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Some factors affecting glassiness in carnation meristem tip cultures

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Abstract

A study was made of the effect of agar concentration on glassiness during meristem tip culture of carnation. Increasing the agar concentration from the usual 6 g/l to 12 g/l decreased glassiness, but at the same time reduced plant growth. A concentration of 8 or 10 g/l reconciled these conflicting effects. The type of closure of the tubes was also found to affect glassiness, the looser types (cotton wool, metal caps, steri stops) being better than the tighter ones (aluminium foil and parafilm). In the first days after excision of the meristem tip a period existed in which a rather high temperature of 26 °C favoured the development of normal plants later. The positive response to high temperature was around 3 days after excision.

Additional keywords: agar concentration, cotton wool plugs, metal caps, steri stops, temperature sensitivity.

Introduction

Meristem tip culture as a means of freeing carnations from viruses has been carried out in the Netherlands for more than twenty years. Established commercial cultivars are kept free from new infections by isolation and intensive testing. New cultivars, however, are subjected to meristem culture in the initial phase of this procedure. They sometimes show glassiness, which in 1977 became an urgent problem.

Glassiness is a physiological disorder of plants growing in tubes. Glassy plantlets lack bloom, their leaves are broad and thick, and, most important of all, they perish after transfer into soil. In carnation the phenomenon is cultivar-bound: some cultivars, such as 'Doranja' and 'Polarthur', suffer badly, whereas others, such as 'Eolo', show it hardly if at all. Notwithstanding the importance of the problem little attention has been paid to it by tissue culturists. In an experiment with 'White Pike's Peak' Sutter and Langhans (1979) obtained only 2 to 4% normal, glaucous plants; the others were glassy. In an attempt to find the cause they studied epicuticular wax formation on carnation plantlets regenerated from meristem tip culture. They attributed low survival of carnation plants regenerated in vitro to lack of epicuticular wax, resulting in excessive desiccation after transfer from the tube to soil under glasshouse conditions.

In 1980 the problem was brought to our attention. This led to an investigation of the effects of agar concentration and the cap type of culture tubes.

Little attention has been paid in tissue culture to the type of closure of the tubes. Often it is not even mentioned. We present some experimental results showing that the type of closure may affect the frequency of glassiness and thus the success of meristem culture.

Finally we draw the attention to a short period in the early days after excision when the developing meristem tip positively responded to high temperature. When properly manipulated in the very beginning, temperature had a profound effect on glassiness which became apparent much later.

Materials and methods

Undisinfected shoot tips consisting of meristem dome and the first set of primordia were excised and placed in glass tubes (16 × 1.7 cm) filled with 10 ml of medium, consisting of ½ MS macro, ½ MS micro, $ZnSO_4.7H_2O$ being reduced to 0.18 mg/l and $MnSO_4.4H_2O$ to 0.1 mg/l. Organic substances were nicotinic acid, pyridoxine-HCl, thiamine, cysteine and Ca-pantothenate (each 1 mg/l), glycine (2 mg/l), biotin (10 mg/l), *m*-inositol (100 mg/l) and casein (500 mg/l); NAA and Kinetin were 0.5 and 0.03 mg/l, respectively; sucrose was 30 g/l; agar was Difco Bacto agar. The pH was adjusted to 5.6 before autoclaving at 108 °C during 20 minutes. The temperature in the growth chamber was 19-20 °C with 16 h of light from fluorescent tubes (Philips 33) in the preliminary experiments and 12 h of light during later experiments. The plantlets completed their development without transfer to a second tube. At two growth stages, glassiness was recorded and plantlets were measured.

Results

Agar concentration. In Table 1 results are presented of an experiment with batches of 24 meristem tips of cv. Sam's Pride, in which we used 6, 8, 10 or 12 g/l agar and five types of caps. Some of the explants did not grow or formed clumps of callus, so that the totals presented per treatment are not always 24. Increasing the agar concentration is shown to result in a decreased number of glassy plants (statistically significant at $P < 0.01$). Table 2 shows that increasing the agar concentrations adversely affected plant length (statistically significant at $P < 0.001$). Similar results were obtained in experiments with cv. Sam's Pride where we compared agar concentrations of 5, 6, 7, 8, 9, and 10 g/l, with cv. Elvira (4, 5, 6, 8 or 10 g/l) and with 16 new cultivars (8 or 10 g/l). In view of the conflicting effects a concentration of 8 or 10 g/l seems a good compromise. At these agar concentrations glassiness is reduced to an acceptable level.

