Bulletin UASVM Horticulture, 68(1)/2011
Print ISSN 1843-5254; Electronic ISSN 1843-5394

In Vitro Propagation of the Thornless Blackberry Cultivar 'Loch Ness'

Alexandru FIRA[1)], Doina CLAPA[1)], Elena RAKOSY -TICAN[2)]

[1)]Fruit Research Station Cluj, 5 Horticultorilor Street, 400457 Cluj-Napoca, Cluj, Romania, www.scdpcluj.ro; firaalexandru@yahoo.com
[2)] Babes-Bolyai University, 1 Mihail Kogalniceanu street, Cluj-Napoca, Cluj, Romania

Abstract. Our paper presents aspects regarding the *in vitro* multiplication rates, the fresh biomass of the resulted plantlets, aspects regarding the incubation of the cultures in the growth room and the rooting and acclimation of the newly formed shoots in the thornless blackberry cultivar 'Loch Ness'. The nutritive medium had, as components Murashige & Skoog 1962 (MS) salts, Myo-Inositol at 100 mg/l, Vitamins B_1- 1 mg/l, B_6 - 0.5 mg/l, Nicotinic Acid - 0.5 mg/l and, as growth regulator, 6-benzylaminopurine at 0.5 mg/l. The rooting of the shoots that resulted from the multiplication medium was done *ex vitro* in the same phase together with acclimation, in floatation hydroculture as well as in solid substrate (acid peat + perlite in 1:1 volume to volume ratio. *Ex vitro* rooting and acclimation in float hydroculture in open air and the subsequent growth and development of the acclimated plants in hydroponics fertilized with 2 g/l Ferticare were also investigated.

Keywords: *Rubus fruticosus***,** micropropagation, acclimation, float hydroponics.

INTRODUCTION

The blackberry can be propagated by seeds, layering, offsets, root cuttings, stem cuttings, division of shoots or grafting and it can also be propagated very effectively *in vitro* vitro (Botez *et al.*, 1984). Blackberry micropropagation has been investigated by many researchers (Gajdosova *et al*, 2006; Meng, 2004; Mihalache, 1996, Zawadska M. and T. Orlikowska 2006).

Bobrowski, *et al*. (1996) tested several variants of Murashige & Skoog media with 1 and 2 mg/l BAP. The presence of the auxin NAA and GA_3 strongly inhibited *in vitro* proliferation in all the cultivars they tested. The variants of MS media with the concentrations of salts reduced to 1/3 with various IBA concentrations (0.3; 0.5 and 0.8 mg/l) ensured 100 % rooting percentages.

Erig *et al*. (2002) experimented with the blackberry cultivar Tupy, testing media with various concentrations of BAP (0; 2; 4; 6; 8 and10 μM – respectively, 0; 0.45; 0.9; 1.35; 1.8 and 2.25 mg/l) and IBA at 0; 0.5 and 1 μM (respectively, 0; 0.1 and 0.2 mg/l) . The best results regarding the multiplication rate were provided by the variant with 2 μM BAP and without IBA, the latter having inhibitory effect, especially at the concentration of 0.5 μM and in the presence of 6 and 8 μM BAP.

Najaf-Abadi A. Jafari şi Y Hamidoghli (2009) experimented with a trailing thornless blackberry variety. Among the variants of media tested for multiplication, the best results were provided by the MS medium with 2 mg/l BAP and 0.3 mg/l GA_3, whereas for rooting, MS with 2 mg/l IBA. Nevertheless, the resulting plantlets were surprisingly small.

