ÎNMULŢIREA *„IN VITRO”* A SOIURILOR DE COACĂZ NEGRU PERLA NEAGRĂ ŞI AMURG

„IN VITRO” PROPAGATION OF BLACK CURRANT ‘PERLA NEAGRA’ AND ‘AMURG’ CULTIVARS

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Abstract

Our paper presents the results obtained in the framework of the in vitro culture laboratory of the Fruit Research Station of Cluj regarding the behaviour on *in vitro* propagation of the black currant cultivars ‘Perla Neagra’ and ‘Amurg’. For initiation, multiplication and rooting the Murashige & Skoog medium was used, with 1 mg/l Vitamin B1, 6 g/l Plant Agar and pH adjusted to 5.8. The in vitro propagation rate was established, as well as the rooting and acclimation percentages. In the multiplication phase, Murashige & Skoog (MS), Woody Plant Medium (WPM) and Driver & Kunyuki Walnut (DKW) were used as basal media. As growth regulators, 6-benzylaminopurine (BAP), Zeatine, Thidiazuron (TDZ) and 2-izopentiladenine (2-iP) were tested. Acclimation was done in solid substrate, respectively perlite or a mixture of soil+perlite in 1:1 volume to volume ratio, as well as in hydroculture, in plastic trays containing water with the pH=7 and using different types of explants.

Cuvinte cheie: coacaz negru, culturi in vitro,
Keywords: black currant, in vitro culture

1. Introduction

The black currant is propagated traditionally by greenwood cuttings, hardwood cuttings, layering and rarely by division or grafting. Propagation by hardwood cuttings is extremely effective, from one hectare of nursery plantation 300,000 cuttings can be obtained, each 20 cm in length (Mircea Botez et. Al., 1984).

There are very few data in literature regarding the *in vitro* propagation of the black currant. Tatiana Lazic and Durdina Ruzic (2007) used MS medium (Murashige & Skoog 1962) and various concentrations of BAP, TDZ and IBA. BAP had inhibitory effect upon the *in vitro* growth of black currant cultivar Cacanska Crna. Better results were obtained regarding *in vitro* growth and multiplication rate after transferring the plantlets to media without cytokinins. Rooting and acclimation rates were of 100 %.

2. Material and methods

We studied the ‘Perla Neagra’ and ‘Amurg’ cultivars bred at the Fruit Research Station of Cluj. For black currant *in vitro* propagation we made the following steps:

The plants used as explants sources for *in vitro* propagation are in the fruit shrub collection of the Fruit Research Station of Cluj. From these plants, shoots (annual growths) were harvested, from which single-node fragments were obtained, which were used for *in vitro* culture initiation.

The single-node fragments were washed with tap water for 60 minutes, then rinsed with clean deionised water, then disinfected for 20 minutes in an antiseptic mixture of sterile deionised water and 20 % ACE bleach and then rinsed with sterile deionised water (6 rinses) in order to eliminate the traces of disinfectant. After disinfection the plant material was fragmented into single-node fragments which were inoculated onto the initiation medium (Table 1).

The plantlets that resulted after initiation were then passed to the multiplication phase, in which Woody Plant Medium (Lloyd G. and McCown,1981) and medium Murashige & Skoog medium were used as basal media, to which other components were added: growth regulators - BAP, TDZ, 2-IP, zeatine, stock solutions of vitamins : B1, B6, Nicotinic acid, crystal sugar as carbon source, Sequestrene 138 or FeNaEDTA as iron source, powder agar (Caisson Laboratories, Inc., catalogue A038, lot 6308) or plant-agar (Duchefa) 6 g/l. The pH of the media was adjusted to 5 (for Woody Plant Medium) or 5.8 (for the MS media). All the components were added to the media before autoclavation.

The media were distributed into Magenta GA7 vessels with polypropylene caps (50 ml/vessel) and autoclaved for 25 minutes at 121^{o} C.

The plant material used for multiplication consisted of shoot fragments about 1 cm in length, containing 1-2 nodes (Fig. 1). 9 microcuttings/vessel were inoculated (Fig. 2). The vessels were sealed with Folpack foil.

Culture incubation was done in the growth chamber, in artificial light provided by fluorescent tubes. Light intensity was around 3000 Lux and temperature was around 24-26 ^{o} C.

The shoot fragments inoculated onto the modified hormone-free MS medium are rooted in 4-6 weeks, after which they can be transferred *ex-vitro* for acclimation.

The rooted plantlets were transferred *ex-vitro* into trays filled with perlite or soil+perlite in 1 :1 volume to volume ratio, after which they were watered and covered with plastic caps in order to maintain humidity. Acclimation in hydroculture was also used. Two explant types were used : whole *in vitro* rooted plantlets, as well as excised plantlet bases containing the root and 2-3 viable nodes together with the leaves grown from the nodes, the rest of the plantlet being used in the process of *in vitro* multiplication. The plantlets were acclimated in 4-5 weeks.

