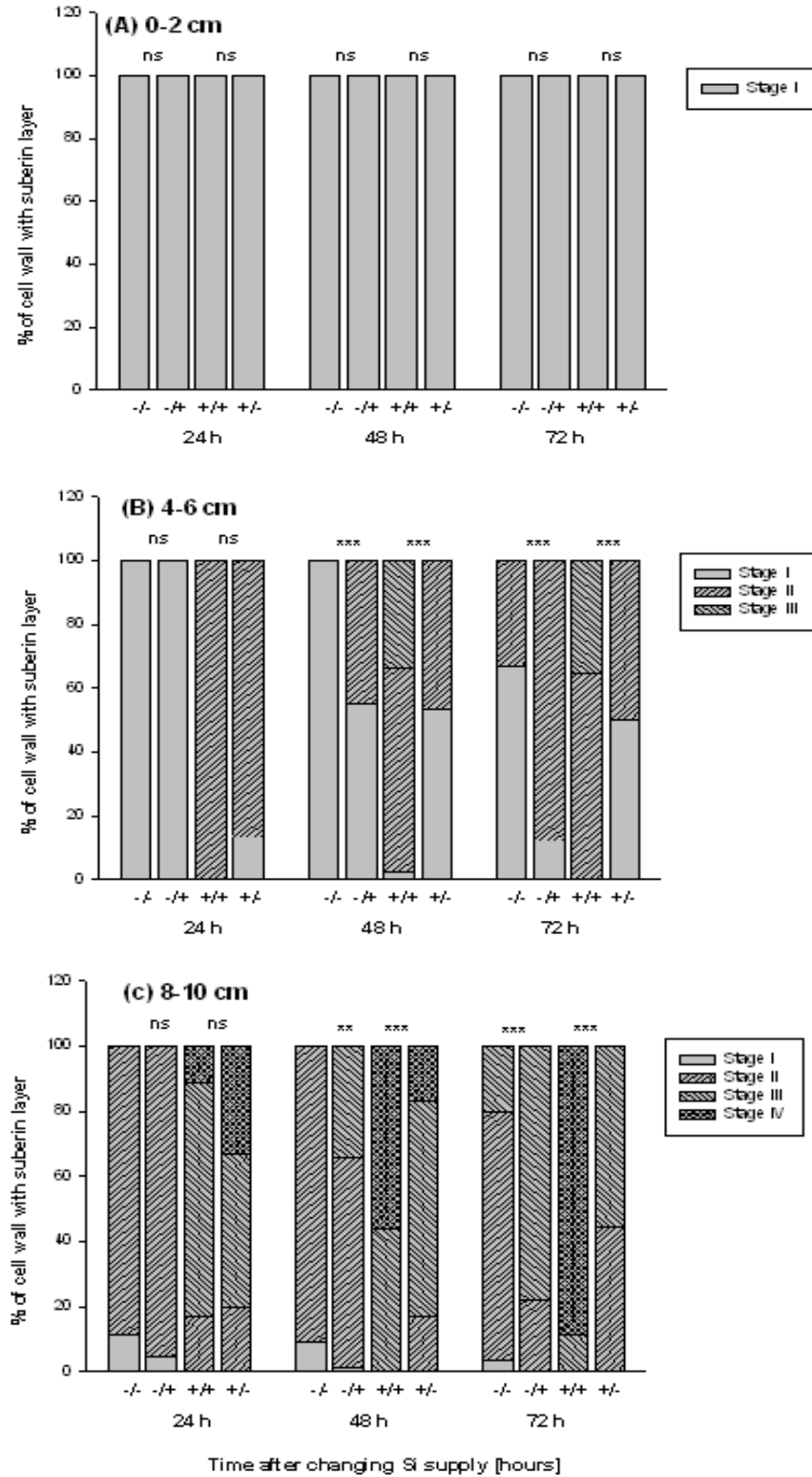


Figure 17: Influence of changing silicic acid supply on suberization of root exodermis in sections 0-2 cm (A), 4-6 cm (B) and 8-10 cm (C) distance from the root tip of rice grown in nutrient solution with four Si treatments (-/-Si, -/+ Si, +/+ Si and +/- Si).