Ružič D. and T. Lazič (2006) carried out experiments of initiation, multiplication and acclimation in the thornless blackberry cultivar `Čačanska bestrna`. The highest multiplication rate was ensured by the variant of MS medium with 1 mg/l BAP, 0.1 mg/l IBA and 0.1 mg/l

GA_3. The longest shoots resulted on the medium with 0.5 mg/l BAP, 0.1 mg/l IBA and 0.1 mg/l GA_3. Rooting was done on MS medium with the concentration of salts reduced to ½, to which 1 mg/l IBA, 0.1 mg/l GA_3 and 1 g/l activated charcoal were added. The rooting percentage was of 100 % after 21 days in culture. The rooted plantlets were acclimated in an artificial fog system, the survival rate being also 100 %.

Villa F. *et al*. (2006, 2009) experimented *in vitro* rooting in the blackberry cultivar Brazos under the influence of NAA at the concentrations of 0; 0.1; 0.5; 1 and 1.5 mg/l and natrium chloride at the concentrations of 0; 25; 50; 75 and 100 mg /l. Natrium chloride at 50 mg/l had stimulating effect upon the length of aerial parts at all the NAA concentrations tested. The concentration of 50 mg/l NAA also strongly stimulated the increase of fresh biomass in the aerial parts, with the highest values at 1.5 mg/l NAA. The combination of 100 mg/l natrium chloride and 1.5 mg/l NAA gave the best results regarding root number/plantlet *in vitro*.

At the Fruit Research Station of Cluj blackberry cultivar 'Thornless Evergreen' was studied (Fira *et al*., 2009, 2010). As basal media, MS and DKW were studied and there were no significant differences regarding multiplication rates. Among the growth regulators tested, BAP proved to be the most effective, at the concentration of 0.5 mg/l, which ensured very high multiplication rates, reaching values of about 100 times/ 2-month multiplication cycle and, also, it ensured the regeneration of shoots with optimal vigour and uniformity. The most effective method of blackberry acclimation proved to be the „float hydroponics" technique.

The blackberry (*Rubus fruticosus*) cultivar that we studied, 'Loch Ness'is the most widespread thornless blackberry cultivar in Great Britain.

MATERIALS AND METHODS

For the initiation of *in vitro* cultures annual shoots were used, which were trimmed eliminating the internodes; the plant material was washed with running water and rinsed with sterilized deionized water. Disinfection was done with a mixture of 20 % ACE bleach for 20 minutes and rinsed with sterile deionized water (5 rinses) in the laminar air flow hood. Then the apical and axillary buds were excised and inoculated on the modified MS medium, with 0.7 mg/l BAP (Tab. 1), 1 bud/test tube.

Tab.1 .

The medium for blackberry *in vitro* culture initiation

Component	Concentration
MS* salts	Full
Myo-inositol	100 mg/l
Vitamin B_1	1 mg/l
Vitamin B_6	0.5 mg/l
Nicotinic acid	0.5 mg/l
BAP	0.7 mg/l
Sugar	30 g/l
Plant Agar (Duchefa)	6 g/l
pH adjusted to 5.8	

* Murashige & Skoog

In the multiplication phase the same medium was used as for initiation, but the BAP concentration was reduced to 0.5 mg/l.

The media were prepared using stock solutions of macro- and microelements and vitamins; allthe components were added before autoclavation. The media were distributed into 720 ml glass jars (100 ml medium/vessel) and were sterilized by autoclavation at 121^{o} C for 20 minutes.

For inoculation, 2 cm long microcuttings were used, 7 inoculi/jar. Incubation was done in the growth chamber in artificial light provided by fluorescent tubes (2400 Lux) at the temperature of 24-26 ^{o}C and 16-hour photoperiod. The vessels were positioned on only one level on the shelves. One experiment was carried out, also, with positioning the jars in two layers on a shelf, placing the upper layer on the caps on the lower layer, in order to test the possibility of economizing space and electricity.

After 3 multiplication cycles of 3 months each, several characteristics were studied: multiplication rates/plantlet taking into consideration the shoots and shoot fragments 2 cm in length, as well as fresh biomass/plantlet and fresh biomass/resulted inoculum.

