Comparative analysis of remodelling of the apoplast in *Lotus corniculatus* L. symbiotic nodules under trace metal contamination

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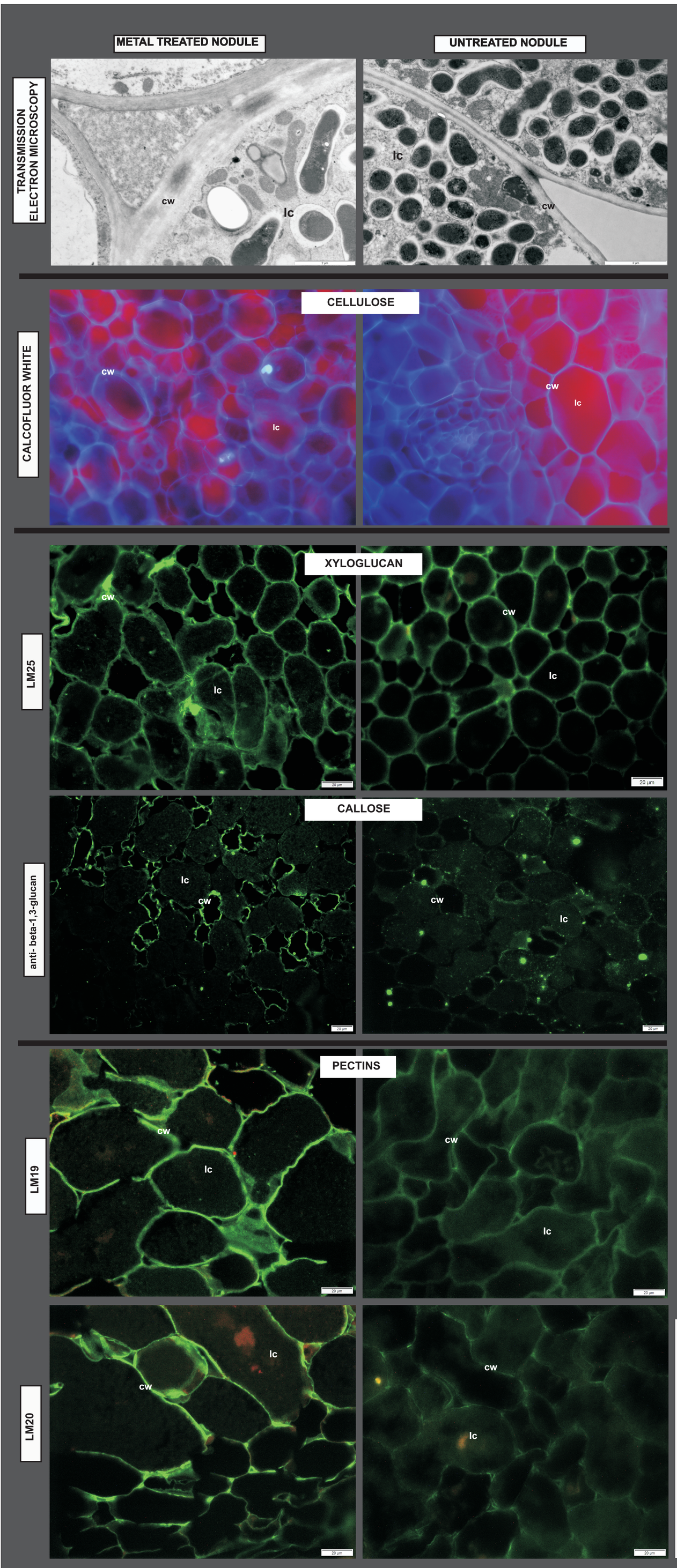
Introduction, Aim and Scope