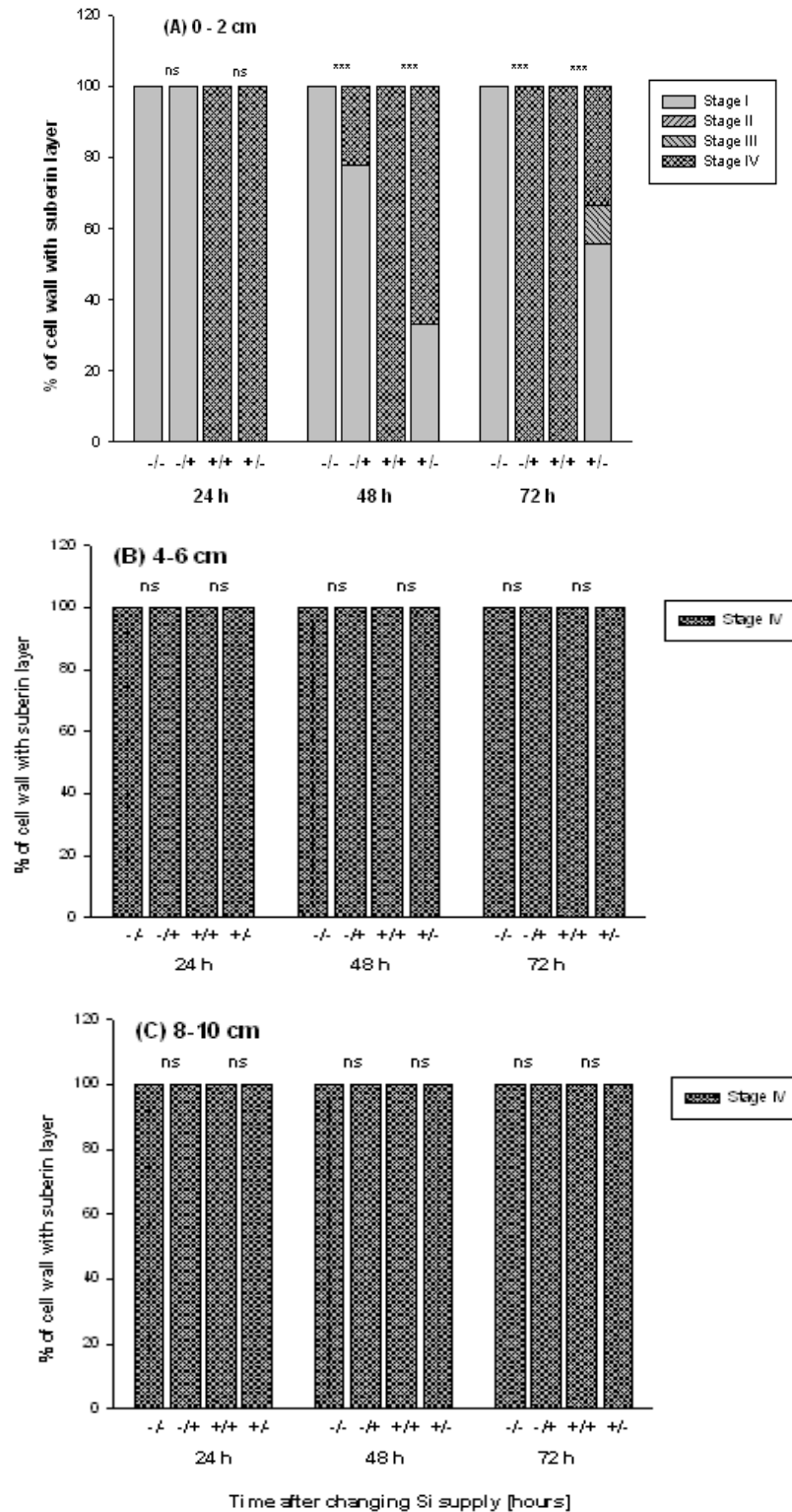


Figure 18: Influence of changing silicic acid supply on suberization of root endodermis in sections 0-2 cm (A), 4-6 cm (B) and 8-10 cm (C) distance from the root tip of rice grown in nutrient solution with four Si treatments (–/– Si, –/+ Si, +/+ Si and +/- Si).

5.1.4 Effect of silicic acid supply on silicon concentration in rice roots and shoots

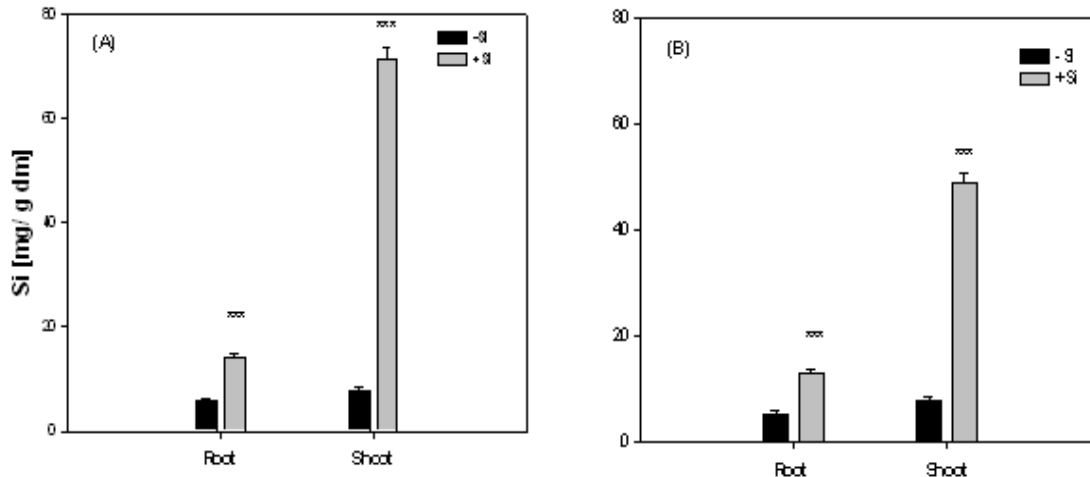


Figure 19: Effect of silicon supply on silicon concentration in roots and shoots of rice plants (A) grown in nutrient solution and (B) grown in quartz sand. Bars = \pm standard errors (SE) of four replicates. *** means significance at $p < 0.001$.

In both rice cultivation systems, Si application enhanced the Si concentration in shoot and root of rice plant (Fig. 19 A and B). This effect was more pronounced for the shoot than for the root. Si concentration in shoot dry matter was several folds higher in + Si treatment than compared to the root of – Si treatment.

5.2 Effect of silicic acid Supply on oxidation power of rice root

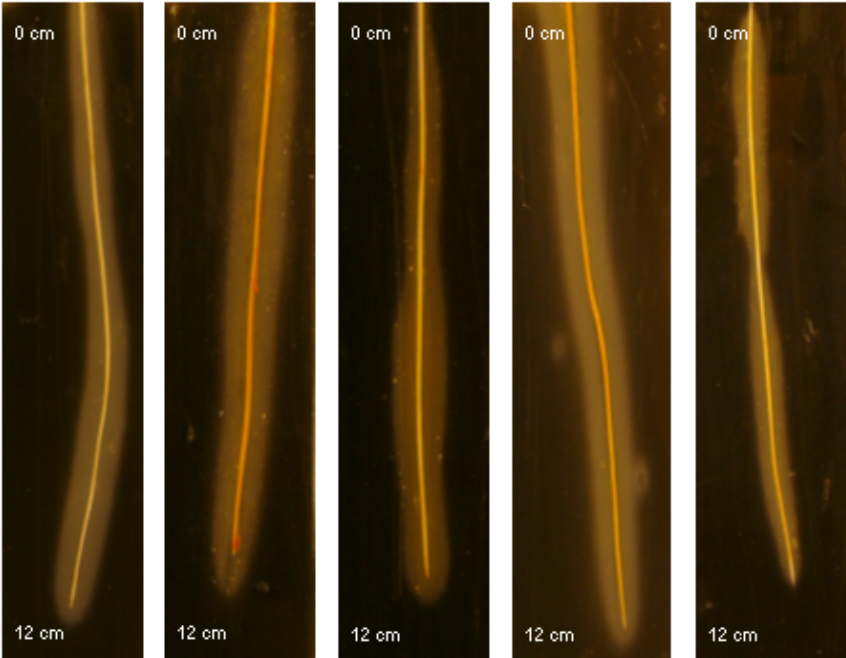
To determine the influence of Si supply on root oxidation, visualization and quantification of oxidation power of root were carried out.

5.2.1 Visualization of oxidation power of rice root

In this study, the extension of the oxidation zone around a single nodal root was clearly observed and each Si treatment was replicated for five times. For – Si treatment, the oxidation power of root was clearly visualized around the entire root length (0-12 cm) after embedding in the agar for 24 h (Fig. 20 A). Similar pattern was found in all other replicates for – Si treatment.

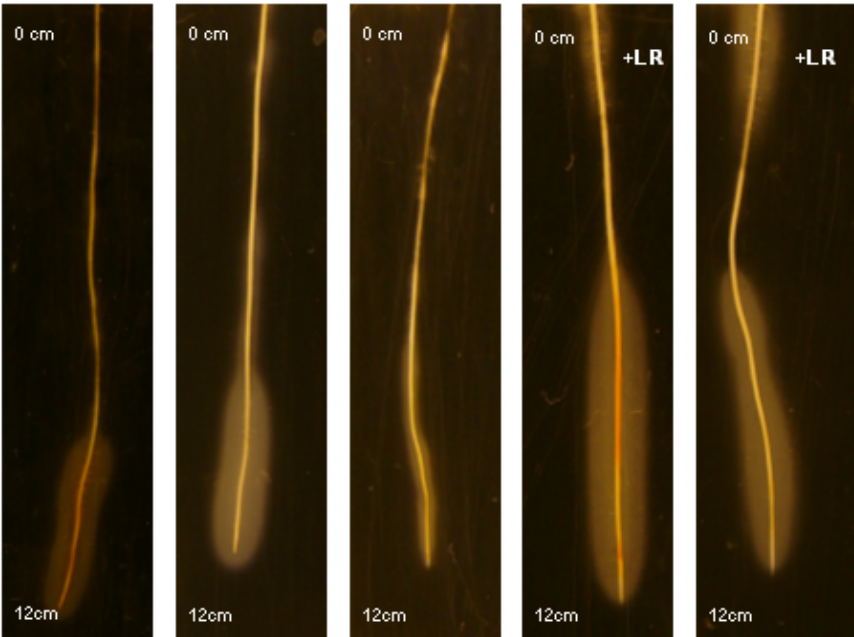
In Fig. 20 B, root oxidation power of + Si treatment was observed only around the root tip zone (~ 0-5 cm). In addition, an oxidation developed where already formed the lateral roots (+LR) appeared (Fig. 20 B (4) and (5)). The pattern of oxidation zone around root after 48 h embedding in FeS- agar was similar to that after 24 h (Fig. 21).