Taking into consideration the acclimation phase, the number of shoots suitable for acclimation that resulted/vessel, obtained from the initial 7 microcuttings/vessel was also established. Only the shoots of at least 2 cm in length were counted.

The non-rooted shoots resulting directly from the multiplication medium were separated and transferred directly *ex vitro* into a mixture of acid peat + perlite 1:1 volume to volume ratio in plastic trays covered with transparent lids. Short shoots, 1-1.5 cm in length were used as well as 3-5 cm long shoots.

Direct *ex vitro* rooting and acclimation in Jiffy pellets was also tested, in transparent plastic trays, either covered with transparent plastic lids or left uncovered, in the growth chamber as well as in the greenhouse.

Ex vitro rooting and acclimation in float hydroponics was also tested. In this case the shoots resulted from the multiplication phase were transferred as bunches of separate shoots into cell trays that were maintained floating in mini-basins in tap water with pH=7 in greenhouse conditions as well as in open air.

From the plantlets rooted and acclimated *ex vitro*, 135 were cultured for 1 month in hydroponic culture, in trays with cells 1 cm in diameter, with the purpose of fortifying them. Ferticare complex fertilizer of the type14:11:25 with microelements was used, at the concentration of 2 g/l.

RESULTS AND DISCUSSION

Initiation was carried out at the end of October on the modified MS medium, with 0.7 mg/l BAP (Tab.1), with a very high regeneration percentage of 75 %. Having in view the high multiplication rates obtained in other blackberry cultivars propagated at the Fruit Research Station of Cluj, in the multiplication phase the BAP concentration was reduced to 0.5 mg/l (Fig. 1).

It was found that in the multiplication phase there were great variations regarding the biomass, sizes and multiplication rates of the 7 inoculi in the same vessel. 1 inoculum/vessel died and the other 6 regenerated plantlets of various sizes, with various multiplication rates, between 4 and 147 microcuttings resulting/plantlet (Tab. 2). Nevertheless, the average multiplication rates/vessel had similar values, between 35.29 and 41.86.

Fig. 1. Blackberry cultivar 'Loch Ness'*in vitro* – 3-month old culture

Tab. 2

Multiplication rates and biomass

Vessel	Plant	Biomass/ plant (g)	Multiplication rate/ plant	Biomass/ Resulted inoculum (g)	Average multiplication rate/vessel	Average multiplication rate/variant
1	1	1.14	12	0.1	41.86	40.18
	2	2.3	45	0.05		
	3	1.87	32	0.06		
	4	3.31	48	0.07		
	5	6.91	108	0.06		
	6	2.39	48	0.05		
	7	-	-			
2	1	5.64	50	0.11	44.29	
	2	2.57	36	0.07		
	3	4.16	49	0.08		
	4	0.81	8	0.1		
	5	11.33	147	0.08		
	6	1.45	20	0.07		
	7	-	-			
3	1	1.77	30	0.06	35.29	
	2	1.63	26	0.06		
	3	0.26	4	0.07		
	4	1.4	17	0.08		
	5	1.47	39	0.04		
	6	6.59	131	0.05		
	7	-	-			
4	1	2.63	48	0.05	39.29	
	2	2.11	37	0.06		
	3	2.1	41	0.05		
	4	0.99	10	0.1		
	5	2.98	42	0.07		
	6	6.99	97	0.07		
	7	-	-			

In the experiment of placing the culture vessels on two layers it was found that the multiplication rates in the lower level were lower than those in the higher level. After a culture cycle of 2 months, in the vessels in the higher level the average multiplication rate was

of 24.11 times, whereas in the lower level 19.52 times. About 20 % hyperhydricity was also found in the vessels in the lower level.

In blackberry cultivar 'Loch Ness'an average multiplication rate of 40.18 times was found in the cultures with a multiplication cycle of 3 months, respectively, 281.25 inoculi resulted/vessel. The average number of shoots suitable for acclimation was of 151.75/vessel, respectively, of 21.69/initial inoculum (Tab. 3).