3. Results and discussions

After initiation, the black currant plants were multiplied for 3 cycles on hormone-free MS medium and the multiplication rate was established after 2 months of *in vitro* culture.

On Variant 1 (hormone-free MS), the two varieties reacted almost identically. The plantlets were extremely vigorous, with 4.5-8 cm long shoots, long internodes at the base and shorter in the middle and apical parts of the plantlets, with large leaves, bright green in colour. On this medium the plants started to root after 4 weeks in culture.

The multiplication rate on the hormone-free MS medium was 2.54 for cultivar ‘Perla Neagra’ and 2.97 for cultivar ‘Amurg’ (Table 3).

In order to establish a nutritive medium which should provide a multiplication rate higher than that on hormone-free MS, the other variants of media from Table 2 were tested, using inoculi from cultivar ‘Amurg’.

On variants 2 and 3, with MS as basal medium and BAP or TDZ as growth regulators, very short and deformed plantlets were obtained, totally unsuitable material for multiplication or acclimation.

In the case of Variants 4 and 5, WPM + 1 mg/l Zeatin or 5 mg/l 2-Ip the results were similar to those obtained on hormone-free MS medium, these cytokinins did not provide *in vitro* proliferation of the 2 black currant cultivars.

In the case of Variants 6 and 7, where DKW (Driver,J.A., Kuniuki,A.H., 1984, Bekir SAN et al., 2007, Mehmet Nuri Nas, 2004) was used as basal medium, without hormones or containing 10 mg/l 2-IP, a slight increase in multiplication rate was observed, but on neither of these variants the proliferation of axillary shoots was stimulated, only one shoot/inoculum resulted. In Variant 6, hormone-free DKW, multiplication rate was of 3.38 and on Variant 7, DKW+10 mg/l 2-IP, multiplication rate was of 3.89.

The multiplication rates in cultivar ‘Amurg’ on the 3 variants that provided the best multiplication rates are presented in Fig. 3.

In cultivar ‘Perla Neagra’, on hormone-free MS the multiplication rates and survival rates of inoculi with 1 node and 2 nodes were compared. The studies were done on 3 Magenta vessels/variant, containing 8-9 inoculi/vessel.

In the case of inoculating single-node fragments, from the initial number of 25 inoculi 16 inoculi survived. Themultiplication rate in this case was of 3.58. In the variant with binodal fragments, from the total of 26 inoculi 24 survived and multiplication rate was of 6.07 (Table 4).

Regarding explant type, in cultivar ‘Perla Neagra’ it is seen that single-node explants are less viable, resulting less vigorous plants as compared to those resulted from binodal explants. From the stem parts containing long internodes and well-developed axillary buds, single-node microcuttings can be excised and used successfully, whereas from the zones containing short internodes it is recommended to excise 2-node fragments.

In the black currant rooting took place at the same time as multiplication, on the same variants of media (Fig. 4). Rooting percentage was over 90 %.

The plants can be transferred *ex-vitro* for acclimation after 6-8 weeks, when they are well rooted. In this phase, the classical method of acclimation in perlite was tested (Fig. 5), in plastic trays covered with transparent lids, using whole rooted plantlets, non-rooted shoots or shoot fragments and rooted plantlet bases containing 2-3 nodes and leaves.

For the whole plantlets rooted *in vitro*, acclimation percentage was of 100 %, for non-rooted shoots acclimation percentage was 0 and the bases had an acclimation rate of over 90 %. Trays containing a mixture of soil+perlite were also used for acclimating rooted plantlets and the acclimation rate was 100 %, also (Fig. 6).

Because the classical acclimation method necessitates much labour, special environments and it is more costly, the method of acclimation in hydroculture was also tested. For this purpose, the *in vitro*-rooted plantlets were transferred *ex-vitro* into trays containing water with neutral pH. The plantlets were acclimated in 3 weeks in a percentage of 100 % and they grew spectacularly, becoming very vigorous (Fig. 7). Because the multiplication rate in the black currant is very low, the method of acclimating the rooted bases resulted from the multiplication phase was also tested, the middle and apical parts of the

plantlets being used for further *in vitro* propagation. From these bases, also, vigorous plants were grown, the acclimation rate also being very high.

The acclimated plants were then planted into pots containing potting mix, survival rate being more than 90 % (Fig. 8).

4. Conclusions

1. The black currant can be successfully propagated *in vitro*, but multiplication rate is very low, varying between 2.60 and 6.02, depending upon the nutritive media and explants type.
2. The basal media that gave good results in the *in vitro* propagation of the black currant are MS, WPM and especially DKW.
3. The growth regulators used, BAP, TDZ, Zeatin and 2-Ip in various concentrations did not lead to the proliferation of axillary shoots, and BAP and TDZ led to the growth of deformed, very short plantlets, with basal callus and deformed leaves, totally unsuitable for further propagation or acclimation.
4. The media on which the best results were obtained were hormone-free MS and hormone-free DKW.
5. In the black currant, *in vitro* rooting is done at the same time with *in vitro* multiplication, on media without growth regulators.
6. Acclimation can be done either by the classical method in perlite or in hydroculture. In both variants, acclimation rate can reach 100 %. Acclimation in hydroculture is more economical, it necessitates little space and labour.