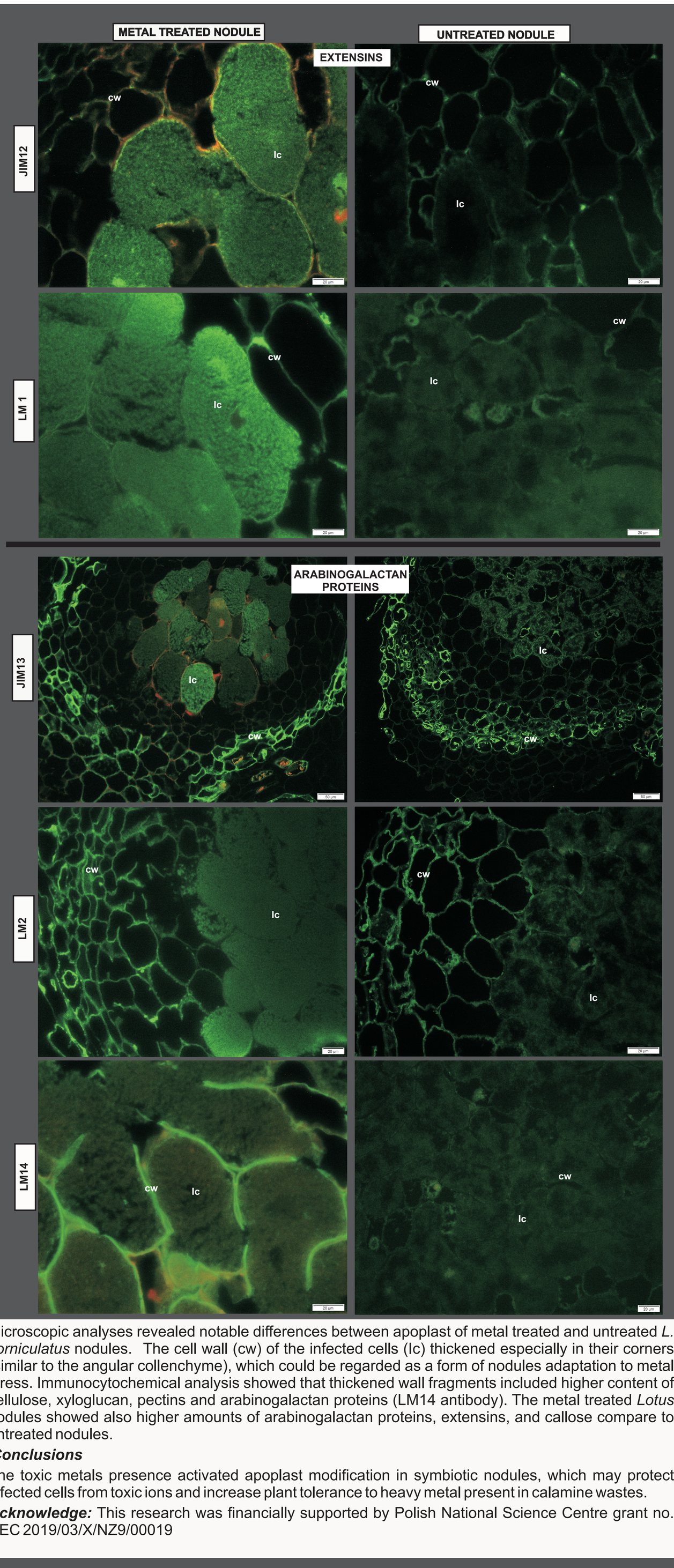
Lotus corniculatus L. is one of the dominant legume species spontaneously colonized calamine tailings in Southern Poland highly contaminated with Zn 42000 mg_xkg, Cd 180 mg_xkg and Pb 2200 mg_xkg (Sujkowska-Rybkowska et al. 2020). This ability to grow in such a demanding environment has been explained by their capacity to symbiotic interactions with nitrogen-fixing rhizobia and nodules formation. Apoplast plays the major role in trace metal resistance, however toxic metals modifies the properties of the cell walls and wall reorganization is one of the most common plant strategy of metal stress avoidance. The aim of this study was to determine this avoidance mechanism in symbiotic nodules of *Lotus* plants growing on calamine wastes.

Materials and Methods

The research material was the root nodules of *L.corniculatus* obtained from plants from seeds of individuals naturally occurring on a calamine heap (a heap in Bolesław 50°17' N 19°29' E in southern Poland), which was grown for 7 weeks in greenhouse on a sterile heap substrate. Three old *Lotus* seedlings were inoculated with isolated metal tolerant *Bradyrhizobium liaoningense* (naturally nodulated *Lotus* plants on calamine heap). The control nodules were obtained from the same seeds inoculated with the same strain of bacteria, growing in pots with expanded clay. Cell wall reorganisation under metal stress was examined by using histochemical methods and specific monoclonal antibodies against cellulose and non-cellulosic wall components in metal treated or untreated *L. corniculatus* nodules.

Results:





Microscopic analyses revealed notable differences between apoplast of metal treated and untreated *L. corniculatus* nodules. The cell wall (cw) of the infected cells (lc) thickened especially in their corners (similar to the angular collenchyme), which could be regarded as a form of nodules adaptation to metal stress. Immunocytochemical analysis showed that thickened wall fragments included higher content of cellulose, xyloglucan, pectins and arabinogalactan proteins (LM14 antibody). The metal treated *Lotus* nodules showed also higher amounts of arabinogalactan proteins, extensins, and callose compare to untreated nodules.

Conclusions

The toxic metals presence activated apoplast modification in symbiotic nodules, which may protect infected cells from toxic ions and increase plant tolerance to heavy metal present in calamine wastes.

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