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THE ROLE OF MEDIUM COMPOSITION AND LIGHT INTENSITY ON *IN VITRO* ROOT FORMATION OF STRAWBERRY*

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Abstract

In vitro propagated strawberry shoots were subjected to *in vitro* rooting under different BA levels, types of media, incubation temperatures and light intensities. Root formation started within 10 days of culture under high light intensity of 13.5 W/m^2. *In vitro* rooting of strawberry shoot tips occurred readily on inorganic salts of Murashige and Skoog medium. The extent of root formation was directly related to sucrose and light intensity. No root formation occurred in the dark, under low light and in sucrose free medium. BA upto 1 µm did not inhibit root formation. Addition of nicotinic acid, pyridoxine-HCl, and thiamine-HCL to the media reduced root formation but their effect was masked jointly by ascorbic acid, biotin, caD-pantothenate, folic acid and riboflavin.

Introduction

In vitro rooting often occurs in the media without hormonal supplement but it may be improved by including a relatively high dose of auxin or on a medium lacking cytokinin and containing an auxin (James, 1981). It is also frequently enhanced by lowering the salt concentration (Skirvin & Chu, 1979; Cockrel *et al.*, 1986), and sometimes rooting was achieved by etiolation (Harrison-Murray, 1981). In studies on the micropropagation of strawberry Boxus (1974, 1978) and Boxus *et al.*, (1977) found that the formation of roots and axillary buds can easily be controlled by the addition or omission of 6-benzylaminopurine (BA) in the medium. Roots were formed when the meristems or *in vitro* rejuvenated plants were cultured on basic medium without BA while 1 mg/1 BA was necessary for axillary bud formation. James (1979) reported that phloroglucinol and IBA reduced root formation in *Rubus* and *Fragaria.*

Light is an important environmental variable which regulates root formation in plant tissue culture through duration, intensity and quality (Read, 1987). Light intensity can either exert its influence on *in vitro* root formation or indirectly through donor cultures. Experiments were carried out to determine the optimum illumination requirements alongwith the effect of culture medium on *in vitro* rooting of strawberry.

*These studies were conducted at the School of Biological Sciences, Bath University, Bath, UK.

Materials and methods

Shoot tips of strawberry (*Fraqaria ananassa* Duch cv Red Gauntlet), 5-10 mm long, were taken from the plants maintained at least for 20 days on inorganic salts of MS medium (Murashige & Skoog, 1962) containing 2% sucrose. Shoot tips were cultured on the modified MS medium containing (mg/1) ascorbic acid (0.18), biotin (0.05) Ca-D-pantothenate (0.5), folic acid (1), riboflavin (0.39). The medium was supplemented with 1 µm 6-benzylaminopurine (BA), 0.5 µm indol-3-butyric acid (IBA), and 3% sucrose with pH adjusted to 5.5 before mixing 0.7% agar and autoclaving.

For *in vitro* root formation studies, BA levels of 0.0, 0.2, 1.0, 5.0, 10.0, 20.0, 40.0 µm and sucrose concentrations of 0.0, 0.75, 1.5, 3.0, 6.0% were tested at an irradiance of 2.0 and 13.5 W/m^{-2}. Similarly the effect of modified MS medium a) with inorganic salts only, b) inorganic salts + glycine, c) inorganic salts + myo-inositol, d) inorganic salts + glycine + myo-inositol, e) inorganic salts + glycine + myo-inositol + nicotinic acid + pyridoxine-HCl + thiamine – HCl and f) complete modified MS medium was studied in *in vitro* root formation at an irradiance of 0.7 and 13.5 W/m^{-2}.

The effect of different regimes of light intensities (0.0, 1.5, 3.0, 6.5, 8.0 W/m^{-2}) obtained by using neutral density filters (Kodak Ltd., Hemel, Hempstead, UK) was also examined.

Sixteen cultures of each treatment were maintained for 30 and 60 days. Visual observations were recorded for extent of rooting and the percentage of cultures that formed roots.

Data were statistically analysed and in a treatment figures with the same letter do not differ significantly at 5% level using Cochran's non-parametric Q test (Cochran, 1937).

Results and discussions

Root formation occurred widely on different types of media in aseptic culture. All the strawberry shoot tips cultured on BA-free medium produced maximum roots after 30 or 60 days of culture under both light intensities (Table 1). The percentage of shoot tips forming roots decreased with corresponding increase in BA levels in the medium except at high light intensity after 60 days of culture where the buds behaved equally upto 1 µm BA. Shoot tips did not form roots on the media containing BA >1 µm (Fig. 1). This corroborates the observation of Boxus (1974, 1978) and Boxus *et al.*, (1977) who found that at 1 mg/1 BA strawberry shoot proliferation occurred but no roots were formed. In the present studies a combination of 0.5 µm IBA and 1 µm BA was used for bud proliferation and this combination did not inhibit root formation and the use of auxin alone was not

Table 1. Effect of different BA levels and sucrose concentrations on root formation of strawberry under two light intensities after 30 and 60 days of culture.