(A) – Si



(1) (2) (3) (4) (5)

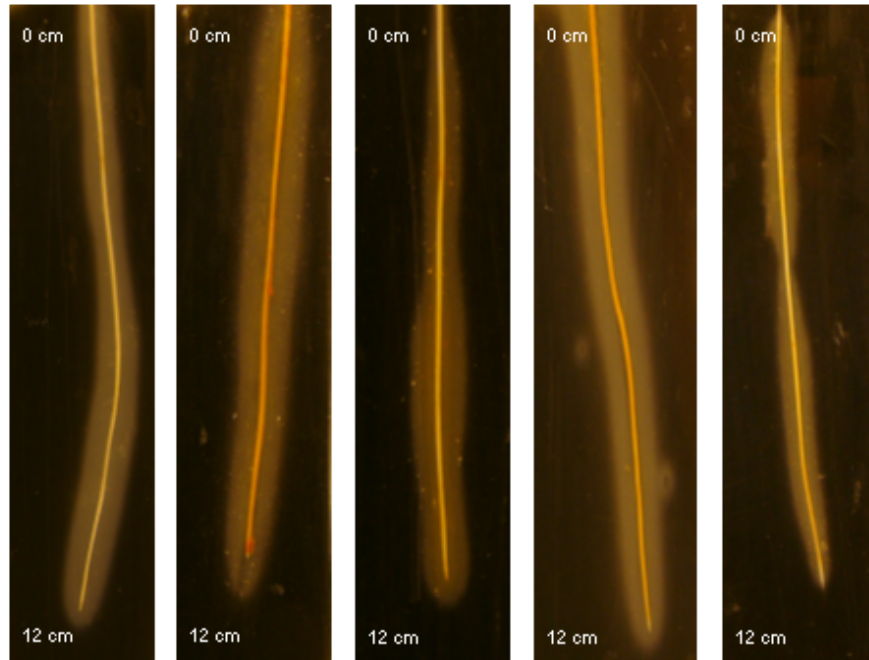
(B) + Si



(1) (2) (3) (4) (5)

Figure 20: The oxidized zone of nodal roots of rice grown in nutrient solution of (A) without and (B) with silicic acid after embedding in a semisolid agar medium for 24 h. Each treatment was replicated for five times. (+ LR means nodal root with lateral roots (+LR)).

(A) – Si



(1)

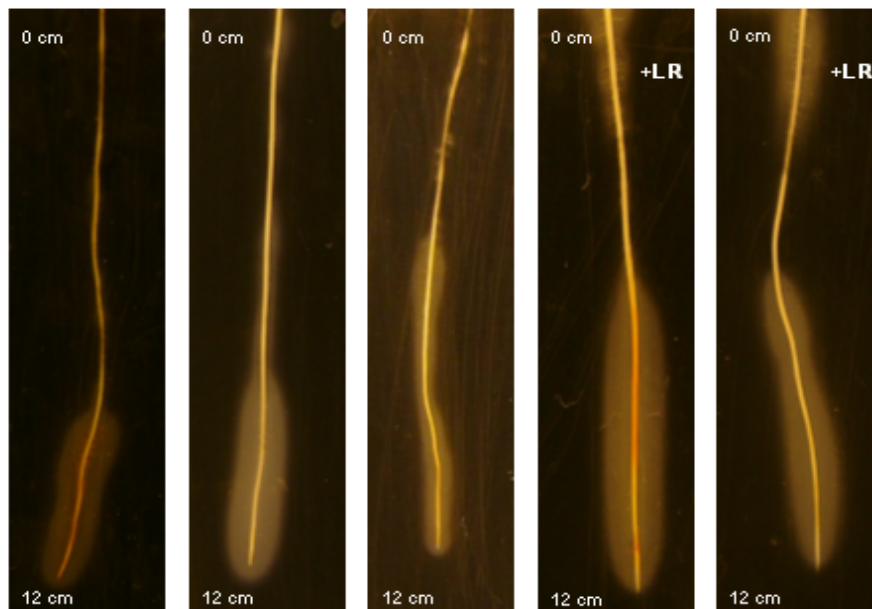
(2)

(3)

(4)

(5)

(B) + Si



(1) (2) (3) (4) (5)

Figure 21: The oxidized zone of nodal roots of rice grown in nutrient solution of (A) without and (B) with silicic acid after embedding in a semisolid agar medium for 48 h. Each treatment was replicated for five times. (+ LR means nodal root with lateral roots (+LR)).

5.2.2 Quantification of Oxidation power of rice root

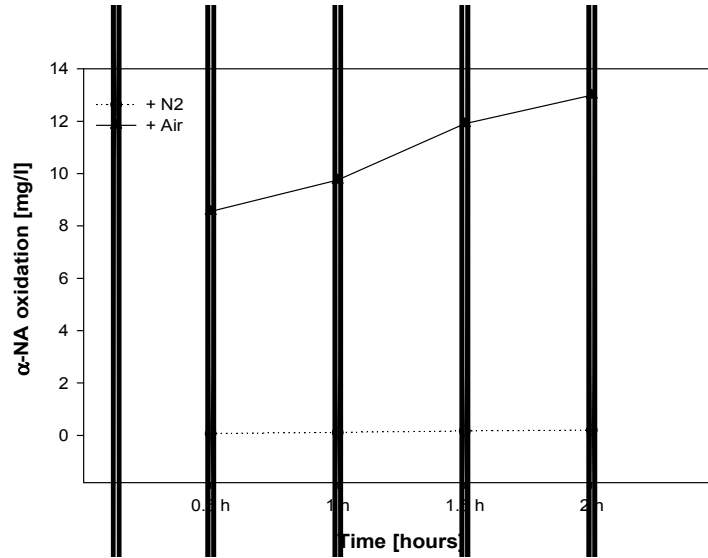


Figure 22: Effect of oxygen supply on α -NA solution.

For evaluation of the method α -NA oxidation by oxygen and dissolved oxygen in α -NA solution was firstly examined. In this experiment, α -NA solution was either bubbled with air or with N_2 gas for 0.5 h, 1 h, 1.5 h and 2 h, respectively. With continuous air supply, α -NA was oxidized, whereas oxidation was negligible when α -NA solution was bubbled with N_2 gas (Fig. 22). This result indicates that oxygen supply influences the α -NA oxidation which has to be considered for the experimental layout.

