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Review Article

Mechanisms of Salt Tolerance in Rice Plants: Cell Wall-Related Genes and Expansins

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Abstract

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Inhibition of crop productivity by salt stress is mainly due to its impact on crop growth. Cell wall is an important component of plant biomass. Salt stress inhibits growth through affecting cell wall loosening. Expansins are cell wall proteins that promote wall loosening. Thus, cell wall-related genes and expansins play roles in regulating salt stress tolerance. The present review summarizes the major research advances in elucidating the importance of cell wall-related genes and expansins in salt stress tolerance.

Key words: Cell wall, Expansin, Rice, Salt stress.

INTRODUCTION

Soil salinity is a severe abiotic stress in agriculture. About 20% of the world's cultivated lands are influenced by salinity (Wu & Cheng 2014). Rice is an important staple food for most if not all Asian people. Rice plants encounter many abiotic stresses, while salt stress is an important constraint limiting rice yield. Generally, rice is sensitive to salt stress. Sensitivity of rice to salt stress varies with the growth stage, very sensitive at young seedling stages and less so at reproduction (Flowers & Yeo 1981; Lutts *et al*. 1995). In contrast, rice is considered to be relatively more salt tolerant at germination (Heenan *et al*. 1988; Khan *et al*. 1997). The problem of salt stress can be improved by irrigation management practices in salt-affected area. However, this approach is not easy to implement on a large scale. Introduction of salt-tolerant varieties in the salt-affected area is another alternative to improve the problem of salt stress. It appears that development of salt-tolerant rice varieties is the most efficient approach to reduce yield caused by salt stress.

When growing in saline soils, roots have to cope with two types of stresses. The first of these is an osmotic stress resulting from salt concentration in the soil that results in lowered water potential and a consequent loss of cell turgor in roots. The second is ionic stress induced by changes in the concentrations of Na^+, Cl^- or both in the root-growing medium and within root tissues. These stresses in turn cause a reduction in the water uptake and inhibition of plant growth (Lin & Kao 2001). The growth inhibition caused by salt stress of plants is due to the restriction of growth by retarding cell extension or expansion. The plant cell wall is a rigid wall responsible for the size and the shape of the cell. It is a polymeric network of crystalline cellulose microfibrils embedded in a hydrophilic matrix of hemicelluloses, pec-

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tins and proteins (Varner & Lin 1989; Cho *et al*. 2009). The plant cell wall has high tensile strength and must be loosened to enable the cell to grow (or enlarge irreversibly) (Cosgrove 2005). Cell extension is driven by turgor generated by water uptake. In other words, wall expansion requires cell turgor pressure to stretch the cell wall and put the network of interlinked cellulose microfibrils under tension. Salt stress may restrict cell expansion by affecting the uptake rates of water, turgor generation and/or cell wall properties. It is now clear that changes in wall properties indeed occur during salt stress. Thus, salt stress may inhibit growth through affecting cell wall loosening. Alternatively, growth inhibition caused by salinity may be associated with cell wall stiffening (Neumann *et al*. 1994). Salt stress has been shown to regulate cell wall-related genes (Fan *et al*. 2013; Zagorchev *et al*. 2014). It is believed that the cell wall may play an important role in salt tolerance. Previously, we have described the regulatory role of compatible solutes, aquaporings, Na^+ transporters, and reactive oxygen species-scavenging systems in salt tolerance of rice plants (Kao 2015a, 2015b, 2017). Here, we briefly describe the possible role of cell wall-related genes in salt tolerance of rice plants. Expansins are proteins that promote wall lossening. Thus, the functional importance of expansns in salt tolerance of rice plants is also discussed in this review.

CELL WALL-RELATED GENES AND RICE SALT TOLERANCE

To understand the processes of cell wall biosynthesis, modification and degradation, Sharma *et al*. (2011) assessed the transcriptional dynamics during enzymatic removal and regeneration of rice cell walls in suspension cells over time. They demonstrated that rice lines carrying Tos17 mutations in genes upregulated during cell wall removal exhibit dwarf phenotype. The observed dwarf phenotypes suggest that these genes might be involved in cell wall-related function.

