Enhancing Aluminium Resistance in Barley

through Over-expression of MATE Genes

by

Gaofeng Zhou

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Degree of Doctor of Philosophy

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DECLARATION

The thesis contains no material, which has been accepted for the award of any other degree or diploma in any tertiary institution, and to the best of my knowledge, contains no material previously published or written by any other person, except where due reference is made in the text of this thesis.

Gaofeng Zhou

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<table>
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<th>Description</th>
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<tbody>
<tr>
<td>2,4-D</td>
<td>2,4-dichlorophenoxyacetic acid</td>
</tr>
<tr>
<td>ALMT</td>
<td>aluminium-activated malate transporter</td>
</tr>
<tr>
<td>Amp</td>
<td>ampicillin</td>
</tr>
<tr>
<td>bp</td>
<td>base pair</td>
</tr>
<tr>
<td>BSA</td>
<td>bovine serum albumin</td>
</tr>
<tr>
<td>BW26</td>
<td>Bobwhite 26</td>
</tr>
<tr>
<td>cDNA</td>
<td>complementary DNA</td>
</tr>
<tr>
<td>d</td>
<td>day</td>
</tr>
<tr>
<td>ddH₂O</td>
<td>didistilled water</td>
</tr>
<tr>
<td>Dicamba</td>
<td>3,6-dichloro-o-anisic acid</td>
</tr>
<tr>
<td>DNA</td>
<td>deoxyribonucleic acid</td>
</tr>
<tr>
<td>DTT</td>
<td>dithiothreitol</td>
</tr>
<tr>
<td>E. Coli</td>
<td>Escherichia coli</td>
</tr>
<tr>
<td>EDTA</td>
<td>ethylene dinitrilotetraacetic acid</td>
</tr>
<tr>
<td>g</td>
<td>gram</td>
</tr>
<tr>
<td>GP</td>
<td>Golden Promise</td>
</tr>
<tr>
<td>h</td>
<td>hour</td>
</tr>
<tr>
<td>IPTG</td>
<td>isopropylthio-β-o-galactopyranoside</td>
</tr>
<tr>
<td>Kb</td>
<td>kilo base</td>
</tr>
<tr>
<td>L</td>
<td>Litre</td>
</tr>
<tr>
<td>MATE</td>
<td>multidrug and toxic compound extrusion</td>
</tr>
<tr>
<td>mg</td>
<td>milligram</td>
</tr>
<tr>
<td>min</td>
<td>minute</td>
</tr>
<tr>
<td>mL</td>
<td>millilitre</td>
</tr>
<tr>
<td>mRNA</td>
<td>messenger RNA</td>
</tr>
<tr>
<td>ng</td>
<td>nanogram</td>
</tr>
<tr>
<td>O.D.</td>
<td>optical density</td>
</tr>
<tr>
<td>oligo（dT）</td>
<td>oligodeoxythymidyl acid</td>
</tr>
<tr>
<td>PCR</td>
<td>polymerase chain reaction</td>
</tr>
<tr>
<td>QTL</td>
<td>quantitative trait loci</td>
</tr>
<tr>
<td>RG</td>
<td>root growth</td>
</tr>
<tr>
<td>RNA</td>
<td>ribonucleic acid</td>
</tr>
<tr>
<td>rpm</td>
<td>rounds per minute</td>
</tr>
<tr>
<td>RRG</td>
<td>relative root growth</td>
</tr>
<tr>
<td>s</td>
<td>second</td>
</tr>
<tr>
<td>SDS</td>
<td>sodium dodecyl sulfate</td>
</tr>
<tr>
<td>Tris</td>
<td>tris (hydroxymethyl) aminomethane</td>
</tr>
<tr>
<td>U</td>
<td>unit</td>
</tr>
<tr>
<td>µg</td>
<td>microgram</td>
</tr>
<tr>
<td>µL</td>
<td>microlitre</td>
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Abstract