The oxidation power of different root zones (1-5 cm and 8-12 cm) of both + and – Si treatments were measured at 0.5 h, 1 h, 1.5 h and 2 h after incubation in α -NA solution. In this experiment, the oxidation of α -NA solution without root was determined and subtracted from the data obtained with roots. Fig. 23 shows the root oxidation power of both + and – Si treatments [root oxidation – control]. The oxidation decreased with incubation time for all treatments was higher

in the section 1-5 cm compared to the section 8-12 cm. Silicic acid decreased α -NA oxidation clearly in both 1-5 cm and 8-12 cm sections. The effect of Si supply on the root oxidation power was more pronounced at 0.5 h as compared to the rest of times (1 h, 1.5 h and 2 h).

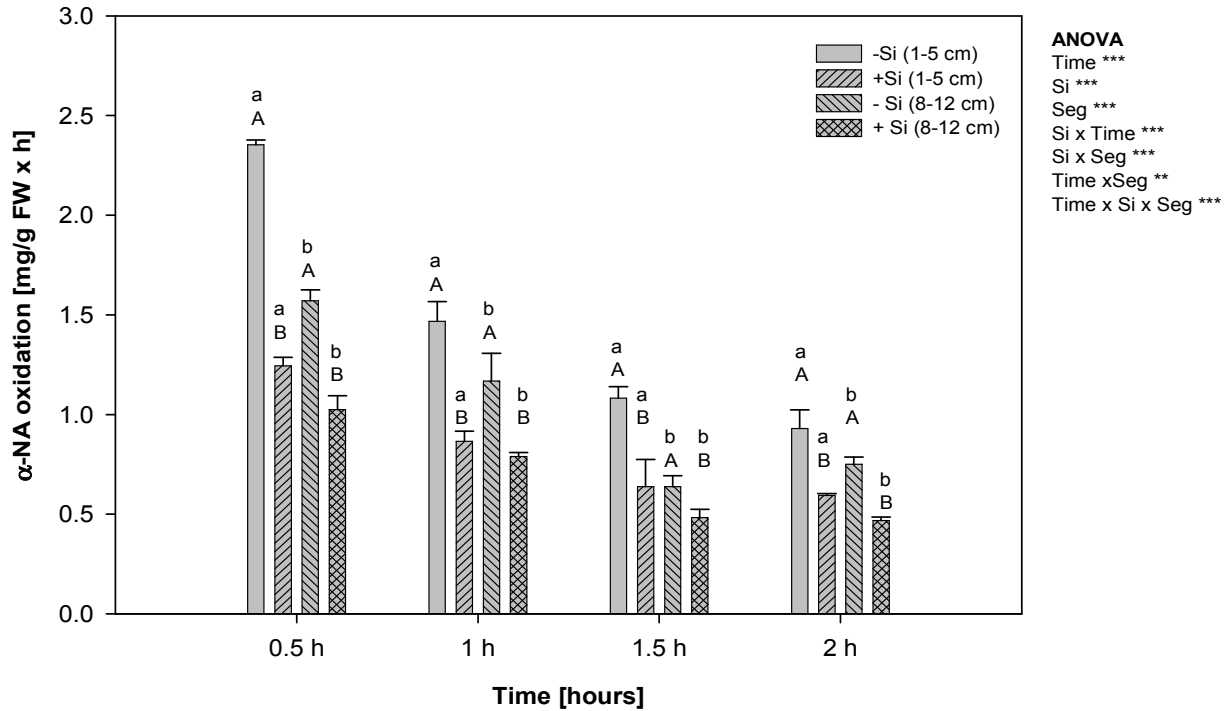


Figure 23: Effect of silicic acid supply on root oxidation power of rice grown in nutrient solution of + and – Si treatments. Bars = \pm standard errors (SE). ** and *** indicate significance at $p < 0.01$ and 0.001 , respectively and ns; not significant. Capital letters stand for comparison of Si treatments at the same segment and same time. Small letters stand for comparison of segment effect at the same Si treatment and same time (Tukey test).

5.3 Effect of silicic acid supply on arsenite Uptake in Rice

5.3.1 Influence of silicic acid supply on expression of arsenite transporters of rice root

To investigate whether silicic acid supply affects on expression of arsenite transporters in different root zones, the transcript level of Lsi1 and Lsi2 of both + and – Si treatments was determined in two different root zones (0-2 cm and 4-6 cm distances from the root tip) by Real Time-PCR. Silicic acid supply reduced the transcription level of both Lsi1 and Lsi2 (Tab. 3 A). This effect was more pronounced in younger root zone (0-2 cm distance from the root tip) than in older root zone (4-6 cm distance from the root tip). Expression of Lsi2 was much higher in the 0-2 cm section compared to the 4-6 cm section (Tab. 3 B). This effect was less pronounced with supply of silicic acid. For Lsi1 the expression was not this much affected by the root section.

Table 3: Influence of silicic acid supply on relative expression arsenite transporter encoding genes in root section 0-2 cm and 4-6 cm distance from the root of both + and – Si treatments. * and *** mean level of significance at $p < 0.05$ and 0.001 , respectively and ns denotes non significant.

(A) Gene expression as affected by silicic acid supply

Gene	Root section (cm)	Ratio -Si/+Si
Lsi1	0-2	6.468*
Lsi2	0-2	27.141***
Lsi1	4-6	2.411*
Lsi2	4-6	5.897*

(B) Gene expression as affected by root section

	Lsi1	Lsi2
Ratio of - Si (0-2 cm)/ - Si (4-6 cm)	1.739 *	16.756 *
Ratio of + Si (0-2 cm)/ + Si (4-6 cm)	0.951 ns	3.744 *

5.3.2 Influence of silicic acid supply on arsenite uptake of rice

Fig. 24 shows arsenite uptake of + and – Si treated roots with both + and - LR (lateral roots) after exposure to arsenite. For nodal roots without lateral roots, silicic acid supply reduced arsenite uptake rate by 40 % and 65 % in the root section 0-4 cm and 8-10 cm, respectively. A similar silicic acid effect was observed for nodal root bearing lateral roots. However, the effect was not this pronounced when arsenite was applied to the section 4-8 cm. Arsenite uptake in section 0-4

cm was similar for nodal root with and without lateral roots for both Si treatments. However, arsenite uptake rate of the root section 4-8 cm was decreased for root having lateral roots when no silicic acid was supplied. There was an interaction between the effect of Si and root type; indicating that the effect of Si depended on the root type.

