**Short Communication**

Organ-Specific Arabinogalactan-Proteins of *Lycopersicon peruvianum* (Mill) Demonstrated by Crossed Electrophoresis

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**ABSTRACT**

Extracts of style, petal, leaf, petiole, stem, and callus derived from stems of wild tomato (*Lycopersicon peruvianum*) contain characteristic sets of arabinogalactan-proteins. This is demonstrated by crossed electrophoresis in which Yariv reagent, which specifically binds to and precipitates arabinogalactan-proteins, is incorporated into the second gel.

Arabinogalactan-proteins are a class of proteoglycans widely distributed in the plant kingdom. They are mainly localized extracellularly, although they are also associated with the plasma membrane (9), and are major components of plant gums and exudates (2, 3). Their carbohydrate moiety consists mainly of galactose and arabinose with other monosaccharides and uronic acids as minor components; the galactosyl residues are organized to form a backbone of 3-linked galactose with branches through C(6); the arabinosyl residues are predominantly in terminal positions. The protein moiety contains hydroxyproline. AGPs specifically bind to and are precipitated by β-glycosyl-Yariv reagents (7) which are red colored dyes formed by coupling diazotized aminophenyl β-glycosides to phloroglucinol (11). The physiological role of AGPs is not known (2, 3). One suggestion is that AGPs may be involved in expression of identity of species, genus, cultivar, tissue, or cell type (1, 2).

To date, two electrophoretic methods for analytical separation of AGPs have been used: cellulose acetate electrophoresis (7) and gel isoelectrofocusing (9). Here we report the development of a new electrophoretic method for separating and comparing AGPs from different plant extracts. The method is related to crossed immunoelectrophoresis (8). AGPs, separated electrophoretically in the first direction are then run electrophoretically into a gel containing β-glucosyl-Yariv reagent in the second direction. Using this method we found that extracts of different organs of *Lycopersicon peruvianum* have characteristic sets of AGPs.

**MATERIALS AND METHODS**

Materials. *Gladiolus* AGP was isolated from styles of *Gladiolus gandavensis* by affinity chromatography according to Gleeson *et al.* (6). The β-glucosyl-Yariv reagent (1,3,5 tris-[4-β-D-glucopyranosyl-oxophenyl azo]-2,4,6 trihydroxy-benzene), a red dye referred to as the Yariv reagent, synthesized according to Yariv *et al.* (11), was a generous gift of Dr. M. A. Jermyn (Parkville, Victoria, Australia). Gum arabic (lot 65C-0226; for analysis see Ref. 10) was obtained from Sigma and agarose (lot 102250; analysis: ash 0.7%, sulfur 0.089%) from Calbiochem.

Plant Material. *Lycopersicon peruvianum* (wild tomato) plants were grown from seed obtained from the Department of Agriculture, Victoria, Australia, and maintained in the glasshouse. All experiments were performed on material clonally propagated from a single plant (plant No. 36). Callus was initiated from stem segments and grown on CSV medium (4) with 0.25 μM kinetin and 10 μM 2,4-D at 25°C in the dark and subcultured every 10 to 14 d.

Plant Extracts. Fresh stem, leaf, petiole, or callus tissue (1.5 g fresh weight) was ground in a mortar and pestle in liquid N and extracted with 4.5 ml extraction buffer (50 mM Tris-HCl [pH 8.0], 10 mM EDTA, 2 mM Na3S2O3, 1% v/v Triton X-100). Fresh styles (0.15 g) and fresh petals (0.3 g) were homogenized in a glass homogenizer with 0.45 and 0.9 ml extraction buffer, respectively. Cell debris was removed from the extracts by centrifugation (10,000g, 5 min, 4°C). The AGP concentration of the supernatant was determined by the method of Van Holst and Clarke (10) and the buffer-soluble AGP content of fresh tissue (Table 1). Five volumes of ethanol were added to the supernatants to precipitate AGPs. After incubation for 3 h at 4°C, the precipitates were pelleted (10,000g, 5 min) and resuspended in 50 ml Tris HCl (pH 8.0). After sonication for 2 min Table 1. Characteristics of Arabinogalactan-proteins in Extracts of L. peruvianum Tissues Separated by Crossed Electrophoresis

