MICROPROPAGATION OF BLACK CURRANT (*Ribes nigrum*)

PAULINE A. COOPER, JAN E. GRANT, AND TONYA J. FREW

Crop Research Division
Department of Scientific and Industrial Research
Private Bag, Christchurch

Abstract. A method is described for the micropropagation of four selections of black currant (*Ribes nigrum*) The optimum multiplication medium contained Murashige and Skoog salts, Gamborg B5 vitamins, 20g l^{-1} sucrose, 0 5g l^{-1} casein hydrolysate, 0 5 mg l^{-1} benzylaminopurine (BAP) and 7g l^{-1} agar Varying levels of inositol, BAP, and indolebutyric acid (IBA) were tested. For shoot elongation and rooting, the BAP concentration was reduced to 0 25 mg l^{-1} and the effect of the addition of activated charcoal (Sigma No C—4386) was investigated For the selection P10, 100% rooting has been achieved, and plantlets transferred to soil. The other selections have been slower to respond and rooting percentages have not yet been assessed

INTRODUCTION

Four Scottish selections (P10, P7, 243, BS) of black currant from the Department of Scientific and Industrial Research (DSIR) Plant Protection collection have been selected by the Ministry of Agriculture and Fisheries (MAF), in conjunction with the New Zealand Black Currant Product Group, for either their high yield, good processing, or other favourable characteristics, such as flavour. These selections, and others, are in the process of being bulked up for field testing, using both conventional propagation techniques (10cm hardwood cuttings) and *in vitro* micropropagation.

The aim of this work was to produce 7000 plantlets for transfer to soil to increase the number of stock plants available for conventional propagation. The NZ Black Currant Product Group and MAF aim to have ¼ million plants by 1991 and 1 million by 1992 for distribution to growers for commercial scale trials.

Several researchers have reported successful techniques for the micropropagation of black currant *in vitro* (2, 5, 7, 8, 9). Our aim was to apply these techniques to obtain the numbers required as rapidly as possible. The protocol of Flegmann and Wainwright (2) for the cultivar Baldwin was initially followed, but much blackening and deterioration of shoots occurred. The addition of ascorbic acid and IBA, as reported by Mokra and Maliarcikova (5), was also unsuccessful. Thus a number of alterations were tested in an attempt to optimize media components.

MATERIALS AND METHODS

Initial explants were taken from dormant, field-grown material of the four selections:-P10, P7, 243 and BS. Nodal pieces were surface sterilized using a 96% ethanol shake (5 secs) followed by agitation

in 0.75% w/w sodium hypochlorite (20 mins) and 2 rinses in sterile distilled water. Dissected buds (5-10 mm) were placed in petri dishes on the initiation media—a modified Murashige and Skoog (MS) (6) basal medium containing 20g l^{-1} sucrose, 0.3 mg l^{-1} or 1.0 mg l^{-1} BAP and 6g l^{-1} Davis Bacteriological agar (2). Some sterilized canes were placed in jars with water, into the culture room to encourage new growth. This material was also surface sterilized, cut into single node pieces and placed on the modified media in vials (4.5 cm diameter, 5.5 cm high). Cultures were maintained at 25 °C, 16 hr day/8 hr night, 30μE m^{-2} s^{-1} light.

For subsequent experiments the medium used was MS salts, B5 vitamins (3) minus inositol, 20 g l^{-1} sucrose, 0.5g l^{-1} casein hydrolysate, 0.6g l^{-1} agar, and pH 5.8, with the following additions for shoot multiplication comparisons (in mg l^{-1}):

Treatment 1: 0.5 BAP + 100 inositol;
Treatment 2: 0.5 BAP + 10 inositol,
Treatment 3: 1.0 BAP + 10 inositol;
Treatment 4: 1.0 BAP + 10 inositol + 0.1 IBA.

For shoot elongation and rooting the additions were (in mg l^{-1}):

Treatment 1: 0.25 BAP + 100 inositol;
Treatment 2: 0.25 BAP + 100 inositol + 1000 activated charcoal.

Culture conditions were as above, and containers used were half pint glass Agee jars (9cm diameter, 10cm high) with clear lids. Shoot multiplication was assessed after the third and fourth subculture on the respective media. Subculture was at 4 weekly intervals. There were a minimum of 10 shoots per treatment for the shoot multiplication assessments, and 25 shoots per treatment for rooting.

