THE MOLECULAR RESPONSE OF WHEAT ROOTS TO ALUMINIUM STRESS

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The most important words in science aren't "Eureka! I have found it!", but "that's funny..."

I. Asimov

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ABSTRACT

Aluminium (Al) toxicity to plants is a significant problem, limiting agricultural production in up to 40% of the world's arable soils. In spite of a large amount of research, there is still no consensus on the physiological mechanisms of Al toxicity in plants. In addition, very little is known about the molecular response of plants to Al stress. This body of research was aimed at identifying the changes in gene expression that occurred in the root tips of plants that had been stressed with Al.

A cDNA library made from the root tips of Al-treated wheat (*Triticum aestivum* L., cultivar Warigal) plants was differentially screened to identify clones whose expression was induced by Al stress. Seven cDNA clones, representing five different genes were identified as being induced in the presence of Al. Initial sequencing and northern analysis revealed that none of the clones isolated were full-length, and that some contained multiple cloning adaptors at their 5' ends. A new cDNA library was then constructed from the root tips of Al-treated Warigal plants, and homologues to each of the original five genes were isolated. These five clones were named wali1 to wali5 (for wheat aluminium induced).

Northern analysis showed that wali1, -3 and -5 were induced 24 to 96 h after Al treatment, and their expression declined when the Al was removed. wali4 had a similar pattern of expression with a transient increase in expression also observed after 0.5 h of Al stress. Each of these four genes was induced by inhibitory concentrations of Al in two wheat cultivars - Warigal, an Al-sensitive cultivar, and Waalt, an Al-tolerant cultivar, - and also in two inbred lines of wheat, RR (Al-tolerant) and SS (Al-sensitive). The fifth gene (wali2) had a bimodal pattern of induction, and was induced by Al only in the Al-sensitive Warigal and the Al-tolerant RR.

The nucleotide sequence of each of the wali clones was determined, and the databases were searched for homologous sequences. wali1 was found to be homologous to a group of metallothionein-like proteins (MLPs) from plants, and wali4 was homologous to phenylalanine ammonia-lyase (PAL). wali3 and wali5 encode related, cysteine-rich proteins with homology to Bowman-Birk proteinase inhibitors, and wali2 encodes a novel protein with a repeating motif of cysteine amino acids.

The induction of the wali genes was investigated in response to a number of other stresses through northern analysis. The expression of wali1, -3, -4 and -5 was induced in root tips of wheat after 2 d treatments with toxic levels of all other metals tested (Cd, Fe, Zn, Cu, Ga, In and La). The expression levels of wali1, -3, -4, and -5 also increased in the root tips of plants grown in the presence of low levels of Ca (10 μM). The transcript levels of wali1, -3 and -5 increased in wounded leaf and root tissue, whereas the transcript levels of wali4 increased only in wounded leaves. The expression of

wali2 was greatly reduced by low concentrations of Ca, and showed no induction, or a variable response with most of the other treatments.

The site of expression of wali1, -2, -3 and -5 in root tips (and wali1 also in leaf tissue) was identified using *in situ* hybridisation. wali1 was expressed predominantly in the meristematic tissue of the root tip, while wali3 and wali5 were expressed predominantly in the cortical tissue of the root. wali2 expression was detected primarily in the epidermis and root cap. Some changes in the site of expression of these genes were evident in the roots of Al-treated plants. In leaf tissue, wali1 expression was found in the mesophyll layer of cells.

The coding sequences for wali1, -2, -3 and -5 were each cloned into the bacterial expression vector pGEX-2T. The resultant fusion proteins between glutathione S-transferase (GST) and the walis were then successfully purified from *E. coli*. Antibodies were made to the wali1-GST fusion protein and purified by immunoaffinity chromatography. However, when used in western analysis, no specific bands corresponding to the native wali1 protein were identified. The wali2-GST protein was used in a south-western procedure to determine if the protein was capable of binding DNA, but no DNA binding to this protein was detected under the conditions tested. The wali3 and wali5 fusion proteins were tested in proteinase inhibitor assays, where no inhibition of either trypsin or chymotrypsin was detected. It is possible that the native wali3 and wali5 proteins may not function as proteinase inhibitors, or that the lack of activity detected for the fusion proteins may be due to incorrect folding or processing in the bacterial system.

This research constitutes the first identification of plant genes whose expression is increased by Al stress. The genes identified are also induced in response to other environmental and nutrient stresses, indicating that they form part of the plant's general response to stress.

ABBREVIATIONS

Standard SI units, chemical abbreviations and the one and three letter codes for amino acids and nucleotides have been used throughout this thesis, as well as the abbreviations that follow:

aa	amino acid(s)
ATP	adenosine triphosphate
bp	base pair(s)
BSA	bovine serum albumin
CNBr	cyanogen bromide
cv	cultivar
C-terminus	carboxy-terminus (of a polypeptide)
dCTP	deoxycytidine-5'-triphosphate
DIG	digoxygenin
DMDC	dimethyl-dicarbonate
DNA	deoxyribonucleic acid
DNAse	deoxyribonuclease I
dNTP	deoxyribonucleotide triphosphate
DMSO	dimethyl sulphoxide
DTT	dithiothreitol
EDTA	disodium salt of ethylenediaminetetraacetic acid
g	gravity
GCG	Genetics Computer Group
GST	glutathione S-transferase
IPTG	isopropyl-β-D-thiogalactoside
kb	kilobase(pair)s
kDa	kilodaltons
MLP	metallothionein-like protein
mRNA	message RNA
NBT	nitroblue tetrazolium chloride
N-terminus	amino-terminus (of a polypeptide)
OD_x	optical density - the absorbance measured at a wavelength of x nm
ORF	open reading frame
PAL	phenylalanine ammonia-lyase
PCR	polymerase chain reaction
pfu	plaque forming units
PIPES	piperazine-N-N'-bis (2-ethane sulfonic acid)

PVDF	polyvinylidene difluoride
PVP	polyvinylpyrrolidone
RH	relative humidity
RNAse	ribonuclease
SDS	sodium dodecyl sulphate
SE	standard error
Tris	Tris(hydroxymethyl)-aminomethane
UV	ultraviolet (light)
v/v	volume to volume (with respect to the percentage of a solution)
X-Gal	5-bromo-4-chloro-3-indolyl-β-D-thiogalactoside
X-phos	5-bromo-4-chloro-3-indolyl-phosphate