<table>
<thead>
<tr>
<th>Tissue</th>
<th>AGP Content</th>
<th>Recovery after Ethanol Precipitation</th>
<th>Rf Values of Peaks (and shoulders)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Style</td>
<td>1.3</td>
<td>69</td>
<td>0.16, 0.44</td>
</tr>
<tr>
<td></td>
<td></td>
<td>100</td>
<td>0.15, 0.44</td>
</tr>
<tr>
<td>Petal</td>
<td>0.72</td>
<td>64</td>
<td>(0.27), 0.50</td>
</tr>
<tr>
<td></td>
<td></td>
<td>61</td>
<td>(0.28), 0.50</td>
</tr>
<tr>
<td>Leaf</td>
<td>0.81</td>
<td>77</td>
<td>0.23, 0.54 (0.74)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>67</td>
<td>0.22, 0.52 (0.73)</td>
</tr>
<tr>
<td>Petiole</td>
<td>0.52</td>
<td>81</td>
<td>0.29, 0.53 (0.73)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>60</td>
<td>0.31, 0.54 (0.74)</td>
</tr>
<tr>
<td>Stem</td>
<td>0.52</td>
<td>68</td>
<td>(0.38), 0.56 (0.77)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>98</td>
<td>(0.37), 0.55 (0.76)</td>
</tr>
<tr>
<td>Callus from stem</td>
<td>0.6 to 0.9</td>
<td>64</td>
<td>0.38</td>
</tr>
<tr>
<td></td>
<td></td>
<td>100</td>
<td>0.39</td>
</tr>
</tbody>
</table>

* The values in brackets represent shoulders. Rf values are relative to the bromophenol blue dye front; the top values are from the gels shown in Figure 4; the bottom values are from gels run from a duplicate tissue extract.

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Abbreviation: AGPs, arabinogalactan-proteins.
FIG. 1. Electrophoresis of gum arabic. Top lane, electrophoresis of gum arabic (7.5 μg) under the same conditions as the first direction of the crossed electrophoresis but directly stained with 0.1 mg/ml Yariv reagent in 1% (w/v) NaCl. Bottom, crossed electrophoresis of gum arabic (7.5 μg loaded). Two components Rv 0.65 and 0.44 separate and account for 83 and 17% of the total area, respectively.

(Revised 8x5 to 604x787)

FIG. 2. Relationship between peak area and amount of gum arabic as a reference arabinogalactan-protein. Top, electrophoresis of gum arabic samples into a gel containing Yariv reagent (30 μg/ml) under the same conditions as the second direction of the crossed electrophoresis. Sample volume is 5 μl in wells of 3 mm diameter. Bottom, relationship between peak area in the gel shown above and amount of gum arabic loaded.

FIG. 3. Electrophoresis of Gladiolus arabinogalactan-protein. Top lane, electrophoresis of Gladiolus AGP (7 μg) under the same conditions as the first direction of the crossed electrophoresis but directly stained with 0.1 mg/ml Yariv reagent in 1% (w/v) NaCl. Bottom, crossed electrophoresis of Gladiolus AGP (7 μg loaded).

(Bransonic 32, Branson, CN) the insoluble material was removed by centrifugation (10,000 g, 5 min), and the AGP concentration of the supernatant determined. The AGP recovered after ethanol precipitation was between 60 and 100% for different samples. The supernatant was finally desalted over a Bio-Gel P-6 DG column (Bio-Rad) and equilibrated with water. The recovery after this step was better than 90%. The desalted sample was analyzed by cross electrophoresis after determination of the AGP concentration. All AGP determinations were performed by the method of Van Holst and Clarke (10) with gum arabic as standard.

Arabinogalactan-Protein-Yariv Reagent Crossed Electrophoresis. A solution of 1% (w/v) agarose, 0.025 M Tris, and 0.2 M glycine (pH 8.3) was heated to boiling. Aliquots (10 ml) were poured onto preheated, level glass plates (8 × 12 cm) covered with Gel Bond film (Pharmacia, Uppsala, Sweden). A Teflon gel comb was used to make two wells (7 × 1.5 mm) in the gel. The top well was filled with 15 μl of bromophenol blue (0.2 mg/ml) in 50 mM Tris-HCl (pH 8.0) and the bottom well with 15 μl sample mixed with 0.8 μl of bromophenol blue (3 mg/ml). Gels were run at 250 V (20 V/cm), 8 mamp, for about 45 min, or until the dye front moved 8 cm. The running buffer was 0.025 M Tris, 0.2 M glycine and gels were connected by sponge cloth wicks (Wettex, Johnson Pty. Ltd., Milsons Point, N.S.W., Australia) (paper wicks may contain interfering substances). The dye fronts from both wells travel the same distance if the plant extract does not contain high levels of salts or other charged compounds. After electrophoresis, a 5.5 × 12 cm slice of gel above the sample lane is removed and replaced by a second gel (1 mm thick), consisting of 1% (w/v) agarose containing 0.025 M Tris, 0.2 M glycine, and 30 μg/ml D-glucosyl-Yariv reagent. The Tris-glycine buffer is chosen for its pH, low conductivity, and high solute concentration which facilitates precipitation of the AGP-Yariv reagent complex. Electrophoresis in the second direction was at 80 V (10 V/cm), 4 mamp for 16 h. The nonprecipitated Yariv reagent was removed by washing with 1% w/v NaCl followed by a rinse with distilled H₂O and the gels were dried in a warm air stream. Peak area was estimated by triangulation.

