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Micropropagation of thornless trailing blackberry (*Rubus* sp.) by axillary bud explants

[1*]A Jafari Najaf-Abadi and [2]Y Hamidoghli

Department of Horticulture. Faculty of Agriculture, University of Guilan, Iran

**Corresponding author: azamjafari83@yahoo.com*

Abstract

The purpose of this study was to establish best condition for *in vitro* propagation of thornless trailing blackberry (*Rubus* sp.). Axillary buds were used as explants. After sterilization, explants were placed into MS medium supplemented with 2 $mg.l^{-1}$ BA. Two weeks after that, in shoot proliferation stage, 12 culture media containing MS supplemented with 3 concentrations of BA (0, 2 and 3 $mg.l^{-1}$), alone or in combination with GA_3 (0, 0.2, 0.5 and 1 $mg.l^{-1}$) were compared. The greatest number of shoots with average of 3.33 and the maximum shoot length with average of 5.87 cm were produced in medium containing 2 $mg.l^{-1}$ BA and 0.5 $mg.l^{-1}$ GA_3. In the rooting experiment, rooting medium comprised MS medium in combination with different concentrations of IBA (0, 0.5, 1 and 2 $mg.l^{-1}$). A concentration of 2 $mg.l^{-1}$ IBA gave a greater number of roots and maximum root length. In this medium four roots with root length average of 7.83 cm were produced.

Key words: axillary bud; blackberry; *in vitro*; micropropagation

Abbreviations: BA_6-benzylaminopurine; GA_3_Gibberelic acid; IBA_Indole butyric acid; MS_Murashige & Skoog Medium.

Introduction

Blackberries, along with red and black raspberries and their hybrids, are fruiting plants from Rosaceae and the *Rubus* genus (Tourn) L. *Rubus* is one of the most diverse genera in the plant kingdom with approximately 740 species (Gu et al., 1993). Blackberries (*Rubus* L. subgenus *Eubatus* Focke) are a native crop in North America and Europe (Crandall, 1995). Interest in blackberry phenolics has increased owing to their roles as antioxidants and the possible beneficial implications in human health, such as in the treatment and prevention of cancer, cardiovascular disease, and other pathologies (Jennings et al., 1990). Blackberries are a rich source of anthocyanins and other polyphenolic antioxidants (Siriwoharn et al., 2004). While a large amount of fruit is sold in the fresh market, most blackberries are first processed into either individually quick frozen (IQF) fruit, puree, or juice that in turn are used as ingredients in processed products such as preservers, jam, jelly, baked goods, dried products, ice cream and yogurt (Hall, 1990).

Blackberry is commercially propagated by the classical methods of vegetative propagation, i.e. by hard wood and soft wood cuttings, by layering and bush division. However, successful application of these methods of vegetative propagation is limited to certain extent. Propagation by layers requires rather large area for a lay-bed, and weed control among the layers is a problem. Propagation by cuttings is simpler although the rooting is not always satisfactory. Soft wood cuttings root easily, but also require much attendance (Busby and Himelrick, 1999). *In vitro* propagation through the development of axillary buds eliminates the seasonal limitations encountered with these methods and needs a small quantity of starting material, the *in vitro* plants propagated in this way in many species have proved to be healthy and true to type (Shen et al., 1990). Micropropagation techniques are capable of

Table 1. Influence of BA and GA_3 on blackberry axillary bud cultures

BA concentration ($mg.l^{-1}$)	GA_3 concentration ($mg.l^{-1}$)							
	0		0.2		0.5		1	
	No. of shoots±SE	Shoot length (cm) ±SE	No. of shoots±SE	Shoot length (cm) ±SE	No. of shoots±SE	Shoot length (cm) ±SE	No. of shoots±SE	Shoot length (cm) ±SE
0	$1±0.3^{c}$	$1.2±0.41^{c}$	$1±0.3^{c}$	$1.47±0.41^{c}$	$2.33±0.3^{b}$	$1.97±0.41^{c}$	$2.33±0.3^{b}$	$2±0.41^{c}$
2	$1.33±0.3^{c}$	$1.9±0.41^{c}$	$2.33±0.3^{b}$	$3.37±0.41^{b}$	$3.33±0.3^{a}$	$5.87±0.41^{a}$	$3.33±0.3^{a}$	$5.03±0.41^{a}$
3	$1.67±0.3^{b}$	$1.23±0.41^{c}$	$2.33±0.3^{b}$	$2.57±0.41^{b}$	$2.33±0.3^{b}$	$2.84±0.41^{b}$	$1.67±0.3^{b}$	$2.53±0.41^{b}$

Means in a column with different small letters are significantly different at $p<0.05$ by Tukey.

Table 2. Influence of IBA on rooting of blackberry shoots produced *in vitro*

IBA concentration ($mg.l^{-1}$)	No. of Roots±SE	Root length (cm) ±SE
0	$0.33±0.52^{b}$	$0.4±0.80^{b}$
0.5	$1±0.52^{b}$	$1.23±0.80^{b}$
1	$2±0.52^{ab}$	$2.9±0.80^{b}$
2	$4±0.52^{a}$	$7.83±0.80^{a}$

Means in a column with different small letters are significantly different at $p<0.05$ by Tukey.

producing many plants in a short period of time (Snir, 1981). Application of *in vitro* propagation has been recorded in great number of blackberry cultivars (Wainwright and Flegmann, 1986; Meng et al., 2004). However, micropropagation requires a great deal of experimental work on optimization of the conditions in all its phases.

The objectives of this study were firstly to determine the optimum balance growth regulators required to induce maximum shoot growth and rooting of thornless trailing blackberry.

