

Plant Gene Register

wali6 and *wali7*¹Genes Induced by Aluminum in Wheat (*Triticum aestivum* L.) Roots

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Al inhibits plant root growth in acid soils (Clarkson, 1965). Despite a large amount of research there is no consensus on the physiological or molecular response of plants to Al (Taylor, 1991). The isolation and characterization of five genes induced by Al has been reported (Snowden and Gardner, 1993). Here we characterize two additional Al-inducible genes, *wali6* and *wali7*. These genes were isolated during differential screening of an Al-resistant (Waalt) and an Al-sensitive (Warigal) wheat cultivar.

The complete nucleotide sequences of *wali6* and *wali7* have been determined (Table I). *wali6* encodes a small protein that is related to the previously isolated *wali3* and *wali5* genes (Snowden and Gardner, 1993). All three proteins have a hydrophobic leader and show homology to Bowman-Birk proteinase inhibitors (Ikenaka and Norioka, 1986). Like most members of the family, *wali3*, 5, and 6 appear to be double-headed inhibitors. The first active site on all three proteins, Phe³⁹ to Ser⁴⁰, should inhibit chymotrypsin proteases (Laskowski and Kato, 1980), whereas the residues in the second site, Thr⁶⁴ to Ser⁶⁵, do not match the specificity of previously characterized inhibitors. The next most closely related protein in the data base is encoded by a wound-inducible gene from maize (Rohrmeier and Lehle, 1993), which has a related hydrophobic leader and two chymotrypsin-inhibiting sites in an overall Bowman-Birk structure.

The reading frame encoded by the nucleotide sequence of *wali7* is incomplete. However, comparison to a "stem-specific" tobacco gene (Seurinck et al., 1990) suggests that it may lack only the first A of the initiation codon. *Wali7* is 39% identical with this tobacco gene product over the first 149 amino acids, beyond which the sequences appear to diverge. The protein encoded by the entire coding region of *wali7* is also homologous to the N-terminal end of plant Asn synthetases (Table I). This region does not contain the active sites of the enzyme, and the significance of this conservation is currently not clear.

Northern hybridization was used to confirm Al induction of *wali6* and *wali7* (Table I). *wali6* mRNA followed the expression pattern of *wali3* and 5, with maximal induction 2

Table I. Characteristics of two Al-induced genes from wheat

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|-------------------------|---|
| Organism: | <i>Triticum aestivum</i> cv Warigal (<i>wali6</i>) and cv Waalt (<i>wali7</i>). |
| Location on Chromosome: | Unknown. |
| Sources: | cDNA library constructed from mRNA isolated from root tips of seedlings, in pSport (<i>wali6</i>) or λZap (<i>wali7</i>). |
| Method of Sequencing: | Subcloning of restriction fragments into plasmid vectors, automated dideoxy sequencing using an Applied Biosystems (Foster City, CA) model 373A sequencer. |
| Gene Products: | <i>wali6</i> : 641-bp cDNA; 88-amino acid open reading frame encoding a 9.4-kD protein including hydrophobic leader; protein is 92% identical with <i>wali3</i> , 78% identical with <i>wali5</i> (Snowden and Gardner, 1993), and 45% identical with maize WIP1 (Rohrmeier and Lehle, 1993); mature protein is homologous to Bowman-Birk proteinase inhibitors, including conserved Cys's, Asp's, and reaction-site Ser's (Ikenaka and Norioka, 1986). <i>wali7</i> : 1014-bp cDNA; ≥270-amino acid open reading frame; the first 150 amino acids are 39% identical with a stem-specific gene from <i>Nicotiana</i> (Seurinck et al., 1990); entire coding sequence is 21 to 23% identical with the N-terminal end of Asn synthetase genes from pea (Tsai and Coruzzi, 1990), asparagus (Davies and King, 1993), <i>Escherichia coli</i> (Scofield et al., 1990), and human (Andrulis et al., 1987). |
| Features/Expression: | Northern hybridizations (RNA samples as in figs. 1 and 2, Snowden and Gardner, 1993) show that both genes are induced in wheat roots by inhibitory Al concentrations. Induction occurs after 1 d exposure to Al; <i>wali6</i> peaks at 2 d; <i>wali7</i> expression continues to increase until at least d 4. |

d after Al exposure. *wali7* mRNA is normally present at low levels in wheat root tips, was induced by 24-h exposure to Al, and continued to increase for at least another 3 d. *wali7* mRNA was also present in leaves, but this expression was not increased when the plant roots were exposed to Al.

Received March 16, 1994; accepted March 26, 1994.

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The GenBank accession numbers for the sequences reported in this article are L28009 (*wali6*) and L28008 (*wali7*).

¹ The research was funded by research grant 93-AGR-06-101 from the Foundation for Research Science and Technology. K.C.S. was a recipient of a postgraduate scholarship from the University of Auckland.

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