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Table and Figures

Table 1. **The nutritive medium for black currant culture initiation**

Components	Variant 1
Salts	MS*
FeNaEDTA	36.7 mg/l
Myo-inositol	100 mg/l
Vitamin B1	1 mg/l
Vitamin B6	0.5 mg/l
Nicotinic Acid	0.5 mg/l
Sugar	30 g/l
Agar	6 g/l
pH	5.8

* Murashige Skoog

Table 2. **Nutritive media for black currant multiplication**

Components	Variant 1	Variant 2	Variant 3	Variant 4	Variant 5	Variant 6	Variant 7
Salts	MS*	MS*	MS*	WPM**	WPM**	DKW***	DKW***
FeNaEDTA	36.7 mg	36.7 mg	36.7 mg	-	-	44.63 mg/l	44.63 mg/l
Sequestrene 138	-	-	-	100mg/l	100mg/l	-	-
Myo inositol	100 mg/l	100 mg/l	100 mg/l	100 mg/l	100 mg/l	100 mg/l	100 mg/l
Vitamin B1	1 mg/l	1 mg/l	1 mg/l	2 mg/l	2 mg/l	2 mg/l	2 mg/l
Vitamin B6	0.5 mg/l	0.5 mg/l	0.5 mg/l	1 mg/l	1 mg/l	-	-
Nicotinic Acid	0.5 mg/l	0.5 mg/l	0.5 mg/l	1 mg/l	1 mg/l	1 mg/l	1 mg/l
Glycine	-	-	-	-	-	2 mg/l	2 mg/l
BAP	-	0,7 mg/l	-	-	-	-	-
Zeatine	-	-	-	1 mg/l	-	-	-
2-IP	-	-	-	-	5 mg/l	-	10 mg/l
TDZ	-	-	0.2 sau 0.7 mg/l	-	-	-	-
Sucrose	30 g/l	30 g/l	30 g/l	30 g/l	30 g/l	30 g/l	30 g/l
Agar	5 g/l	5 g/l	5 g/l	5 g/l	5 g/l	6 g/l	6 g/l
pH	5.8	5.8	5.8	5	5	5.8	5.8

*****MS – Murashige & Skoog
** WPM - Woody Plant Medium
*** Driver & Kunyuki Walnut

Table 3. **Multiplication rate in black currant cultivars 'Perla neagra' and 'Amurg'**

Vessel no.	Perla neagra			Amurg		
	Initial number of inoculi	No. of resulted inoculi	Multiplication rate	Initial number of inoculi	No. of resulted inoculi	Multiplication rate
1	9	19	2.05	9	31	3.50
2	9	25	2.72	9	23	2.88
3	9	25	2.77	9	22	2.44
4	9	21	2.63	9	27	3.05
Average	9	22.5	2.54	9	25.75	2.97

Table 4. **The influence of explant type upon multiplication rate in black currant cultivar 'Perla neagra'**

Variant	Initial no. of inoculi/3 vessels	No. of viable inoculi/3 vessels	No. of nodes/3 vessels	Multiplication rate (single-node fragments)	Multiplication rate (two-node fragments)
Single-node explants	25	16	179	7.16	3.58
Two-node explants	26	24	313	12.04	6.02

Fig. 1. Plant material used for multiplication

Fig. 2. Inoculation into the Magenta GA7 vessel

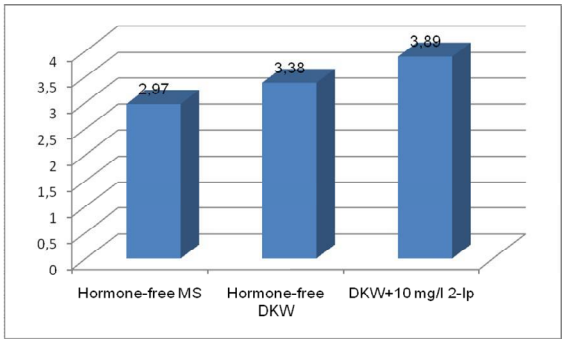

Fig. 3. Multiplication rate for cultivar Amurg

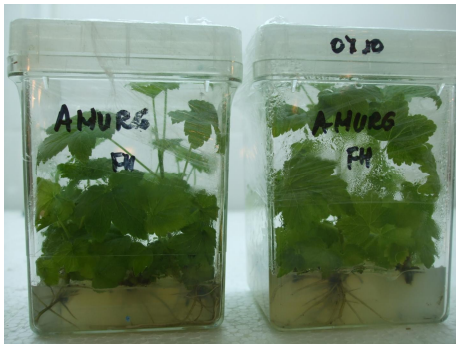

Fig. 4. *In vitro* rooting of the black currant

Fig. 5. Acclimation in perlite

Fig. 6. Acclimation in soil+perlite mixture

Fig. 7. Acclimation in hydroculture

Fig. 8. Acclimated plants planted to pots