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The production of strawberry plants from callus cultures

O.P. JONES, BARBARA J. WALLER & M.G. BEECH
Institute of Horticultural Research, East Malling, Maidstone, Kent, ME19 6BJ, UK

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Abstract. Shoots were regenerated from callus of the commercially important strawberry varieties Bogota, Brighton, Cambridge Favourite, Hapil, Ostara, Rapella, Red Gauntlet and JILA33 which is a promising selection from a current breeding programme.

The callus was initiated from explants of petiole or lamina of leaves of micropropagated shoots in vitro or of lamina or peduncle from greenhouse plants. There was more shoot regeneration with callus from lamina than from petiole although with the variety Hapil, regeneration occurred only with callus from peduncle.

With seven of the varieties, shoot regeneration occurred on culture media with BAP and 2,4-D whilst with the remaining variety, Cambridge Favourite, it occurred only with medium which contained 1AA-β alanine conjugate in place of 2,4-D.

Regenerated shoots rooted readily and the plants produced are being studied for somaclonal variation.

Introduction

Plant regeneration from callus tissue cultured in vitro can play an important role in the propagation and improvement of crop plants. This paper reports on plant regeneration from callus of eight commercially important varieties of strawberry. This is preliminary to a study of somaclonal variation in strawberry [4]. Regeneration from callus of the strawberry variety Hokowase has already been reported [5].

Materials and methods

Plant material

This was all virus-free. Callus cultures were initiated from explants of in vitro micropropagated shoots of the varieties Bogota, Brighton, Cambridge Favourite, Hapil, Ostara, Rapella and Red Gauntlet and also JILA33 which is a promising selection from the strawberry breeding programme at the

Institute of Horticultural Research, East Malling. Callus cultures were also initiated from explants of greenhouse plants at full bloom of Hapil and Cambridge Favourite. Micropropagated shoot and callus cultures were maintained under sterile conditions. All sterile manipulations were carried out in a laminar flow cabinet.

Micropropagated shoot culture

This was based on methods which have been described previously [1]. Cultures were initiated from small shoot tips consisting of meristem plus 2 or 3 leaf primordia which were dissected from stolons of greenhouse plants. The tips were sterilised prior to dissection by immersion in 10% Domestos solution (Lever Bros. Ltd.) for 25 minutes followed by four washes in sterile distilled water. They were then cultured in 15 × 2.5 cm test-tubes each with 10 ml of culture medium and closed with plastic caps. The shoot tips of all the varieties grew rapidly at the initial stage with the exception of JILA33. However, these tips grew rapidly subsequently following the addition of 0.5–1.0 ml of sterile distilled water to the surface of the culture medium [3]. The later stages of producing large proliferating shoot cultures, which were multiplied by sequential monthly subculture to fresh medium, were carried out in conical flasks each with 30 ml of culture medium and closed with aluminium foil. At this later stage of culture, the shoots of JILA33 grew as rapidly as those of the other varieties in the absence of any water addition to the culture medium.

All the shoot cultures were maintained in a growth room at 22°C with 16 h light daily from 'Crompton White' tubes giving 14 Wm^2 at the surface of each culture vessel. Shoots used as sources of explants were from culture lines that had been maintained for between five and ten months by sequential subculture.

Shoot culture medium

The medium consisted of Murashige and Skoog mineral salts [6] with the following additions (μM): inositol, 555; nicotinic acid, 4.0; pyridoxine-HCl, 2.5; thiamine-HCl 0.3; 6-benzylaminopurine (BAP), 0.9; indole-3-butyric acid (IBA), 0.1; gibberellic acid (GA_3), 0.3. Sucrose and agar (oxoid No. 3) were added at 30 $g\,l^{-1}$ and 7 $g\,l^{-1}$ respectively. The pH of the medium was adjusted to 5.6 before sterilizing by autoclaving at 121°C for 15 min.

Callus culture

Cultures were initiated from petiole sections, 0.5 cm in length, or portions of leaf laminae, ca 25 mm^2, from micropropagated shoot cultures of all the

varieties. Cultures were also initiated from leaf discs, 0.5 cm diam., or peduncle section, 1 cm in length, from greenhouse plants of Hapil and Cambridge Favourite at full bloom. These latter explants were sterilized by immersion for 30 min in 10% Domestos solution followed by four washes in sterile water. Each explant was cultured on 3 ml of medium in a compartment of 25 compartment petri dish (Sterilin Ltd.) under the same growth room conditions as the shoot cultures. Batches of about 100 explants of each type were taken in 1984/85 from the micropropagated shoots and greenhouse plants.

Callus culture media

Medium A
This consisted of Murashige and Skoog mineral salts with the following additions (μM): inositol, 555; thiamine-HCl, 0.3; BAP, 0.9; 2,4 chlorophenoxyacetic acid (2,4-D), 0.9; sucrose and agar (oxoid No.3) were added at 20 g l^{-1} and 7 g l^{-1} respectively. The pH was adjusted to 5.6 with 0.1 N NaOH followed by autoclaving at 121°C for 15 min.

Medium B
This was of the same composition as medium A except that the 2,4-D was replaced by indole-acetic acid-β alanine conjugate (IAA-β alanine) at 1.0 μmol [2].

