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Short Communication

Progeny from the crosses of two antisense potato plants exhibit ectopic xylem differentiation

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Progeny from the crosses of two transgenic potato lines csr2-1 and csr4-8, containing two different antisense constructs, csr2 and csr4 had been previously characterized to exhibit altered tuber production. Histochemical staining and microscopic examinations of the tubers were made to investigate cellular phenotype in the tubers. We observed ectopic proliferation of xylem, which is most pronounced in the csr2 tubers. Light microscopy of csr2 tubers revealed that the proliferation of xylem was associated with lignification of their cell walls. This paper provides evidence of cellular phenotype as a consequence of the presence of the antisense construct.

Key words: Genetic crossing, microscopy, potato, ectopic xylem, lignin.

INTRODUCTION

Genetic engineering and conventional breeding through genetic crossing are complementary approaches being used for plant improvement. Oomen et al. (2004) had previously used antisense approach to generate a number of antisense potato plants with reduced cellulose content in the tuber cell walls. These transgenic potato tuber clones were obtained by transforming the potato plant with antisense constructs of the class-specific regions (CSR) of four corresponding potato cellulose synthase genes (CSR1, 2, 3 and 4). To explore the possibility of influencing cellulose-hemicellulose networks in potato, with respect to cellulose reduction (Obembe et al., 2006), we have carried out genetic crossing of two antisense potato lines csr2-1 and csr4-8, which were identified to exhibit reduced cellulose content as compared to the wild type (Oomen et al., 2004). Our working hypothesis was that greater reduction in crystalline cellulose content in the double transformant would have greater effect on cellulose-hemicellulose networks, as would be revealed by more severe cell wall and/or cellular phenoltypes. The ultimate aim was to investigate the threshold limit for cellulose reduction that the potato tuber could tolerate. It was also hypothesised that lower cellulose content might lead to higher pectin content. It was observed previously that the tubers of the two antisense lines that have crossed in this study had about 5 and 10% higher uronic acid content than those of the control plants (Oomen et al., 2004). Hence it was thought that a much-reduced cellulose content in the double transformant tubers might lead to even higher pectin content than in either of the parents. Supplementary synthesis of pectin in the cellulose-depleted potato cell wall might give added value to it and thus enhance its potential use in food industrial applications. In this paper, we report remarkable proliferation of xylem cells and abnormal lignin formation in the progeny containing single csr2 construct.

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MATERIALS AND METHODS

Lignin and starch staining

Tuber slices perpendicular to the stolon axis were hand cut with a razor blade from potato tubers obtained from the four previously characterized clones (csr2, csr4, csr2/csr4 and control) of the pro-geny from the crosses of csr2-1 and scr4-8 (Obembe et al., 2008). Slices were stained for lignin with 1% (w/v) phloroglucinol in 96% ethyl alcohol, and for starch with 0.01% iodine potassium iodide. Each histochemical staining was performed in triplicates (tubers of three individual plants from each clone).Photograph was taken with

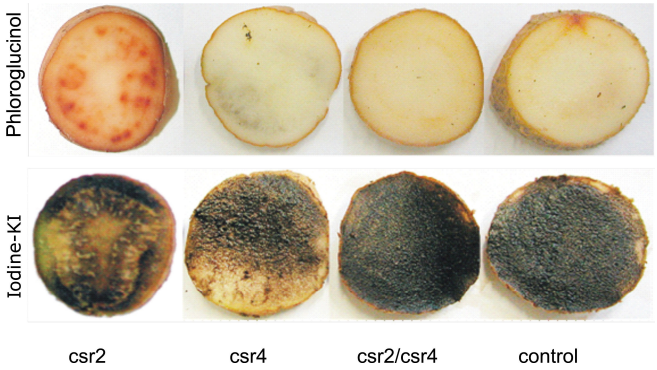

Figure 1. Histochemical staining for lignin and starch. Potato tuber slices in the upper panel were stained with phloroglucinol, showing intense red staining for lignin of the csr2 tuber slices, which was absent in other slices. Tuber slices in the lower panel were stained with 0.01% iodine-potassium iodide, showing differential staining pattern between the csr2 tuber slices and others.

