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Short communication

Pectins esterification in the apoplast of aluminum-treated pea root nodules

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ABSTRACT

Aiming to elucidate the possible involvement of pectins in aluminum-mediated growth inhibition the distribution of pectins in the apoplast of root nodules was investigated. Experiments were performed on the pea (*Pisum sativum* L.) root nodules treated with aluminum (50 μM $AlCl_3$, for 2 or 24 h). For histochemical acidic pectin localization we used ruthenium red staining. Immunolabeling techniques with monoclonal antibodies specific to high methyl-esterified pectin (JIM7), low methyl-esterified pectin (JIM5) and calcium cross-linked pectin (2F4) were used to re-examine the pattern of pectin esterification and distribution. After immunolabeling the samples were observed using a fluorescent and transmission electron microscope.

Ruthenium red staining showed that acid pectin content increased in the apoplast of Al-treated nodules and immunolocalization of pectin epitopes revealed that the fraction of de-esterified pectins increased significantly under Al stress. JIM5 and 2F4 epitopes were located on the inner surface of the primary cell wall with higher intensity at cell corners lining the intercellular spaces and at infection threads (ITs) walls. By contrast, JIM 7 labels all walls uniformly throughout the nodule. In the presence of Al, the increase of JIM5 and 2F4 labeling in thick plant and IT walls, together with a decrease of JIM7 labeling was observed.

These results indicate a specific role for pectin de-esterification in the process of wall thickening and growth inhibition. In particular, Al-dependent increase in pectin content and their low methyl esterification degree correlate with wall thickness and higher rigidity, and in this way, may affect IT and nodules growth.

Introduction

One of the most obvious symptoms of aluminum (Al) toxicity in plants is the rapid inhibition of root growth (Kochian et al., 2005). Exposure to Al stress modifies cell wall composition and properties (Tabuchi and Matsumoto, 2001) causes accumulation of polysaccharides, glycoproteins and lignin, resulting in the typical thick and rigid cell wall (Horst et al., 1999; Sivaguru et al., 2000; Ma et al., 2004; Jones et al., 2006; Zhu et al., 2012). One of the factors increasing wall rigidity is pectin (Jarvis, 1992; Horst, 1995; Taylor et al., 2000; Ma et al., 2004). Pectins are abundant in the primary cell walls, where they function in regulating intercellular adhesion, and are greatly reduced or absent in non-extendable secondary cell walls (Willats et al., 2001). Pectins are secreted into the wall as highly methyl esterified forms and wall loosening seems to be related to the high degree of esterification, which is modulated through pectin methylesterase (PMEs) (Micheli, 2001; Willats et al., 2001; Maron et al., 2008). The removal of methyl ester groups results in the pectin's capability of being cross-linked by calcium ions and formation gels, which changes the pH and charge in the cell wall (Carpita and Gibeaut, 1993). In the presence of Al, calcium ions are substituted by Al^{3+}, thereby reducing cell wall extensibility and inhibiting root growth (Horst, 1995; Chang et al., 1999; Taylor et al., 2000; Ma et al., 2004; Tabuchi and Matsumoto, 2001). Schmohl and Horst (1999, 2000) found a close negative correlation between the degree of methylation of pectin and Al accumulation in maize suspension cells. Cell wall pectin of Al-resistant genotypes may have a higher degree of methylation and thus a lower Al accumulation than Al sensitive cultivars (Eticha et al., 2005).

Al has been shown to adversely affect the nodulation process through inhibition of lateral root extension (Silva et al., 2001). Al stress affects nitrogen fixation and assimilation (Balestrasse et al., 2006). Moreover, it has been demonstrated that Al induce

Abbreviations: HG, homogalacturonan; Al, aluminum; MX, infection thread matrix; IT, infection thread; W, infection thread wall.