RESULTS

Upon initiation, contamination rates were high (80%), not unexpected when using field-grown material. Shoots did not thrive on the initiation media. There was much blackening of shoots and leaves, and considerable exudation of phenolic-like substances. The addition of 50mg l^{-1} ascorbic acid to reduce blackening was detrimental, and the use of other salt media such as B5 and Woody Plant medium (4) resulted in further deterioration in growth. The addition of 0.5g l^{-1} casein hydrolysate—possibly supplying added nitrogen or essential amino acids—was beneficial in establishing, healthy multiplying cultures.

Table 1 shows the comparison of the multiplication media after the third subculture. When comparing inositol levels at 0.5 mg l^{-1} BAP, the selections showed very little difference in multiplication rates. For P10 the multiplication rate was 3.4 fold on both 10mg l^{-1} and 100 mg l^{-1} inositol, while for P7 it was 2.1 fold on 10mg l^{-1}, and 1.3 fold on 100 mg l^{-1} inositol. After the fourth subculture the multiplication

rates were 3.0 and 3.1 for P10, 2.4 and 2.5 for P7, and 2.7 and 2.8 for BS at 10 mg l^{-1} and 100mg l^{-1} inositol, respectively.

Table 1. Effect of inositol, benzylaminopurine, and indolebutyric acid on multiplication rate of four selections of black currant (minimum 10 shoots per treatment)

	Selection			
Treatment*	P10	243	BS	P7
1	3 4	2.1	1 5	1 3
2	3 4	1 9	1 8	2 1
3	3 7	2 0	1 9	3 5
4	3 1	2 0	1 8	**

* Treatments (mg l^{-1}) (1) 0 5 BA + 100 inositol, (2) 0 5 BA + 10 inositol, (3) 1 0 BA + 10 inositol, (4) 1 0 BA + 10 inositol + 0 1 ± BA

** No treatment due to lack of material

For the comparison on the two levels of BAP at 10mg l^{-1} inositol, again the multiplication rates showed little difference, except for the selection P7 which had a higher rate on 1.0mg l^{-1} BAP at 3.6 fold compared with 2.1 fold on 0.5mg l^{-1} BAP.

However, the use of 1.0mg l^{-1} BAP caused the shoots to become very suppressed and difficult to manage, particularly after the fourth subculture on this medium, when multiplication rates dropped to 1.6 fold.

Treatment 4 was only tested on selections P10, 243 and BS, due to the lack of material of P7. The addition of IBA did not improve multiplication. Moreover, the shoots looked abnormal with pale, narrow, crinkled leaves and petioles that pointed downwards.

Table 2 shows the effect of charcoal on rooting of P10 plantlets *in vitro*. There was 100% rooting on both plus and minus charcoal. However, on the medium without charcoal there were 7.7 roots per shoot with mean length 2.3 cm, while with charcoal there were 3.8 roots per shoot with mean root length of 1.1 cm.

Table 2. Effect of 1g l^{-1} charcoal on rooting *in vitro* of P10 black currant plantlets (Values are means ± standard error, n=25)

Treatment	Percent rooting	No roots per shoot	Mean root length (cm)
1. – charcoal	100	7 7 ± 1 7	2 3 ± 1 3
2 + charcoal	100	3 8 ± 1 9	1 1 ± 0 7
		ts = 7.65***	ts = 4.07***

*** represents significant differences among means at the 0 1% probability level, as determined by a t-test

DISCUSSION

Of the four selections P10 was the most vigorous in all the tissue culture regimes tested. In contrast, P7 and BS were much less vigorous. Other workers have had difficulties with blackcurrants using *in vitro* micropropagation (1; J. Seelye, pers. comm.). The only other successful reports were by Flegmann and Wainwright (2, 7, 8) but they used only one cultivar—Baldwin, and their medium was not suitable for our selections. Selection 243 has shown evidence of internal "white ghost" bacterial contamination which consequently affected its performance. The antibiotic cefotaxime (100mg l^{-1}) has controlled the contamination but this caused the multiplication rate to decrease.

Clearly, rooting was not a problem for the selection P10, as it rooted both with and without charcoal, and also without auxin. In fact P10 rooted better (i.e., more roots and longer) on the medium without charcoal that had 2.5 mg l^{-1} of cytokinin. The action of charcoal used in the medium is unclear, but in this case had an inhibitory effect on root number and length. Wainwright and Flegmann (7) used a two-step process for rooting their cultivar, Baldwin. The first step involved 4 days on a medium containing auxin followed by 37 days on a medium with no auxin. Our process effectively saves one subculture step thus making propagation of black currants in tissue culture more economically viable. However, further work will be necessary to optimize the tissue culture environment for the other selections.

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