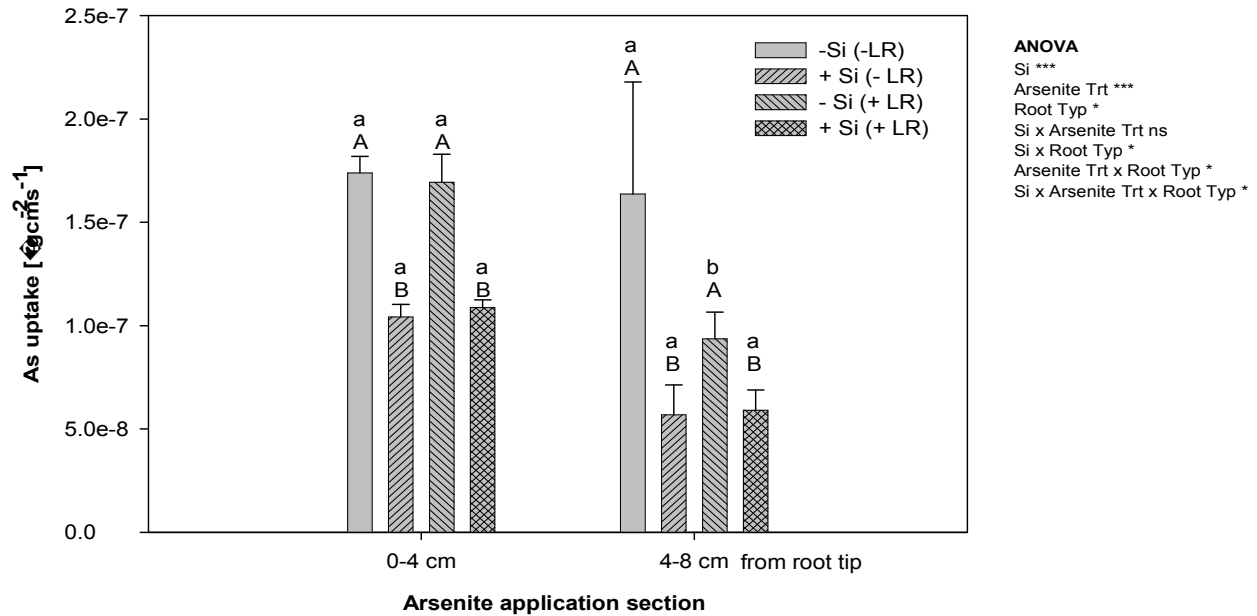


Figure 24: Influence of silicic acid supply on arsenite uptake by nodal roots with and without lateral roots (+, - LR) grown in the nutrient solution with + and – Si supply. Bars = ± standard errors (SE). * and *** denote level of significance level at $p < 0.05$ and 0.001 , respectively; ns, not significant. Capital letter stands for comparison of Si treatments of same root type. Small letter stands for comparison of root type at the same Si treatment.

5.3.3 Influence of silicic acid supply on arsenic concentration of rice root

Fig. 25 shows arsenic concentration of + and – Si treated roots with and without lateral roots. Silicic acid supply decreased the As concentration for both nodal root types with and without lateral roots for both applications. Over all treatments, As concentration in root fresh matter was lower if arsenite was applied to root section 4-8 cm.

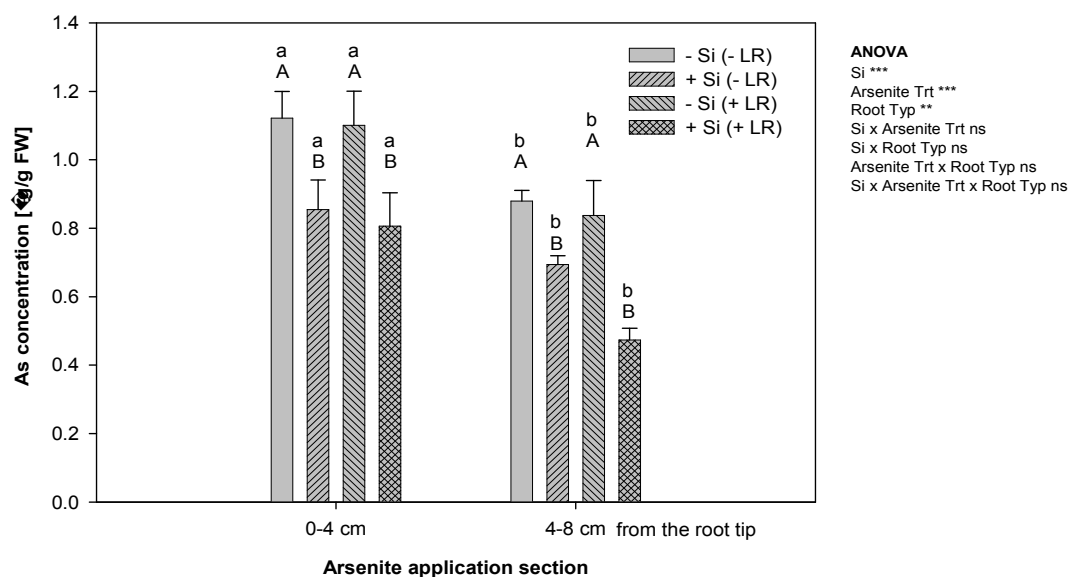


Figure 25: Influence of silicic acid application on arsenic concentration of nodal roots with and without lateral roots (+,- LR) grown in nutrient solution with + and – Si supply. Bars = \pm standard errors (SE). Capital letter stands for comparison of Si treatments of the same root type. Small letter stands for comparison of root types at the same Si treatment (Tukey test).

5.3.4 Influence of silicic acid supply on arsenic efflux of rice root

Tab. 4 A and B show As concentration of + and – Si treated roots with both + and – LR when arsenite applied to the first compartment (0-4 cm). For nodal root without lateral root, Si supply reduced As concentration of root as well as in nutrient solution. However, change was more pronounced in the nutrient solution. This is observed in both root sections 4-8 cm and 8-10 cm. For nodal root with lateral roots, silicic acid application reduced the concentration in root and nutrient solution in the section 4-8 cm but not in 8-10 cm distance from the root tip.

Table 4: Influence of silicic acid supply on arsenic concentration in root fresh matter and nutrient solution of + and – Si treated roots when arsenite applied to the first compartment (0-4 cm). + and - LR means nodal root with and without lateral root, respectively. Bars = ± standard errors (SE). Capital letter stands for comparison of Si treatments of the same root type. Small letter stands for comparison of root types at the same Si treatment (Tukey test).

(A) Root section 4-8 cm distance from the root tip

Si treatment	Root As concentration (µg/L/g FW)	As concentration in Nutrient solution (µg/L)
- Si (- LR)	0.211 Ab	1.188 Ab
+ Si (- LR)	0.104 Bb	0.822 Bb
- Si (+ LR)	0.2325 Aa	2.325 Aa
+ Si (+ LR)	0.1869 Aa	1.869 Aa

(B) Root section 8-10 cm distance from the root tip

Si treatment	Root As concentration (µg/L/g FW)	As concentration in Nutrient solution (µg/L)
- Si (- LR)	0.632 Aa	2.033 Aa
+ Si (- LR)	0.369 Bb	1.401 Bb
- Si (+ LR)	0.535 Aa	2.339 Aa
+ Si (+ LR)	0.544 Aa	2.342 Aa

Silicic acid supply also decreased the As concentration in the two other root sections when arsenite was applied to the section 4-8 cm for both nodal root types (with and without lateral roots) as shown in Tab. 5 A. Only in the section 8-10 cm no Si effect occurred if root had laterals (Tab. 5 B).

Table 5: Influence of silicic acid supply on arsenic concentration in root fresh matter and nutrient solution of + and – Si treated roots when arsenite applied to the second compartment (4-8 cm). + and - LR means nodal roots with and without lateral roots, respectively. Bars = ± standard errors (SE). Capital letter stands for comparison of Si treatments of the same root type. Small letter stands for comparison of root types at the same Si treatment (Tukey test).