Tab.3

Proliferation rates – shoots suitable for acclimation – blackberry cultivar 'Loch Ness'

Vessel	No. of inoculi/vas	No. of shoots/vessel	Proliferation rates (shoots suitable for acclimation)
1	7	140	20
2	7	165	23,57
3	7	157	22,48
4	7	145	20,71
Average	7	151.75	21.69

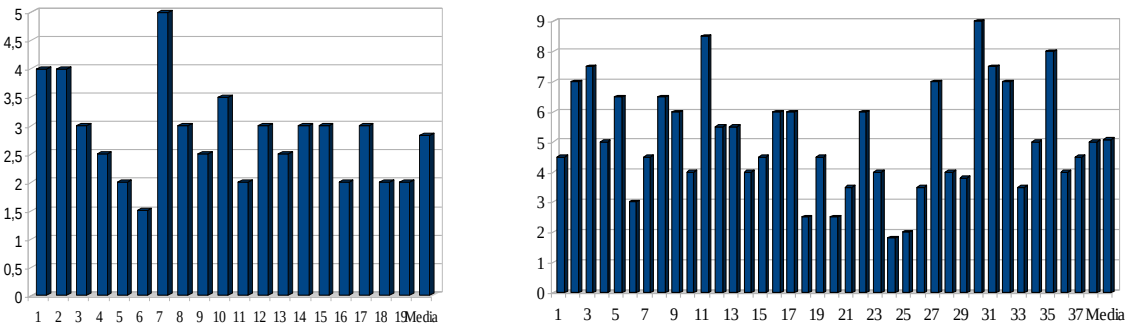
Rooting and acclimation were carried out in the same phase, *ex vitro*. In the experimental variants of *ex vitro* rooting and acclimation in peat+ perlite mixture the survival percentage was of 100 % in the 1-1.5 cm long shoots as well as the 3-5 cm long shoots (Fig. 2)

Fig.2. *Ex vitro* rooting and acclimation in peat + perlite

From the 3-5 cm long shoots more vigorous plantlets resulted (3-8 cm in length), with large leaf area and well developed root system. In the two variants, the average root length was of 2.82 cm in the 1-1.5 cm long microcuttings and 5.08 cm in the 3-5 cm long shoots (Fig. 3).

The shoots transferred *ex vitro* for rooting and acclimation in Jiffy pellets had 100 % survival rate in the covered vessels incubated in the growth chamber, whereas in the non-covered vessels most of the microcuttings did not survive. In the greenhouse, high survival rates were registered only for the variants where the Jiffy pellets were maintained in vessels covered with lids (Fig. 4).

The fastest and most effective *ex vitro* rooting and acclimation method proved to be float hydroculture. In the greenhouse (in May-June) the first roots appeared after 10 days in culture and after 1 month the plantlets were rooted and acclimated and then were planted into pots, in potting mix. After 6 weeks of culture in float hydroculture the percentage of well-rooted plantlets was of 74 %, 16.43 % dead and 9.57 % non-rooted (Fig. 5).



a b

Fig.3. Root length in the plantlets rooted *ex vitro* in peat+perlite mixture
(a- 1-1.5cm long shoots; b- 3-5 cm long shoots)

a b c

Fig. 4. Shoots acclimated in Jiffy pellets: a-without lids, b- with lids, c- acclimated plants .

Fig.5. Shoots rooted and acclimated in float hydroculture

In the case of acclimation in float hydroculture in open air, the percentage of well-rooted plantlets was of 73 %, 24 % non-rooted and 3 % dead. Average root length was of 13 cm.

The plantlets acclimated *ex vitro* in hydroculture and then cultured for 1 month in hydroponics fertilized with Ferticare at 2 g/l were well grown and developed, reaching stem lengths of 22 cm and root lengths of 14 cm and fresh biomass of over 6 g (Fig. 6).

