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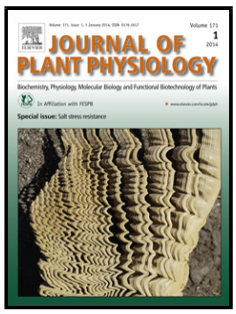

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Localization of calreticulin and calcium ions in mycorrhizal roots of *Medicago truncatula* in response to aluminum stress

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Abstract

Aluminum (Al) toxicity limits growth and symbiotic interactions of plants. Calcium plays essential roles in abiotic stresses and legume-*Rhizobium* symbiosis, but the sites and mechanism of Ca^{2+} mobilization during mycorrhizae have not been analyzed. In this study, the changes of cytoplasmic Ca^{2+} and calreticulin (CRT) in *Medicago truncatula* mycorrhizal (MR) and non-mycorrizal (NM) roots under short Al stress [50 µM $AlCl_3$ pH 4.3 for 3h] were analyzed.

Free Ca^{2+} ions were detected cytochemically by their reaction with potassium pyroantimonate and anti-CRT antibody was used to locate this protein in *Medicago* roots by immunocytochemical methods.

In MR and NM roots, Al induced accumulation of CRT and free Ca^{2+}. Similar calcium and CRT distribution in the MR were found at the surface of fungal structures (arbuscules and intercellular hyphae), cell wall and in plasmodesmata, and in plant and fungal intracellular compartments. Additionally, degenerated arbuscules were associated with intense Ca^{2+} and CRT accumulation. In NM roots, Ca^{2+} and CRT epitopes were observed in the stele, near wall of cortex and endodermis.

The present study provides new insight into Ca^{2+} storage and mobilization in mycorrhizae symbiosis. The colocalization of CRT and Ca^{2+} suggests that CRT is essential for calcium mobilization for normal mycorrhiza development and response to Al stress.

Key words: aluminum; arbuscule; calreticulin; intracellular Ca^{2+}; mycorrhizae

1. Introduction

Aluminum (Al) is one of the prevalent metals generating phytotoxicity due to its strongly negative effects on plant root growth (Kochian et al. 2005), and symbiotic interactions with arbuscular mycorrhizal (AM) fungi (Borie and Rubio 1999; Cumming and Ning 2003), plant growth-promoting bacteria (Burd et al., 2000) and *Rhizobium* bacteria (Pajuelo et al., 2011). Al causes primary injury in the apoplast of peripheral root cells, where it interferes with essential processes such as cell wall assembly, ion fluxes, plasma membrane (Kochian et al., 2005) and plasmodesmata properties (Sivaguru et al., 2000). Al also has a negative effect on symplastic targets such as the cytoskeleton of root cells (Barlow and Baluška, 2000) and nuclei of meristematic root cells (Silva et al., 2000).

AM symbiosis occurs between the majority of plants and fungi of the *Glomeromycota* (Wang and Qiu 2006). The main function of AM is related to the acquisition of water and nutrients for the plant (Clark and Zeto 2000; Jeffries et al. 2003; Harrison 2005) through extraradical hyphae. In *Arum*-type mycorrhizas, the intraradical hyphae elongate in the apoplastic spaces between cortical cells, in which tree-like arbuscules are formed. A host-derived membrane, the periarbuscular membrane (PAM), surrounds arbuscule branches, and an apoplastic space (interface) forms between this membrane and hyphal cell walls (Gianinazzi-Pearson 1996). The symbiotic interface is a specialized apoplastic zone composed of fungal and plant cell-wall material and is specialized for nutrient exchange between the symbionts (Gianinazzi-Pearson 1996; Peterson and Massicotte 2004; Smith and Read 2008). In addition, AM symbiosis is thought to be essential for plant survival in harsh environments and heavy metal polluted areas (Borie and Rubio 1999; Cumming and Ning 2003; Finlay 2008; Smith and Read 2008; Bunn et al. 2009; Bano and Ashfaq