Rooting and growing-on

Shoots regenerated from callus were excised when they were 2–3 mm in length and were subsequently rooted by placing them singly in 10 ml of culture medium contained in 15 × 2.5 cm test-tubes which were kept in the same growth room conditions as the callus cultures. The medium for rooting was of the same composition as for shoot culture except that the BAP was omitted. After three weeks the small plantlets were transferred to pots of compost under polythene hoods in a greenhouse. The hoods were removed after four weeks and the plantlets were grown-on in the greenhouse.

Results

Medium A

Explants from micropropagated shoots
Results with the petiole and lamina explants of Bogota, Brighton, Red Gauntlet, Ostara and Rapella are summarized in Table 1. Compact green

Table 1. Callus production and shoot regeneration after 16 weeks on medium A by explants from micropropagated shoots of strawberry varieties.

Variety[a]	Explant	No. of replicates	Callus cultures (values ± standard errors)		
			Mean fresh wt (mg) per culture	Percent with regenerated shoots	No. of shoots per regenerating culture
Bogota	lamina	58	237 ± 50	37	4.2 ± 1
	petiole	50	137 ± 28	10	7.3 ± 2
Brighton	petiole	94	–	50	10.0 ± 2
Red	lamina	100	–	31	4.0 ± 2
Gauntlet	petiole	100	–	0	0
Ostara	lamina	98	245 ± 91	53	8.0 ± 3
	petiole	62	294 ± 97	20	7.0 ± 2
Rapella	lamina	60	217 ± 65	43	4.0 ± 1
	petiole	58	52 ± 19	6	10.0 ± 2

[a] There was no shoot regeneration with callus from lamina or petiole explants from micropropagated plants of Cambridge Favourite, Hapil or JILA33.

callus tissue was produced from the cut surfaces of all the explants within 4–8 weeks and within a further eight weeks shoots were regenerated from the callus of up to 50% of the cultures (Fig. 1). Explants from petiole exhibited some polarity with more callus production and shoot regeneration from the proximal end than from the distal end. However, there was generally more shoot regeneration with cultures from lamina than from petiole.

There was no regeneration whatsoever on medium A from any type of explant from Cambridge Favourite, Hapil or JILA33 although callus was produced as with the other varieties. However, in following experiments with medium with various concentrations of BAP and 2,4-D, 6 out of 54 cultures from lamina of micropropagated JILA33 shoots each regenerated 2 or 3 shoots within sixteen weeks when cultured on a medium which was identical to medium A except that the concentration of 2,4-D was reduced to 0.2 μmol. There was no regeneration with Hapil or Cambridge Favourite on media with modified levels of BAP or 2,4-D.

Explants from greenhouse plants

Explants of petiole, lamina and peduncle from greenhouse plants of Hapil and Cambridge Favourite also produced callus within 4–8 weeks. This callus was similar to that produced from the micropropagated shoots. However, 28 out of 102 cultures from peduncle of Hapil each regenerated between two and four shoots within a further eight weeks. Furthermore, in repeat experiments with second and third batches of explants, shoot regeneration again occurred in ca 30% of the cultures from peduncle of Hapil but there was never any regeneration with Cambridge Favourite.

Fig. 1. Callus with regenerated shoots from leaf lamina explants from micropropagated shoots of Red Gauntlet. After 16 weeks of culture.

Medium B

Explants from the lamina of leaves of greenhouse plants of Cambridge Favourite were cultured for twelve weeks on medium A with 0.9 μmol 2,4-D and then transferred to medium B. Of 50 cultures transferred, one produced four shoots from callus within four weeks. A repeat of this transfer from medium B with a second batch of fifty cultures again resulted in a single culture which regenerated some shoots.

Subculture of callus

Some of the cultures of each of the varieties that had regenerated shoots on medium A or B were sequentially subcultured at two-monthly intervals for eight months by dividing the callus into portions which were then transferred to fresh batches of the respective medium. The subcultured callus continued to grow and produce new shoots which appeared to arise de novo from callus or from lateral buds of pre-existing shoots.

Rooting

This was readily achieved with between 90 and 100% of shoots from all the types of callus becoming well-rooted within 2–3 weeks. The small plantlets survived readily and grew-on when transferred to pots of compost.

Discussion

Plants have been regenerated from the callus cultures of all eight strawberry varieties that have been studied. This regeneration depended to some extent upon the type of explant used to initiate cultures. However, with seven of the varieties the regeneration occurred on media with BAP and 2,4-D. With the remaining cultivar, Cambridge Favourite, there was only a low level of regeneration and this occurred on medium with IAA-β alanine conjugate in place of 2,4-D. Auxin-amino acid conjugates should perhaps be investigated further in relation to callus types that are presently recalcitrant to organogenesis [2].

The methods that have been described are being applied routinely to the regeneration of plants from callus of all eight varieties. Thus far, about 10 000 plants have been produced. Many of these plants exhibit marked somaclonal variation, much of which is detrimental, such as reduced plant vigour, thickening and splitting of petioles and leaf variegation. However, some of the variation may be of potential value such as enhanced cropping and changing and changed response to *Verticillium* wilt disease. Selected variants with desirable characteristics have been propagated from runners and are presently being studied in long-term field trials. The results of these field trials will be described in other papers together with other details of the somaclonal variation that has been produced.

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