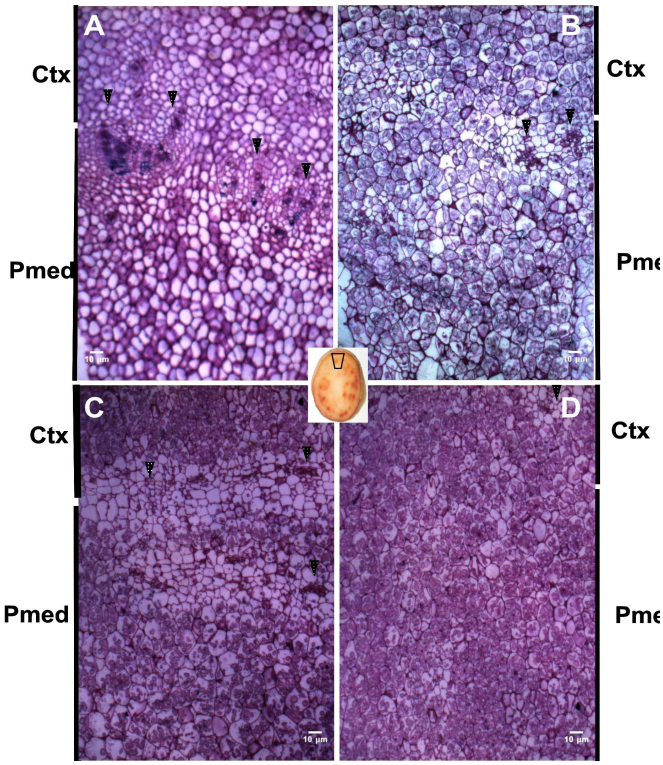

Figure 2. Toluidine staining of technovit-embedded potato tuber sections. Bright field micrographs of 4 µm transverse sections of cortex and perimedullary tissues of (A) csr2, showing distinct vascular ring of xylem, (B) csr4, (C) csr2/csr4 and (D) the control tubers, showing lesser numbers of xylem, with no distinct vascular ring. Ct, cortex; Pmed, perimedullary

a Nikon digital Camera.

Light microscopy

1 mm-thick potato tuber sections were fixed in 3% glutardialdehyde and 3% paraformaldehyde in 0.1 M phosphate buffer containing 0.1% Triton x 100 for 2 h. The samples were then washed and dehydrated in ethanol series. After dehydration, they were embedded in Technovit 7100 resin (Kuroiwa et al., 1990). 4 micron-thick tissue sections were stained with calcofluorwhite (0.04%) and toluidine Blue (0.1%) and examined by light microscopy. Each microscopic examination was done in triplicate (tuber sections of three individual plants from each clone). To verify our observation for the calcofluorwhite-stained csr2 tuber sections, we made fresh staining of csr2 and the control tuber sections with 0.1% aniline blue in 0.1 M K_3PO_4 and examined under a fluorescence microscope. We then did calcofluor white staining of the aniline blue-stained tuber sections and examined under a fluorescence microscope for differential staining patterns. Lignin staining was also repeated for fresh csr2 and the control sections to verify our earlier observations on tuber slices stained with phloroglucinol.

RESULTS

Lignin and starch staining

For fast screening for changes in cellular morphology and physiology, we stained tuber slices for lignin. The idea of this fast screening step was informed by our prior observation of a prominent vascular ring on the transverse surface of csr2 tuber slices. After staining with phloroglucinol and HCl, we found that only csr2 tuber slices stained intense red for lignin as shown in Figure 1 (upper panel). This is indicative of considerable lignification of the tuber cell walls. Similarly, for starch staining with iodine-potassium iodide, there appeared to be differential starch staining patterns between the csr2 tuber slices and others (Figure 1, lower panel). The csr2 tuber slices stained less intensely for starch than others, which is suggestive of lower starch content.

