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Research article

Promoter activities of genes encoding β-galactosidases from Arabidopsis a1 subfamily

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ABSTRACT

Promoter regions of each of the six At*BGAL* gene of the subfamily a1 of *Arabidopsis thaliana* were used to drive the expression of the β-glucuronidase gene. The pattern of promoters (pAt*BGAL*) activity was followed by histological staining during plant development. pAt*BGAL1*, pAt*BGAL3* and pAt*BGAL4* showed a similar activity pattern, being stronger in cells and organs in expansion, and the staining decreasing when cell expansion decreased with age. That indicates a consistent involvement of the encoded β-galactosidases in cells undergoing cell wall extension or remodelling in cotyledons, leaves and flower buds. These promoters were also active in the calyptra cells and in pollen grains. pAt*BGAL2* activity showed a clear relationship with hypocotyl elongation in both light and dark conditions and, like pAt*BGAL1*, pAt*BGAL3* and pAt*BGAL4*, it was detected during the expansion of cotyledons, rosette leaves and cauline leaves. Its activity was also intense in the early stages of flower and fruit development. pAt*BGAL5* was the only one among those from the subfamily a1 that was active in the trichomes that appear throughout the plant, indicating a high specificity of the AtBGAL5 protein and its involvement in the cell wall changes that accompany the formation of the trichome. The activity of pAt*BGAL5* was also high in radicles and roots, except in the meristematic area of these organs, and during seed formation. Finally, the activity of pAt*BGAL12* was mainly detected in meristematic zones of the plant: the leaf primordium, emerging secondary roots and developing seeds, which indicates an involvement in the differentiation process.

1. Introduction

β-Galactosidases are enzymes that hydrolyze the glycosidic bond between a non-reducing galactose residue and another sugar or alcohol. They can act on numerous substrates such as lactose, glycolipids, proteoglycans, oligosaccharides or polysaccharides. Glycosyl hydrolases (GH) have been classified as families on the basis of structural similarity [1]. β-galactosidases fall into four glycoside hydrolase (GH) families, GH-1, GH-2, GH-35, GH-42, which are part of superfamily A (or Clan A) [2]. GH-1, GH-2 and GH-42 are mostly found in microorganisms, whereas GH-35 enzymes are found in both prokaryotes and eukaryotes. The plant β-galactosidases that have been described belong to GH family 35. In higher plants, the GH-35 family (EC 3.2.1.23) has been implicated in carbohydrate reserve mobilization and in cell wall biogenesis and modification [3,4]. The substrate specificities of plant β-galactosidases can vary widely, indicating functional diversity in their activity.

β-Galactosidases have been described in several plant species as multigene families, suggesting that GH-35 gene multiplicity is ubiquitous in plants. Thus, in tomato a multigene family with seven members has been described, some of which are involved in fruit development and ripening [5]. In chickpea, a family of at least four β-galactosidases is expressed differentially during seedling and plant growth [6]; one of then, the βIII-Gal protein, is associated with cell wall loosening during epicotyl elongation and acts on the β-(1,4) galactan side chain of rhamnogalacturonan I [7].

In the Arabidopsis genome, 17 GH-35 *BGAL* genes have been identified and designated from At*BGAL1* to At*BGAL17* [8]. The 17 Arabidopsis β-galactosidases (AtBGAL) fall into two groups and seven subfamilies [8]. The majority of these hydrolases may be glycoproteins and, with the exception of AtBGAL14, have an N-terminal signal peptide, and many of them appear targeted to the cell exterior, suggesting their potential involvement in cell wall modification.

The classification in two groups is mostly based on molecular masses. Group 1 members are 721–731 amino acids in length.

Abbreviations: GH, glycosyl hydrolases; AtBGAL, Arabidopsis β-galactosidases; pAt*BGAL*, At*BGAL* gene promoter.

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Group 2 BGALs are far longer (832-888 amino acids), mainly due to the existence of a C-terminal domain that is structurally related to a sea urchin egg lectin (SUEL-lectin), which was first identified as a D-galactose-binding lectin [9], although it was later shown that it preferentially binds to L-rhamnose [10]. With the exception of AtBGAL14 and AtBGAL17, Arabidopsis BGALs fall into either Group 1 (AtBGAL2, 4, 5, 6, 10 and 12) or Group 2 (AtBGAL1, 3, 7, 8, 9, 11, 13, 16 and 17).

Most of the subfamilies contain one to three members, except for the largest subfamily, a1, which consists of six proteins, AtBGAL1, AtBGAL2, AtBGAL3, AtBGAL4, AtBGAL5 and AtBGAL12, exhibiting 60–81% sequence identity [11]. It has been predicted that all have signal peptides and basic pIs (7,2-8,6) consistent with a cellular destination in the cell wall [8]. This localization has been confirmed for AtBGAL2 and AtBGAL5 [4] and for AtBGAL1 and AtBGAL12 [11].

The differential tissue-specific expression patterns and stress responsiveness suggest that AtBGALs are involved in diverse biological processes. Several studies to determine the expression levels of genes encoding β-galactosidases in Arabidopsis using RT-PCR and microarray techniques have been carried out in different organs and during several developmental stages. However, the results obtained by both methods are sometimes contradictory.

Specifically regarding subfamily a1, Ahn et al. [8], using relative RT-PCR analysis, reported that At*BGAL1*, At*BGAL2* and At*BGAL3* were expressed constitutively across the different organs and developmental stages, with the highest transcript levels in leaves, roots and flowers, while At*BGAL4* transcripts accumulate primarily in roots. At*BGAL4* and At*BGAL5* were not expressed in green seedlings. In these experiments, no expression of At*BGAL12* was detected.

