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The Rapid Method of Blackberry Propagation

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Abstract: Blackberry cv Čačanska Bestrna was successfully propagated by using the method of *in vitro* tissue culture. After obtaining aseptic culture and rosette formation, multiplication and rooting stages took place simultaneously, i.e. the shoots were cultured on the rooting medium which enabled the successive acclimatization of half of the plants while the other half was subcultured through shoot division into one- and two-node cuttings. The process of micropropagation is thus speeded up by merging the two stages, multiplication and rooting into one.

Key words: blackberry, micropropagation, active charcoal.

Introduction

Blackberries are commercially propagated mostly by tip layers or stem cuttings. Tip layering requires a sizeable planting for the layering bed, only few tips are available per plant, and weed control among the layers is a problem. Propagation by hardwood stem cuttings is simpler but rooting is not always satisfactory (Broom and Zimmerman, 1978). Softwood cuttings require considerably more care but could root readily, especially when using one-node cuttings under mist treatment (Zimmerman et al., 1980; Ružić and Stanisavljević, 1986).

A considerable number of blackberry cultivars has been propagated by *in vitro* method so far (Broome and Zimmerman, 1978; Harper, 1978; Kiss and Zatyko, 1978; Skirvin et al., 1981; Babić and Nešković, 1984).

However, if there is a lack of plant material or a newly-developed cultivars and hybrids need to be rapidly propagated for further *in vivo* studies,

the method of tissue culture, which is still too expensive for commercial application, becomes fully justified.

The aim of the paper is to speed up the propagation method, to introduce promptly newly-bred blackberry cultivar into production.

Material and Method

Thornless blackberry cv Čačanska Bestrna used in our experiment was developed at the Fruit and Grape Research Centre, Čačak, by crossing thornless blackberry cultivars Dirksen Thornless and Black Satin. It showed exceptional results as regards cropping, fruit quality and resistance to diseases and winter frost (Stanisavljević, 1994). It is thornfree like the parental pair.

Tips 10-20 mm long were taken from actively growing shoots. After surface sterilization by the standard method, explants 1-2 mm long were isolated and established on the Murashige and Skoog (MS) (Murashige and Skoog, 1962) mineral salts with (in mgl^{-1}): pyridoxine-HCl, 0.5; thiamine-HCl, 0.4; nicotinic acid, 0.5; myoinositol, 100; glycine, 2; N_6-benzylaminopurine (BAP), 2; indolyl-3-byturic acid (IBA), 0.5; gibberellic acid (GA_3), 0.1; sucrose, 20000 and agar, 7000. Before autoclaving the pH of the medium was adjusted to 5.7 with 0.1 N KOH. Sterilization was done at 120^0C for 20 minutes. Cultures were maintained in growth chamber at $25^0C \pm 1^0$C with 16/8 hr of light photoperiod (light/dark). After obtained aseptic culture and rosette formation, the shoots were cultured on MS media with different hormonal content presented in table 1, and then on rooting medium containing MS salts reduced to one-half with $FeSO_4$ x $7H_2O$, Na_2EDTA and vitamins unchanged and in mgl^{-1}: active charcoal (AC), 1000; IBA, 1; GA_3, 0.1; sucrose, 20000 and agar, 7000; and the same medium but without active charcoal.

Half of the rooted cultures were transplanted every 20-25 days (duration of one subculture) by shoot division into inetnodes with one node and root rejection, while the rest was planted in (2:1) peat: perlite and acclimatized under mist system.

Results and Discussion

After 20 days in culture the isolated tips developed into leafy rosettes and started branching, i.e. started to multiplicate.The highest index of multiplication was echieved on the medium with 0.1 mgl^{-1} IBA, whereas the greatest shoot length was on the medium with 1 mgl^{-1} IBA (Fig. 1). On the media with α-naphthalenacetic acid (NAA), especially with the higher concentration there was greater callus production at the shoot base - 0.025g/shoot (Tab. 1).