Ectopic proliferation of xylem and lignification in csr2 tubers

In order to observe cellular morphology, 4 µm transverse sections of technovit-embedded tubers were stained with toluidine blue and examined under a light microscope. Figures 2A and 3C show a large region of xylem that formed a well defined ring in the csr2 tuber section. For the double csr2/csr4 transformant, the single csr4 transformant, and the control tubers, lesser numbers of xylem, with no distinct vascular ring, were spotted on their sections (Figure 2B-2D). Additionally, there is an indication that the csr2 tubers exhibit a smaller cell size than the other tubers (Figure 2). This reduction in cell size may have impacted on the overall size of the tubers, thus leading to the production of smaller tubers. Several cellulose-deficient mutants of *Arabidopsis* have been shown to exhibit a reduced cellulose content leading to a reduced cell size (Arioli et al., 1998). We next verified the ectopic proliferation and lignification of xylem of the csr2 tubers by staining fresh tuber sections with phloroglucinol-HCl. Figure 3A revealed an ectopic proliferation of xylem and a consequent lignification in csr2 tubers as compared to the control tuber. A closer look at the sec-

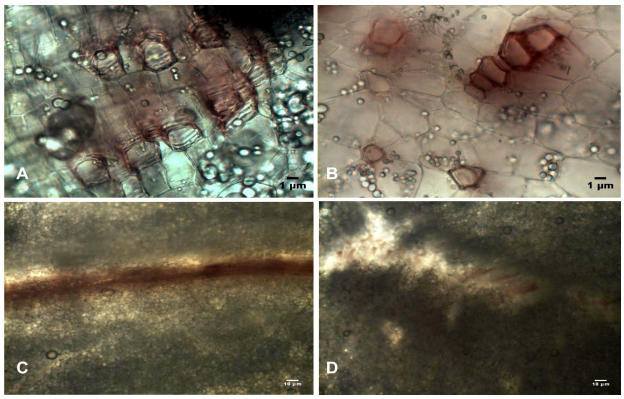

Figure 3. Phloroglucinol staining of fresh potato tuber sections. In upper panel are bright field micrographs of hand-cut thin sections of the vascular regions of csr2 tubers (A), showing ectopic xylem and (B) the control, showing normal xylem formation. In lower panel are bright field micrographs of hand-cut thick sections, showing the vascular rings of csr2 tubers in perpendicular orientation to the stolon axis (C) and xylem (red dots) in the control in normal, parallel orientation to the stolon axis (D).

tions revealed milder staining of the ectopic xylem as compared to the control, which is indicative of recent differrentiation and development. It was also remarkable to observe that the ectopic xylem of csr2 tubers were laid down perpendicularly to the stolon axis (Figure 3C) whereas those of the control tubers ran paralleled to the stolon axis (Figure 3D).

DISCUSSION

Phloroglucinol staining revealed extensive xylem prolixferation coupled to considerable lignification in the csr2 tubers. The milder phloroglucinol staining intensity that was observed for the csr2 fresh tuber slices as compared to the control indicates that the xylem differentiation in the csr2 was recent, as such the xylem were not as well developed as in the control tuber. It is plausible that the ectopic xylem differentiation may have resulted from inhibition of cell expansion (Lee and Roberts, 2004). Cano-Delgado et al. (2000) opined that inhibition of cell division itself could be as a result of altered cellulose deposition. To a varying extent, similar observations of ectopic lignin and xylem formation have been reported for several cellulose-deficient *Arabidopsis* mutants. *Eli1* (ectopic lignification), a mutant of AtCesA3 (Cano-Delgado et al., 2003), exhibited ectopic production of heavily lignified xylem (Cano-Delgado et al., 2000). Ectopic xylem differentiation and lignification were also observed in *apl* (altered phloem development) and *mux* (multiple xylem) (Bonke et al., 2003). Apart from the xylem, ectopic lignification was also reported in cells that are not normally lignified as in *rsw1* mutants of AtCesA1 (Arioli et al., 1998), *kor1* (Nicol et al., 1998), a mutant of KORRIGAN (a membrane-bound endo-β-1,4-glucanase) that has been implicated in cellulose biosynthesis and deposition (Molhoj et al., 2002) and *lit* (Lion's tail) mutants (Cano-Delgado et al., 2000). However, it remains to be determined, whether or not cellulose deposition in the csr2 tubers correlates with the observed cellular events as with the works cited above. It was surprising to observe, however, that the csr2 phenotype was not exhibited by csr2/csr4 clone. This may have been as a result of the different effects the antisense contructs had on cellulose synthesis and/or deposition in the tuber cell walls, leading to varying levels of either crystalline or armophous cellulose microfibrils. Further analyses on the tuber cell wall for cellulose deposition and content will be carried out to investigate this likelihood.

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