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Effect of Silicon on the Formation of Cell Walls in Rice and Tomato Plants

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Summary

The effects of silicon on cell wall formation in rice (*Oryza sativa* L.) and tomato (*Solanum lycopersicum* L.) plants were determined. Rice and tomato plants were grown in culture solution without silicon or with 50mg L^{-1} silicon, and then the cell wall was extracted from the rice straw and 3 plant parts of tomato (roots, upper leaves and stem, lower leaves and stem). The cell wall was fractionated into soluble pectin substances in hot water (F1), pectin (F2), oxidized lignin (F3), hemicellulose (F4) and cellulose (F5). In rice straw, silicon addition increased F2 weight after heading. In all plant parts of tomato, silicon supply increased F2 and F3 weights. In spite of silicon deficiency, silicon content of rice straw cell wall before heading and those of F2 and F3 at heading were higher in silicon deficiency than in silicon supply. Addition of silicon markedly increased silicon content of F1. In all plant parts of tomato, silicon supply markedly increased total cell wall silicon content, as shown by the increase in F1. The silicon content of F2 was higher with than without silicon addition. The absorption bands of infrared spectroscopy of F1 from roots of rice plant by silicon addition showed a shoulder at 940 cm^{-1} and an increase in intensity at 465 cm^{-1}, indicating an association of Si-O with $-CH_2-CH_2-$, and the existence of silicon, respectively. These results suggest that the later growth of rice straw and curling of tomato leaves as a result of silicon deficiency may be due to insufficient formation of primary cell wall, pectin and lignin, and that silicon absorbed during plant growth accumulates in F1, binding with organic compounds.

Key words: silicon, cell wall, pectin, lignin, plants

Introduction

A deficiency of silicon in culture solution was shown to delay heading in rice because of the later growth of straw during the panicle stage, the appearance of brown spot on the hull and a decrease in spikelet number [1]. Miyake et al. found curling in newly formed leaves of tomato, cucumber and soybean [2, 3, 4], and in cotton plants, silicon deficiency was shown to reduce fiber length [5]. In rice, silicon deficiency reduced the number of leaf vascular bundles [6]. We found that silicon might combine with lignin- or phenol-carbohydrate complexes [7]. These results suggest that the physiological damage

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resulting from silicon deficiency may be due to inadequate cell wall formation.

In this study we aimed to determine the effects of silicon on cell wall formation, using rice (*Oryza sativa* L.) and tomato (*Solanum lycopersicum* L.) plants.

Materials and Methods

Experiment 1. Change in cell wall components of rice straw during growth stage.

Seventy-two seedlings sown on 20 May 1998 were transplanted on 28 June to a 20-L container filled with a culture solution described previously [1], containing 50mg L^{-1} silicon, and were then grown in the culture solution without silicon (treatment I) or with 50mg L^{-1} silicon (treatment II) from 31 July. Straw from the main stem was harvested before heading on 15 August, at heading day (0), and 10 and 40 days after heading.

Experiment 2. Culture of tomato plants

One tomato plant seedling was transplanted to a 4-L pot filled with Kimura culture solution without silicon (treatment I) or with 5ee (the upper parts) and lower leaves and stem below node three (the lower part) 0mg L^{-1} silicon (treatment II) on 20 April 1999. After harvesting on 5 June, the tomato plant was separated into roots, upper leaves and stem from the top leaf to node thr.

Preparation of cell wall and cell wall fractionation

After freeze-drying the plant parts, the cell wall was prepared by Ito and Fujiwara's method [7], and then fractionated. First, the cell wall was extracted by hot water (80℃) (fraction 1, F1), followed by 0.25% ammonium oxalate (f2-1), 0.25% ammonium oxalate and 0.25% oxalic acid solution (f2-2), 50mL water with 0.5mL acetic acid and 0.5 g sodium chlorite at 80℃ for 4 hours (F3), 5% potassium hydroxide (f4-1) and 24% potassium hydroxide (f4-2). f2-1, f2-2 and F3 were dialyzed against water, and then, together with F1, freeze-dried and weighed. f4-1 and f4-2 were acidified with acetic acid, and 9% ethanol was added. The resulting white precipitate was washed with ethanol, acetone and ether, and then dried and weighed. The extracted residue (F5) was washed with ethanol, acetone and ether, and weighed. After weighing each fraction and decomposition by the wet method, silicon was determined by the flameless atomic absorption method. f2-1 and f2-2 summed are shown as F2, while f4-1 and f4-2 summed are shown as F4. F1, F2, F3, F4 and F5 correspond to pectin substances soluble in hot water, pectin substances, oxidized lignin, hemicelluloses and cellulose, respectively.