(A) 0-4 cm distance from the root tip

Si treatment	Root As concentration (µg/L/g FW)	As concentration in Nutrient solution (µg/L)
- Si (- LR)	0.145 Ab	1.304 Aa
+ Si (- LR)	0.078 Bb	0.944 Ba
- Si (+ LR)	0.184 Aa	1.078 Ab
+ Si (+ LR)	0.133 Ba	0.783 Bb

(B) 8-10 cm distance from the root tip

Si treatment	Root As concentration (µg/L/g FW)	As concentration in Nutrient solution (µg/L)
- Si (- LR)	0.333 Ab	1.491 Ab
+ Si (- LR)	0.240 Bb	1.024 Ba
- Si (+ LR)	0.544 Aa	2.339 Aa
+ Si (+ LR)	0.535 Aa	2.342 Aa

6. DISCUSSION

6.1 Effect of silicic acid supply on suberin layer formation in rice root

Most studies have been performed on how Si supply alleviates metal toxicities, several stress conditions and infection by fungi. However up to date, no precise information has been reported about a beneficial effect of Si supply on anatomy of rice roots. It is well known that exodermis and endodermis may serve as the major sites for the deposition of Si in root of rice (Ma and Yamaji, 2008; Gong et al., 2003). They are structurally specialized cell layers that develop Casparian bands in their cell walls (Peterson and Perumalla, 1990). These Casparian bands are composed of lignin and suberin (Hose et al., 2001) which serve as a barrier against both the entering of water and dissolved ions as well as the loss of oxygen into the rhizosphere of rice. Thus, the effect of silicic acid supply on suberization of root exodermis and endodermis and radial loss of oxygen as well as arsenite uptake was studied.

6.1.1 Influence of silicic acid supply on suberin layer formation in root of rice grown in nutrient solution

Microscopic examination revealed that the application of silicic acid could enhance the suberization of root exodermis and endodermis when rice was grown in nutrient solution (Fig. 9 and 11). This finding is in contrary to results with maize (Vaculik et al., 2009) where no effect of Si supply was observed on the development of suberin layer in root endodermis. Suberin biosynthesis according to Franke and Schreiber (2007) is related with the functions of acyltransferases, ATP binding cassette (ABC) transporters and peroxidases. Therefore, silicic acid supply may affect these functions. The result of the current study suggests that silicic acid supply might influence the expression of genes which are involved in the suberin biosynthesis in rice roots. However, it is unknown how silicon mediates suberization in roots of rice and thus further studies on this aspect need to be done.

In principle, the degree of suberization in root exodermis is higher in the regions far away from rice root tips (Kawata and Lai, 1968 cited in Yoshida, 1981). This statement appears reasonable

because in the present study, suberization of root exodermis was more pronounced in the basal part as compared to the apical part in both Si treatments (Fig. 8 and 10). Moreover, this finding is in agreement with the results from *Phragmites australis* and *Glyceria maxima*, where suberin content of the root apical part was higher than in the basal part (Soukup et al., 2007).

No formation of suberin lamellae in exodermis cell walls was found at less than 3 cm distance from the root tip of both Si treatments (Fig. 8 and 9). Schreiber et al. (2005) also reported that the development of suberin layer in root exodermis of rice is formed at a distance of 3 cm behind the root tip. For onion and corn, the formation of Casparian bands with suberin lamellae in root exodermis appears at ~ 6 cm from the root tip (Lux, 2008; Enstone and Peterson, 1997). In the current study, suberization was fully developed in root endodermis of rice without Si supply at 4-5 cm behind the root tip (Fig. 11 g). However with silicic acid supply, cell walls of root endodermis were already fully suberized in the first cm behind on the root tip (Fig. 11 c). For suberin development of root endodermis, Casparian bands with suberin lamella formed at a distance of ~ 1cm from the root tip in *Arabidopsis* (Ma and Peterson, 2003) and ~ 6 cm in corn (Schreiber et al., 2005). Schreiber et al. (2005) also mentioned that all root endodermis of rice have fully suberized at 5 cm behind the root tip.

The formation of suberization in root endodermis of both Si treatments was observed at less distance from the root tip as compared to root exodermis (Fig. 8 and 10). It was well documented that Casparian bands with suberin lamellae in root endodermis usually appear at a closer distance from the root tip than that of root exodermis of maize plants (Lux, 2008). Conversely, a reverse sequence of differentiating was found in some wetland plants (Seago et al., 1999; Soukup et al., 2002) and in tea plants (Homma et al., 2000; Tanimoto et al., 2004 both cited in Lux, 2008), suberin layer has formed closer to the root tip of root exodermis than that of root endodermis.

6.1.2 Influence of silicic acid supply on suberin layer formation in root of rice grown in quartz sand

In order to determine the effect of silicic acid supply on root suberization of rice grown in a different growing media, the development of suberin layer by silicic acid supply was also investigated in root of rice grown in quartz sand. It was interesting to notice that cell walls of

both root exodermis and endodermis were fully suberized beginning from the first cm behind the root tip of with and without silicic acid supply, respectively (Fig. 14 and 16). Although root Si concentration was higher by silicic acid supply (Fig. 19 B), no influence of silicic acid supply on suberization was found in root exodermis and endodermis, respectively. Generally, the formation of root suberization is increased by environmental stress factors (North and Nobel, 1995; Reinhardt and Rost, 1995). Salt stress, e.g. enhanced in maize plants the degree of suberization in root apoplast, which leads to a reduction of apoplastic uptake of sodium chloride or water losses during salt stress (Schreiber et al., 2005). In this study, rice roots needed to penetrate in quartz sand, whilst roots of plants grown in the nutrient solution had not to develop such a pressure. Consequently, rice roots might get stress when growing in quartz sand. This might be the reason that early formation of suberization was observed in both root exodermis and endodermis when rice was grown in sand.

Furthermore, the faster a root grows, the longer the distance from the tip to the regions where Casparian bands with suberin lamellae are formed under specific conditions (Enstone et al, 2003; Ma and Peterson, 2003). In other words, any stress that affects root growth may result in a reduced distance of Casparian band formation from the root tip (Reinhardt and Rost, 1995). This statement is fairly supported by the data of this study where suberization of root exodermis and endodermis of rice grown in quartz sand was formed at the closer distance from the root tip than that of rice grown in the nutrient solution with and without silicic acid supply, respectively.

6.1.3 Influence of changing silicic acid supply on suberin layer formation in root of rice grown in nutrient solution

In the present experiment, the effect of silicic acid supply was observed at 48 h and 72 h after changing silicic acid supply (Fig. 17 and 18). Additionally, this effect was more pronounced at 72 h. This might be linked to root growth rate of *Selenio* rice variety where in both Si treatments root growth rate was about 1 cm per day (Fig. 5). Obviously 48 h were necessary to make silicic acid effect on suberin formation visible by the staining method.

