The formation of symbiotic interface in root nodules of *Pisum sativum* L. and *Medicago truncatula* Gaertn

Tsyganova A.V.¹, Seliverstova E.V.^{1,2}, Brewin N.J.³, Tsyganov V.E.^{1,4}

¹All-Russia Research Institute for Agricultural Microbiology, Saint Petersburg, Russia; ²Sechenov Institute of Evolutionary Physiology and Biochemistry of the Russian Academy of Sciences, Saint Petersburg, Russia; ³John Innes Centre, Norwich, United Kingdom; ⁴Saint Petersburg Scientific Center RAS, Saint Petersburg, Russia

E-mail: isaakij@mail.ru

Key message. The infection of root cells of legumes with rhizobia involves the gradual remodelling of the plant-microbial interface. General and species-specific features of symbiotic interface remodelling during nodule development were demonstrated.

Keywords: symbiotic nodule, cell wall, extracellular matrix, pectin, arabinogalactan protein

In legumes during the symbiotic interaction with rhizobia, the plant cell walls, extracellular matrix and bacterial surface polysaccharides are part of the intimate interface for developmental coordination and nutrient exchange. Plant symbiotic interface remodelling is a fundamental aspect of the *Rhizobium*-infection process. The aim of this work was to analyze modification and remodelling of the plant cell surface during nodule development in two legume species. We used monoclonal antibodies to characterize the distribution of the homogalacturonan (HG), rhamnogalacturonan-I (RG-I), extensin and arabinogalactan proteins (AGP) in wild-type and ineffective mutant nodules in *Pisum sativum* L. and *Medicago truncatula* Gaertn. Immunocytochemical analyses, fluorescence and transmission electron microscopy were used. During nodule development in *P. sativum* and *M. truncatula*, high methyl-esterified HG recognized by JIM7 was demonstrated throughout both the cell wall and the infection thread wall at all nodule developmental stages. Low methyl-esterified HG recognized by JIM5 participated in increased rigidity of cell walls and walls of infection threads, especially in nodules of *P. sativum* mutant SGEFix-2 (*sym33-3*) and *M. truncatula* mutant TR3 (*ipd3*). RG-I was present in meristematic cell walls, vascular bundles in both species and in infection thread walls in nodules of *P. sativum* only. The membrane anchored AGP recognized by JIM1 was present not only on the plasma membrane of *P. sativum* nodules, but also on the symbiosome membrane. The increase in JIM1 labelling with aging suggests that AGP recognized by JIM1 participates in the maturation of symbiosomes in *P. sativum* wild-type nodules. LM6-M, which recognize both AGP and arabinan side chain of RG-I was present in the cell walls at all nodule developmental stages as well as in juvenile symbiosomes in the infection zone and early nitrogen fixation zone in wild-type nodules of both *P. sativum* and *M. truncatula*, and in mutants of *P. sativum* Sprint2Fix⁻ (*sym31*) and *M. truncatula* *dnf-1* characterized by undifferentiated bacteroids. Subsequently, in the nitrogen fixation zone, the label from the symbiosomes disappeared. In the mutants of *P. sativum* SGEFix-2 (*sym33-3*) and *M. truncatula* TR3 (*ipd3*), characterised by the absence of bacterial release, the LM6 label was localized exclusively in the cell walls. Extensins – hydroxyproline-rich proteins – are part of the extracellular matrix. Extensin labelled with JIM11 was localized mainly in the matrix of infection threads and droplets and in the intercellular matrix in both *P. sativum* and *M. truncatula* nodules. An increase in the accumulation of extensin in the extracellular space matrix, especially at the cell junctions, was observed in nodules of mutants Sprint2Fix⁻ (*sym31*) and *dnf-1* characterized by undifferentiated bacteroids. Differences in the localization of RG-I and AGP with a GPI anchor between *P. sativum* and *M. truncatula* indicate that in different plants the cell walls and intercellular matrix are formed by various components and are species-specific.

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