Plant Cell Reports (1989) 8:358–360

Plant Cell Reports

Vitrification and soluble carbohydrate levels in *Petunia* leaves as influenced by media Gelrite and sucrose concentrations

Thomas W. Zimmerman and B. Greg Cobb

Department of Horticultural Sciences, Texas A&M University, College Station, TX 77843-2133, USA

Received May 19, 1989/Revised version received June 22, 1989 – Communicated by I.K. Vasil

Summary. Normal nodal segments of *Petunia hybrida* were grown on Murashige and Skoog salts containing varied levels of Gelrite and sucrose. Higher concentrations of Gelrite decreased vitrification while increased sucrose concentrations promoted vitrification. Leaves of vitreous plants had higher levels of reducing sugars and sucrose but lower or undetectable levels of inositol as compared to normal plants. Normal plants on medium void of inositol have the ability to synthesize inositol and maintain levels equal to that found in plants from inositol containing media.

Key words: Vitrification - Inositol - Carbohydrates - Petunia - Gelrite

Introduction

Vitrification is a physiological disorder which affects numerous plants propagated *in vitro* (Kevers et al. 1984). Vitrification has been reduced by increased agar concentrations (Debergh 1983; Debergh et al. 1981; Hakkaart and Versluijs 1983; Leshem 1983; Ziv et al. 1983) or agar in combination with Gelrite (Pasqualetto et al. 1986). Gelrite, when used to obtain the same firmness as agar, promotes vitrification (Bottcher et al. 1988; Bornam and Vogelmann 1984).

Varied concentrations and sources of carbohydrates have been used in agar solidified media (Rugini 1986; Orlikowska 1987) to control vitrification. Orlikowska (1987) obtained inconclusive results when 30 or 45 g l^{-1} sucrose was used on quince cultures. By replacing sucrose with 45 g l^{-1} fructose, Rugini (1986) drastically reduced vitrification in almond.

Although vitrification can be attenuated by altering the carbohydrate source and/or gelling agent, the effect on leaf carbohydrate metabolism is not known. The purpose of this work was to examine the influence of media containing varied Gelrite and sucrose concentrations on the development of normal (non-vitrified) and vitrified plants. In addition, soluble carbohydrate levels of leaves from both plant types were determined. Here we report that even though inositol is in the medium and in normal plants, it was absent or present at very low concentrations in vitreous plants.

Materials and methods

Nodal segments from normal *Petunia hybrida* (Monsanto V23 x R51) were obtained from plants maintained on Murashige and Skoog (MS) (1962) salts containing 30 g l^{-1} sucrose, 100 mg l^{-1} myo-inositol, 0.5 mg l^{-1} thiamine-HCl and 2 g l^{-1} Gelrite. The source plants had been maintained in culture for 5 months and nodal segments had been subcultured monthly before the start of this experiment.

The treatment medium was MS as above with varying levels of sucrose (15, 30, or 45 g l^{-1}) and Gelrite (1, 2 or 3 g l^{-1}). The treatment media were adjusted to pH 5.7 with 1N KOH and dispensed into Magenta GA7 vessels (40 ml/vessel) and autoclaved for 15 min at 120 °C, 20 psi. There were 8 vessels per treatment, each with 5 normal nodal segments. The cultures were grown in a 26°C growth room under cool white fluorescent lights (12 W m^{-2}) under a 16 hr photoperiod.

After 4 weeks, the development of normal and vitrified plants was recorded. Leaf samples were collected from normal and vitrified plants for determination of soluble carbohydrate, chlorophyll and protein content. Fresh weights were recorded and each sample was ground (1:5 w/v) in 50 mM Tris-HCl (pH 7) on ice. After centrifugation, aliquots of the supernatant were collected for use in soluble protein determination (Bradford 1976) and carbohydrate analysis. Other leaf samples were ground in 80% acetone and chlorophyll content determined by the method of Arnon (1949).

For carbohydrate analysis, 1 ml of the supernatant was lyophilized and dissolved in 500 µl STOX™ reagent (pyridine solution containing 25 g l^{-1} hydroxylamine-HCl and 400 mg l^{-1} phenyl-ß-D-glucopyranoside) and heated in a 70°C sand bath. After heating 1 hr, 500 µl hexamethyldisilazane and 50 µl trifluroacetic acid were added. Samples were analyzed on a 30m x 0.75 mm ID Supelco SPB-1 glass capillary column. The temperature program for the column was begun at 100°C held 1 min, to 182°C at 10°C min^{-1}, to 209°C at 1.5°C min^{-1}, to 305°C at 8°C min^{-1}, and held at 305°C for 5 min. The carrier gas was He at a flow rate of 12 ml min^{-1}.