Tab. 1. Effect of different growth regulator concentrations in a nutrient medium on the index of multiplication, shoot length and fresh weight (FW) of calli

BAP* (mgl^{-1})	NAA (mgl^{-1})	IBA (mgl^{-1})	Index of multiplication Average ± S.D.	Shoot length (mm) Average ± S.D.	FW of callus g/shoot Average ± S.D.
1	1	-	1.50 ± 0.5	5.0 ± 1.4	0.025 ± 0.008
1	0.5	-	2.00 ± 1.2	5.5 ± 2.1	0.022 ± 0.014
1	0.1	-	1.50 ± 0.6	6.8 ± 2.9	0.014 ± 0.005
1	-	1	1.92 ± 0.6	6.9 ± 2.4	0.021 ± 0.007
1	-	0.5	1.89 ± 0.8	5.2 ± 1.8	0.012 ± 0.004
1	-	0.1	2.78 ± 1.0	5.8 ± 2.3	0.012 ± 0.004

* Media tested contain 0.1 mgl^{-1} GA_3

After several subcultures, to accelerate the process of propagation the shoots were established on the above mentioned rooting media. The greatest rooting percentage (100%) was obtained with the first occurrence of roots after 7 days in culture on the medium with active charcoal (Tab. 2).

Tab. 2. Rooting parameters of blackberry cultivar Čačanska Bestrna

MS* IBA	(in mgl^{-1}) AC	Rooting %	Average root number	Average root length (mm)	Average plant length (mm)
1	1000	100	1.97 b**	44.89 a	16.66 a
1	-	64.27	3.18 a	18.60 b	9.10 b

* GA_3 present in both media at 0.1 mgl^{-1}

** Means followed by the same letter do not differ at 5% level of significance: Duncan multiple range test

On the medium with no active charcoal root induction started after 15 days with the rooting percentage 64.2%, after 25 days in culture. All the rooting parameters showed significant differences on the medium with active charcoal when compared with the shoots rooted on the medium with no active charcoal (Tab. 2).

The parental pair of this blackberry cultivar also showed the tendency to be rooted *in vitro*, but with the poor multiplication rate. This proclivity might be controlled by changing quantity and/or proportions of growth regulators (Broom and Zimmerman, 1978). Cultivar Dirksen Thornless, one of the parents, was highly sensitive to BAP concentration, which had to be reduced from 1 to 0.5 mgl^{-1} in order to get suitable plantlets (Babić and Nešković, 1984). However, it is not sometimes possible to increase index of multiplication by changing the type or the concentration of hormones, but other factors are also important, such as mineral composition. (Ružić, 1999).

On the medium with active charcoal roots were well-developed, with individual roots up to 80 mm long (Fig. 2).

