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Research article

Expression of arabinogalactan proteins during tomato fruit ripening and in response to mechanical wounding, hypoxia and anoxia

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ABSTRACT

Arabinogalactan proteins (AGPs) are highly glycosylated members of the superfamily of hydroxyproline-rich glycoproteins (HRGPs). Despite their implication in many aspects of plant growth and development little is known about their role in tomato fruit ripening (*Solanum lycopersicum*) and their response to abiotic stress in tomato fruits. A search of the currently available tomato genome database resulted in the identification of 34 genes encoding putative AGPs, with at least 20 of them being expressed in fruit. We monitored the abundance of AGPs bound by JIM8 and JIM13 monoclonal antibodies as well as the gene expression profiles of the Lys-rich LeAGP1 and two classical AGPs, SlAGP2 and SlAGP4. The JIM8- and JIM13-bound AGPs showed constitutive expression during fruit ripening and under hypoxic conditions, slight up-regulation to mechanical wounding in excised tomato fruit pericarp discs and up-regulation under anoxia indicating functional roles for these proteins in the developmental program of ripening and in response to abiotic stresses. Moreover, the SlAGP2 mRNA was significantly up-regulated during fruit ripening following the climacteric ethylene production, a pattern of expression similar to that of tomato fruit PG. The SlAGP4 and LeAGP1 mRNAs were up-regulated in response to mechanical wounding while under anoxia only the SlAGP4 transcript was induced. The protein and mRNA levels of these AGPs were induced under mechanical wounding while only JIM8-bound AGPs and SlAGP4 expression were induced under anoxic conditions. Our results indicate that selected tomato AGPs seem to play a role in fruit ripening as well as in response to mechanical wounding and anoxia.

1. Introduction

Arabinogalactan proteins (AGPs) are highly glycosylated members of the superfamily of hydroxyproline-rich glycoproteins (HRGPs) found in cells throughout the plant kingdom [1]. AGPs are present on the plasma membrane, cell wall, apoplastic space and in secretions [2].

Their protein moiety is rich in proline/hydroxyproline, alanine, serine and threonine and accounts for the 1–10% of total mass of the proteoglycan while more than 90% consists of arabinogalactan polysaccharides [3]. Most of the proline residues are hydroxylated by prolyl 4-hydroxylases in the endoplasmic reticulum [4] and subsequently are *O*-glycosylated in the Golgi by glycosyltransferases [5]. Both proline hydroxylation and hydroxyproline arabinogalactosylation motifs have been experimentally determined [6].

AGPs are classified based on amino acid sequence analysis as classical, Lys-rich, Asn-rich, fasciclin-like (FLAs), chimeric plastocyanin (PAGs) and chimeric with extensins, while AG peptides are AGPs with shorter, less than 90 amino acids, sequences [7–9]. Many AGPs are predicted to have secretory signal sequence at their N-terminus and hydrophobic domain at the C-terminus which directs the addition of glycosylphosphatidylinositol (GPI) anchor allowing the transmission of information between the intracellular and extracellular space [10,11].

AGPs are involved in a plethora of developmental processes such as seedling growth [12], cell expansion [13,14] and division [15,16], wounding [17], programmed cell death [18] and biotic and abiotic stresses such as *Agrobacterium tumefaciens*-mediated infection and salt tolerance [19,20]. Moreover, AGPs interact with plant growth regulators [21] as was demonstrated for the tomato LeAGP1 mRNA which is differentially regulated by auxin and

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Abbreviations: AGP, arabinogalactan protein; BR, breaker; FLA, fasciclin-like AGP; GPI, glycosylphosphatidylinositol; HAE, hybrid arabinogalactan and extensin; HRGP, hydroxyproline-rich glycoprotein; IG, immature green; MG, mature green; NFDM, non fat dry milk; PAG, chimeric plastocyanin AGP; PAST, proline, alanine, serine and threonine; PK, pink; RR, red ripe; RT-PCR, reverse transcriptase polymerase chain reaction; SDS, sodiumdodecyl sulfate; SDS-PAGE, sodiumdodecyl sulfate polyacrylamide gel electrophoresis; TBS, tris buffered saline; TU, turning.

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cytokinin, while its overexpression results in plants phenotypically similar to cytokinin overproducing [22].

AGPs are bound by certain synthetic phenylglycosides, such as β-D-glucose or β-D-galactose Yariv reagents [23]. The use of Yariv reagents has allowed the purification, quantification and localization of AGPs, while Yariv reagents have also been used as probes for the functional characterization of AGPs *in vivo* [24]. Yariv induced a gene expression profile which was most similar to that of wound-like responses indicating the involvement of AGPs in this abiotic stress [17]. Although AGPs were found in different plant organs including fruits [3] little is known about their role in fruit ripening with the exception of the well-studied Lys-rich LeAGP1 protein which was found to be more abundant in young fruits than roots, young stems, flowers and leaves [25].

We identified 34 putative AGPs with 20 of them being expressed in fruit tissue using the currently available annotated tomato genome database (http://solgenomics.net) and initiated a preliminary study on the role of selected AGPs on fruit ripening as well as abiotic stresses such as oxygen deficiency and mechanical wounding.

2. Results

2.1. Identification and classification of tomato AGPs

The search of the annotated tomato genome led to the identification of 34 putative AGPs comprising two classical, two Asn-rich and one Lys-rich AGPs, four AG peptides, 22 fasciclin-like AGPs (FLAs), three chimeric plastocyanin AGPs (PAGs) and two hybrid proteins having arabinogalactan and extensin modules (HAEs). Twenty of them were identified from tomato fruit ESTs while sixteen and fifteen were predicted to have signal peptide sequence and glycosylphosphatidylinositol (GPI) anchor sites, respectively (Table 1).