RESULTS AND DISCUSSION

The method of crossed electrophoresis was established using two well characterized AGPs, gum arabic and Gladiolus style AGP. Crossed electrophoresis of gum arabic is shown in Figure 1 (bottom). A red precipitation line is formed by the interaction
between the Yariv reagent and AGP. The gum arabic sample is
separated into two AGPs with Rf values relative to the bromo-
phenol blue dye front of 0.65 ± 0.01 and 0.44 ± 0.02. The areas
of the major and minor peaks are 83 and 17% of the total area.
In the absence of AGP in the sample, a continuous red straight
line is formed. The physical basis for this phenomenon is not
understood; however, it is useful as a reference base line. The
peak area is linearly related to the amount of AGP present; this
is shown by running samples (5 µl) from a dilution series of gum
arabic in wells punched into the first dimension gel. Electropho-
resis of the samples into the Yariv reagent-containing second gel
was under the same conditions as those used for crossed elec-
trophoresis (Fig. 2).

Analysis of AGPs by crossed electrophoresis depends on their
capacity to migrate to the anode at pH 8.3 in the gel system used.
AGPs are generally negatively charged or neutral at this pH. The
net charge of AGPs is due to uronic acid groups present in an
unmethylated form as part of the carbohydrate moiety, as well
as any contribution from the protein moiety, which is usually a
minor component (<10%) of AGPs. Uronic acid residues might
thus dominate the charge behavior of the AGP and allow migra-
tion to anode at pH 8.3. An AGP known to have a low uronic
acid content (<1%), *Gladiolus* style AGP (5) was examined to
test the range of application of the present method. In the first
gel the *Gladiolus* style AGP remained close to the sample well,
but migrated a short distance towards the anode (Fig. 3), although
it is reported to migrate towards the cathode at pH 8.8 in a
different electrophoretic system (6). Different behavior might be
expected from AGPs which have a significant content of basic
proteins, although such AGPs have not been described (2, 3).

Tissue extracts of *L. peruvianum* contain AGP with the style
having a particularly high AGP content (Table I). The values for
the AGP concentration in organ extracts were reproducible, but
those for callus varied for different samples. Whole tissue extracts
of *L. peruvianum* contained material which interfered with the
crossed electrophoresis. AGPs from the extracts were therefore
precipitated with ethanol before examination with crossed elec-
trophoresis. This procedure removed most interfering substances
and concentrated the AGP sample, with recoveries greater than
60% (Table I). The recovered AGPs of each tissue extract had a
characteristic set of AGPs (Fig. 4). The Rf values of peaks and
shoulders relative to the bromophenol blue dye front of duplicate
gels are listed in Table I. Duplicate gels from extracts from
different clonal plants prepared on different d were almost iden-
tical. Identical gels were also obtained from replicate experiments
in which the recoveries varied between 60 and 100%, indicating
that AGPs were not selectively lost during the precipitation and
recovery procedure. The peaks shown in Figure 4 are in many
cases broad and probably represent more than one AGP.

In general, 7 to 16 µg total AGP was loaded per gel to ensure
detection of minor AGPs in the extracts although 0.25 µg is
easily detected. For the petal extract (Fig. 4B) 4 µg AGP was
loaded and the Yariv reagent concentration in the second gel
was halved to 15 µg/ml. The precipitation line formed was less
dense than that formed under standard conditions, but was quite
distinct.

By this method, which requires only small tissue samples, we
have demonstrated that tissues of *L. peruvianum* contain differ-
et and separable groups of AGPs. The finding supports the
suggestion that AGPs may be involved in the expression of
identity of tissues (2).

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**LITERATURE CITED**

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