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学　位　論　文　要　旨

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題目：Physiological and Biochemical Characterization of Root Cell Wall in *Suaeda salsa* and *Spinach oleracea* under Saline Condition

（塩分条件下における*Suaeda　salsa*と*Spinach　oleracea*の根細胞壁の生理と生化学的特性）

Soil salinization is one of the serious environmental factors limiting agricultural productivity. Improving crop salt tolerance on the basis of tolerance mechanisms will ultimately contribute to the crop production in the world. The growth response of halophytes and glycophytes to salinity can differ significantly. The study of the physiological responses of glycophytes and halophytes to salinity is useful for understanding the mechanisms underlying plant salinity tolerance. Recent studies on the mechanism of salt tolerance in plants have focused on the symplast pathway, while studies on the apoplast pathway are relatively limited. Root cell wall directly interacts with the salts present in the soil and plant. But the functions of root cell wall in plant salt tolerance are still unclear.

In this study we investigated cell wall composition, extensibility, and viscosity in the root elongation zone of young seedlings of halophyte (*Suaeda salsa*) and glycophyte (*Spinach oleracea* cv. ‘Akinokagayaku’), which both belong to the Amaranthaceae family. Furthermore, we investigated the salinity tolerance of three spinach cultivars (Helan 3, Prius β and R7) with a focus on pectin content, such as: pectin polysaccharides, the degree of pectin methy-lesterification (PMD) and pectin-related wall parameters in the cell walls. The objectives of this study were elucidating the interactions of cell wall composition, extensibility and root growth under salinity stress and pectin characteristics of root cell wall that contribute to root growth under salinity.

1. Chemical composition of root cell wall in relation with cell wall extensibility and root growth under salinity stress

2 days after germination, young seedlings of *Suaeda salsa* and *Spinach oleracea* were treated with a series concentration of NaCl (0, 100, 200 and 300 mM). After the seedlings were subjected to the salinity treatments for 8 days, root samples were collected and relative parameters were determined.

Root growth was inhibited by increased salinity in both species. The pronounced inhibition in the root growth in *S. oleracea* grown under salt stress showed relatively greater sensitivity to salinity when compared with *S. salsa*. The cell wall contents were much less in *S. oleracea* than *S. salsa*. With the increase in NaCl concentration, pectin contents were significantly decreased in *S. oleracea*. There was a significantly positive correlation between pectin contents and root growth in this species. In *S. salsa*, there was no decrease in the pectin content with salinity treatments. There was no difference in the contents of hemicellulose I or II in *S. oleracea* among all treatments. In *S. salsa*, there was a significant increase in the contents of hemicellulose I and II under 300 mM NaCl condition. Cellulose contents had not change after salinity treatments in both species. Salt treatments decreased the uronic acid contents in pectin fraction in *S. oleracea*. There was a significantly positive correlation between uronic acid contents in pectin fraction and root growth. In *S. salsa*, the uronic acid contents in pectin fraction were maintained at 100 mM NaCl but significantly decreased when NaCl concentrations elevated above 200 mM. In general, the tendencies of uronic acid in each cell wall fractions were similar to those fractions in response to the series of salinity treatments in either of the species. These results suggested that cell wall pectin plays important roles in root growth in both species under salinity, and that the salt tolerance of glycophyte *S. oleracea* is affected by the pectin. Cellulose limits root elongation under saline conditions in both species, but in halophytes, a high cell wall content and the proportion of cellulose in cell walls may be a salt tolerance mechanism that protects the stability of cell structure under salt stress.

Salinity treatments decreased the cell wall extensibility in *S. oleracea*. There was a significantly positive correlation between the extensibility and root growth in this species. While NaCl, up to 200 mM, increased the cell wall extensibility in *S. salsa*. Under 200 mM NaCl concentrations, cell wall extensibility was significantly higher in *S. salsa* as compared with *S. oleracea*. The similar tendency could be observed in the viscosity of root cell wall of the two species. The negative correlation observed in this study between E_0 and root growth in *S. salsa* and *S. oleracea* indicated that cell extensibility in the root elongation zone is an important limiting factor of root growth in both halophyte and glycophyte species under saline conditions.

In *S. salsa*, the plastic extension showed negative values, except in the 200 mM treatment. The negative value of plastic extension means that the root shrunk even shorter than the original length after extension. In contrast, this phenomenon was not found in *S. oleracea.* To date, there are no reports of root shrinkage exceeding the original measured root length, which indicates a negative plastic deformation in halophyte roots. This phenomenon might be due to the high cellulose content of the cell walls in this species. Cellulose chains can expand in a direction that is perpendicular to the cellulose chains after a tensile force is applied. This response of the cellulose chains to tensile force may have caused the vertical shrinkage observed in the *S. salsa* roots. A high amount of cellulose may provide tensile strength and crosslink sites that increase the cell wall stiffness and ensure the cell structure of *S. salsa* under salinity stress.

2. Pectin characteristics in relation with cell wall extensibility and root growth in spinach under salinity stress

2 days after germination, young seedlings of spinach cultivars, Helan 3, Prius β and R7 were treated with a series concentration of 0 and 200 Mm NaCl. After the seedlings were subjected to the salinity treatments for 6 days, root samples were collected and relative parameters were determined.

Cultivar R7 showed higher root growth under salinity stress compared with Helan 3 and Prius β. This indicated that the R7 cultivar had higher salt tolerance than the other two cultivars. The pectin content was significantly increased across the cultivars under salinity stress, whereas the molar proportion of uronic acid in pectin was significantly decreased in Helan 3 and R7 cultivars. There was a positive correlation between the molar proportion of uronic acid in pectin and the root length of the two cultivars. This result showed that pectic uronic acid was consistent with root growth in the two cultivars. A similar trend was observed in *S. salsa* and *S. oleracea* 'Akinokagayaku'. These results indicate that pectic uronic acid plays important roles in root growth in both species under salinity.

Salinity significantly reduced cell wall extensibility in all cultivars, and increased cell wall viscosity in Helan 3 and R7 relative to Prius β. Pectin content, the molar proportion of uronic acid in pectin and HG:RG-I ratio were significantly correlated with E_0 under salinity stress in Helan 3 and R7 cultivars. These correlations indicate that cell wall extensibility was affected by pectin in Helan 3 and R7 cultivars, which may have affected cell expansion under the stress condition. The molar proportion of uronic acid in pectin and the HG:RG-I ratio were significantly correlated with η_N across the cultivars; this is an indication that uronic acid in pectin and the RG-I backbone may have regulated cell wall viscosity during salinity stress in spinach.

In comparing with Helan 3 and Prius β cultivars, the high salt tolerance of the R7 cultivar was significantly correlated with the pectin characteristics. The length and degree of pectin methy-lesterification of neutral side chains were significantly decreased in the R7 cultivar, with no significant changes in the other two cultivars. The demethylation and increased side chains of pectin under salinity stress may lead to changes in cell wall elongation, and thus root growth, which fundamentally enhances plant growth under salt tolerance.

This study has shown that the root cell wall plays a crucial role in regulating root growth, and that differences in the properties of the root cell wall among different cultivars can affect their tolerance to saline stress. Specifically, characteristics of root cell wall such as higher amount of uronic acids and pectin, higher amount of cellulose, and demethylation and increased side chains of pectin were of importance for root growth under salinity stress.