Cap type. With the cvs Elvira and Sam's Pride cotton wool, metal caps and steri stops were found to yield more normal plants than were obtained with aluminium foil. In the experiment with cv. Sam's Pride (Table 1) parafilm was also included. It is clear that the looser types of closure like cotton wool, metal caps and steri stops gave better results than tight types like aluminium foil and parafilm (statistically significant difference at $P < 0.001$).

Table 1. Effect of agar concentration and cap type on glassiness in cv. Sam's Pride[1].

Cap type	Number of plantlets on agar concentration (g/l)										Statistical difference[2]
	6		8		10		12		total		
	normal	glassy	normal	glassy	normal	glassy	normal	glassy	normal	glassy	
cotton wool	16	7	18	5	16	8	18	3	68	23	a
metal cap	9	14	19	4	19	4	21	2	68	24	a
steri stop	10	14	18	5	16	7	22	0	66	26	a
aluminium foil	12	10	7	15	10	14	9	11	38	50	b
parafilm	6	17	3	19	8	13	9	15	26	64	b
total	53	62	65	48	69	46	79	31	266	187	

[1] Batches of 24 meristem tips per treatment.
[2] Same letters: no statistically significant difference; different letters: statistically significant differences ($P < 0.001$).

Tabel 1. Effect van agarconcentratie en type afsluiting op glazigheid bij cv. Sam's Pride.

Table 2. Effect of agar concentration and cap type on length of developed plantlets of cv. Sam's Pride.

Cap type	Number of plantlets on an agar concentration (g/l)									
	6		8		10		12		average	
	normal	glassy	normal	glassy	normal	glassy	normal	glassy	normal	glassy
cotton wool	6.1	0.9	5.9	1.0	5.1	0.9	3.6	0.7	5.2	0.9
metal cap	7.7	0.8	7.2	1.2	5.2	0.6	5.9	0.4	6.5	0.7
steri stop	6.2	0.8	6.0	0.7	4.5	0.9	3.3	–	5.0	0.8
aluminium foil	6.8	1.1	6.5	1.6	6.5	2.4	7.5	2.6	6.8	1.9
parafilm	7.2	1.4	7.4	1.7	6.7	2.6	5.6	3.4	6.7	2.3
average	6.8	1.0	6.6	1.2	5.6	1.5	5.2	1.8		

[1] Averages of lengths of all plantlets indicated in Table 1.

Tabel 2. Effect van agarconcentratie en type afsluiting op de lengte van de geproduceerde plantjes van cv. Sam's Pride.

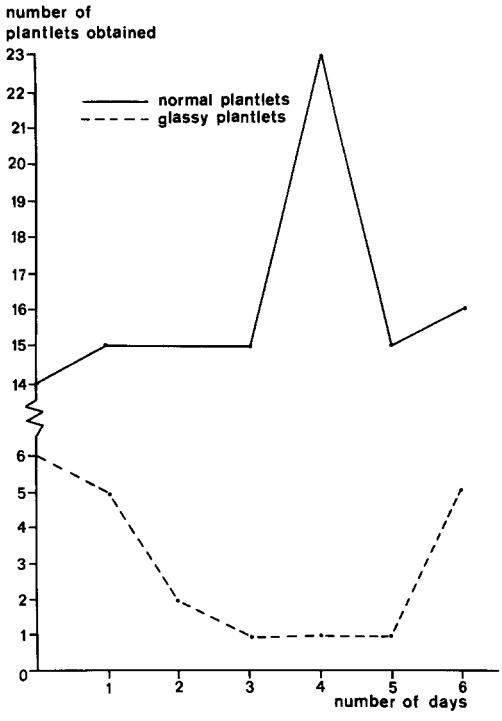

Fig. 1. Number of normal and glassy plantlets (cv. Sam's Pride) obtained from 24 meristems per treatment after 0, 1, 2, 3, 4, 5, or 6 days exposure to 26 °C after excision of the meristems.

Fig. 1. Aantal normale en glazige plantjes (cv. Sam's Pride) verkregen uit 24 groeipunten per behandeling na blootstelling gedurende 0, 1, 2, 3, 4, 5 of 6 dagen aan 26 °C na het uitprepareren.