In a subsequent experiment, normal nodal segments were placed on MS media with 0, 10, 100 or 1000 mg l^{-1} myo-inositol. Tissues from new growth were harvested weekly for 4 weeks and analyzed for soluble carbohydrate content as above.

Results and Discussion

Vitrified plants had water-soaked translucent stems and leaves. In addition, the leaves were elongated and concave. Vitreous petunia plants had significantly elevated relative water content, but lower protein and chlorophyll levels than normal *in vitro* grown petunias (Table 1).

Vitrification was reduced in media at higher Gelrite concentrations (Fig. 1). Similar results have been found for agar gelled media (Bornman and Vogelman 1984; Hakkaart and Versluijs 1983; Pasqualetto et al. 1986; Ziv et al. 1983). It has been suggested by Debergh (1981) that agar, when

Offprint requests to: T.W. Zimmerman

Table 1. Comparison of normal and vitreous petunia leaf tissue for relative water content, protein and chlorophyll.

Plant	Relative Water Content (%)	Protein (mg g^{-1} FW)	Chlorophyll ($\mu g\ g^{-1}$ FW)
Vitreous	96.30 a [a]	3.92 ± 0.66	168.49 ± 15.92
Normal	93.57 b	7.12 ± 1.58	663.07 ± 7.30

[a] t-test performed on arcsin transformed data (P>0.01).

used at higher concentrations, reduces the incidence of vitrification by increasing media matrix potential which may also be occurring here. Furthermore, increased sucrose concentrations, except at 1 g l^{-1} Gelrite, promoted vitrification (Fig. 1).

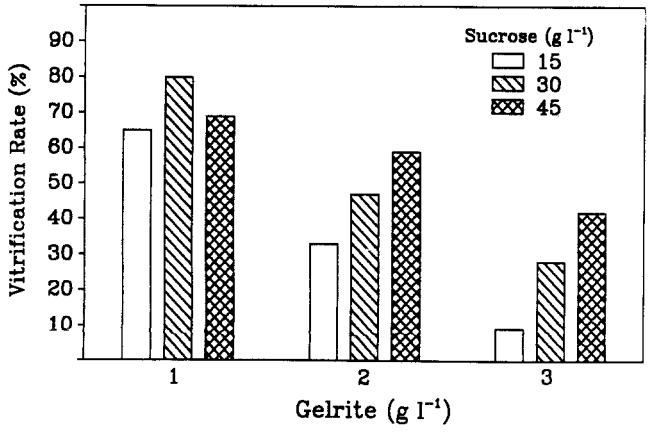

Fig. 1. Vitreous plant development after 4 weeks *in vitro* on media containing varying concentrations of sucrose and Gelrite.

Greater amounts of reducing sugars were present in vitrified leaves than in normal leaves in nearly all treatment combinations (Table 2). The concentration of sucrose was twice as high in vitreous leaves at 2 and 3 g l^{-1} Gelrite as compared to normal leaves. Inositol levels in vitreous leaves were below the levels found in normal leaves.

The reduced level of inositol in vitrified plants could be due to an inability to transport inositol from the media. To test whether this was occurring, plants were grown on media without inositol and on varying levels of inositol (Fig 2). Inositol levels from plants on media without inositol were similar to levels found in plants grown on media with 10 or 100 mg l^{-1} inositol (Fig 2). A significant increase in leaf inositol content was only obtained when 1000 mg l^{-1} (10 times the level normally added to the media) was added to the medium. This indicates that normal petunia plants were able to synthesize and accumulate inositol. The growth and appearance of normal plants were the same on media with or without inositol and adding inositol to the media did not have an effect on controlling vitrification (data not shown).

Loewus and Loewus (1983) indicated that inositol is a significant component of every plant. It is interesting that differences exist between normal and vitreous plants for inositol content even when inositol is added to the media (100 mg l^{-1}). Vitreous plants contain higher levels of reducing sugars and sucrose but contain low levels of inositol. Inositol synthesis is not limited by lack of substrate because inositol is synthesized from reducing sugars (Loewus and Loewus 1983) which are not limiting in vitreous plants.

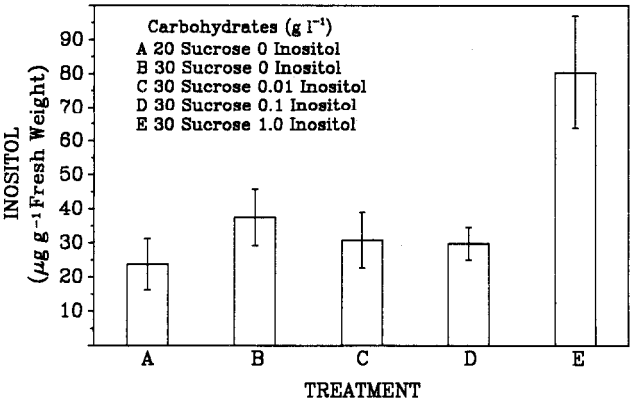

Fig. 2. Inositol levels in normal petunia plants grown on media with variable inositol content after 4 weeks.