6.1.4 Effect of silicic acid supply on silicon concentration in rice roots and shoots

Rice is a typical plant of high Si demand (Savant et al., 1997; Ma et al., 2003) and thus it contains Si in the range between 1 to 10 % of dry matter (Epstein, 1994) which is several fold higher compared to essential macro nutrients in plants (Ma et al., 2006). This is in line with the data of the present study where Si concentration in shoot of + Si treatment was about 7.1 % of shoot dry matter (Fig. 19 A). Rice plant without silicic acid supply exhibited a Si concentration of around 0.8 % of shoot dry matter, which is in the same dimension of some macronutrients such as S and Mg (Epstein, 1994; 1999).

Si is generally deposited in greatest quantities in the tissues from which water is lost in greatest quantities (Jones and Handreck, 1967). For this reason, the concentration of Si in roots of both Si treatments was lower than in shoot of rice grown in all culture systems.

6.2 Effect of silicic acid supply on oxidation power of rice root

When root system of rice plants is embedded in FeS agar, the extension of oxidation zone around root system is observed (Trolldenier, 1988). This finding was also found in current study, where the oxidation power of root was shown by embedding nodal roots (Fig. 20). This suggests that rice root is permeable to oxygen (Colmer, 2003).

On the other hand, the oxidation pattern of nodal root with silicic acid supply differed clearly from that of root without silicic acid supply. In without Si treated plant, root formed a bright zone at the whole investigated root length (0-12 cm distance behind the root tip), which suggests that the root is permeable to oxygen along the whole root. However, silicic acid supplied root developed an oxidation zone around the root tip (0-5 cm distance behind the root tip), whereas the basal root part (5-12 cm distance from the root tip) did not show the oxidation zone. This result was in line with the findings of Nordheim (2007). This experiment was confirmed by the results of quantification of oxidation power by α -NA assay (Fig. 23). The reason of decreased oxidation power by silicic acid supply might be due to the increased suberization of root

exodermis by silicic acid supply (Fig. 26) since exodermis suberization serves a barrier to ROL from the root to the rhizosphere (Soukup, 2005; Clark and Harris, 1981; Zier et al., 1999). Another possibility might be an enhancement of lignified sclerenchyma cells by silicic acid supply since lignification in sclerenchyma of root is also thought to serve a barrier formation (Kotula and Steudle, 2009). However, the observation is in contrast to literature, where it is claimed that silicon application might promote the oxidation power of root (Okuda and Takahashi, 1961 cited in Jian and Takahashi, 1993).

The lateral roots generally contribute to the loss of oxygen to the rhizosphere (Amstrong et al., 1990 cited in Kirk, 2003). This statement is agreed with the findings of current study where nodal root with lateral roots developed an oxidation zone in the surrounding agar (Fig. 20 B (4) and (5)). Probably the lateral roots not only crack the epidermis but also the exodermis and this enhances leakage of oxygen (Kirk, 2003).

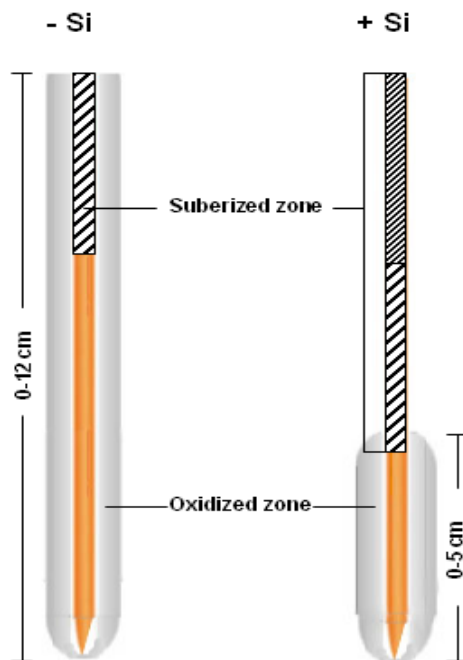


Figure 26: A schematic drawing of comparing suberization and oxidation power of rice root with and without silicic acid supply.

The data of quantification of oxidation power by α -NA assay showed that the root oxidation power declined with time (Fig. 26). The reason might be limited supply of oxygen from the root

aerenchyma since gas exchange of excised roots was interrupted (Colmer, 2003; Videmš, et al., 2006). Another reason might be insufficient carbohydrate supply that provides energy for oxidizing metabolism (Egging, 2003).

The oxidation power of rice root may include the release of oxygen and enzymatic oxidation of root as measured by α -NA assay (Ando et al., 1983). Both FeS agar and the α -NA oxidation are oxidized by the presence of oxygen and hydrogen peroxide and both are much more sensitive to the latter because hydrogen peroxide is a stronger oxidation substance than molecular oxygen (Mitsui and Kumazawa, 1961). The results of the present study showed that α -NA oxidation was influenced by oxygen supply (Fig. 22). Thus α -NA oxidation method is not suitable to distinguish between O₂-oxidation of α -NA assay and metabolic controlled oxidation.

6.3 Effect of silicic acid supply on arsenite uptake in rice

Arsenite is the dominant As species in soils under flooded paddy soil conditions (Bogdan and Schenk, 2008; Marin et al., 1993; Takahashi et al., 2004; Xu et al., 2008). Therefore, this experiment focused on the effect of silicic acid supply on arsenite uptake of rice.

6.3.1 Influence of silicic acid supply on arsenite uptake rate of rice

In the present study, for the nodal root without lateral roots the arsenite uptake rate of younger root part (0-4 cm distance behind the root tip) was higher in both without and with silicic acid supply compared to that of older root part (4-8 cm distance from the root tip) shown in Fig. 24. A possible reason might be the decreased expression of arsenite transporters (Lsi1 and Lsi2) in the older root part (Fig. 27). For the nodal root with lateral roots, similar effect was also observed.

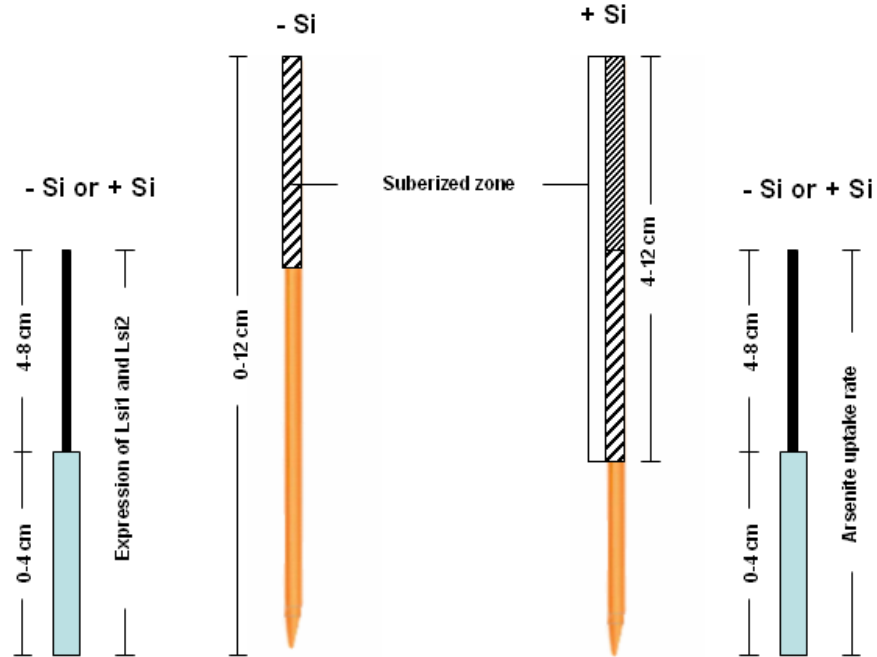


Figure 27: A schematic drawing of comparing suberization, expression of arsenite transporters and arsenite uptake rate of root with and without silicic acid supply.

