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GENE NOTE

RS2: a sugar beet gene related to the latex allergen Hev b 5 family

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Abstract

A novel gene (*RS2*) has been isolated from a *Beta vulgaris* (cv. Regina) cDNA library. The expression of this gene was enhanced in the mature storage organ as compared to leaf tissue. The protein encoded by this gene was found to be alanine- and glutamic acid-rich and it resembles members of the latex allergen Hev b 5 family.

Key words: *Beta vulgaris*, storage organ, latex allergen, parenchymatous cells.

Sugar beet (*Beta vulgaris*) stores sucrose in a large storage organ that develops from root and hypocotyl tissue. This storage organ displays an anomalous form of secondary thickening from a series of supernumerary cambia arranged in concentric rings. These cambia are responsible for the production of alternating zones of vascular tissue and parenchyma cells in which the sugar is stored. In order to obtain information about gene expression in the various cell types of the storage root and to isolate storage organ enhanced genes a cDNA library (cDNA synthesis kit and λgt10 cloning kit, Amersham) was created. For this purpose RNA was isolated from a mature sugar beet (cv. Regina) storage organ (Slater, 1988). *RS2* was obtained by differential screening of the library with photobiotin-labelled root and leaf RNA (McInnes *et al*., 1987). Root-enhanced sequences were selected on the basis that their plaques gave a positive signal with a root RNA probe, but not with the leaf probe. The sequence obtained for *RS2* was shown to be 816 nucleotides long. It contained an open reading frame of 474 nucleotides (158aa) coding for a protein with a predicted molecular mass of 16.098 kDa. The protein was rich in alanine (42aa, 26.6%) and also, being rich in glutamic acid (40aa, 25.3%), it had a predicted pI of 3.72. The glutamic acid was arranged in a significant number of the repeated motif XEEX (where X was often alanine) throughout the protein, but particularly in the carboxy terminal region. *RS2* showed homology with other proteins of a similar size, pI and containing this arrangement of glutamic acid (Table 1). The proteins included the major latex allergen Hev b 5 (Slater *et al*., 1996; Akasawa *et al*., 1996), a putative allergen protein from *Prunus armeniaca* (unpublished results), the kiwi fruit protein pKIWI501 (Ledger and Gardner, 1994), and TUB8 from *Solanum tuberosum* (Taylor *et al*., 1992). *RS2* also showed some homology with a grape-ripening related protein, GRIP68 (unpublished results), and a buckwheat seed protein (unpublished results). Hev b 5 is one of over a dozen major allergenic proteins found in latex. The others include an endochitinase and several proteins that are involved in rubber biosynthesis. These allergens are responsible for natural rubber latex (NRL) allergy (Niggemann and Breiteneder, 2000) and persons with NRL are often sensitized against various fruits that are believed to have proteins with similar epitopes. This cross-reaction is also seen in the reverse direction and the phenomenon has become known as the latex-fruit syndrome. The apparent structural relationship of the predicted protein pKIWI501, with Hev b 5, led to the suggestion that it might be the corresponding fruit protein involved in the latex-Kiwi fruit cross-reaction (Slater *et al*., 1996; Akasawa *et al*., 1996). The functions of *RS2* and the other proteins listed in Table 1 remain unknown, but, they are all believed to be cytoplasmic structural proteins. The similarity of *RS2* with these proteins raised the question whether it too had the potential to elicit a cross-reaction within the context of the latex-fruit syndrome. Hypersensitivity to beet is not a common disease, very few reports have been made of allergies related to beet components (Hohenleutner *et al*., 1996). However, examination of the *RS2* protein sequence indicated that there were

Table 1. *Summary of* RS2 *homologues*

Organism	Gene	Accession number	Identity (DDBJ tblastn)
Beta vulgaris	*RS2*	AJ278989	100%
Vitis vinifera	Grip68	AJ237987	46% (72/155)
Prunus armeniaca	Putative allergen protein	AF134731	46% (65/135)
Hevea brasiliensis	Hev b 5	U51631	43% (65/150)
Solanum tuberosum	TUB8	Z11679	47% (61/169)
Actinidia deliciosa	pKIWI501	L27810	41% (60/146)
Fagopyrum esculentum	Seed protein	D87983	49% (58/118)

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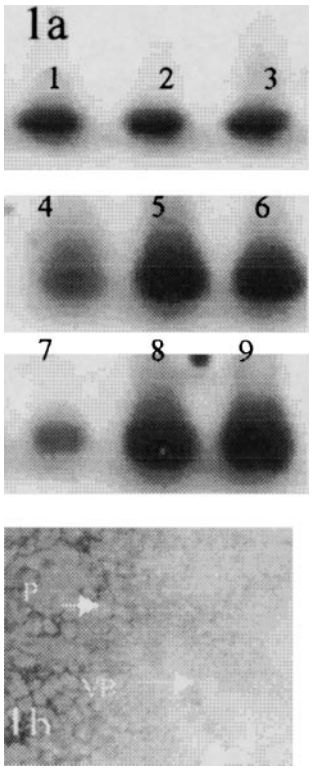

Fig. 1. (a) Total RNA was isolated from sugar beet (cv. Roberta) at various stages of development using the Wizard SV^{+} Total RNA extraction kit (Promega). 12 μg of each sample was separated on a 1.1% agarose gel (MOPS/formaldehyde) and then blotted onto a nylon membrane. This was then hybridized using with Psoralen-biotin labelled *RS2* probe (Ambion). Samples: 14 d seedling; 1, cotyledon, 2, hypocotyl, 3, root; 46 d plant; 4, leaf, 5, petiole, 6, root; 90 d plant; 7, leaf, 8, petiole, 9, root. (b) WISH analysis of a 46-d-old beetroot (cv. *Albina vereduna*) storage organ (using the method of de Almeida Engler *et al*., 1994). P, parenchymatous cells; VB, vascular bundles.

possible sites of interaction with antibodies to Hev b 5. Six human IgE-binding regions have been identified in Hev b 5, two of which contained the sequence EEXXA, where X was P, T or K (Beezhold *et al*., 1999). It was interesting to find that the *RS2* predicted protein sequence contained several examples of this motif (positions 46–50; 103–107).

RS2 was isolated because it was identified as being storage root enhanced during the library screening. This was confirmed by using Northern blot hybridization (Fig. 1a). In sugar beet seedlings the gene was expressed in root, hypocotyl and cotyledons in equal amounts. In older plants the relative amount of expression in the developing storage root increased in comparison with the leaf. The explanation for this enhancement was uncovered by the use of whole mount *in situ* hybridization with tissue from a beetroot storage organ (WISH, de Almeida Engler *et al*., 1994). This showed that *RS2* was expressed in the large number of parenchymatous storage cells that were produced in the mature storage organ (Fig. 1b). Although it is not root specific, this pattern of expression is likely to make the *RS2* promoter useful for targeting to the storage organ.

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