Acid soils limit crop yields around the world due to nutrient deficiencies and mineral toxicities. Non-adapted plants grown on acid soils typically have shorter and thicker root systems because high concentrations of soluble aluminium (Al\(^{3+}\)) inhibit root elongation. This restricts their ability to acquire water and nutrients. An important mechanism of Al\(^{3+}\) resistance discovered in many plant species relies on the release of organic anions from roots. The gene controlling this trait are members of two gene families called the aluminium activated malate transporter (ALMT) family and multidrug and toxic compound exudation (MATE) family. Members of the ALMT family encode anion channels which release malate anions from roots while the MATEs encode co-transporter proteins which facilitate citrate release from roots. Although barley (*Hordeum vulgare*) is more sensitive to Al\(^{3+}\) toxicity than many other cereals including wheat (*Triticum aestivum*), rye (*Secale cereale*) and rice (*Oryza sativa*) significant genotypic variation in resistance does occur. This variation is controlled by citrate efflux from the root apices which is encoded by a MATE gene called *HvAACT1*. In this study three MATE genes from barley, *Arabidopsis* and sorghum (*Sorghum bicolor*) were transformed into the Al\(^{3+}\)-sensitive barley cultivar ‘Golden Promise’ with a constitutive promoter. These genes include the major Al\(^{3+}\)-resistance genes from barley and sorghum (*HvAACT1* and *SbMATE* respectively) and the *Frd3* gene from *Arabidopsis thaliana* which is important for iron nutrition. All three are known to encode transport proteins that facilitate citrate efflux from cells. The resulting transgenic lines were assessed for transgene expression, citrate efflux from root apices, and Al\(^{3+}\) resistance in hydroponic solution and acid soil. The control plants included in these experiments were null segregant lines and the parental barley cultivar. The Al\(^{3+}\)-resistant barley cultivar Dayton was also included as a positive control.

Barley cultivar “Golden Promise” was transformed separately with the MATE genes using the *Agrobacterium* method. Several independent T2 or T3 barley lines homozygous for each transgene were generated as well as null segregant lines. The transgenic lines released significantly more citrate from their root apices than the null controls. Plants expressing the *HvAACT1* and *SbMATE* genes required Al\(^{3+}\) in the external solution to activate citrate efflux while plants expressing *Frd3* released
citrate in the presence and absence of Al$^{3+}$. This is consistent with previous studies showing that HvAACT1 and SbMATE are Al$^{3+}$-activated proteins. The citrate efflux from the transgenic lines was similar to, or greater than, the efflux detected from cv. Dayton.

Transgenic and control seedlings were grown in an aerated hydroponic culture containing a simple nutrient solution with 0, 1, 2, or 4 µM AlCl$_3$ (pH 4.3). Net root growth was measured after 4 d. Relative root growth (growth in the Al$^{3+}$ solution relative to control solution) was significantly greater in the transgenic lines than the null controls for most Al$^{3+}$ treatments and similar results were obtained for the three MATE genes. The Al$^{3+}$ resistance of the transgenic lines was similar to the Al$^{3+}$ resistance of cv. Dayton.

Al$^{3+}$ resistance of the transgenic and control lines was also assessed in short-term soil experiments. The acidic ferrosol was either unamended (pH 4.33 with aluminium being 21% of exchangeable cations) or limed so that pH increased to 5.18 and only 1% of exchangeable cations was aluminium. After 6 d growth the following measurements were made: length of the longest and second-longest roots, total root length, total root weight, shoot weight and distribution of root diameters. In the unamended acid soil root growth of the null lines was inhibited compared to the limed soil and the roots became thicker. Expression of each of the MATE genes significantly increased Al$^{3+}$ resistance with relative length of the longest roots (root length in acid compared to limed soil) and relative total root length (total root length in acid compared to limed soil) providing the greatest differences between the transgenic and null lines. The transgenic lines also maintained a greater percentage of thinner roots in the acid soil than the null lines.

These results demonstrate that Al$^{3+}$ resistance in barley can be enhanced by heterologous expression of the ShbMATE and Frd3 genes or by over-expression of the endogenous HvAACT1 gene. Biotechnology provides important options for increasing the Al$^{3+}$ resistance of crop plants which can complement traditional breeding practices. Both strategies will be important for maintaining and even increasing food production on acid soils in the future.

Keywords: HvAACT1, citrate transporter, aluminium tolerance, transgene
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