Fractionation was repeated three times except for the cell wall from rice straw before heading, and all plant parts of the tomato.

Infrared spectroscopy

Infrared (IR) spectra were recorded using KBr disks from the freeze-dried F1 from the roots of tomato and rice plants at 40 days after heading [8].

Results

The heading day of the rice was 23 August in treatment I and 20 August in treatment II.

Figure 1 shows the changes in cell wall components of rice straw during growth. Most cell wall components were F4 and F5 through the growth stages, but in both treatments, both fractions were lower before than after heading. In both treatments, F5 increased after heading.

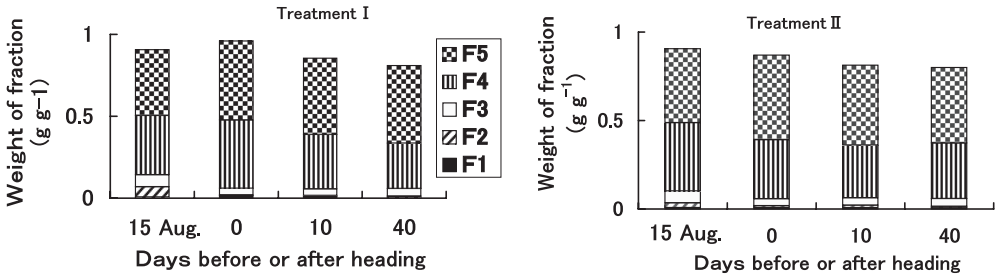

Figure 1. Effect of silicon addition on weight of each fraction of cell wall from rice straws.

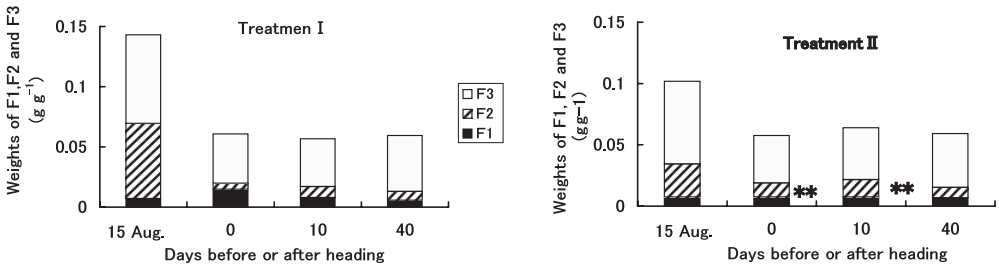

Figure 2. Scale up F1, F2 and F3 in figure 1.
**: significantly difference at 5% level from treatment I

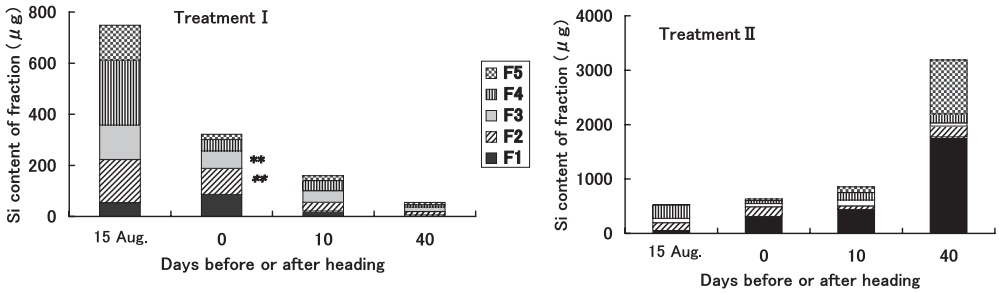

Figure 3. Change in silicon content of each fraction in cell wall from rice straw through growing stage.
**: significantly difference at 5% from treatment II.

