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PATTERNS OF PECTIN EPITOPE EXPRESSION DURING SHOOT AND ROOT REGENERATION IN ANDROGENIC CULTURES OF TWO WHEAT CULTIVARS

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Spatiotemporal patterns of expression of pectic epitopes during organogenesis were compared in androgenic cultures of two wheat cultivars: Apollo and Kaspar. The LM8 epitope (against xylogalacturonan) was found in the cell walls of loosely attached peripheral cells of Apollo and Kaspar callus. The LM8 binding signal was much stronger in Kaspar than in Apollo culture, and was localized in conspicuous extracellular material covering almost all surface cells of Kaspar callus. The LM8 epitope was also expressed in extracellular strands and layers that coated the root caps of adventitious roots in the two cultivars studied. The JIM7 epitope (against methyl ester-rich pectins) was not detected in Kaspar culture, but the JIM5 epitope (against methyl ester-poor pectins) was abundant in the cell walls lining the intercellular spaces of callus parenchyma. The LM5 epitope (against galactans) was scattered in the epidermis of shoot buds and in inner parenchymatic cells of Kaspar and Apollo callus; the LM6 epitope (against arabinans) was not found in either of the cultivars. The type and location of particular pectin epitopes are discussed in relation to their possible function in wheat androgenesis.

Key words: *Triticum aestivum*, wheat, androgenesis, cell wall, extracellular surface matrix network (ECMSN), immunolabelling, pectins.

INTRODUCTION

Pectins are heterogenous acidic polysaccharides that occur in the matrix of the primary cell walls of all land plants. Pectic substances account for much of the mechanical properties of the cell wall, but also have signaling roles in cell-to-cell communication, development and defense (for review see: Willats et al., 2001). Several studies have shown that morphogenic processes such as somatic embryogenesis and organogenesis are accompanied by differential expression of specific pectic epitopes (e.g., Kikuchi et al., 1996; Chapman et al., 2000; Verdeil et al., 2001; Willats et al., 2004). In grasses, having significantly lower cell wall pectin content than in other species, the role of these polysaccharides in developmental processes is poorly documented (Wiethölter et al., 2003; Šamaj et al., 2006). Recently we found that shoot formation from androgenic callus of Polish wheat cv. Apollo was accompanied by abundant expression of two homogalacturonan epitopes (JIM5 and JIM7) at cell-cell adhesion sites and in the outer extracellular surface matrix network (ECMSN) (Konieczny et al., 2007). The experiment described here continues that study of the distribution of pectic epitopes and ECMSN formation in androgenic culture of the Apollo cultivar. We also studied another Polish wheat cultivar, Kaspar, by immunolabelling, and compared the pectic epitope expression patterns of these two cultivars. The data from immunolabelling are supplemented with scanning electron microscope (SEM) observations and discussed in terms of the explants' ability to form ECMSN.

MATERIALS AND METHODS

The Polish wheat cultivars *Triticum aestivum* (L.) cv. Apollo and *T. aestivum* cv. Kaspar were used in the experiment. The conditions for donor plant growth and the procedure for culture in vitro were as described previously (Konieczny et al., 2003). Briefly, anthers containing microspores at the mid

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to late uninucleate stage were cultured in darkness for 6 weeks on induction medium, followed by transfer of the calluses to regeneration medium. Regenerative cultures were kept in light for 4 weeks. Calluses maintained for 6 weeks on induction medium and after 1, 2, 3 and 4 weeks of subculture on regeneration medium were taken for microscopic studies. Sample preparation for conventional light microscopy, scanning electron microscopy and the immunolabelling procedure were as described earlier (Konieczny et al., 2005, 2007). The following antipectin antibodies were used in this study: LM5 (1→4)-α-galactan (Jones et al., 1997); LM6 (1→5)-β-arabinan (Willats et al., 1999) and LM8 binding to xylogalacturonan (Willats et al., 2004) for labelling of both Apollo and Kaspar callus, and JIM5 and JIM7 reacting with methyl ester-poor and methyl ester-rich homogalacturonan epitopes (Willats et al., 2000) for labelling of Kaspar callus. The results of immunolabelling of the Apollo cultivar with JIM5 and JIM7 were described elsewhere (Konieczny et al., 2007). The JIM5 and JIM7 antibodies were obtained from PlantProbes (UK), and the LM5, LM6 and LM8 antibodies were kindly provided by Dr. Jozef Šamaj (University of Bonn, Germany).

