

Composition of the Walls of Stem and Leaves of Vitrifying Carnation

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Abstract. Vitrification of stem explants of carnation was brought about by culturing in liquid medium. Cellulose and lignin levels were decreased in vitrified stems and leaves. Isolated cell walls of vitrified tissues were also characterized by low calcium content, low Ca^{2+} /uronic acids ratio, low ratio of uronic acids to neutral sugars due to higher amounts of the latter. All these characteristics may account for the high wall plastic potential previously measured in vitrifying internodes.

Passage of carnation explants consisting of nodes with leaves from a solid to agitated liquid medium induces vitrification (SUTTER and LANGHANS 1979, KEVERS *et al.* 1984). Such vitrified tissues were characterized by hypolignification (KEVERS *et al.* 1987) apparently due to a deficiency in the activity of syringaldazine-peroxidases (KEVERS and GASPAR 1985). It has also been shown that liquid culture induced an increase in the *in vitro* immediate and total deformation capacities of the walls. Measurements indicated that these deformation capacities involved plastic properties rather than elastic ones (KEVERS *et al.* 1987). These internode wall properties could not be related to wall composition since previous analyses concerned whole explants. The present paper examines the wall composition of separated stem and leaves comparatively in normal (solid medium) and vitrifying (liquid medium) conditions.

MATERIALS AND METHODS

An *in vitro* culture of *Dianthus caryophyllus* was initiated from nodes following the technique of EARLE and LANGHANS (1975) on solid agar (0.75 %) medium containing kinetin (0.5 mg l^{-1}) and naphthaleneacetic acid (0.1 mg l^{-1}) at pH 5.7. Growth regulators were added before autoclaving (20 min, $121 \text{ }^\circ\text{C}$, 1.2 kg cm^{-2}). Subcultures were compared on similar solid and liquid

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TABLE 1

Lignin and cellulose levels in stems and leaves of carnation explants after 9 days culture on solid (9S) and liquid (9L) media compared to explants (from solid cultures) at zero (0) time

	Stems			Leaves		
	0	9S	9L	0	9S	9L
Lignin						
% fresh m.	0.21±0.02	0.31±0.03	0.16±0.01	0.14±0.01	0.15±0.01	0.09±0.01
% dry m.	1.41±0.10	2.05±0.17	1.11±0.10	0.95±0.07	1.07±0.09	0.66±0.05
Cellulose						
% fresh m.	1.96±0.15	2.25±0.15	1.41±0.10	1.82±0.12	1.79±0.12	1.16±0.08
% dry m.	14.5±0.8	15.3±1.2	10.1±0.9	13.0±1.1	12.5±1.0	8.3±0.6

(non agar) media. Explants were made from 10 mm long nodes taken from the upper portion of the shoots. In both cases (solid or liquid medium) 50 ml were dispensed in 100-ml Erlenmeyer flasks. The cultures were maintained in a growth chamber at 24 °C with 16 h photoperiod and irradiance of 3.22 W m⁻² (Sylvania Gro-lux fluorescent lamps). The liquid cultures were continuously agitated horizontally (80 displacements min⁻¹). They brought about 100 % vitrification in 9 days with first visible signs after 6 days.

Extraction and Spectrophotometric Determination of Lignin Content

were made using the modified techniques of JOHNSON *et al.* (1961) and ALIBERT and BOUDET (1979). Lyophilised nodes with leaves were ground in a Braun homogeniser, then thoroughly extracted first with distilled H₂O, successively with 1M NaCl containing 2 % Triton × 100 and finally with ethanol-benzene (1 : 2, v/v). The aqueous extracts were centrifuged (3000 g for 10 min), the organic ones filtrated on frittered glass.

Alkaline hydrolysis of the dried residue with 2M NaOH under N₂ atmosphere for 1 h was indispensable; the final residue was dried in a desiccator. To solubilise lignins 2.5 ml 25 % acetylbromide was added to 25 mg of the dry powder. After heating at 70 °C for 30 min, the tubes were rapidly cooled and 2.5 ml 2M NaOH was added. The mixture was filtered through filter paper into a 50 ml volumetric flask. 0.25 ml 7.5 M hydroxylamine was added to remove bromine and polybromide. The absorbance of the final solution diluted to 50 ml with acetic acid was measured at 280 nm; the blank was run along with the sample in the same manner. Results were expressed as a percentage of the final dry matter according to the formula:

$$\frac{A_{280\text{nm}}/20 \times 100}{\epsilon \times \text{final dry matter [g]}}$$

with a coefficient ϵ of lignin standard = 24 (JOHNSON *et al.* 1961).