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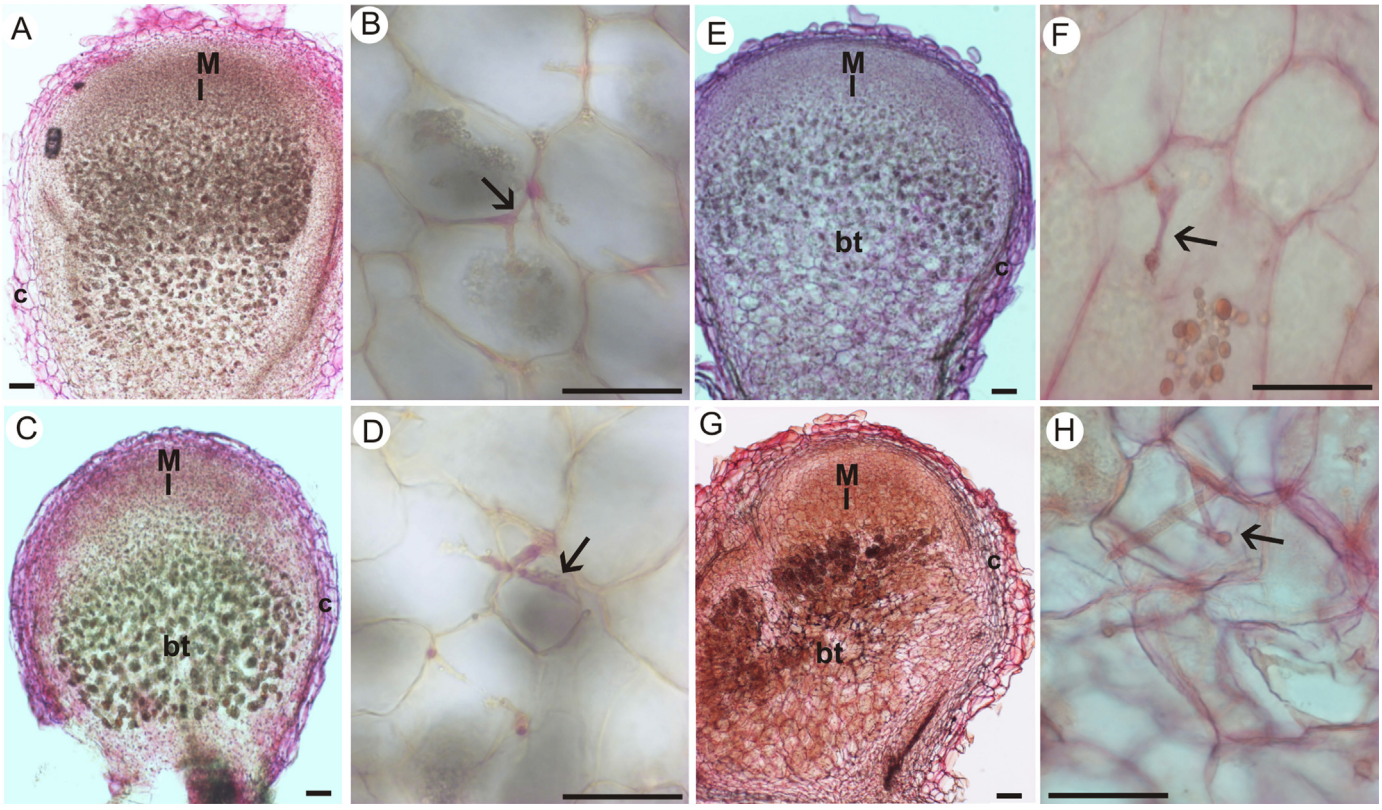

Fig. 1. Ruthenium red staining for detection of unesterified pectins in the pea root nodules after 0 h (A and B), 2 h (C and D) and 24 h (E and F) of Al stress and after alkali de-esterification of control (G and H). Red or pink color developed by staining indicates presence of acidic pectins. An increase of unesterified pectins content was observed in cell walls of nodule cortex (c), bacteroidal tissue (bt) and infection threads (arrow) after Al treatment. Chemical de-esterification performed on control nodule sections increased the intensity of ruthenium red staining. M – meristem; I – infection threads penetration zone. (A, C, E and G) Scale bars = 100 μm. (B, D, F and H) Scale bars = 50 μm.

oxidative stress in root nodules (Sujkowska-Rybkowska, 2012) and changed the root nodule apoplast (Sujkowska-Rybkowska et al., 2012; Sujkowska-Rybkowska and Borucki, 2014). These changes included thickening and stiffening of plant cell walls and walls of infection threads (ITs). The IT is one of the morphological structures unique to the *Rhizobium*-legume symbiosis. The thread is a tubular ingrowth, occurring due to invagination of the root hair cell wall, bound by a cylindrical wall, which comprises esterified and de-esterified pectins, xyloglucans and cellulose fibrils (Rae et al., 1992). The thread wall encloses the matrix containing invading rhizobia. The lumen of the thread is topologically equivalent to an intercellular space, and the luminal matrix (including glycoprotein and embedded rhizobia) apparently shares many components with the extracellular matrix (Rae et al., 1992; Brewin, 2004). However, it has been noted that IT walls have more resistance to digestion than other cell walls, suggesting additional modification (Higashi et al., 1987). Extension growth of the thread depends on IT walls elongation and the secretion of matrix glycoproteins into the lumen, and is confined to the tip region of ITs (Gage, 2002). Pectins as components of IT walls (Rae et al., 1991, 1992) may modulate IT growth. Thus, ITs wall thickening and stiffening may influence the process of IT growth and tissue and cell colonization by *Rhizobium* bacteria (Sujkowska-Rybkowska and Borucki, 2014).

In the present paper we use cytochemical methods and monoclonal antibodies to provide more precise information about the molecular components of the nodule apoplast and contribute to a better understanding the mechanisms of the IT growth inhibition to aluminum. Therefore, we focused on the range of pectin epitopes, in particular de-esterified pectins, which are known to be able to change wall extensibility (Willats et al., 2001).

Material and methods

Biological material

Nodules infected with *Rhizobium leguminosarum* bv. *viciae* wild-type strain 248 were grown using established methods (Sujkowska-Rybkowska and Borucki, 2014). Two-week-old pea plants were treated with 50 μM $AlCl_3$ for 2 or 24 h, pH 4.5. After treatment, all the plants were washed with distilled water and root nodules were collected for the investigation.

Staining for pectin

Ruthenium red staining was used for acidic pectin localization (Ruzin, 1999). Nodule hand sections were treated with Javel water for 30 min and afterwards were stained for 10 min in a 0.02% solution of ruthenium red in distilled water, rinsed several times with distilled water and observed for red or pink staining under light microscopy (AX Provis, Olympus). In some cases, nodule sections were submitted to chemical treatment before ruthenium red staining for the de-esterification of pectins. The control sections were incubated with 2 M NaOH for 30 min at room temperature and then thoroughly washed with deionized water. Five nodules of each treatment were analyzed for pectin staining. The experiments were repeated three times to confirm repeatability of results.

Immunolocalization

For immunolocalization experiments the tissue samples were embedded in butyl-methylmethacrylate resin (BMM) for