Micropropagation as Means of Rapid Multiplication of Newly Developed Blackberry and Black Currant Cultivars

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Summary

Newly developed blackberry cultivar Čačanska bestrna was successfully micropropagated. For black currant, cv Čačanska crna further research should be done to optimize conditions for succesful multiplication.

Buds from the branches cut during dormancy (end of January) were used as the initial explants and set to develop under laboratory conditions at room temperature. Aseptic culture was established on Murashige and Skoog (MS) medium with BA 2.0, IBA 0.5 and GA_3 0.1 mg 1^{-1}. MS media with BA and IBA or NAA and GA_3 were used for multiplication phase, whereas medium MS with mineral salts reduced to 1/2, organic complex unchanged according to MS, with 1.0 mg 1^{-1} IBA, 0.1 mg 1^{-1} GA_3 and 1g 1^{-1} of active charcoal was used in the rooting phase.

Well developed root system and high quality of rooted plants induced a high percentage of acclimatization (100%) of cv Čačanska bestrna under the 'myst' system in greenhouse whereas the percentage of acclimatization of cv Čačanska crna was low, 40%.

Key words

blackberry, black currant, micropropagation, *in vitro*

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Introduction

Breeding work on blackberry and black currant with application of the method of planned hybridization was initiated at the Fruit Research Institute in Čačak in the 1980s. Blackberry (*Rubus* sp.) cv Čačanska bestrna and black currant (*Ribes nigrum* L.) cv Čačanska crna were developed as the result of the breeding program.

Blackberry cv Čačanska bestrna derived from a cross of Dirksen Thornless and Black Satin in 1984. It was selectioned in 1987 and tested as the hybrid 1/III/87. This cultivar is thorn-free as well as its parent combination. Its performance in respect of bearing, fruit quality and resistance to diseases and early spring frosts is extraordinary (Stanisavljević, 1999).

Black currant cv Čačanska crna originated from open fertilization of cv Malling Jet. It was selectioned in 1984 as hybrid I/75. It is a heavy cropper, resistant both to early spring frosts and anthracnose (*Pseudopeziza ribis*). It is suitable for mechanized harvesting and its fruits may be used both for fresh consumption and various forms of processing (Stanisavljević et al., 2002). Both cultivars are named and released and have been produced since 1997.

Blackberry and black currant are commercially propagated by the classical methods of vegetative propagation, i.e. by hard wood and soft wood cuttings, by layering, bush division and the like. However, successful application of these methods of vegetative propagation is limited to certain extent. Thus, for example, propagation by layers requires rather large area for a lay-bed, controlling weed incidence among lay-beds arising as a difficulty. 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).

Application of contemporary methods of vegetative propagation, such as micropropagation *in vitro* is more justified and proves economical, especially in respect of propagation of small fruit varieties. Besides providing production over the whole year this method is suitable for rapid propagation of newly developed cultivars and hybrids as well as for their introduction into production and obtainment of healthy planting material which is a must in successful production.

Application of tissue culture *in vitro* has been recorded in great number of blackberry and black currant cultivars (Orlikowska, 1984; Wainwright and Flegmann, 1986; Ružić and Cerović, 1998; Meng et al., 2004). It has also been applied in other *in vitro* methods, such as adventitious organogenesis (Reed and Tsao, 2002), or cryopreservation (Kusharenko et al., 2004). However, micropropagation *in vitro* requires a great deal of experimental work on optimalization of the conditions in all its phases and for each cultivar.

The objective of this investigation was precisely the establishment of the efficient method of rapid propagation of newly developed blackberry cv Čačanska bestrna and black currant cv Čačanska crna.

Material and methods

Branches of blackberry cv Čačanska bestrna and black currant cv Čačanska crna with dormant buds (January 24, 2005) were cut and placed to develop under the laboratory conditions, at room temperature. Upon activation of lateral and apical buds the standard procedure of surface sterilization was performed involving the following: washing the explants in the running water (1.5 – 2 hours), rinsing in 70% ethanol and 10% bleach for 1 min and 10 min, respectively, followed by triple rinsing with the sterile water. Buds 0.3 – 0.8 cm sized were isolated under the stereomicroscope and placed onto the nutritive medium (Murashige and Skoog, 1962) with the following hormones in mg l^{-1}: N^6-benzyladenine (BA) 2.0; indole-3-butyric acid (IBA) 0.5 and gibberellic acid (GA_3) 0.1.