The 34 AGPs were grouped into three clusters according to a dendrogram constructed with their deduced amino acid sequences (Fig. 1). The two classical AGPs, SlAGP2 and SlAGP4, were positioned in different clusters with the SlAGP2 in cluster B comprising the Asn-rich AGPs, SlAGP7 and SlAGP8 and the SlAGP4 in cluster A comprising the Lys-rich LeAGP1, HAEs and PAGs (Fig. 1). The FLAs and the two AG peptides are grouped in cluster C (Fig. 1).

Both classical AGPs, SlAGP2 and SlAGP4, comprise putative signal peptide and several AP, PA, SP and TP repeats while only in SlAGP4 was a GPI-modification site predicted (Fig. 2). This AGP protein structure is similar to the previously identified Lys-rich domain of LeAGP1 [26] (Fig. 2).

2.2. Protein and gene expression profiles during fruit ripening

Antibodies raised against AGP glycan epitopes may provide a means to probe the AGPs profile during fruit ripening. To this direction, soluble AGP levels were monitored at six different stages of tomato fruit ripening using two monoclonal antibodies recognizing AGP glycan epitopes, JIM8 [27] and JIM13 [28]. However, it should be noted that these antibodies may react with carbohydrate epitopes which are displayed on different AGP core proteins, on certain glycoforms of a particular AGP core protein or even in other molecules such as pectins [29].

The soluble AGPs comprising the JIM8 and JIM13 epitopes ranged from 55 to 210 kDa and from 45 to 300 kDa, respectively

Table 1
Identification, characterization, and classification of putative tomato AGP genes.ND, not determined.

Unigene	Name	Class	AP/PA/SP/TP repeats	PAST	Amino acids	Signal peptide	GPI anchor	Expressed in fruit
SGN-U577484	*LeAGP1*	Lys-rich	21/15/7/7	61%	215	Yes	Yes	Yes
SGN-U577100	*SlAGP2*	Classical	6/5/5/7	42%	153	Yes	No	Yes
SGN-U581381	*SlAGP4*	Classical	5/3/8/4	61%	131	Yes	Yes	Yes
SGN-U577101	*SlAGP7*	Asn-rich AGP	6/5/6/8	33%	253	Yes	No	ND
SGN-U594284	*SlAGP8*	Asn-rich AGP	6/7/6/8	43%	143	No	No	ND
SGN-U569525	*SlAGP5*	AG Peptide	2/1/1/0	36%	72	Yes	No	ND
SGN-U575573	*SlAGP6*	AG Peptide	3/3/0/0	36%	72	Yes	Yes	Yes
SGN-U566489	*SlFLA1*	Chimeric	0/1/5/0	32%	233	No	Yes	Yes
SGN-U567096	*SlFLA2*	Chimeric	8/7/5/0	38%	264	Yes	Yes	Yes
SGN-U567097	*SlFLA3*	Chimeric	5/5/4/0	42%	142	No	Yes	Yes
SGN-U567735	*SlFLA4*	Chimeric	5/3/7/2	40%	262	Yes	Yes	ND
SGN-U571051	*SlFLA5*	Chimeric	3/3/0/1	37%	166	No	Yes	ND
SGN-U571367	*SlFLA6*	Chimeric	2/3/2/0	30%	337	Yes	ND	Yes
SGN-U571368	*SlFLA7*	Chimeric	4/6/2/2	37%	181	No	Yes	ND
SGN-U573243	*SlFLA8*	Chimeric	4/6/5/1	39%	249	Yes	Yes	Yes
SGN-U574500	*SlFLA9*	Chimeric	5/11/8/2	36%	411	Yes	Yes	Yes
SGN-U576059	*SlFLA10*	Chimeric	6/4/1/0	30%	409	Yes	Yes	Yes
SGN-U579149	*SlFLA11*	Chimeric	6/9/6/3	36%	312	No	Yes	Yes
SGN-U580259	*SlFLA12*	Chimeric	11/3/1/1	27%	439	Yes	No	Yes
SGN-U580319	*SlFLA13*	Chimeric	12/10/8/5	41%	414	Yes	Yes	Yes
SGN-U580810	*SlFLA14*	Chimeric	10/3/1/0	27%	449	Yes	No	Yes
SGN-U581555	*SlFLA15*	Chimeric	6/4/2/0	32%	247	Yes	Yes	Yes
SGN-U583299	*SlFLA16*	Chimeric	4/1/1/2	35%	209	Yes	No	ND
SGN-U583300	*SlFLA17*	Chimeric	10/4/1/3	40%	206	No	Yes	ND
SGN-U585149	*SlFLA18*	Chimeric	4/5/1/3	35%	221	Yes	No	ND
SGN-U590038	*SlFLA19*	Chimeric	6/4/2/0	32%	209	Yes	Yes	Yes
SGN-U592034	*SlFLA20*	Chimeric	1/2/2/0	38%	130	Yes	No	ND
SGN-U593256	*SlFLA21*	Chimeric	7/6/6/2	41%	226	No	Yes	Yes
SGN-U598308	*SlFLA22*	Chimeric	1/1/3/0	32%	199	No	No	ND
SGN-U564300	*SlPAG1*	Chimeric	3/2/6/1	38%	191	No	No	ND
SGN-U565967	*SlPAG2*	Chimeric	1/1/2/0	27%	200	Yes	Yes	Yes
SGN-U578700	*SlPAG3*	Chimeric	10/10/8/11	47%	345	Yes	No	Yes
SGN-U578586	*SlHAE1*	AGP/EXT hybrid	4/3/9/5	61%	152	Yes	Yes	ND
SGN-U579186	*SlHAE2*	AGP/EXT hybrid	4/3/6/3	56%	96	No	Yes	ND