Porteresia coarctata is a halophyte which can grow in salt conditions corresponding to 500–650 mM NaCl (Sengupta *et al*. 2008). It is a close wild relative of rice and represents an important source of genes related to salt tolerance (Latha *et al*. 2004; Sengupta *et al*. 2008). There are reports showing that engineering of some genes related to inositol metabolic pathway from *Porteresia* can confer salt tolerance in tobacco (Majee *et al*. 2004) and in cultivated rice (Sengupta *et al*. 2008). In order to identify the candidate genes involved in salt tolerance, Garg *et al*. (2014) analyzed gene expression of *Porteresia* under salt stress conditions and demonstrated that one of the key metabolic pathways involved in salt tolerance is cell wall structures. These results suggest that genes related to cell wall structures are important for designing salt tolerance rice plants.

The knowledge of drought stress transcriptomes in rice are mostly relying on the comparative studies of diverse genetic background under drought conditions. A more reliable approach is to use near-isogenic lines (NILs) with a common genetic background but contrasting levels of tolerance to drought stress under water deficit. Moumeni *et al*. (2011) examined two pairs of NILs in IR64 background with contrasting drought tolerance. They found that genes, such as cellulose synthase-like family, are highly activated under drought stress in two tolerant NILs. Their data suggest that these genes in root tips of tolerant rice under drought stress resulted in enhanced root growth and elongation.

All the experiments discussed above seem to strengthen the idea that cell wall plays a central role in enhancing salt tolerance in rice plants.

EXPANSINS

Discovery

Growing plant cells characteristically exhibit acid growth. Isolated cell walls also exhibit this phenomenon. At neutral pH, the cell walls soon stop extension, but they rapidly extend when pH is lowered (Cosgrove 2000). This acid-induced extension is not merely a physical property of wall polysaccharides, but requires active wall proteins (Cosgrove 1989). When the acid-growth behavior of isolated walls is eliminated by heat or protease treatment, it can be almost fully restored by the addition of purified wall proteins (McQueen-Mason *et al*. 1992). The active wall proteins were named as expansins, which are hypothesized to stimulate cell wall extension by disrupting non-covalent interactions between hemicelllo-ses and crystalline cellulose microfibrils (Cosgrove 2005).

Nomenclature

Expansins were first identified from cucumber hypocotyl walls (McQueen-Mapson *et al*. 1992). The availability of the full-genome sequences of Arabidopsis and rice permits the genome-wide search of expansin gene sequences. The results of genome-wide search have revealed that expansin genes contain four subfamilies (Choi *et al*. 2006). They are α-expansin (*EXPA*), β-expansin (*EXPB*), expansin-like A (*EXLA*), and expansin-like B (*EXLB*). In rice, there are 34 *OsEXPA*, 18 *OsEXPB*, 4 *OsEXLA*, and 1 *OsEXLB* (AbuQamar 2014). It is now clear that expansin-like genes evolved from the common ancestor with *EXPA* and EXPB. However, we do not know the actual function of expansin-like genes. Recently, Boron *et al*. (2015) demonstrated that AtEXLA2 may function as a positive regulator of cell elongation in the dark-grown hypocotyl of Arabidopsis by possible interference with cellulose metabolism.

Role in growth of rice plants

Rapid intermodal elongation of deepwater rice occurs upon submergence (Kende 1998). The expression of α- and β-expansin genes is correlated with internodal growth (Cho & Kende 1997; Huang *et al*. 2000; Lee & Kende 2001). Rice coleoptile is also able to elongate under submergence. Expression of *OsEXPA4* and *OsEXPA2* in rice coleoptile is greatly induced by submergence (Huang *et al*. 2000). Choi *et al*. (2003) generated transgenic rice plants that expressed sense and antisense expansin gene *OsEXPA4*. They found that the overexpression and antisense of *OsEXPA4* enhance and decrease growth of rice seedlings, respectively. *OsEXPA8* is a root-specific α-expansin gene. Ma *et al*. (2013) provided direct evidence that overexpression of this root-specific expansin gene increases plant height, enhances leaf number, enlarges leaf size, and improves root system architecture (longer primary roots, more lateral roots and root hairs) (Ma *et al*. 2013). Atomic force microscopy (AFM) technology is now able to measure living cell wall stiffness alternation. They clearly demonstrated that overexpression of *OsEXPA8* enhances cell wall extensibility of suspension rice cells. In another experiment of the same group, the importance of *OsEXPA3* gene in root system development in rice was examined via RNAi strategy (Qiu *et al*. 2014). They provided strong evidence showing that RNAi rice plants exhibit root growth inhibition, which is mostly due to the poorer root system architecture. Recently, Zou *et al*. (2015) identified a rice β-expansin gene, *OsEXPB2*, which is predominately in roots of rice. Based on the results of subcellular localization assay, they found that *OsEXPB2* is localized in the cell wall. In order to know the functions of *OsEXPB2*, RNA interference (RNAi) was used to inhibit gene expression. They clearly showed that RNAi transgenic lines had less-developed root systems. In other words, *OsEXPB2* plays key role in regulation of root system architecture and has the potential to be utilized in transgenic root breeding to improve abiotic stress tolerance. It appears that expansins are indispensable for the growing tissues of rice seedlings.

