(Format No. 13)

SUMMARY OF DOCTORAL THESIS

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Title: Physio-Biochemical Characteristics of Root Cell Wall in Salinity Tolerance Mechanisms in Wheat

(コムギの耐塩性機構としての根細胞壁の生理生化学的特性)

Wheat (*Triticum aestivum* L.) production has been severely affected by soil salinization. There is need to improve wheat production in salinized soils. The importance of studying salinity tolerance mechanisms in wheat cannot be overemphasized. So far, wheat salinity tolerance has been studied from the aspects of osmotic adjustment, membrane transportation, hormone regulation, signal transduction, etc. However, studies on root cell walls were very limited. This study was conducted to investigate the salinity tolerance mechanisms of wheat, focusing on the function of root cell wall. The objectives of this study were to elucidate the interactions of cell wall composition, extensibility, expansin expression, root extension, and root growth under salinity stress and characteristics of root cell wall that contribute to root growth under salinity. Two salt tolerant (JS-7, Xinchun-31 (XC-31)) and two salt sensitive (YL-15, GS-6058) spring wheat cultivars were selected as experimental materials. These cultivars were cultivated at 0 (control), 40, 80 and 120 mM NaCl concentrations. When root length showed significant difference among the cultivars, chemical compositions (pectin, hemicellulose I and II, cellulose, and uronic acid in each composition), extensibility, expansin expression and apoplastic pH in apical root (0-10 mm) cell walls, as well as cation exchange capacity of the whole root were investigated. The main results are described as follows:

1. Chemical compositions and properties of root cell wall in relation with root growth under salinity stress

Cultivars of JS-7 and XC-31 had higher root growth under salinity stress compared with YL-15 and GS-6058. This confirmed that the former is more tolerant to salinity stress than the latter. Salinity stress significantly decreased the pectin content in the elongation zone in all cultivars except JS-7. Hemicellulose I and II were significantly increased in the elongation and adjacent zones in sensitive cultivars under salinity stress. Similarly, the cellulose content increased significantly across the cultivars in both root zones. This increment was more pronounced in the sensitive cultivars than in the tolerant cultivars. The uronic acid content in pectin in the elongation zone was decreased significantly in the sensitive cultivars relative to the tolerant cultivars, conversely, the uronic acid content in hemicellulose showed a reversed tendency. The cation exchange capacity of the root cell wall was significantly lower in sensitive cultivars than the tolerant cultivars. A positive correlation existed between root growth and relative content of pectin in elongation zone, and cation exchange capacity of the whole roots. However, root growth and relative content of cellulose were negatively correlated. These results indicate that a high pectin content and cation exchange capacity, as well as low hemicellulose and cellulose contents in the cell wall benefit root growth and thus, tolerance under salinity stress conditions.

2. Extensibility of root cell wall in relation with root growth under salinity stress

The extensibility of root cell wall was significantly decreased in sensitive cultivars, whereas, that in tolerant cultivars was maintained at the same level as that in the control. Root extension and the differences between cultivars were largely dependent on elastic extension, which accounted for one-half to two-thirds of the total extension. Viscosity and the plastic extension of the root cell walls had no difference across the treatments and cultivars. The significant decrease in cell wall elasticity in the root elongation region was one of the factors that depressed root growth in sensitive cultivars under salt stress. The well-maintained elasticity of tolerant cultivars alleviated the depression of root growth by NaCl. Cell wall elasticity was positively correlated with the relative pectin and hemicellulose I contents and negatively correlated with the relative cellulose content. Under saline conditions, the relative hemicellulose II content was not altered in the sensitive cultivars; however, it decreased significantly in the tolerant cultivars. Therefore, changes in chemical composition of cell wall corresponded with the cell wall extensibility and root growth in wheat cultivars at different levels of salinity tolerance.

Salinity decreased the root cell wall extension significantly, especially in sensitive cultivars through an increased extension resistance, but, there were no significant effects on the tolerant cultivars. The elastic properties of root cell wall of wheat under salinity were more pronounced in root elongation as compared with the plastic properties. The increment in pectin and hemicellulose I better improved the elastic extension in the root cell wall, relative to the deposition of cellulose.

3. Specific expression of expansins in response to apoplastic pH under salinity stress

Salinity treatment significantly reduced apoplastic pH in apical root in both tolerant and sensitive cultivars. The apoplastic pH in elongation zone was about 6.28 under non-saline condition, while it decreased to about 5.3 under salinity in both cultivars. For the roots grown under the non-saline condition, the optimal pH for cell wall extension was 6.0 and 4.6 in tolerant and sensitive cultivars, respectively. In contrast, roots grown under salinity showed that the optimal pH for cell wall extension was 5.0 in the tolerant and 6.0 in the sensitive cultivars. Therefore, the apoplastic pH (5.3) under salinity was favorable to root extension in tolerant cultivars, but not in the sensitive ones. Expansin gene expressions in root cell wall were generally suppressed by salinity. Gene expressions of *TaEXPA3*, *TaEXPA6, TaEXPB1* and *TaEXPB10* were reduced in both cultivars under salinity stress. However, those of *TaEXPA5* and *TaEXPA8* in tolerant cultivars were increased under salinity. This increment may improve root extension under salinity. The expansin activity of the tolerant cultivar was significantly higher than that of the sensitive one. *TaEXPA8* mediated cell wall loosening especially at pH 5.0, whereas, *TaEXPA5* activated especially at pH 6.0. Under salinity stress with lowered apoplastic pH, expansins in the tolerant cultivar resulted in the maintenance of cell wall extensibility, whereas those in the sensitive cultivars had no such activity.

This study investigated the root cell wall from the aspects of chemical composition, physical property and expansin expression. Each component of the root cell wall has its own effect on root extension. The extension of root cell wall under saline conditions, with reduced turgor pressure, was adversely depressed in the sensitive cultivars, but maintained to some extent in the tolerant ones. The wall loosening corresponded to the elastic extension, which involved wall expansins and all other components. When the cell wall loosens, new wall materials fill in the space or bind to the old wall. These materials correspond with the plastic nature, i.e. the final elongation of the root. The present study revealed the regulation role of cell wall in root growth. Cultivar differences in salinity tolerance can be related to the property of root cell wall. Characteristics of root cell wall such as higher amount of uronic acids and pectin, lower amount of cellulose, and specific expansin expression were of importance for root extension and growth under saline stress.