Upon establishment of the aseptic culture, i.e. rosette induction, shoots of these cultivars were multiplied on media of different hormonal composition shown in the Table 1.

Forty explants [2 repetition x (5 explants x 4 culture vessels)] were placed on each medium. The subculture lasted 20 days and the following parameters of multiplication were monitored: multiplication index, length of axial and lateral shoots.

The rooting phase involved utilization of MS medium with mineral salts reduced to 1/2, organic complex remained unchanged according to MS and with 1.0 mg l^{-1} IBA, 0.1 mg l^{-1} GA_3 and 1 g l^{-1} of active charcoal. Percentage of the rooted plants was determined after 21 days along with other rooting parameters (number and length of roots, height of the rooted plants). All media consisted agar and sucrose at concentrations of 7 g and 20 g l^{-1}, respectively.

The cultures were grown in the growth room under controlled temperature (23±1°C), light photoperiod (16/8 h, light/dark) and 8.83 Wm^{-2} light intensity on culture surface, with white fluorescent lamps of 40W, 6.500°K.

The rooted plants were planted into the sterile peat, Steckmedium (Klasmann) and acclimatized under the 'mist' system in a green house.

Table 1.
Hormonal content of media used in multiplication phase

Cultivar	No of medium	Mineral salts	$FeSO_4$ x 7 H_2O	BA (mg l^{-1})	IBA (mg l^{-1})	NAA (mg l^{-1})	GA_3 (mg l^{-1})
Čačanska bestrna	I	MS	MS	1.0	0.1	-	0.1
blackberry	II	MS	MS	0.5	0.1	-	0.1
	III	MS	MS	1.0	-	0.1	0.1
	IV	MS	MS	0.5	-	0.1	0.1
Čačanska crna	IV	MS	MS	0.5	-	0.1	0.1
blackcurrant	V	MS	MSx2	0.5	-	-	-

Results and discussion

Lateral and apical buds used as explants were developed on the branches of blackberry cv Čačanska bestrna and black currant cv Čačanska crna upon 20 days. Over the period spontaneous rooting of all branches of cv Čačanska crna was observed whereas in cv Čačanska bestrna roots emerged directly out of the lateral bud at basal section of branches (Fig. 1a, b). Placing of explants lasted one month, as the incidence of rather intensive bacterial and fungal infection was observed, which induced extremely high percentage of infected cultures, especially in cv Čačanska crna (Tab. 2). Aseptic cultures of both cultivars were obtained in the middle of March (Fig. 1c). Regenerated shoots emerging from each bud were designated separately resulting in 16 and 6 lines in blackberry cv Čačanska bestrna and black currant cv Čačanska crna, respectively.

The highest values of the multiplication index in cv Čačanska bestrna were obtained on medium with in mg l^{-1}: BA 1.0, IBA 0.1 and GA_3 0.1 (Tab. 3). The shoots had small nodular callus and great number of bud rudiments in the basal section of shoots (Fig. 2a). Ružić and Cerović (1998) also found that in this cultivar the highest multiplication index is obtained on the same medium.

The longest shoots, both axial and lateral ones have been obtained (0.89 cm and 0.59 cm, respectively), by reducing BA concentration to 0.5 mg l^{-1} with IBA 0.1 mg l^{-1} and GA_3 0.1 mg l^{-1}. The plants had wide green leaves, stem had red pigmentation and callus was dark and of firm consistency. Slight multiplication has been observed at the shoot base also (shoot length 0.1-0.3 cm).

