4-3-14 Isolation and characterization of aluminum tolerance genes in pigeonpea

Pigeonpea [Cajanus cajan (L.) Millsp.] is an important legume crop; sources of protein and a cash crop for millions of resource poor farmers. The STOP1 (Sensitive TO Proton rhizotoxicity 1), a C2H2 type zinc finger protein, regulates multiple genes (MATE, ALMT and ALS3) for Al\(^{3+}\) and H\(^{+}\) tolerance. In the present study, Al resistance in pigeonpea was examined by measuring root elongation of 3-d-old seedlings grown in hydroponically. The inhibition of root elongation was increased with increasing Al concentration and time. Additionally, first time we are reporting the major organic acid (Citrate) secretion responsible for acid soil tolerance during Al stress in pigeonpea. We isolated \textit{ASTOP1}, \textit{AIMATE1} and \textit{AtALS3} orthologous genes from pigeonpea by using degenerate PCR.

The CcSTOP1 amino acid sequence contained four conserved zinc-finger domains that were highly homologous to those found in previously identified STOP1 orthologues from various plant species. We characterized CcSTOP1 and CcMATE1 by using transient expression assay in tobacco leaf to study its sub-cellular localization, suggesting that CcSTOP1 and CcMATE1 localized to the nucleus and plasma membrane, respectively. Furthermore, we investigated the CcMATE1 expression pattern in root by qRT-PCR; revealed that Al rapidly induced the CcMATE1 expression. These results could be useful to establish breeding strategies for pigeonpea crops to develop an acid soil tolerance.

4-3-15 Characterization of two Al-induced half-size ABC transporters, FeALS1 with different size in buckwheat

Buckwheat is an Al-accumulator, which detoxifies Al by sequestering it into vacuoles in the leaves. However, the underlying molecular mechanisms are unknown. In rice and Arabidopsis, a tonoplast-localized half-size ABC transporter, OsALS1/AtALS1 has been reported to be involved in sequestration of Al into the vacuoles in their roots. Our previous RNA-seq data showed that there is a homolog of \textit{OsALS1}/\textit{AtALS1} in buckwheat, \textit{FeALS1}. However, 5'-RACE experiment revealed that there were two transcripts with different sizes: 1941 bp (\textit{FeALS1}-long) and 1308 bp (\textit{FeALS1}-short). \textit{FeALS1}-short was a truncated transcript of \textit{FeALS1}-long. \textit{FeALS1}-long was expressed in both the shoot and root, but \textit{FeALS1}-short was only expressed in the shoot, not in the root. Both \textit{FeALS1}-long and -short were up-regulated by Al but not affected by low pH or other metals. Transient assay using GFP fusion showed that \textit{FeALS1}-long was localized to the tonoplast, whereas \textit{FeALS1}-short was mainly localized to the plasma membrane in the buckwheat leaf protoplasts. Yeast assay showed that expression of \textit{FeALS1}-long resulted in increased sensitivity to Al, but accumulated similar Al compared with empty vector. However, expression of \textit{FeALS1}-short did not alter the sensitivity of the yeast to Al. These results suggest that \textit{FeALS1}-long and \textit{FeALS1}-short have different roles in internal detoxification of Al in buckwheat. Further characterization is being undertaken.

4-3-16 Functional characterization of an Al-inducible expansin gene, OsEXPA10 in rice

High aluminum (Al) tolerance of rice is controlled by a C2H2-type zinc finger transcription factor, ART1 (Yamaji et al. 2009), which regulates 31 genes. Here, we functionally characterized one of them, OsEXPA10, which encodes an expansin. OsEXPA10 was expressed in both the roots and shoots, but only the expression in the roots was up-regulated by Al. Furthermore, spatial expression analysis showed that the Al-induced expression was only found in the root tips (0.3 mm), but not in the mature root zones. The expression was neither induced by other metals including Cd and La nor by low pH. Immunostaining showed that OsEXPA10 was localized at all cells of the root tips.

Analysis with two independent T-DNA insertion lines showed that knockout of \textit{OsEXPA10} resulted in a significant decrease in the root growth and grain yield in the absence of Al. However, knockout of \textit{OsEXPA10} did not alter Al sensitivity. There was also not correlation between expression level of \textit{OsEXPA10} and Al tolerance in different rice cultivars. Expansins are cell wall proteins implicated in the control of plant growth via loosening of the extracellular matrix. Our results indicate that \textit{OsEXPA10} is required for normal growth of rice. The Al-induced upregulation of \textit{OsEXPA10} is probably due to the Al-induced inhibition of the root elongation.