It was known that silicic acid supply can inhibit on arsenic uptake by rice (Bogdan and Schenk, 2008). In agreement to this, the current study showed that the arsenite uptake rate of root without lateral roots in the root section 0-4 cm distance behind the root tip was reduced by silicic acid supply (Fig. 24). This can be explained by the decreased expression of arsenite transporters by silicic acid application (Tab. 3 A). Moreover, for root section 4-8 cm distance behind the root tip arsenite uptake rate was decreased in root without lateral roots by silicic acid supply (Fig. 24). There may be two possible explanations for decreased arsenite uptake by silicic acid supply: (1) The decreased arsenite uptake of root might be due to the higher degree of suberization in root exodermis and endodermis by silicic acid supply (Fig. 9 and 11) where suberized cell walls of root influence on the uptake of ions (Franke and Schreiber, 2007). (2) The expression of arsenite transporters was reduced in the Si treated root (Ma et al., 2006; 2007). This is also in line with the previous finding (Tab. 3 A). Moreover, the arsenite uptake rate of root with lateral roots was reduced by silicic acid supply in both root sections (0-4 cm and 4-8 cm distance behind the root tip).

Moreover, the effect of silicic acid supply on arsenite uptake rate of rice root was less pronounced in root with lateral roots as compared to that of root without lateral roots at root section 4-8 cm distance from the root tip. Since the lateral roots crack the exodermis of root (Kirk, 2003), this might reduce the effect of silicic acid supply on arsenite uptake rate of root.

The effect of silicic acid supply on arsenite uptake rate was more pronounced in the root section 4-8 cm distance from the root tip as compared to the root section 0-4 cm for nodal root without lateral roots (Tab. 6). For root with lateral roots, this effect was not much difference between the root sections 0-4 cm and 4-8 cm distance from the root tip. Although reduction of expression of arsenite transporters by silicic acid supply was more pronounced in the root section 0-4 cm than that of 4-8 cm distance from the root tip, reduction of arsenite uptake rate by silicic acid supply was more pronounced in the root without laterals at 4-8 cm distance from the root tip. This might be the increased suberization of root exodermis by silicic acid application at 4-8 cm distance from the root tip (Fig. 9). However, lateral roots might crack the exodermis of root (Kirk, 2003) and thus effect of silicic acid supply might not be affected on suberization of root with laterals. Accordingly, for root with lateral roots reduction of arsenite uptake rate by silicic acid supply was not much pronounced in the root section 4-8 cm distance from the root tip.

Table 6: Reduction of arsenite uptake rate and reduction of arsenite transporter expression by silicic acid supply in both roots with (+LR) and without (-LR) lateral roots.

Root section	Reduction of arsenite uptake rate (%)		Reduction of arsenite transporter expression for both root (- and + LR) (%)	
	Root (-LR)	Root (+LR)	Lsi1	Lsi2
0-4 cm	40	36	85	96
4-8 cm	65	38	59	83

The result of present experiment also showed that application of silicic acid can reduce the arsenic concentration of both root with and without lateral roots (Fig. 25). This statement is in line with Guo et al. (2005; 2007) where Si supply to the growing medium reduced As concentration of rice roots. Bogdan and Schenk (2008) also stated that Si application might

decrease the As concentration of rice plants. This might be explained by the decreased arsenite uptake rate of root by silicic acid supply (Fig. 24) due to the decreased expression of arsenite transporters by silicic acid supply (Tab. 3 A).

Furthermore, As concentration of root with and without lateral roots in both Si treatments was lower when arsenite was applied to the root section 4-8 cm behind the root tip as compared to that of root when arsenite was applied to the root section 0-4 cm. This might be the decreased arsenite uptake of root section 4-8 cm distance from the root tip in both Si treatments (Fig. 24).

6.3.2 Influence of silicic acid supply on arsenic efflux of rice root

In the present experiment, arsenic was observed in nutrient solution of the compartments where arsenite was not applied (Tab. 4 and 5). It is known that arsenite efflux from the plant roots to the external solution is a basal mechanism to detoxification of As in plants (Logoteta et al., 2008 cited in Zhao et al., 2009; Zhao et al., 2009). Zhao et al. (2009) stated that some arsenite is lost from the cells to the external medium following arsenite taken up by root. The efflux of arsenite by roots was also found in rice, barley, wheat (Su et al., 2009) and *Peteris vittata* (Su et al., 2008) as well as in tomato root (Xu et al., 2007). The reason of As efflux to As free medium might be due to arsenite efflux transporter Lsi2 which actively transports As to the xylem as well as to the external medium (Zhao et al., 2009). Lsi2 is located on proximal side of cells (Ma et al., 2007) and thus arsenite can be transported from exodermal cells to cortex and from endodermal cells to xylem. Xu et al. (2007) suggested that As efflux might be linked to the proton gradient across the plasma membrane.

For nodal root without lateral roots, arsenic efflux from the roots to the nutrient solution of the compartments that was not supplied with arsenite was reduced by silicic acid supply. The reason of this decreased As efflux might be the higher degree of suberization in root by silicic acid supply since suberization of root exodermis is a barrier to the movement of ions (Enstone et al., 2003). Another possible reason might be the decreased expression of Lsi2 by silicic acid supply (Tab. 3 A) where the decreased expression of Lsi2 might mediate the As efflux to the external medium (Zhao et al., 2009). However, no effect of silicic acid supply on As efflux was observed in root with laterals. Since the exodermis was cracked by the lateral roots, this might lead to no

barrier function in the root. Therefore, the effect of silicic acid supply was not found in the root with lateral roots although the expression of Lsi2 was decreased by silicic acid supply. Only in the root section 0-4 cm from the root tip the As efflux was reduced by silicic acid application in roots without and with laterals. Since no lateral roots did appear in this root section the lower efflux was probably occurred by the reduced expression of Lsi2 by silicic acid supply.

Generally, arsenite is transported by arsenite transporters from the external medium towards the xylem where it is translocated acropetally (Ma et al., 2008). However, some of arsenic was found in the root section 0-4 cm distance from the root tip when arsenite was applied to the root section 4-8 cm distance. The reason of this might be the diffusion of arsenite from the higher concentration to the lower concentration within the root.

In summary, silicic acid application to the nutrient solution might influence on suberization of both root exodermis and endodermis in rice. The effect of silicic acid supply on suberization of root might need at least 48 h. Moreover, silicic acid supply decreased the oxidation power of root. The results of current study suggest that silicic acid supply to nutrient solution resulted in decrease of both arsenic concentration and arsenite uptake by both roots with and without lateral roots. This might be due to the higher degree of suberization and lower expression of arsenite transporters by silicic acid supply. In addition, As efflux was reduced by silicic acid supply in root without lateral roots. However, there are still remaining many questions on the influence of silicon on rice root suberization. Future research is needed to bring conclusive new information on involvement of Si on suberization in root.

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