MECHANISMS OF MN EFFICIENCY IN BARLEY

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The mechanisms of manganese (Mn) efficiency (genetic tolerance to Mn-deficient soils) in barley (*Hordeum vulgare* L.) were investigated at both physiological and molecular levels. The restriction of expression of Mn efficiency was observed in small pots. By using a pot of adequate size, genotypic differences in dry matter production and shoot Mn concentration were demonstrated in controlled conditions over a wide range of Mn supply. Thus, measurement of Mn concentrations of youngest expanded leaf blade or shoots was applied in soil-based pot screening as an index of Mn efficiency. This newly developed laboratory procedure has been proven to be robust, with low sensitivity to high seed Mn content and in variations in available soil Mn.

Soil culture experiments indicate that the basis of Mn efficiency is higher Mn acquisition from soil. Mn$^{2+}$ absorption was investigated further in a chelate-buffered nutrient solution. The results showed that unlike the soil culture, no clear genotypic differences in Mn accumulation of shoots were detectable over a range of Mn supply and over a range of pH. However, genotypic differences in Mn concentration of roots were observed at high pH in the same nutrient solution. These results show that the mechanism of Mn efficiency is likely to be a genotype ability in Mn mobilisation from soil. Thus, genotypic differences in Cu$^{2+}$ and Fe$^{2+}$ reductions were assessed, but no genotypic difference could be observed. Therefore, molecular aspects of Mn efficiency were explored to find genes which may be related to Mn efficiency. Two barley genes, *Idsl* and *Idsl2* from Japan, which are implicated in Fe acquisition, were tested for their connection with Mn efficiency. No genetic difference in *Idsl* expression was found between Mn-efficient and Mn-inefficient cultivars, but differential expression of *Idsl2* was found, which is inversely related to Mn efficiency. Attempts were made to isolate Mn efficiency-related genes. A root cDNA library was constructed from a Mn-efficient genotype and differentially screened with root cDNAs from a Mn-inefficient
More than one hundred putative clones were isolated. One of these clones, *Mne-1* was characterized because it appeared to be more abundant in the Mn-efficient plant under the low Mn conditions than in the Mn-inefficient plant by RNA gel blot analysis. DNA sequencing indicated that *Mne-1* encoded a zinc finger protein, novel in higher plants, showing a possible role in Mn efficiency through Mn binding or transcriptional regulation. For further insights into the functions of *Mne-1*, *Mne-1* recombinant protein was expressed in *E. coli*, and polyclonal antibodies to the recombinant *Mne-1* protein were raised. Protein gel blot analysis showed that the higher accumulation of *Mne-1* protein in roots of Mn-efficient plants was consistent with higher accumulation of *Mne-1* mRNA. Under low Mn conditions, the higher expression of *Mne-1* at both mRNA and protein levels is correlated to greater Mn efficiency. The analysis of metal contents showed that the recombinant *Mne-1* protein contained Zn but not Mn. This suggests that *Mne-1* may function as a transcriptional factor in adaptive response to low available Mn in soil to regulate genes responsible for Mn efficiency. Further applications of the *Mne-1* recombinant protein and anti-*Mne-1* antibodies will enable us to determine the transcriptional function of *Mne-1* gene, and thus increase the understanding of the role of the *Mne-1* in Mn efficiency. *Mne-1* is the first gene associated with differences in micronutrient efficiency traits, and a molecular marker for this gene may be useful for future breeding programs for South Australian soil conditions.