As the signs of chlorosis were traced on the regenerated shoots in black currant cv Čačanska crna upon induction of rosette (Fig. 1) these shoots were placed on the MS medium with doubled content of $FeSO_4$ x 7 H_2O and 0.5 mg l^{-1} BA. This medium proved highly efficient in first subcultures in raspberry cv Willamete (Ružić et al., 2003). However, multiplication index in the second subculture was considerably lower (1.93), compared to the results obtained in raspberry, although numerous tiny rudiments 0.4 cm long were observed at the basal section of shoots (Fig. 2b). The shoots were also placed on the medium with the same BA concentration, though accompanied by NAA at concentration of 0.1 mg l^{-1}. Adding auxin into the medium, however, gave no positive

Table 2.
Obtainment of aseptic culture – rosette formation

Cultivar	% of infected cultures	% of tarnished cultures	Induced rosette (%)
Čačanska bestrna	42.8	-	57.2
Čačanska crna	82.4	-	17.6

Table 3.
Multiplication parameters of cvs Čačanska bestrna and Čačanska crna on the media with different hormonal content

Medium/designation	Multiplication index	Length of axial shoots (cm)	Length of lateral shoots (cm)
Cv Čačanska bestrna			
I	2.45 a*	0.73 b	0.56 ab
II	2.04 ab	0.89 a	0.59 a
III	1.46 b	0.75 b	0.51 b
IV	2.05 ab	0.72 b	0.54 ab
Cv Čačanska crna			
IV	1.17 a	0.59 a	0.50 a
V	1.93 a	0.57 a	0.50 a

*Means within columns followed by the same letter do not differ at P = 0.05 according to Duncan's Multiple Range Test.

Table 4.
Rooting parameters of cvs Čačanska bestrna and Čačanska crna upon 21 days (Average ± S.D.)

Cultivar	Rooting %	Number of roots	Root length (cm)	Plant length (cm)
Čačanska bestrna	100	2.7 ± 1.3	2.74 ± 1.95	1.36 ± 0.18
Čačanska crna	30	1.3 ± 0.5	1.07 ± 0.65	1.12 ± 0.35

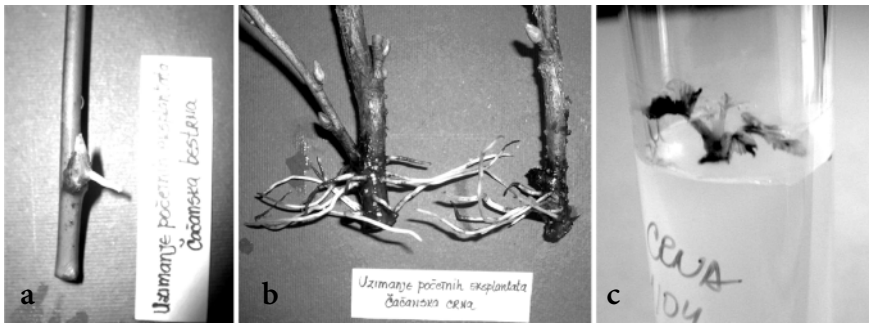

Figure 1.
Taking initial explants of cvs Čačanska bestrna (a), Čačanska crna (b) and rosette formation (c)

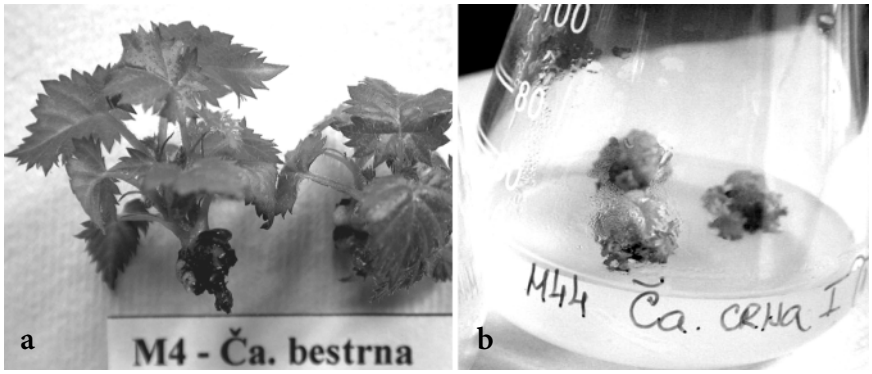

Figure 2.
Shoots of cvs Čačanska bestrna (a) and Čačanska crna (b) in multiplication stage