By semi-quantitative RT-PCR using a different set of primers than in the experiments of Ahn et al. [8], Gantulga et al. [11] reported that At*BGAL1* has an essentially constitutive expression, while the other five genes from subfamily a1 are expressed in most, but not all, tissues. The At*BGAL1*, At*BGAL2*, At*BGAL5* and At*BGAL12* genes were the only ones expressed in seedlings; in the petiole, only At*BGAL1* and At*BGAL3*, and in siliques, only At*BGAL1*, At*BGAL3* and At*BGAL4*.

Perez-Almeida [12] found At*BGAL2* expression with moderate levels of transcripts in all organs. In the case of At*BGAL5*, they found higher levels of expression in roots than Gantulga et al. [11]. While Gantulga [11] and Perez-Almeida [12] agree that At*BGAL5* is not detectable in mature roots, only Perez-Almeida [12] found At*BGAL5* expression in root elongation and the root hair zones of juvenile plants.

In the publicly-available microarray data, all six genes appear to be expressed to some extent in all tissues examined although there are notable differences in expression levels in different organs and developmental stages. In addition, the use of microarrays has revealed that some genes are induced by biotic or abiotic stress, such as At*BGAL1*, which is induced by hypoxia, salt stress or pathogen attack.

Another method for determining the levels of expression of a gene is to analyse the activity of its promoter. Thus, the study of promoters of genes encoding different β-galactosidases provides valuable information about the places at which their genes are transcribed, which can help to elucidate their function. The aim of the present work was to study the activity of the gene promoters At*BGAL1* (*At3g13750*), At*BGAL2* (*At3g52840*), At*BGAL3* (*At4g36360*), At*BGAL4* (*At5g56870*), At*BGAL5* (*At1g45130*) and At*BGAL12* (*At4g26140*) from the Arabidopsis a1 subfamily that encode β-galactosidases by construction of transgenic plants of *Arabidopsis thaliana* producing the enzyme β-glucuronidase (GUS) driven by At*BGAL* gene promoters (p*AtBGAL*) and later histological localization of GUS activity to determine the pattern of gene expression during plant development. We decided to study the members of the subfamily a1 of β-galactosidase since they have been described as exo-galactanases able to act on the pectic polysaccharides of the cell wall, and might therefore play important roles in cell wall remodelling during plant growth and development [11], although their functions have not yet been determined.

2. Results and discussion

2.1. Activity of the β-galactosidase promoters of the a1 subfamily

Arabidopsis transgenic plants in which the gene promoter drives the expression of reporter proteins such as GUS were produced and further histological localization of GUS activity during plant development was carried out to determine the activity of these promoters. The study was carried out in plants growing under both dark and light conditions. Our results indicate that three of the At*BGAL* promoters studied, pAt*BGAL1*, pAt*BGAL3* and pAt*BGAL4*, showed a similar pattern of activity in the different plant tissues and organs analysed; accordingly, these results will be discussed together. In separate sections we shall analyse the activity of the pAt*BGAL2*, pAt*BGAL5* and pAt*BGAL12* promoters. Wild type plants used as controls never showed GUS activity, and hence the images obtained are not included here.

2.1.1. Activity of pAtBGAL1, pAtBGAL3 and pAtBGAL4

The activity of *pBGAL1*, *pBGAL3* and *pBGAL4* indicates a consistent involvement of the encoded β-galactosidases in cells undergoing wall extension or remodelling in cotyledons, leaves and flowers. These promoters exhibited the same pattern of activity, and hence for discussion as an example we shall present only the results regarding pAt*BGAL1*.

pAt*BGAL1* displayed strong activity in cotyledons during the first days after germination, progressively disappearing along the development of cotyledons (Fig. 1a,b,c). Cotyledons develop during embryogenesis and their growth after germination is determined by cellular expansion and not by division [13]. Thus, a degradation of cell wall pectin has been described during the expansion of cotton cotyledons [14], probably mediated by an endopolygalacturonase [14] and a rhamnogalacturonan lyase [15]. Although the action of β-galactosidases has not yet been described in cotyledons, these proteins have been related to the elongation process [16–18] and could also participate in cell expansion. Similarly to what happens in cotyledons, pAt*BGAL1* shows strong activity in the leaf primordia in a development-related manner. Thus, pAt*BGAL1* activity was high in leaf primordia from very young seedlings (Fig. 1a), remained high in young leaves (Fig. 1b), and progressively decreased as the leaves matured until it finally disappeared completely (Fig. 1c). Each new developing rosette leaf, as well as the cauline leaves (Fig. 1d), followed the same pattern of GUS activity, regardless of the age of the plant.

pAt*BGAL1* also displayed a strong activity in roots that persisted throughout root development in an age-independent manner and was located in radicles (Fig. 1e) and primary and secondary roots (Fig. 1f), except in the root elongation zone, suggesting a function in maturation rather than in expansion in this organ. Furthermore, GUS activity was not observed in all root tissues, being limited to the central cylinder, which stained an intense blue (Fig. 1f). It is worth noting the activity of these promoters in the root cap or calyptra (Fig. 1f,g), although in secondary roots this activity only started after they had emerged from the main root and it was not visible when secondary roots started their development (Fig. 1h). The root cap protects the meristem by the production of mucilage, composed primarily of pectins, and by the detachment of its own cells to reduce mechanical friction against the ground, which