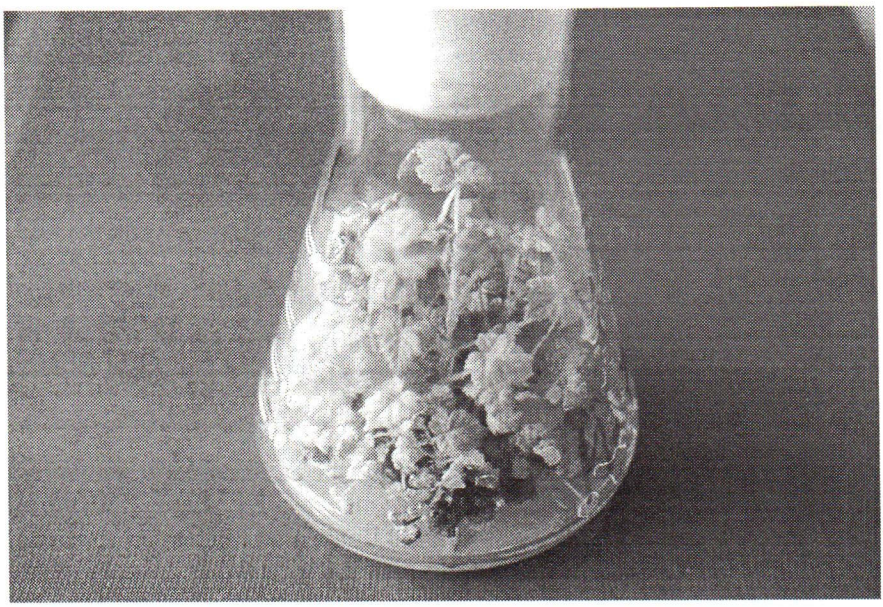

Fig. 1. Shoots of blackberry cv Čačanska Bestrna on multiplication medium

Roots were thin, with numerous secondary roots 3-5 mm long, and no callus at the base of rooted plantlets. The average length of rooted shoots on the same medium was 16.7 mm, which was considerably less when compared to this modified method of micropropagating of grapevine (Ružić and Cerović, 1991). However, in this case the shoots could be transplanted by shoot division into two internodes with one node for further subculture. In this way the same number of shoots is gained in comparison with the greatest multiplication rate (2.78) obtained on the medium with 1 mgl^{-1} BAP and 0.1 mgl^{-1} IBA, and the procedure is considerably shortened.

In order to increase the rooting percentage and to obtain bertter-quality plants at the rooting stage, the medium with no hormones was used in *Rubus* (Babić and Nešković, 1984) followed by direct planting into substrate (Zimmerman and Broom, 1978) or active charcoal adding into medium (Anderson, 1980; Snir, 1981; Sobczykiewicz, 1984; Welander, 1985), which showed some positive results in more cases, as well as in our paper. It is considered that active charcoal as absorbent, removes toxic oxidative products and contains some possible rooting factors (phenolamine, putrescine, spermidine and spermine). It may also have shading effect in the medium on shoot bases and developing roots (Donnely and Daubeny, 1986).

A high rooting percentage and a good quality of plants which were obtained in this paper, indicate that active charcoal, i.e. the whole composition of the medium selected, had the positive effect. Well-developed root and above ground part of plantlets enabled the greatest percentage (100%) of surviving plants at acclimatization stage.

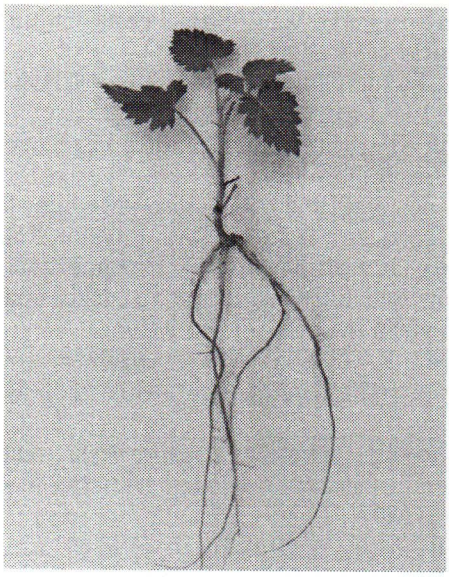

Fig. 2. Shoots of blackberry cv Čačanska bestrna rooted on medium with AC

Conclusion

Nowadays, in fruit growing, micropropagation is mostly used in the propagation of small fruits and fruit rootstock. It is also applied in the production of virus free propagation material for the establishment of mother plantations as well as in cultivar transferring on its own root. This method is also applied in the propagation of plants, which cannot be easily propagated by standard methods of vegetative propagation of the newly developed cultivars and hybrids, as is the case in this paper.

By joining multiplication and rooting stages, the method of micro-propagation was considerably improved and it becomes much faster commercially justified.

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BRZI METOD RAZMNOŽAVANJA KUPINE

-originalan naučni rad-

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Rezime

Nova sorta kupine Čačanska bestrna, uspešno je razmnožena metodom kulture tkiva *in vitro*. Regeneracija je postignuta već posle 20 dana na medijumu Murashige i Skoog (1962) (MS) sa u mgl^{-1}: BAP, 2; IBA, 0.5; GA_3, 0.1; saharoza, 20000 i agar, 7000. Izdanci su se posle nekoliko supkultura uspešno kultivisali na medijumu za ožiljavanje koji je sadržavao MS mineralne soli smanjene na 1/2 sa u mgl^{-1}: IBA, 1; GA_3, 0.1; aktivni ugalj, 1000; saharoza 20000 i agar, 7000.

Faza multiplikacije je istovremeno tekla sa fazom ožiljavanja, tako što je polovina ožiljenih biljaka sađena u supstrat treset : perlit (2:1) i aklimatizovana pod mist izmaglicom, a polovina je dalje supkultivisana.

Na taj način, spajanjem dve faze u jednu, metod mikropropagacije kupine je mnogo brži i ekonomičniji.