Materials and methods

Actively growing shoots were excised from field-grown plants. Initially expanded leaves were removed and single-node explants were dipped for 30 sec. in ethanol (70% v/v) and in a solution containing 30 % commercial bleach for 20 min. 0.1 % Tween 20 was used as a wetting agent. These explant materials were washed three times with sterile distilled water. Ends of explants (0.5 cm) were cut off and segment was placed on initiation medium containing major and minor salts as in Murashige and Skoog (1962) (MS) supplemented with 2 $mg.l^{-1}$ BA, 3% sucrose and 4 $g.l^{-1}$phytagel. Two weeks after initiation, the shoots were transferred to proliferation medium but enriched with different contents of BA (0, 2, 3 $mg.l^{-1}$) and GA_3 (0, 0.2, 0.5, 1 $mg.l^{-1}$) and ascorbic acid (100 $mg.l^{-1}$).

Shoots longer than 10 mm were used as micro cuttings and were transplanted to MS basal medium containing four different concentrations (0, 0.5,1 and 2 $mg.l^{-1}$) of IBA. The pH of all media was adjusted to 5.7 before the media were autoclaved at 121°C for 15 min at 142kPa. Cultures were routinely transferred every 2 weeks into fresh medium. Plantlets were maintained at 24±2° C with a 16-h photoperiod. The irradiance at the level of the cultures was 57 $\mu mol.m^{-2}.s^{-1}$. After 6 weeks, the plantlets were transferred to a plastic container containing a mixture of sterilized peat and perlite (v/v=1/1). The plantlets were grown initially under glass and were gradually exposed to normal greenhouse conditions. After a 3 week period of hardening-off, the surviving plants were transplanted into pots.

Data were taken from three replicate experiments and analysed following Analysis of Variance (ANOVA) technique. Means were compared by using Tukey test at 5% level of significance.

Results and discussion

Establishment of in vitro culture and shoot multiplication

Almost all blackberry axillary buds placed on culture initiation medium were established and transferred to proliferation medium 2 weeks later (Fig. 1).

The interaction of BA and GA_3 had a significant effect on the number of shoots produced and shoot length (Table 1). The greatest number of shoots was obtained with MS medium containing 2 $mg.l^{-1}$ BA and 0.5 or 1$mg.l^{-1}$ GA_3 (Fig. 2). The least number were produced with MS medium without growth regulators (control) and MS medium containing 0.2 $mg.l^{-1}$ GA_3. The maximum shoot length was produced in medium containing 2 $mg.l^{-1}$ BA and 0.5 $mg.l^{-1}$ GA_3 and the minimum shoot length was obtained in the absence of exogenous growth regulators.

In tissue cultures (as well as in intact plants and plant organs), cytokinins appear to be necessary for plant cell division. Cytokinins are very effective in promoting direct or indirect shoot initiation. To encourage the growth of axillary buds, and reduce

Fig 1. Initial explant grown on MS+2mg.l^{-1}BA after 2 weeks

Fig 2. Axillary shoots formed on MS+2mg.l^{-1}BA+0.5 mg.l^{-1} GA_3 medium

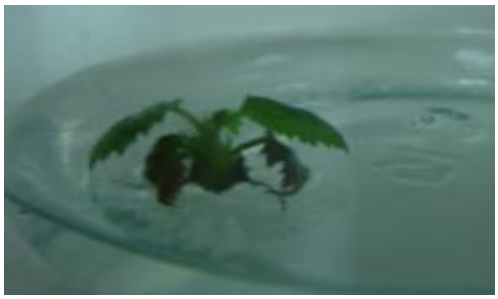

Fig 3. Adventitious roots formed on MS +2 mg.l^{-1}IBA medium

Fig 4. In vivo acclimated plantlets of thornless blackberry

apical dominance in shoot cultures, one or more cytokinins are usually incorporated into the medium at proliferation stage (George et al., 2007).

Our shoot proliferation is comparable with those reported by Bobrowski et al. (1996). They reported that the best medium for shoot proliferation was MS medium containing 1 or 2 mg.l^{-1} BA, but GA_3 was not effective on multiplication rate. Wei et al (1992) reported that if the concentration of BA was decreased from 2 mg.l^{-1} to 1 mg.l^{-1}, axillary bud proliferation was not induced and when the concentration of BA increased to 4.0 mg.l^{-1}, proliferation of axillary buds was reduced. They found that GA_3 was essential for proliferation in three of the four species used and it has profound effects on the complex hormone system, which apparently controls the proliferation. It was known that GA_3 can synergistically act with auxins (Stowe, 1957). While Villa et al. (2005) reported that the greater numbers of shoots were produced with 1 mg.l^{-1} BA in WPM basal medium, in our experiment the best result was obtained with 2 mg.l^{-1} BA in MS basal medium.

Rooting and acclimatization

The rhizogenic activity of IBA compared with the control was confirmed. The addition of IBA had a significant effect on the number of roots produced and root length (Table 2). An IBA concentration of 2 $mg.l^{-1}$ gave a greater number of roots and maximum root length than 0, 0.5 or 1 $mg.l^{-1}$. In this medium four roots with root length average of 7.83 cm were produced (Fig. 3). The least number of roots and minimum root length were produced with MS medium without growth regulators (control). This agrees with the study of James et al. (1980) and Anderson (1980) for rooting. James et al. (1980) reported that IBA stimulate rooting of red raspberry explants. In contrast of our result, Donnelly et al. (1980) reported that MS medium supplemented with 0.5-0.7 $mg.l^{-1}$ IBA was the best medium for rooting of *Rubus* sp. shoots.

At the acclimatization stage about 85% of rooted microcuttings survived in the field for a month after transplanting (Fig. 4).

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