RESULTS AND DISCUSSION

By light microscopy the six-week-old calluses of Apollo and Kaspar cultivars appeared similar in structure, consisting of loosely attached parenchymatic cells at the periphery and more compact tissue inside the callus (Fig. 1a). Strong fluorescence due to LM8 binding was observed at the surface cells of Kaspar callus, while the peripheral cells of Apollo callus were only weakly labelled by it (Fig. 1b,c). SEM showed almost all peripheral cells of Kaspar to be covered by coarse strands and fibrils which occasionally formed a continuous layer over a large area of callus (Fig. 1d,e). Extracellular material (ECMSN) of similar appearance accompanies early stages of somatic embryogenesis and organogenesis in situ and/or in vitro (for review see: Šamaj et al., 2006). The composition of the extracellular network might be different; it can contain proteins such as arabinogalactan proteins (e.g., Šamaj et al., 2008). The presence of pectic substances in ECMSN has been reported in chicory (Chapman et al., 2000), coconut (Verdeil et al., 2001) and maize (et al., 2006). In wheat cv. Apollo also, pectic epitope JIM7 was abundantly expressed at sites of ECMSN formation (Konieczny et al., 2007). In all the above-mentioned reports, however, the occurrence of ECMSN was found to be restricted to the meristematic regions of explants and young embryos/shoot buds, while nonregenerative cells were devoid of any fibrils or strands. Studies on different plant tissues showed that LM8-binding epitopes are specifically associated with plant cell detachment and appear when the cells separate from each other, as was observed in carrot suspension cell cultures or the parenchyma cells of maturing seeds (Willats et al., 2004). Notably, after one week of subculture, large, LM8 epitope-bearing peripheral cells dissociated from Apollo and Kaspar callus, which became meristematic at the periphery and parenchymatous in its inner region (Fig. 1b,c,f).

As in the Apollo culture (Konieczny et al., 2007), in Kaspar callus, shoot buds regularly formed from peripherally located meristematic clumps or layers of callus (Fig. 1g). Interestingly, unlike the Apollo cultivar in which the JIM7 epitope was abundantly expressed at sites of ECMSN deposition (Konieczny et al., 2007), Kaspar callus showed no signal resulting from JIM7 binding (Fig. 1j). Moreover, the meristematic area of Kaspar callus as well as the whole shoot bud surface were smooth, with no visible extracellular outgrowths (Fig. 1h,i). Instead, the LM5 antibody only bound weakly to the epidermis of young buds and the inner parenchyma cells of callus at this stage of culture (Fig. 1k). The rhamnogalacturonan I epitope recognized by LM6 was not detected throughout culture of both Kaspar and Apollo (data not shown). The homogalcturonan epitope JIM5 showed similar distribution patterns in the two cultivars and was localized at the cell walls lining the intercellular spaces between inner parenchyma cells of callus (Konieczny et al., 2007).

In both cultivars, roots originating from meristematic centers deep in the callus were formed after 1 to 2 weeks of subculture. Immunolabelling revealed that in Apollo and Kaspar callus the cell walls within root initials were not labelled by any of the antibodies used, while the walls of surrounding parenchyma cells showed similar scattered distributions of LM5 (Fig. 1l). Root cap cells of newly formed Apollo and Kaspar roots were highly reactive with the LM8 antibody and were coated by layers and strands of extracellular material (Fig. 1m–p). Willats et al. (2004) reported abundant expression of LM8 pectins in cell walls of root caps and in some regions of mucilage coating the root cap surface in several species. Based on the above findings, we suggest that the extracellular layers and strands observed over the apices of Apollo and Kaspar roots might represent mucilage secretions containing the LM8 xylogalacturonan epitope.

This study highlights one role of pectic substances in wheat androgenesis, that is, their possible involvement in regulation of cellular adhesion. Earlier the dissociation of large, loosely attached parenchyma cells from the callus periphery was observed to be a prerequisite for shoot regeneration in the Apollo cultivar (Konieczny et al., 2007). Here we found that the walls of dissociating nonregenera-

