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A possible role of sphingolipids in the aluminium resistance of yeast and maize ☆

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Summary

The plasma membrane is most likely the major target for sensing of aluminium (Al), leading to inhibition of plant root-growth. As a result of high external Al, alterations in plasma membrane composition may be expected in order to maintain its properties. As sphingolipids are characteristic components of this membrane, their involvement in membrane adjustment to increased Al concentrations was investigated. Heterologous expression of a stereounselective long-chain base (LCB) (8*E*/*Z*)-desaturase from *Arabidopsis thaliana*, *Brassica napus* and *Helianthus annuus* in *Saccharomyces cerevisiae* improved the Al resistance of the transgenic yeast cells. This encouraged us to investigate whether Al affects the LCB composition, and whether genetic engineering of the LCB profile modifies the Al resistance of the Al-sensitive plant species maize (*Zea mays*, L.). Constitutive expression of the LCB (8*E*/*Z*)-desaturase from *Arabidopsis thaliana* in maize roots led to an 8- to 10-fold increase in (8*E*)-4-hydroxysphing-8-enine in total roots. Less marked but similar changes were observed in 3 mm root apices.

Abbreviations: Al, aluminium; CMedium, complete minimal drop-out uracil medium; DNP, 2,4-dinitrophenyl; PE, Pachyman equivalents; PM, plasma membrane; SL, sphingolipids; SE, standard error; t18:1^{8Z}, (8*Z*)-4-hydroxysphing-8-enine; t18:1^{8E}, (8*E*)-4-hydroxysphing-8-enine; d18:$2^{4E,8Z}$, (4*E*,8*Z*)-sphinga-4,8-dienine; t18:0, 4-hydroxysphinganine; d18:$2^{4E,8E}$, (4*E*,8*E*)-sphinga-4,8-dienine

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Al treatment of the Al-sensitive maize cv Lixis resulted in a significant increase in the proportion of (8*Z*)-LCB and in the content of total LCBs in root tips, which was not observed in the Al-resistant cv ATP-Y. When root tips of transgenic plants were exposed to Al, only minor changes of both (8*Z*)- and (8*E*)-unsaturated LCBs as well as of the total LCB were observed. Al treatment of the wild type parental line H99 decreased the (8*Z*)-unsaturated LCBs and the total LCB content. Based on Al-induced callose production, a marker for Al sensitivity, the parental line H99 was as Al-resistant as cv ATP-Y, whereas the transgenic line became as sensitive as cv Lixis. Taken together, these data suggest that, in particular, the loss of the ability to down-regulate the proportion of (8*Z*)-unsaturated LCBs may be related to increased Al sensitivity.

Introduction

Aluminium (Al) makes up 7.3% of the earth's crust, and is the third most abundant element. When soils become acidic as a result of natural processes or human activities, Al is solubilized in the form of the phytotoxic trivalent cation Al^{3+}. Micromolar concentrations of Al^{3+} inhibit root growth of many agriculturally important plant species such as maize, wheat and barley (Kochian, 1995, 2004). Therefore, Al toxicity is one of the major factors limiting plant productivity in acidic soils (von Uexküll and Mutert, 1995). Because of the rapidly growing world population, future attention must be focused on improvement of the agricultural production in acidic soils by developing Al-resistant crops and pastures. However, efficient breeding strategies aiming at the rapid improvement of Al resistance through genetic engineering are limited by a lack of knowledge regarding the physiological and molecular mechanisms of Al toxicity.

Several studies have shown that Al^{3+} binds to carboxyl and phosphoryl groups of the cell wall and the plasma membrane (PM) (Horst, 1995; Rengel, 1996). Binding of Al^{3+} to negatively charged phosphate groups of phospholipids leads to a decrease of membrane fluidity in the microorganism *Thermoplasma acidophilum* and of unilamellar phospholipid vesicles (Vierstra and Haug, 1978; Deleers et al., 1986; Akeson and Munns, 1989). Staß and Horst (1995) proposed that binding of Al induces a stronger association of membrane phospholipids and a higher packing density of phospholipids, reducing PM permeability. Furthermore, a rapid decrease of PM permeability following temporal exposure of protoplasts isolated from root-tip cells to Al ions is correlated with Al sensitivity among a variety of plant species (Ishikawa and Wagatsuma, 1998). After exposing roots to Al, a higher proportion of phosphatidylcholine at the expense of phosphatidylethanolamine and a lower content of free sterols were observed in PMs isolated from roots, and appear to be characteristic features of Al-resistant lines (Lindberg and Griffiths, 1993; Zhang et al., 1997). These changes in lipid composition may lead to less ordered PMs, and may compensate for the Al-induced decrease in membrane fluidity. Additionally, an Al-induced decrease of the proportion of less ordered PM domains has been observed by electron paramagnetic resonance spectroscopy *in situ* in mycelia of an Al-sensitive fungus (*Amanita muscaria*, Zel et al., 1993a). On the other hand, an increase in the proportion of less ordered PM domains, i.e. in lipid fluidity, was observed in an Al-resistant fungus (*Lactarius piperatus*, Zel et al., 1993b). Combined, these studies suggest that specific changes in the lipid composition of the PM may contribute to maintenance of root growth under Al stress.

Glucosylceramides and related sphingolipids (SLs) are characteristic components of plant PM (Warnecke and Heinz, 2003). Animal ceramides and sphingomyelin are known to increase the stability and decrease the permeability of membranes as a consequence of intra- and intermolecular hydrogen bonding between amide and hydroxyl groups of the ceramide moiety (Massey, 2001). Moreover, the glycosylceramides are greatly enriched in lipid rafts of animal cells with important structural and signaling functions (Brown and London, 2000). Rafts are also present in plant PM (Peskan et al., 2000; Mongrand et al., 2004).

In contrast to mammals and baker's yeast, plant SLs contain additional Δ^8-unsaturated LCB of (*E*)- and (*Z*)-configuration such as 4-hydroxy-8-sphingenines (t18:1^8) and sphinga-4,8-dienines (d18:$2^{4,8}$). Though plant SLs show this characteristic and predominating structural feature of a Δ^8-unsaturation differing from modifications found in mammals and *Saccharomyces cerevisiae*, much less is known about its function (Sperling and Heinz, 2003). To date, SLs have not been taken into consideration in studies dealing with Al-induced