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Physiological characterisation of genes contributing to enhanced aluminium resistance in yeast

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Abstract

A cDNA library of *Saccharomyces cerevisiae* was overexpressed in the yeast strain INVSc2. The transfomants were screened for increased Al resistance and several of the overexpressed cDNAs were found to confer Al resistance. These transformants were tested for their ability to secrete organic acids. None of the clones that conferred Al resistance showed an increased efflux of malate, suggesting that, unlike for some plant species, the complexation of Al by malate was not responsible for the Al-resistance. All of the cDNAs that conferred Al resistance were sequenced. Apart from genes of yet unknown function, several genes were found to increase Al resistance. One gene encodes the protein for a Δ 9 acyl desaturase, which altered the fatty acid composition of yeast. Preliminary experiments were conducted to assess the effect of Al on Arabidopsis mutants with altered fatty acid synthesis or desaturation.

Introduction

Aluminium is the major factor reducing plant growth on acid soils. The first visible sign of Al toxicity is a reduction in root growth. Mechanism(s) through which Al toxicity or resistance are achieved are not yet fully understood. Yeast (*Saccharomyces cerevisiae*) is frequently used as a model system for plant cells as it shares many similar mechanisms for ion uptake.

We therefore tried to identify genes that could confer Al resistance to yeast. We also assessed if similarities can be found between the way yeasts and plants cope with high Al supplies.

Materials and methods

The yeast strain INVSc2 (Invitrogen; MATa his3-Δ1 ura3-52) was transformed with a cDNA library from the yeast strain INVSc1 (provided by F. Smith, CSIRO, Queensland, Australia), using the expression vector pYES2 (Invitrogen, 2µ, URA3, GAL1p), which uses the gal-promotor to induce the inserted genes. Genes that were found to increase Al resistance were isolated and partially sequenced.

Malate efflux: overnight cultures of the transformants were incubated for one day in a medium that contained 2% (w/v) galactose, to express the inserted gene. The cultures were then adjusted to an OD_{600} of 3-4, centrifuged and resuspended in the same volume of fresh nutrient solution. After 4 h, malate in the incubation medium was determined enzymatically (Gutman and Wahlefeld, 1974).

Determination of Al toxicity in Arabidopsis mutants: seeds were germinated under sterile conditions in transparent plastic containers filled with 0.5% agarose in nutrient solution (pH 4.5) with the various Al concentrations. The containers were wrapped in black plastic foil to protect the roots from light. The nutrient solution had the following composition (µM): KNO_3 500, $CaCl_2$ 500, NH_4NO_3 500, $MgSO_4$ 150, KH_2PO_4 10, $FeCl_3$ 1,6, $MnSO_4$ 1, H_3BO_3 5, $CuSO_4$ 0,05, $ZnSO_4$ 0,2, $NaMoO_4$ 0,02, $CoCl_2$ 0,001, pH 4,5.

Arabidopsis mutants CS8041 (deficient in 18:1 fatty acid desaturase), CS8035 (slight increase in linolenic acid), CS8034 and CS808036 (reduction in desaturation of storage and membrane lipids) were acquired from the Arabidopsis Biological Research Center, Ohio State University.

Results

Clones in which overexpression of yeast cDNAs lead to increased Al resistance were selected and used for a second transformation to ensure that the observed increase was plasmid encoded and not due to a spontaneous mutation in the yeast genome. Mutants that still showed an increased Al resistance after this second transformation were sequenced (Table 1). Three of these cDNAs encoded for proteins of unknown function. Hydrophylicity plots using the computer program TM with a window size of 20 were performed and they showed that the cDNAs YLR 350w and YLR 157w code for probable membrane proteins.

Organic acid anion efflux is a well documented Al-resistance mechanism in plants and it is also known that yeast secrete organic acid anions (Carmelo *et al.*, 1997). Salmon (1987) has suggested that the secretion of organic acid anions by yeast involves active transport. All transformants with an increased Al resistance were therefore tested for their ability to secrete organic acid anions (Fig. 1). However, none of the transformants showed a strong increase in malate efflux. Some even showed a slight decrease in malate efflux compared to the control.

The transformants expressing the OLE1 cDNA, that encodes a Δ 9 acyl desaturase, were investigated further.