Figure 2 shows values for F1, F2 and F3 scaled up from those in figure 1. Before heading, F2 was higher in treatment I than in treatment II, but at heading and 10 days after heading F2 was higher in treatment II than in treatment I.

Silicon contents of each fraction are shown in figure 3. In treatment I, total silicon content of the cell wall on 15 August (before heading) was higher than that in treatment II, and was the highest among the growth stages, then decreased dramatically. In contrast, in treatment II total silicon content increased with the growth of the rice straw.

On 15 August (before heading), silicon contents of all fractions were higher in treatment I than in treatment II and silicon content of F4 was highest among all fractions, followed by F2, F3 and F5. At heading, silicon contents of F2 and F3 were higher in treatment I than in treatment II. In treatment II, silicon content of F1, which was markedly lower in weight than in F2, F3 and F4 before heading, increased with the growth of the rice straw, as did its proportion of total silicon content. At 40 days after

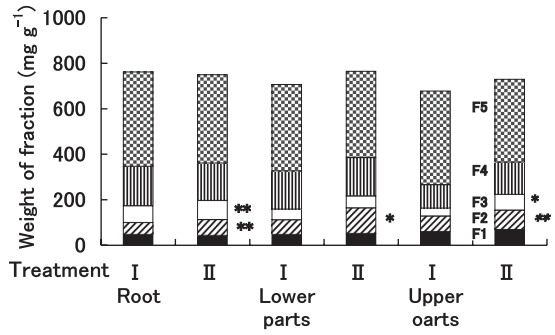

Figure 4. Weight of each fraction from cell wall of tomato plant.
*: significantly difference at 1% level from treatment I
**: significantly difference at 5% level from treatment I

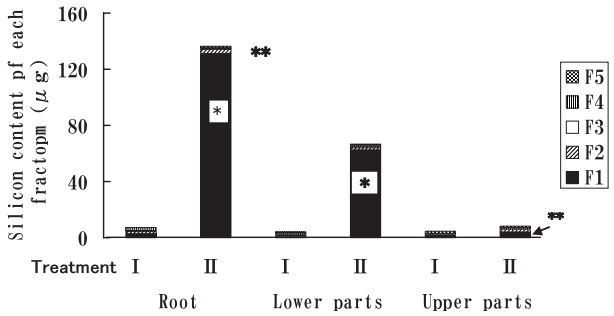

Figure 5. Silicon convent in each fraction of cell wall from tomato plant.
*: significantly difference at 1% level from treatment I
**: significantly difference at 5% level from treatment I

heading, silicon content of F5 had also increased.

Figure 4 shows the effects of silicon on cell wall components in each plant part of tomato. In all plant parts in both treatments, F5 was the highest among all fractions, followed by F4. In the upper plant parts and roots, F2 and F3 were higher in treatment II than in treatment I.

The silicon content of each fraction of tomato cell wall is shown in figure 5. Total cell wall silicon content in treatment II was highest in roots among all plant parts, followed by lower plant parts. The silicon content of all plant parts in treatment I was markedly lower than in treatment II. In both treatments, the silicon content of F1 was higher than that of other fractions, and the silicon content of lower plant parts and roots markedly increased in treatment II. Silicon addition increased the silicon content of F2 in roots and upper plant parts.

Figure 6 shows IR spectra of F1 from rice roots at 40 days after heading, and of tomato roots. F1 IR spectra were similar among all treatments. However, in rice the spectra were shifted to a longer absorption band, at 3369 cm^{-1}, had a stronger signal at 467 cm^{-1} and a shoulder near 960 cm^{-1} in treatment II. In the tomato, only a shift to a longer absorption band, at 1614 cm^{-1}, was observed.