Effect of higher initial temperature. In a preliminary experiment with cv. Emir meristem tips were grown at 26 °C in the dark during the first 2 weeks and then transferred to 20 °C in the light. They developed fewer glassy plants than meristem tips kept at 4, 9, 17 or 20 °C during the first 2 weeks. When the cultures were kept at a constant temperature of 26 °C their growth was poor. The temperature effect was limited to the initial growth period. Meristem tips of cv. Red Lily Ann were kept for 0, 1, 2, 3, 4, 7, 8, 9, 10, 13 or 14 days after excision, respectively, at 26 °C in darkness. The best effect had a 4-days' treatment. In another experiment with cv. Sam's Pride cultures were kept for 0, 1, 2, 3, 4, 5 or 6 days at 26 °C in darkness, respectively. In this case the 3-days' treatment proved best. Therefore, it seems that 3 to 4 days after excision the meristem tips responded more positively to higher temperature, the effect becoming apparent much later. In Fig. 1 some data are presented of meristem tips of cv. Sam's Pride kept in light. Subjecting them 4 days to 26 °C immediately after excision, resulted in more normal and fewer glassy plants.

Discussion

Problems of glassiness occur whenever carnation tissue is cultured in liquid medium. Hauzinska (1974) obtained leafy shoots with a glassy green colour ('vert vitreux') from carnation callus cultures in liquid medium. Davis et al (1977), in their studies of clonal multiplication of carnation by micro-propagation, encountered problems using the revolving flask technique. After removal from the flasks the leaves were excessively broad and thick and always lacked the characteristic blue-green bloom. The

plants were extremely vulnerable. By using a solid stationary medium some of the problems were eliminated, but our experience with solid media shows that this is by no means sufficient.

Sutter and Langhans (1979) studied epicuticular wax formation in meristem tip culture. They found wax plates on leaves of normal, glaucous plantlets, but no structured wax on glassy plantlets. When transferred to the greenhouse, increased amounts of structured epicuticular wax developed on leaves of glaucous plantlets but not on those of glassy plantlets. This resulted in excessive desiccation and death after transfer from in vitro conditions.

Debergh et al. (1981) refer to in vitro problems of glassiness in globe artichoke, leek, celery, cauliflower, carnation, *Ficus, Malus, Prunus, Pinus, Raphanus* and *Sequoia*. It is not clear if there is a relationship with epicuticular wax formation in all these instances. With globe artichoke the authors concluded that the only way to overcome glassiness in tissue-cultured artichokes is raising the agar concentration of the medium from 6 to 11, 15 or even 20 g/l. At the same time, however, increasing the agar concentration decreased the propagation rate. They concluded that the matric water potential, which depends on the water-binding colloids such as agar, and not the osmotic potential, depending on dissolved substances like carbohydrates, was responsible for the phenomenon.

The results of our experiments with carnation lead to the same conclusion. Increasing the agar concentration decreased glassiness, but at the same time reduced the length of the plantlets. Here the water relations in the medium could play a role, but they were certainly not exclusively responsible, as the results with different types of caps indicate. We attribute the effects of the different caps to differences in rates of ventilation: the loose types, such as cotton wool, metal caps and steri stops, allowed a better gas exchange than the tight types, such as aluminium foil and parafilm. However, no data are available about the relative humidity inside the tubes.

The effect of higher temperature in the initial phase is interesting. Because tissue cultures commonly are incubated either at a constant temperature or at fluctuating day/night temperatures there is little information about periods in which the explants specifically respond to a temporary rise in temperature. Further studies are required to shed light on the events occorring in the first days of growth of the freshly excised meristem tip.

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Samenvatting

Enkele maatregelen tegen glazigheid bij meristeemcultuur van anjers

In verband met het urgente probleem van glazigheid bij de meristeemcultuur van anjers werden drie factoren onderzocht. Het verhogen van de agarconcentratie in het medium van 6 tot 12 g per liter verminderde de glazigheid, maar verminderde tegelijkertijd de groei. Een concentratie van 8 of 10 g agar per liter wordt als compromis

tussen deze tegenstrijdige effecten aanbevolen. De wijze van afsluiting van de buizen speelde ook een rol bij het optreden van glazigheid. De lossere typen afsluiting, zoals watteproppen, metalen doppen en steristoppen, voldeden beter dan de meer gesloten typen, zoals aluminiumfolie en parafilm. Voorts werd gevonden dat er in het begin van de groei van het explantaat een fase bestond, waarin dit gevoelig was voor een verhoging van de temperatuur tot 26 °C. De piek in gevoeligheid lag 3 tot 4 dagen na het uitprepareren. Indien dan een temperatuur van ongeveer 26 °C werd aangehouden, verschenen er enkele weken later minder glazige plantjes.

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