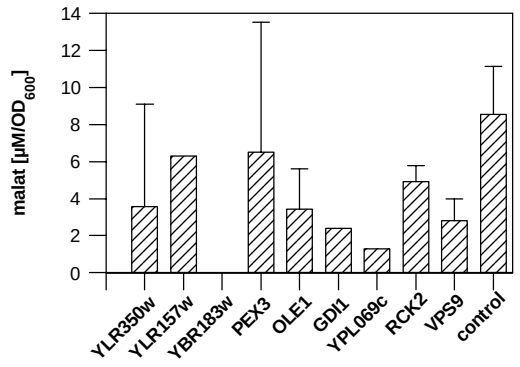

Figure 1. Malate effux of yeast transformants. Net-efflux was measured after 4 h of incubation in nutrient solution.

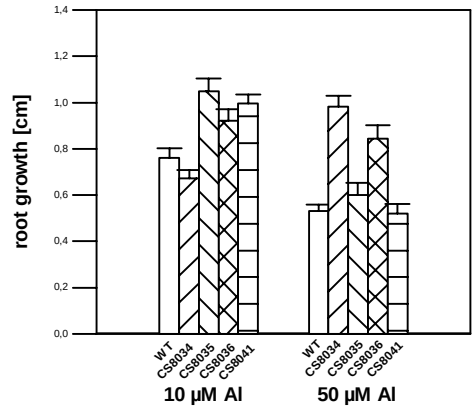

Figure 2. Growth of *Arabidopsis thaliana* in the presence of Al. Root growth was measured 35 days after sowing.

Analysis of their fatty acid composition showed a considerable change in fatty acids (data not shown). A large increase in oleic acid was accompanied by a large decrease in stearic acid. We hypothesised that Al resistance of yeast cells expressing OLE1 was due to a change in desaturation of membrane lipids.

To test the effect of membrane fatty acid composition on the Al-resistance of plants Arabidopsis mutants with defects in fatty acid synthesis or desaturation were tested for their Al resistance. However, no difference in their Al resistance consistent with their changes in fatty acid composition could be found (Fig. 2). Due to the high proton sensitivity of Arabidopsis the germination rate without Al was strongly reduced, so that only different Al concentrations could be compared.

Discussion

Expression of the OLE1 cDNA in yeast lead to a considerable increase in Al resistance which was accompanied by an increase in oleic acid content. However, yeast was able to counteract these changes by reducing other fatty acids, including both saturated and non saturated forms (data not shown).

However, for the Arabidopsis mutants in fatty acid synthesis and desaturation, no difference in their Al sensitivity consistent with their changes in fatty acid composition could be found. This lack of differences could be due to the high proton sensitivity of Arabidopsis, that might mask existing differences between the mutants. Alternatively, the mutants may be able to compensate for the defects in fatty acid synthesis by altering the activity of other enzymes involved in fatty acid biosynthesis and desaturation.

The genes found to increase Al resistance in yeast point to the plasma membrane and to the cytoplasm as the most likely sites of Al resistance mechanisms. Changes in plasma membrane characteristics as a result of exposure to Al are well documented in plants (Horst, 1995). The observations that the site of Al toxicity may be the same in plants as in yeast, make yeast a useful tool to identify genes involved in Al resistance mechanisms of plants.

References

Carmelo V, Santos H and Sa-Correiea I 1997 Biochim. Biophys. Acta 1325, 63-70.

Cassio F, Leao C and Uden N van 1987 Appl. Envrionm. Microbiol. 53 (3), 509-513.

Gutman I and Wahlefeld AW 1974 *In* Methods of Enzymatic Analysis. Ed. HU Bergmeyer. pp. 1585-1589. Academic Press, New York.

Salmon JM 1987 Biochim. Biophys. Acta 901, 30-34.

Horst WJ 1995 Z. Pflanzenernähr. Bodenkunde 158, 419-428.

Table 1. Genes found to increase Al resistance in yeast

Gene	Possible function
YLR350w	Unknown function, possible membrane protein
YLR157w	Unknown function, possible membrane protein
YBR183w	Unknown function
PEX3	Involved in peroxisome biogenesis and synthesis
OLE1	Δ 9 acyl desaturase
GDI1	GDP dissociation inhibitor, involved in yeast secretory pathway
YPL069c	Farnesyltranstransferase
RCK2	Protein kinase
VPS9	Involved in golgi to vacuole traffic