Discussion

Plant cell wall consists of primary and secondary cell walls. The former contains mainly pectin

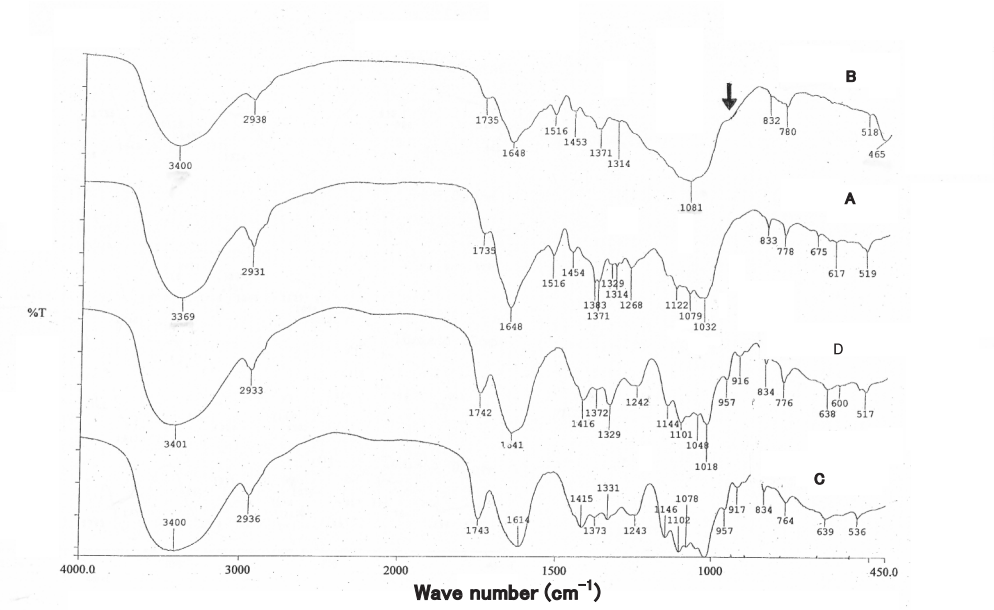

Figure 6. Infrared absorption spectra of soluble pectin substances from cell wall of rice or tomato plant. A: rice treatment Ⅰ, B: rice treatment Ⅱ, C: tomato treatment Ⅰ, D: tomato treatment Ⅱ

substances, which make up the middle lamella, and lignin, which is the main component of the vascular bundle, while the latter has hemicelluloses and cellulose. First, pectin and lignin are formed in the cell wall, and then the secondary cell wall. The cell wall of peanut shell before enlargement is composed mainly of pectin and lignin fractions, and then hemicelluloses and cellulose contents increased with aging [9]. The cell wall of young rice leaves contained more pectin and lignin than hemicelluloses and cellulose [7]. In this study, the heading date of the rice was 23 August in treatment I and 20 August in treatment II. As shown in figures 1 and 2, in the cell wall on 15 August before heading, the primary cell wall components were higher in treatment I than in treatment II, mainly because silicon deficiency delayed the development of rice straw.

Any physiological disorders caused by silicon deficiency might be due to insufficient light reaching the leaves and a reduction in translocation of photoassimilate [10]. However, when keeping leaves in sufficient light conditions in rice, short straw was observed in the main stem 9 days after the supply of silicon was stopped after the panicle stage [1]. Furthermore, silicon deficiency decreased the number of vascular bundles in rice [6] and the content of lignin-carbohydrate complexes was lower in vegetative organs [8]. These results suggest that late heading of rice and curling of tomato leaves as a result of silicon deficiency may be due not only to a reduction in photosynthetic ability and translocation of photoassimilate but also to other factors.

Calcium, which plays an important role in the cell wall by binding with pectin substances, did not affect the formation of cell wall components in rice [7], and calcium deficiency decreased the cellulose content in the cell wall of peanut shell [9]. As shown in figures 1 and 4, silicon deficiency did not affect the secondary cell wall of rice straw after heading and all plants of parts of tomato. However, silicon addition increased the pectin fraction of rice straw after heading and pectin and lignin fractions of tomato (figures 3 and 4). In spite of silicon deficiency, as shown in figure 3, the cell wall silicon content of rice straw before heading was markedly higher than after heading. Furthermore, the silicon content in the

pectin and lignin fractions at heading were more than those in silicon addition (figure 3). In all plant parts of the tomato, silicon addition increased silicon content of the pectin fraction (figure 5). These results suggest that silicon is necessary for the formation of the primary cell wall, pectin and lignin, and that the physiological disorders caused by silicon deficiency, such as the late heading of rice, curling of tomato leaves and short fibers of cotton, may be the result of insufficient formation of primary cell wall.