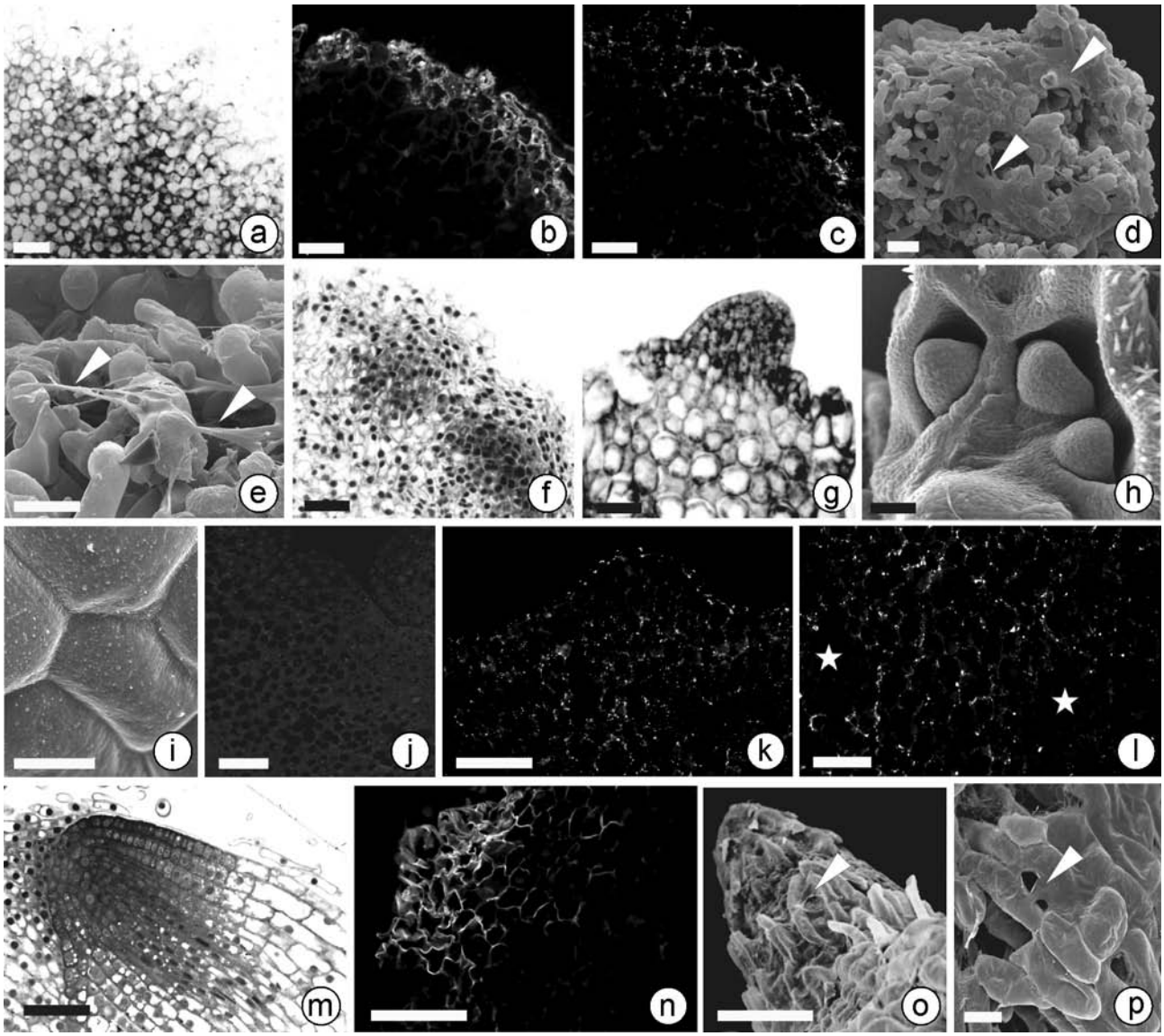

Fig. 1. Shoot and root regeneration from androgenic callus of two Polish wheat cultivars. (**a**) Toluidine blue-stained section of six-week-old callus of Kaspar cultivar, (**b,c**) Immunofluorescence of peripheral cells of Kaspar (**b**) and Apollo (**c**) callus due to binding of LM8 antibody, (**d,e**) SEM images of Kaspar callus after 6 weeks of culture on induction medium, (**f,g**) Toluidine blue-stained section through regenerative region of Kaspar callus after one (**f**) and two (**g**) weeks of subculture, (**h,i**) SEM images of whole shoot buds (**h**) and their surface cells after two weeks of subculture of Kaspar callus (**i**), (**j,k**) Immunofluorescence due to JIM7 (**j**) and LM5 (**k**) binding within adventitious meristems of Kaspar callus, (**l**) Immunofluorescence of inner parenchyma cells of Kaspar callus due to LM5 binding, (**m–p**) Root regeneration of Apollo. Toluidine blue-stained section (**m**), Immunofluorescence due to LM8 binding (**n**) and SEM images of apex (**o**) and surface cells of cap (**p**). Arrowheads on (d), (e), (o) and (p) point to extracellular strands and layers of ECMSN; stars on (l) show groups of meristematic cells within parenchyma unlabelled by LM5. Bars in (a, b, d, f-h, m-o) = 50 μm, in (j-l) = 20 μm and in (i,p) = 5 μm.

tive cells of both Apollo and Kaspar callus bore the same LM8 xylogalacturonan epitope, specifically associated with cells detached from or only barely connected to the callus. Thus we suggest a relation between expression of the LM8 epitope and the subsequent onset of regeneration in wheat callus culture. The most striking difference observed between Apollo and Kaspar callus was the lack of detectable fluorescence of the JIM7 epitope in Kaspar tissue. In previous work on the Apollo cultivar, the JIM7 homogalacturonan epitope was seen to be abundant in the cell walls of almost all meristematic cells

(Konieczny et al., 2007). Pectins are developmentally regulated, and changes in their composition have been found to accompany the growth and differentiation of various plant organs (e.g., Ermel et al., 2000; Majewska-Sawka et al., 2004).

We cultured Apollo and Kaspar callus under the same in vitro conditions; they followed the same developmental pathways, so the observed discrepancy in the expression of JIM7 epitope between these two cultivars seems genetically based. Additionally, the peripheral meristematic cells of Apollo callus showed a conspicuous extracellular coat (Konieczny et al., 2007). That structure was never observed over regenerative areas of Kaspar tissue; instead, extracellular material of similar appearance was found to cover LM8-reactive parenchyma cells of Kaspar callus. These differing observations indicate tissue-specific secretory activity in the studied cultivars. The question of whether the role of the extracellular coat in wheat is related only to its specific pectic composition requires further studies.

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