We mention that there were great variations regarding these characteristics, due to the fact that density was too high per culture area and reciprocal inhibition appeared (Fig. 7). Survival percentage was only 55 %. It was found that the procedure of hydroponic culture with fertilizer could not be applied to the non-rooted and non-acclimated shoots transferred directly *ex vitro*, as about 75 % of these underwent necrosis in a period of 20 days and rooting was also stopped.

Fig. 6. Hydroponic culture, fertilized with Ferticare at 2 g/l

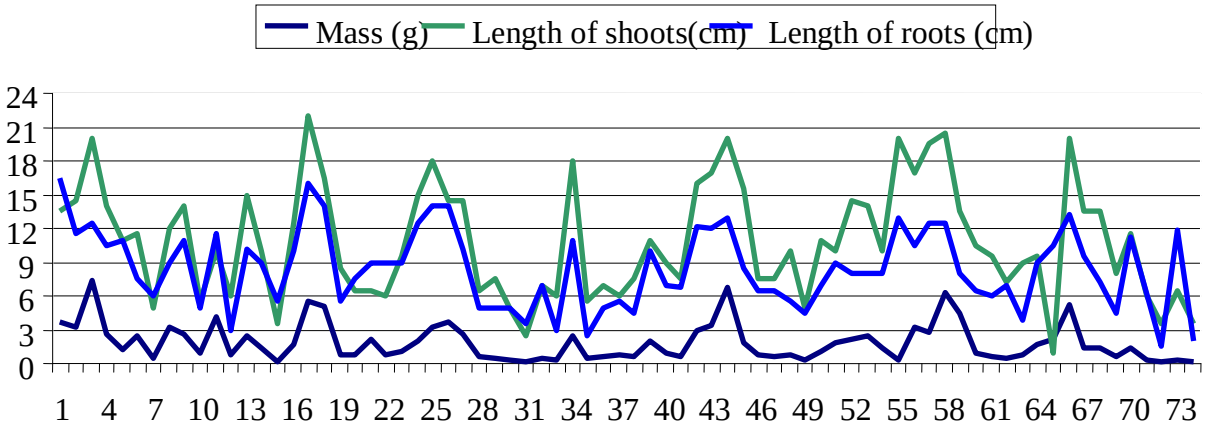


Fig. 7. Mass, stem length and root length in hydroponic culture fertilized with Ferticare

CONCLUSIONS

Blackberry cultivar ’Loch Ness’can be successfully propagated *in vitro*.

For the initiation and *in vitro* multiplication of this blackberry cultivar we recommend the culture medium with the following components: Murashige & Skoog 1962 (MS) salts, Myo inositol-100 mg/l, Vitamin B1- 1 mg/l, Vitamin B6 - 0.5 mg/l, Nicotinic Acid - 0.5 mg/l and, as growth regulator, 6-benzylaminopurine at the concentration of 0.5 mg/l.

Blackberry cultivar ’Loch Ness’does not require *in vitro* rooting, as it can be successfully done directly *ex vitro* in solid substrate as well as in hydroculture, in the same phase with acclimation.

Rooting and acclimation can be carried aut *ex vitro* using, as plant material, non-rooted shoots resulted from the multiplication medium. Rooting and acclimation in solid substrate can be done in plastic trays with lids using a mixture of peat+ perlite in 1:1 ratio. In this case, rooting and acclimation percentage can reach 100 %.

A method that is more economical in terms of materials, necessary space and, especially, workforce is float hydroculture. Through this method, the first roots appear after 10 days, and after 1 month the plants are rooted and acclimated and can be transplanted into potting mix. The percentage of rooted plantlets was of 74 %. This percentage can reach 100 % if well-developed shoots several cm in length are used.

Acknowledgements. This work was possible with the financial support of the Sectoral Operational Programme for Human Resources Development 2007-2013, co-financed by the European Social Fund, under the project number POSDRU/107/1.5/S/76841 with the title

„Modern Doctoral Studies: Internationalization and Interdisciplinarity”.