As shown in figure 3, silicon continued to increase in F1 of the rice cell wall during growth. As shown in figure 5, silicon was very high in F1 in all plant parts of the tomato, and the silicon content of F1 was higher in the lower than in the upper parts, indicating that silicon incorporated into the plant was accumulated in soluble pectin substances. No absorption band or signal due to organo-silicate compounds was observed in IR or NMR spectroscopy of rice plants [11, 12]. However, the high content of silica gel in rice might suppress organo-silicate signals. Silicon accumulated in F1 of cell wall may exist in the form of silica gel. In the cell wall of diatoms, much silicon is polymerized on hydroxyl-amino acid complexes [13]. The results of the UV and IR spectra of cell wall extracts indicate that silicon might exist in association with lignin- or phenol-carbohydrate complexes [8]. IR absorption bands were not observed at 790 and 1092 cm^{-1}, indicating the existence of silicon gel. However, a shoulder at 940 cm^{-1}, suggesting the association of Si-O with -CH_2-CH_3-, was observed in the IR spectrum as a result of silicon supply, which intensified strongly at 465 cm^{-1}, suggesting the existence of silicon (figure 6). The results obtained suggest that silicon in soluble pectin substances might exist as organic silicon because no signal due to silica gel was observed in the IR absorption band.

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水稲およびトマト細胞壁の形成に及ぼすケイ素の影響

稲永醇二† ・樗木直也・陳能場

要　約

ケイ素が不足すると，水稲では出穂が遅れ，またトマト，キュウリなどでは新葉にカーリングなどの奇形が生じる。水稲とトマトの細胞壁の形成に及ぼすケイ素の影響について検討を行った。水稲およびトマトをケイ素無添加または50mg L^{-1}のケイ素を含む水耕液で栽培し，水稲桿およびトマトの根，茎葉部から細胞壁を調整し，熱水可溶性ペクチン（F1），ペクチン（F2），酸化リグニン（F3），ヘミセルロース（F4），セルロース（F5）に分画し，質量とケイ素を測定した。

出穂日以降，水稲桿の細胞壁は約95％がF4およびF5であったが，両画分には処理間に有意差はなかった。しかし，F2はケイ素欠如により減少した。出穂前８月15日の細胞壁では，出穂の遅れによりF2およびF3がケイ素欠如により高くなった。ケイ素欠如にも拘わらず，出穂前の細胞壁および出穂日のF2F3のケイ素含量は無添加区が高い値を示した。一方，トマトでは，根，下・上茎葉部の細胞壁ともF2およびF3はケイ素欠如により低下した。また，F2のケイ素含量はケイ素添加により高くなった。

細胞壁のケイ素含量についてみると，水稲では出穂日以降，またトマトでは根，下茎葉部でF1にケイ素が著しく集積した。水稲およびトマトの根細胞壁からのF1をIRで調べると，シリカゲルの存在を示す790, 1090cm^{-1}に吸収スペクトルは両種とも観察されず，水稲ではケイ素の添加により467cm^{-1}の強度が増加し，また960cm^{-1}にショルダーが現れた。

これらの結果は，植物の細胞壁中のペクチン，リグニンの一次細胞壁の形成にケイ素は関与しており，ケイ素欠如により観察される水稲の出穂の遅れやトマトなどのカーリングは一次細胞壁の不十分な形成によるものと推定される。また根から吸収されたケイ素は，細胞壁では熱水可溶性ペクチン物質に集積されるが，その存在形態はシリカゲルではなく，有機物との結合が考えられた。

キーワード：ケイ素，細胞壁，ペクチン，リグニン，植物

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