TABLE 2

Composition of the pectic fractions PF₁ and PF₂ (see methods) of stems and leaves of carnation explants after 9 days culture on solid (9S) and liquid (9L) media compared to explants (from solid cultures) at zero (0) time. U.A. and N.S. in μmol per mg cell walls, Ca^{2+} and Mg^{2+} as μeq per 100 g walls

	Stems			Leaves		
	0	9S	9L	0	9S	9L
PF ₁ Uronic acids (U.A.)	0.33±0.02	0.18±0.01	0.32±0.01	0.19±0.02	0.44±0.05	0.27±0.02
Neutral sugars (N.S.)	0.10±0.01	0.11±0.01	0.25±0.02	0.09±0.01	0.25±0.01	0.35±0.04
U.A./N.S.	3.32	1.62	1.27	2.23	1.79	0.76
Ca^{2+}	0.49±0.02	3.90±0.30	1.05±0.06	0.84±0.09	0.63±0.04	0.58±0.03
Mg^{2+}	0.21±0.01	0.17±0.02	0.30±0.01	0.28±0.02	0.30±0.02	0.31±0.03
PF ₂ Uronic acids (U.A.)	0.70±0.02	0.54±0.05	0.73±0.02	0.40±0.01	0.52±0.02	0.37±0.02
Neutral sugars (N.S.)	0.07±0.01	0.10±0.01	0.26±0.02	0.048±0.005	0.13±0.01	0.13±0.02
U.A./N.S.	9.94	5.20	2.76	8.33	4.14	2.79
Ca^{2+}	2.73±0.05	4.04±0.08	2.68±0.11	2.03±0.20	1.48±0.07	0.95±0.06
Mg^{2+}	0.59±0.03	0.51±0.05	0.69±0.03	0.37±0.02	0.42±0.03	0.48±0.02

Cellulose Content

of nodes with leaves was estimated according to MARIGO and BOUDET (1980). Cellulose was isolated after different extractions successively eliminating chlorophylls (80 % acetone), proteins (300 mM NaOH, 24 h at 27 °C), pectic substances (0.5 % ammonium oxalate-0.5 % oxalate, v/v) and hemicellulosis substances (10 % NaOH). All supernatants were discarded by centrifuging at 3000 g for 10 min and the final preparation was lyophilised. Cellulose was then extracted with 72 % H₂SO₄ for 2 h at 20 °C followed by 2 % H₂SO₄ for 2 h at 115 °C. The residue was lyophilised and the cellulose content was estimated by weight difference before and after extraction of the cellulose.

Composition of Pectic Material

Cell walls were obtained from lyophilised internodes and leaves previously treated with methanolacetone (1/1) according to GOLDBERG *et al.* (1986). Two pectic fractions (PF₁ and PF₂) were successively extracted from the cell walls with hot water (2 × 1 h, 100 °C) and 1 % EDTA (6 h, 60 °C). Uronic acids were estimated by the *m*-diphenol method (BLUMENKRANZ and ASBOE-HANSEN 1973) and total carbohydrates detected with anthrone. Ca^{2+} and Mg^{2+} contents of the pectic fractions were determined by atomic absorption spectrophotometry with La²⁺ and Sr²⁺ as internal standards, respectively for Ca^{2+} and Mg^{2+} .

RESULTS AND DISCUSSION

The levels of lignin and cellulose in stem and leaves of 9 day-old explants of solid and liquid (vitrifying) cultures are recorded in Table 1. The lignin levels were increased in both stem and leaves of carnation from the solid cultures while decreased in the corresponding organs from explants in liquid cultures. The relative cellulose content of stems increased during the culture on solid medium but decreased in liquid conditions. It did not change significantly in the leaves of normal plants but was reduced to two thirds in vitrified plants. Table 2 compares the uronic acid and neutral sugar contents of normal and vitrified carnation stems and leaves. After 9 day-culture the uronic acids content ($PF_1 + PF_2$) of stems and leaves of vitrifying material was approximately the same as the controls at zero time, while it had decreased in stems and increased in leaves of normal plants. The contents of neutral sugars increased in stems and leaves of both normal and vitrifying carnations but more in liquid conditions. This led to a lower uronic acids/neutral sugars ratio in stem and leaves of vitrifying plants.

Table 2 also presents the Ca^{2+} and Mg^{2+} levels in the same wall fractions. It appears clearly that stems and leaves of the glassy plants exhibited lower calcium and higher magnesium contents than those of normal plants.

The present results confirm the deficiency in lignification observed in many types of vitrifying explants (VIETH *et al.* 1983, DAGUIN and LETOUZÉ 1985). They also indicate a reduction of the cellulose content in the cell wall, which had not been estimated yet in vitrifying materials. Cellulose, as well as lignin, thus could be involved in the changes of cell wall mechanical properties of vitrifying carnation tissues. Stem walls of vitrified tissues contained more uronic acids than those of normal tissues but inverse relation holds for the leaves. More spectacular was the high level of neutral sugars in both organs of vitrified tissues, which led to a low ratio of uronic acids to neutral sugars. Vitrifying internodes were also characterized by a relatively low calcium content. The ratio of cations to uronic acids was obviously higher in normal than in vitrifying internodes; this low value of the Ca^{2+} /uronic acids ratio would induce high electrostatic potential which, according to RICARD and NOAT (1986), enhances cell wall plasticity. Due to their strong interactions with the carboxylic groups of the pectic acids, the Ca^{2+} ions would behave in the wall somewhat like the H^+ ions. Therefore, they would control the charge density of the wall, which factor is directly correlated to the ionic selectivity of the wall and to its degree of swelling (DEMARTY *et al.* 1984). All these data are in good agreement with the high plastic potential observed in vitrifying internodes (KEVERS *et al.* 1987). As already noticed with pea internodes (NAKAJIMA *et al.* 1981) and mung bean hypocotyls (GOLDBERG *et al.* 1986), low cell wall plasticity is accompanied with high Ca^{2+}/Mg^{2+} ratios and high calcium content.

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