Improvement of rice plants to salt stress

Halophytes are land plants that are able to grow under high salt conditions. Hwang *et al*.

(2013) isolated three α-expansin genes (*SjEXPAs*) from *Suaeda japonica*, a halophyte. They demonstrated that expression of *SjEXPAs* does not show any remarkable change at different NaCl concentrations, suggesting that *EjEXPAs* may play a role in the unaffected seedling growth of *S. japonica* in a high-salt environment. There are only a few reports have evaluated the effect of salt stress on the expression of expansin genes and the abundance of expansin proteins. From the two-dimensional proteome analysis, Pitann *et al*. (2009) have shown that a decrease in β-expansin protein in a salt-sensitive maize cultivar, whereas β-expansin level in a resistant cultivar is less affected by salt stress. Similarly, Geilfus *et al*. (2010) demonstrated that reduced expression β-expansin protein correlates positively with reduced shoot growth of salt-sensitive maize cultivar under salt stress. The decrease of this expansin protein is due to the down-regulation of *ZmEXPB2*, *ZmEXPB6*, and *ZmEXPB8* expression. In contrast, the maintenance of shoot growth in salt-tolerant maize cultivar is related to an unaffected β-expansin protein in the shoot. Improvement of salt tolerance has been shown in overexpression of a wheat expansin gene, *TaEXPB23*, and a rose expansin gene, *RhEXPA4*, in tobacco and Arabidopsis, respectively (Han *et al*. 2012; Lu *et al*. 2013). Recently, Qin *et al*. (2014) demonstrated that the expression of *OsEXPA3* is upregulated by NaCl stress, suggesting that *OsEXPA3* might have great potential in improving salt tolerance of rice plants. Further work will be required to examine the response of *OsEXPA3* or other expansin genes by transgenic and genetic studies.

CONCLUSIONS AND PERSPECTIVES

The adverse effect of Na^+ and/or Cl^- on plants is called salt stress. Crop yield is severely affected by salt stress. This occurs directly due to the impact of salt stress on crop growth. An important element to plant biomass is the cell wall. The role of the plant cell wall in growth is emerging in recent years. Endler *et al*. (2015) identified a protein family whose members are components of the cellulose synthesizing machinery in Arabidopsis. Their results revealed that mutation of these proteins led to salt-sensitive phenotypes and proposed a mechanism for how plants maintain the biomass producing capacity under salt stress conditions. It is probably that investigations into the role of sustained cellulose synthesis in rice plants during salt stress will be initiated. There is no doubt that expansins have the potential to be utilized as targets for improving stress tolerance of rice plants. However, much remains to be discovered about the role of expansins in salt stress tolerance of rice plants. Future research needs to emphasize on the genetic modification of expansin expression in rice plants.

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水稻耐鹽之機制：細胞壁相關基因與擴展蛋白

高景輝 [1,*]

摘要

高景輝。2017。水稻耐鹽之機制：細胞壁相關基因與擴展蛋白。台灣農業研究 66(2):87–93。

鹽分逆境影響作物生長進而降低產量。細胞壁是生質量 (biomass) 之主要成分。鹽分逆境經由影響細胞壁鬆軟而抑制生長。擴展蛋白 (expansins) 是鬆軟細胞壁之蛋白。因此，細胞壁相關基因與擴展蛋白對作物調控鹽分逆境耐性扮演重要功用。本文說明細胞壁相關基因與擴展蛋白在水稻耐鹽重要性之主要研究進展。

關鍵詞：細胞壁、擴展蛋白、水稻、鹽分逆境。

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