Table 2. Analysis of leaf tissue for soluble carbohydrates from normal and vitrified plants developed on MS medium with 15, 30 or 45 g l^{-1} sucrose in combination with 1, 2 or 3 g l^{-1} Gelrite.

Gelrite g l^{-1}	Sucrose g l^{-1}	Plant	Reducing Sugars $\mu g\ g^{-1}$ F W	Sucrose $\mu g\ g^{-1}$ F W	Inositol $\mu g\ g^{-1}$ F W
1	15	Normal	200.3 ± 26.9	77.7 ± 7.4	22.8 ± 7.5
		Vitreous	384.7 ± 58.9	80.6 ± 26.1	nd
	30	Normal	168.9 ± 8.7	72.4 ± 10.9	32.7 ± 13.1
		Vitreous	473.0 ± 66.2	96.5 ± 14.0	nd
	45	Normal	207.8 ± 40.8	114.3 ± 16.1	54.1 ± 16.5
		Vitreous	197.1 ± 46.0	125.5 ± 32.1	nd
2	15	Normal	213.3 ± 5.6	113.1 ± 23.7	25.4 ± 7.9
		Vitreous	1299.9 ±186.0	677.0 ± 67.4	14.0 ± 0.5
	30	Normal	639.3 ± 49.5	163.3 ± 23.1	41.0 ± 7.3
		Vitreous	1422.2 ± 93.5	449.6 ± 33.8	4.0 ± 0.8
	45	Normal	1079.4 ± 87.9	252.1 ± 33.6	129.0 ± 31.2
		Vitreous	1033.7 ±179.0	586.9 ± 46.2	20.0 ± 1.8
3	15	Normal	964.5 ±175.0	394.2 ± 83.2	80.1 ± 28.3
		Vitreous	2435.4 ±283.7	1182.8 ±149.6	nd
	30	Normal	1027.1 ± 57.7	498.2 ± 82.2	90.1 ± 16.8
		Vitreous	1624.2 ± 83.5	1947.1 ± 87.5	6.6 ± 2.6
	45	Normal	1150.6 ±210.1	320.9 ± 58.5	159.8 ± 30.6
		Vitreous	3051.0 ±164.6	2257.3 ±155.8	nd

Inositol has been suggested to be involved in osmoregulation of plant cells. Sacher and Staples (1985) found that inositol levels increased during drought stress. Normal plants had increases in inositol levels as the media Gelrite or sucrose concentrations increased (Table 2). Inositol may be involved in the mechanism by which normal plants adjust to the different water potentials of these media. Thus, the higher content of water and soluble carbohydrates in vitreous plants may eliminate the need for osmotic control from inositol.

These results indicate that metabolism is altered in vitrified plants. In addition to reduced protein and chlorophyll levels, there are shifts in carbohydrate metabolism especially in regard to inositol. It is not known at this point whether the altered carbon metabolism in these vitreous petunias is a result of vitrification or is in some way controlling the onset of vitrification.

Acknowledgements. Texas Agricultural Experiment Station Journal Series No. TA 24786.

References

Arnon DI (1949) Plant Physiol 24:1-15
Bornman CH, TC Vogelmann (1984) Physiol Plant 61:505-512
Bottcher I, K Zoglauer, H Goring (1988) Physiol Plant 72:560-564
Bradford MM (1976) Anal Biochem 72:248-254
Debergh PC (1983) Physiol Plant 59:270-276
Debergh PC, Y Harbaoui, R Lemeur (1981) Physiol Plant 53:181-187
Hakkaart FA, JMA Versluijs (1983) Neth J Plant Path 89:47-53
Kever C, M Coumans, MF Coumans-Gilles, T Gaspar (1984) Physiol Plant 61:69-74
Leshem B (1983) Ann Bot 52:413-415
Murashige T, F Skoog (1962) Physiol Plant 15:473-497
Loewus FA, MW Loewus (1983) Ann Rev Plant Physiol 34:137-161
Orlikowska T, (1987) Acta Hort 212:237-244
Pasqualetto PL, RH Zimmerman, I Fordham (1986) J Amer Soc Hort Sci 111:976-980
Rugini E (1986) Almonds. In:Handbook of Plant Cell Culture, Vol 4 (DA Evans et al.) Macmillan Pub. Co, London, pp 574-611
Sacher RF, RC Staples (1985) Plant Physiol 77:206-210
Ziv M, G Meir, AH Halevy (1983) 2:55-6