The Putative (1,3)-β-D-Glucan Synthase Gene Family in *Hordeum vulgare*

Submitted by

Michael Scott Schober

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Discipline of Plant and Pest Science
School of Agriculture and Wine
Faculty of Sciences
University of Adelaide, Waite Campus
Glen Osmond, South Australia, 5064, Australia

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Abstract

(1,3)-β-D-Glucan, which is often referred to as callose, is deposited in numerous locations and at various stages during normal growth and development in higher plants, as well as in response to abiotic and biotic stresses. For example, (1,3)-β-D-glucan is the main polysaccharide component of the forming cell plate during mitosis, and is deposited at various stages during the meiotic events of micro- and megasporogenesis. (1,3)-β-D-Glucan is also a structural component of plasmodesmatal canals, sieve plate pores and pollen tube walls, and is deposited during cellularisation of the developing endosperm. (1,3)-β-D-Glucan is deposited in response to mechanical wounding, metal toxicity, and in response to viral, bacterial, nematodal and fungal infection.

There are several lines of evidence that link **g**lucan **s**ynthase-**l**ike, or *GSL*, genes to the deposition of (1,3)-β-D-glucan in higher plants. In the work described in this thesis the role of individual barley (*Hordeum vulgare*) *GSL* genes in the deposition of (1,3)-β-D-glucan was investigated. There was an emphasis on the identification of the barley *HvGSL* gene involved in the deposition of (1,3)-β-D-glucan in the response to fungal infection, in the anticipation that disruption of the barley orthologue might lead to increased resistance to fungal diseases in an important cereal crop species.

In addition to the barley *HvGSL1* gene that had been characterised previously, an additional six (possibly seven) *HvGSL* genes were identified here from mRNA preparations of various barley tissues. The genes were placed on a high density barley genetic map, and are distributed across the genome. The deduced amino acid sequence of the *HvGSL8* gene, for which a near full-length cDNA was isolated, was predicted to have a large hydrophilic region located on the cytoplasmic side of the plasma membrane, flanked by six to eight transmembrane helices towards the COOH-terminus and five to seven transmembrane helices towards the NH_2-terminus. There were no obvious UDP-glucose binding motifs identified in the deduced amino acid sequences of any of the *HvGSL* genes. It was observed that the barley *HvGSL7* gene had the highest sequence identity to the Arabidopsis *AtGSL5* gene.

Potential *HvGSL* gene functions were investigated using quantitative real-time PCR and through the analysis of microarray data. It was assumed that genes that were up-regulated in particular tissues were candidates for the deposition of (1,3)-β-D-glucan specific for that tissue, and that co-transcribed genes might be involved in the overall process of (1,3)-β-D-glucan deposition. With this approach to *HvGSL* gene characterisation, it was concluded that the *HvGSL2* gene was potentially involved in the deposition of (1,3)-β-D-glucan during meiotic division. The analyses also suggested that *HvGSL3* might be involved in (1,3)-β-D-glucan deposition during endosperm cellularisation. The *HvGSL6* gene was also implicated in endosperm cellularisation, and in addition was also the most likely candidate for (1,3)-β-D-glucan deposition during cell plate formation in dividing cells. The barley *HvGSL4* gene might be required for deposition of (1,3)-β-D-glucan that is associated with closure of plasmodesmata. Finally, it was shown that *HvGSL7* was the only *HvGSL* gene that was up-regulated in epidermal tissue in response to *Blumeria graminis* infection.

To determine whether *HvGSL7* was required for (1,3)-β-D-glucan deposition in papillary structures that arise during fungal infection, an *HvGSL7* dsRNAi vector was generated and delivered into excised barley leaf blades by micro-projectile bombardment. Cells bombarded with the dsRNAi vector deposited less (1,3)-β-D-glucan in papillary structures resulting from *B. graminis* infection when compared with the experimental negative control. Based on sequence identity, transcription profile and post-transcriptional gene silencing, *HvGSL7* appears to be the barley orthologue of *AtGSL5* in Arabidopsis. It is uncertain at this stage if silencing *HvGSL7* results in increased resistance against virulent barley fungal pathogens. Transgenic *HvGSL7* dsRNAi barley lines were therefore generated, but are yet to be analysed.