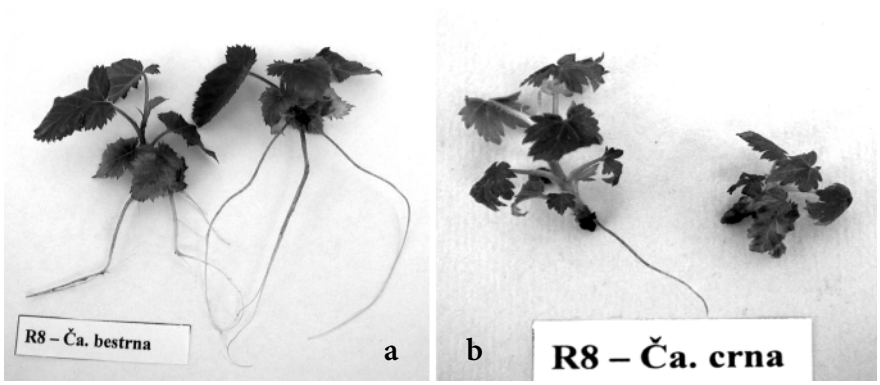

Figure 3.
Rooted shoots of cvs Čačanska bestrna (a) and Čačnska crna (b) on medium with active charcoal, IBA and GA_3

effect on the multiplication parameters of this cultivar. Moreover, it brought about reduction in multiplication index (Tab. 3). Due to low quality of shoots and small and black callus at the basal section these shoots could not be used for further growing. Orlikowska (1984) ascertained that shoots of black currant cv Roodknop did not react positively on auxin added into the media for multiplication, regardless of type and concentration of the applied auxin.

During the rooting phase the shoots of both cultivars were placed on the aforementioned medium for rooting. Active charcoal had positive effect during the rooting phase in many representatives of the *Rubus* genus (Donnelly and Doubeny, 1986; Ružić and Cerović, 1998). In the shoots of blackberry cv Čačanska bestrna first roots were observed 8 days upon placing on the medium, whereas on day 21 the percentage of rooting amounted to 100% (Tab. 4). Tiny roots of shoots placed on this medium were well developed, sometimes individually attaining length up to 7.6 cm (Fig. 3a). Average length of the rooted plants was 1.36 cm which corresponds with the results obtained by Ružić and Cerović (1998) with the same cultivar.

Well developed root system and high quality of the rooted plants induced high percentage of acclimatization (100%) under the 'myst' system in greenhouse. However, the process of micropropagation in this cultivar may be considerably accelerated by simultaneous performance of phases of rooting and multiplication by nodal transplantation of one part of the rooted plants and its return to multiplication, and planting of the other part and placing it on acclimatization (Ružić and Cerović, 1998).

In black currant cv Čačanska crna low percentage of rooting was obtained on the medium for rooting with active charcoal, shoots having rather poorly developed roots (Tab. 4; Fig. 3b). Low percentage of root-

ing, though resulting with substantially higher quality of rooted plants on medium with active charcoal, was obtained in raspberry cv Willamette (Ružić et al., 2003). Investigations conducted by Orlikowska (1984) showed, however, that the percentage of rooting in black currant cv Roodknop on media with IBA was also low, going below 40%, which suggested the authors that the exogenous auxins were indispensable only for the initiation of rhizogenesis.

Rooted plantlets of cv Čačanska crna were planted into peat and placed under the 'mist' system in greenhouse. The percentage of acclimatization was low, 40%.

Conclusion

Micropropagation has been widely used recently as mean of rapid propagation as well as for introduction of newly developed cultivars and hybrids into production, especially when propagation by standard vegetative methods is difficult and when obtainment of healthy planting material is the objective.

Newly developed blackberry cv Čačanska bestrna has been successfully propagated by this modern method of vegetative propagation in only 4 months with the initial explants placed on the nutritive medium in the middle of February and first rooted and acclimatized plants obtained in the middle of June.

Multiplication phase in cv Čačanska crna requires elaboration, i.e. introduction of media with other types and concentrations of cytokinins, with study of hormone-free media for the rooting phase, etc. Direct planting of multiplied shoots into the soil substrate should also be tested.

Results obtained in this work suggest full justification of micropropagation for rapid and efficient blackberry propagation. For black currant, further research should be done to optimize conditions for succesful multiplication.

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