REFERENCES

1. Botez, M., Ghe. Badescu and A. Botar (1984). Cultura arbustilor fructiferi, Ed. Ceres, Bucuresti
2. Bobrowski, V. L., P. C. Mello-Farias and J. A. Peters (1996). Micropropagation of blackberries *(Rubus sp.)* cultivars. Rev. Bras. De Agrociencia, 2(1):17-20;
3. Erig, A.C., A. de Rossi and G. R. de Luces Fortes (2002). 6-Benzilaminopurina e acido indolilbutirico na multiplicacao in vitro da amoreira-preta *(Rubus idaeus L.), cv. Tupy*. Ciência Rural, Santa Maria, v.32, n.5, p.765-770, ISSN 0103-8478;
4. Fira, Al. and D. Clapa (2000). *Ex-Vitro* Acclimation of some Horticultural Species in Hydroculture. Bulletin UASVM, nr. 66 (1), Horticulture, pp 44-51
5. Fira, Al. D. Clapa and C. Plopa (2009). Micropropagarea soiului de mur Thornless evergreen. Proceedings RIFG Piteşti Vol. XXV;
6. Fira, Al., D. Clapa and C. Plopa (2010). New Aspects Regarding the Micropropagation of Blackberry Cultivar ‘Thornless evergreen’. Bulletin UASVM Horticulture, 67(1)/2010,
7. Gajdosova, A., M. G. Ostrolucka, G. Libiakova, E. Odruskova and D. Simala. (2006). Microclonal Propagation of Vaccinium sp. And Rubus sp. and detection of genetic variability of culture *in vitro*, Journal of Fruit and Ornamental Plant Research, 14 (1):103-119
8. Meng, R. (2004). Improving in Vitro Plant Regeneration from Leaf and Petiole Explants of 'Marion' Blackberry. HortScience 39 (2): 316-320;
9. Mihalache, S. (1996). Contribuţii la îmbunătăţirea tehnologiei de micropropagare “*in vitro*” a zmeurului şi murului fără ghimpi (teză de doctorat). Universitatea de Stiinţe Agricole şi Medicină Veterinară Cluj-Napoca;
10. Murashige, T. and F. Skoog, (1962). A revised medium for rapid growth and bioassays with tabacco tissue culture, Physiol. Plant 15: 473-497.
11. Najaf-Abadi, A. Jafari and Y. Hamidoghli (2009). Micropropagation of thornless trailing blackberry (Rubus sp.) by axillary bud explants. Australian Journal of Crop Science 3 (4): 191-194,
12. Ružić, D. and T. Lazić (2006). Micropropagation as Means of Rapid Multiplication of Newly Developed Blackberry and Black Currant Cultivars. Agric. Conspec. Sci. Vol. 71, No. 4;
13. Ruzic, D. and T. Lazic (2007). Organogenesis *In Vitro* from the Leaf of Blackberry cv . Čačanska Bestrna. Genetika, 39(1): 69 -78;
14. Villa, F., P. Moacir, L. A. Salles Pio and G. Sales Teodoro (2009). Cloreto de sodio e acido naftalenoacetico no enraizamento de microestacas de amoreira-preta cv. Brazos *in vitro* . Ciênc. agrotec., Lavras, v. 33, Edição Especial, p. 1819 -1824;
15. Villa, F., P. Moacir, L. A. Salles Pio and G. Sales Teodoro (2006). Multiplicacao *in vitro* de amoreira-preta cultivar Brazos. Ciênc. agrotec., Lavras, v. 30, n. 2, p. 266-270;
16. Zawadska, M. and T. Orlikowska (2006). Factors modifying regeneration *in vitro* of adventitious shoots in five red raspberry cultivars, Journal of Fruit and Ornamental Plant Research, 14: 5-5.