	2.0 W/m²				13.5 W/m²			
	30 days		60 days		30 days		60 days	
Varibles	Extent of rooting	%age	Extent of rooting	%age	Extent of rooting	%age	Extent of rooting	%age
BA concentrations (µm)								
0.0	+++	100 c	+++	100 c	+++	100 c	+++	100 c
0.2	++	50 b	+++	100 c	++	71 b	+++	100 c
1.0	++	50 b	+++	50 b	++	50 b	+++	100 c
5.0	–	0 a	–	0 a	–	0 a	–	0 a
10.0		Not tested			–	0 a	–	0 a
20.0		Not tested			–	0 a	–	0 a
40.0		Not tested			–	0 a	–	0 a
Sucrose concentrations (%)								
0.0	–	0 a	–	0 a	–	0 a	++	29 b
0.75	–	0 a	–	0 a	–	13 a	+++	14 ab
1.5	++	14 ab	+++	50 d	++	13 b	+++	29 b
3.0	++	50 d	+++	100 f	++	50 d	+++	100 f
6.0	++	50 d	+++	100 f	+++	43 c	+++	80 e

Extent of rooting: no root formed (–), low root formation (+), medium root formation (++) and high root formation (+++).

necessary for *in vitro* root formation in strawberry as in blackberry (Babic & Neskovic, 1984) and camellias (Smartin *et al.*, 1985).

For root formation carbon source seems indispensible as rooting did not occur in the absence of sucrose (Table 1). Even high light intensity was ineffective in inducing roots and only 20% of the explants formed roots. Rooting was directly related to sucrose concentration in the medium as in rose (Hyndman *et al.*, 1982). Sucrose seems to interact with light as at high light intensity the root formation increased with corresponding increase in sucrose level. At high light intensity (13.5 W/m^{-2}) the rooting percentage reduced to 80% in the presence of 6% sucrose in the medium. It would suggest that the optimum sucrose requirement for rooting is around 3% which corresponds to chestnut (Jose *et al.*, 1984), apple (Chong & Pua, 1985), but differs from sugarcane where it is 9% (Maretzki & Hiraki, 1980). Exact role of sucrose in root formation is not clear but it could be osmotic level in the medium, which is maintained by 3% sucrose and disturbed at other levels.

Fig. 1. Effect of different BA levels (μm) on *in vitro* root formation of strawberry.

The concentration of medium constituents has been found to influence the root formation (Table 2), which is similar to apple (Travers *et al.*, (1986) and papaya (Drew, 1987). For rooting few salts of MS medium are required than for the shoot proliferation. The results show that inorganic salts without any plant growth hormone in the medium induce *in vitro* rooting and on this medium shoots did not proliferate. Rooting in the shoots left on any of the media was not noticed upto 60 days under an irradiance of 0.7 W/m^{-2} except in the medium which contained complete modified MS medium. At high light intensity of 13.5 W/m^{-2} shoot tips showed better rooting after 60 days of culture. Myo-inositol or glycine alone or in combination favoured root formation under high light intensity of 13.5 W/m^{-2}, in complete modified MS medium 100% explants produced roots. The limiting factor for rooting was nicotinic acid, thiamine-HCl, and pyridoxine-HCl. The effect of the individual vitamins was not studied but when they were withdrawn from the medium, percentage of shoot forming root decreased to 71-83%. Their effect was masked by a cumulative action of ascorbic acid, biotin, Ca-D-pantothenate, folic acid and riboflavin. Composition of the medium affecting rooting has been demonstrated in apple where 92% cultures developed roots in the presence of BA, when the medium did not contain coconut milk and malt extract (Kouider *et al.*, 1984).

The irradiance appears to influence *in vitro* rooting during bud proliferation stage. High light itensity was found to promote root formation in strawberry and total darkness or low light inhibited root formation (Table 3). These findings do not correspond with the results obtained in apple (Jones *et al.*, 1985) and camellias (Smartin *et al.*, 1986) where incubation of cultures in dark was found necessary for the first few days for the initiation

Table 2. Effect of different medium components on root formation of strawberry under two light intensities after 30 and 60 days of culture.

Medium components	0.7 W/m²				13.5 W/m²			
	30 days		60 days		30 days		60 days	
	Extent of rooting	%age	Extent of rooting	%age	Extent of rooting	%age	Extent of rooting	%age
Inorganic salts	–	0 a	–	0 a	+	25 a	+++	57 b
Inorganic salts + glycine		Not tested			+	13 a	++	71 c
Inorganic salts + my-inositol		Not tested			++	29 a	+++	83 c
Inorganic salts glycine + my-inositol	–	0 a	–	0 a	++	50 b	+++	71 c
Basic MS medium + microinorganics	–	0 a	–	0 a	–	0 a	–	0 a
Complete modified MS medium	–	0 a	++	50 b	–	50 b	++	100 c

Extent of rooting: no root formed (–), low root formation (+), medium root formation (++) and high root formation (+++).

Table 3. Effect of light intensities on root formation of strawberry after 30 and 60 days of culture.

Light intensity W/m⁻²	30 days		60 days	
	Extent of rooting	%age	Extent of rooting	%age
0.0	–	0 a	–	0 a
1.5	–	0 a	–	0 a
0.3	++	88 b	+++	88 b
6.5	++	100 c	+++	100 c
8.0	++	100 c	+++	100 c

Extent of rooting: no root formed (–), low root formation (+), medium root formation (++) and high root formation (+++).

of root primordia. The minimum irradiance required was 3.0 W/m^{-2} and at 6.5 or 8.0 W/m^{-2} all the cultures yielded roots. The initiation of root primordia takes place within 10 days of culture and all the shoots developed roots by 30 days of culture. Irradiance higher than 13.5 W/m^{-2} was not tested and at this light intensity the shoot tips showed extensive rooting although the medium contained BA (1 µm) and IBA (0.5 µm). This cytokinin: auxin ratio has yielded maximum bud proliferation of strawberry during these studies (unpublished data) indicating that cultures left for longer period under high light intensity will produce roots. It would appear that *in vitro* rooting of strawberry shoot tips occurs readily on MS inorganic salts without growth hormones. The present method of producing roots is thus reliable and convenient since roots can be produced on wide range of media.

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