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► To cite this version:

Marie-Christine Ralet-Renard. A new monoclonal antibody to pectic type I arabinogalactan. 14th cell wall meeting, Jun 2016, Chania, Greece. 2016. <hal-01606553>

HAL Id: hal-01606553

<https://hal.science/hal-01606553v1>

Submitted on 3 Jun 2020

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A new monoclonal antibody to pectic type I arabinogalactan

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Rhamnogalacturonan I (RGI) accounts for 20% to 35% of pectic polysaccharides and is one of the most heterogeneous polysaccharides found in plant cell walls. Its backbone is composed of [2)- α -L-Rhap-(1,4)- α -D-GalAp-(1] repeats. Depending on the plant source, 20-80% of the Rhap residues can be substituted, mainly at O-4, with neutral side chains consisting primarily of galactan, arabinan or type I or II arabinogalactan. RGI side chain composition, fine structure, length and degree of branching are highly variable according to plant sources, organs, tissues and developmental stages. The functional role of the complex and versatile RGI in the context of cell biology remains to be clarified. For doing so, the structural diversity, occurrence, and dynamics of RGI subpopulations needs to be investigated.

Tremendous efforts have been made to study cell wall components directly in muro using monoclonal antibodies (MAbs), and these have proven useful to indicate the diversity and dynamics of cell wall architectures. A large set of MAbs against pectin motifs is already available among which some are specifically directed to RGI motifs. However, in order to extend the existing libraries of MAbs and to increase probe coverage of potential oligosaccharide features found in cell walls, additional MAbs recognising novel epitopes are required. In particular, antibodies directed to complex and branched pectic side chains or to the junction between the side chains and the RGI backbone have not been isolated yet.

Here, we describe the production and characterization of a novel MAb that binds to type I arabinogalactan (Buffetto et al., 2015). Its immunogen was engineered from RGI isolated from potato pulp by designing through enzymatic catalysis highly branched RGI oligosaccharides with monomeric or very short Ara and Gal-containing side-chains and conjugating these oligomers to a carrier protein. Subsequent to immunization with this conjugate we have identified a mouse monoclonal antibody, designated INRA-AGI-1. A screening of its antigenicity recognition using glycan microarrays and competitive-inhibition ELISA assays has shown that INRA-AGI-1 has a novel binding pattern compared to the available RGI-related MAbs. INRA-AGI-1 did bind to potato, carrot, lupin and soybean polymeric RGI and to potato RGI oligosaccharides. INRA-AGI-1 did not react with any of the other polymeric and oligomeric pectin-related substrates tested; notably, homogalacturonan, type II arabinogalactan and oligosaccharides arising from (1,4)-linked galactan, (1,5)-linked arabinan and unbranched RGI backbone were not recognized. INRA-AGI-1 was shown to bind to a peculiar type I arabinogalactan motif consisting of (1,4)-linked Galp residues interspersed with internal (1,5)-linked Araf residues. The INRA-AGI-1 epitope is present within cell walls of potato tuber and

carrot root. Pre-treatment with galactanase was required to unmask the epitope, and no strict co-localization with any of the established RGI antibody epitopes was observed. Based on our findings, we will discuss RGI structure, its integration within the pectic and cell wall architectures and how MAbs coupled with enzymatic deconstruction can provide information on the biosynthesis, deposition and remodelling of plant cell wall polymers.

Buffetto et al., *Plant and Cell Physiology*, **56**, 2181-2196 (2015)