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Direct *ex Vitro* Rooting and Acclimation in Blackberry Cultivar 'Loch Ness'

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Abstract. Our paper presents the results of several direct *ex vitro* rooting and acclimation experiments in blackberry (*Rubus fruticosus*), thornless cultivar 'Loch Ness'. The plant material consisted of plants propagated on Murashige & Skoog (MS) medium with 0.5 mg/l benzyladenine (BAP). The shoots excised from the plantlets were rooted directly *ex vitro* in various substrates: floating perlite, plastic sponge inserted in floating cell trays, rockwool in plastic trays covered with transparent lids, as well as potting mixes available commercially: Florasol, Sol Vit G, Florimo. These experimental variants yielded good results regarding the rooting and acclimation percentages. Rooting in Jiffy pellets placed in floating cell trays as well as the use of rockwool in non-covered plastic trays yielded negative results. The experiments regarding *ex vitro* rooting and acclimation in aeroponics or by suspending the shoots in air saturated with vapour also yielded negative results.

Keywords: *Rubus*, micropropagation, *in vitro*, proliferation

INTRODUCTION

Ex vitro acclimation is the final phase of micropropagation, during which the micropropagated plantlets, are gradually accustomed to living conditions, which are similar to the natural environment (greenhouse, field). *In vitro* propagation of blackberry was be studied by several authors (Villa *et al*., 2006, 2009; Najaf-Abadi and Hamidoghli, 2009; Bobrowski *et al*., 1996; Erig *et al*., 2002; Gajdosova *et al*., 2006; Ružić *et al*., 2006; Lepse and Laugale, 2009). The direct *ex vitro* rooting of shoots in conventional potting mixes, in a combined phase of *ex vitro* rooting and *ex vitro* acclimation, without the use of an *in vitro* rooting stage was mentioned in an early work (Botar, 1985, pp. 56) and practical results regarding this procedure were obtained with several Rubus cultivars also (Mihalache S., 1996). At the Fruit research Station Cluj an effective method was set up for the direct *ex vitro* rooting and acclimation of blackberry shoots in float hydroculture (Fira *et al.*, 2011) based on the technology of float hydroponics (Ross and Teffeau, 1995, Tyson *et al.*, 1999; Reed, 2009) and carried out *in vitro* propagation and *ex vitro* acclimation in blackberry cultivar 'Loch Ness' (Fira *et al.*, 2011). The method of *ex vitro* acclimation in float hydroculture was modified and adapted by Frank Tromble, who combined the method of acclimation in solid substrate with the method of float hydroculture (Tromble, 2011) by adding a layer of perlite on the surface of the water in the acclimation vessel. The plants were transplanted *ex vitro* into the layer of perlite, thus acclimation was performed in a floating perlite bed.

The aim of the research presented in this paper was to test various alternative methods for the *ex vitro* rooting and acclimation in blackberry cultivar 'Loch Ness', such as *ex vitro* rooting and acclimation in floating perlite beds as well as the use of some commercially

available potting mixes.

MATERIALS AND METHODS

The 'Loch Ness' blackberry plantlets were propagated *in vitro* according to the standard blackberry *in vitro* culture protocol used at the Fruit Research Station Cluj. The explants consisted of microcuttings 2 cm in length. The culture vessels were 720 ml glass jars with vented metal screw caps. The vessels contained 100 ml of culture medium/vessel and 5 microcuttings/vesse. The microcuttings were inserted about 2/3-3/4 of their length into the medium, in orthotropic position, in order to establish good direct contact between the buds and the medium. The multiplication medium was MS (Murashige & Skoog, 1962) modified according to Tab. 1. The culture cycles were of 2 months. For experiments 1, 2, 4 and 5, media gelled with agar were used. For experiment 3, media gelled with starch were used, as the use of agar for blackberry *in vitro* culture was abandoned due to the effectiveness of starch.

For *ex vitro* transfer, the plantlets were taken out of the jars and their base was washed with warm water in order to melt away the medium and the base of the plants was cut off, resulting bunches of separate shoots.

Tab. 1

The nutritive medium used in the multiplication phase for blackberry cultivar 'Loch Ness'

Component	Concentration
MS* salts	Full concentration
Myo-inositol	100 mg/l
Vitamin B1	1 mg/l
Vitamin B6	0.5 mg/l
Nicotinic acid	0.5 mg/l
BAP	0.5 mg/l
Sugar	30 g/l
Plant Agar or wheat starch	6 g/l, respectively 50 g/l

*Murashige & Skoog

For the direct *ex vitro* rooting of blackberry shoots, several experiments were carried out:

1. Testing the method of *ex vitro* rooting and acclimation in floating perlite bed. The blackberry shoots were inserted into the perlite floating on the surface either singly (one by one) or in bunches of shoots (Fig. 1). The small tub was maintained in the greenhouse for one month at temperatures that varied from about 15°C to 20-40 °C during daytime, without being covered for maintaining humidity. The volume of the tub was 10 liters. 5 liters of water and 5 liters of perlite were used.
2. The experiment at point 1 was replicated in similar conditions but with the tub being incubated in the growth room at the temperature of 23±3°C, light intensity of about 2400 Lux and 49-52% air humidity. The volume of the tub was 5 liters. 3 liters of water and 3 liters of perlite were used.
3. Classical solid substrates and commercially available potting mixes were tested for the direct *ex vitro* rooting and acclimation of blackberry shoots resulted from the multiplication phase. Jiffy7 pellets were used, as well as Sol Vit G, Florasol and Florimo potting mixes. As culture vessels, transparent polypropylene Multi Purpose Trays were used, which were

covered with the same type of vessels in order to maintain high air humidity. In case of using Jiffy pellets, 40 pellets/tray were used. For the potting mixes, a cell tray with 48 cells was used in each Multi Purpose Tray. The experiment was replicated twice in the growth room. One-way ANOVA was used for statistical analysis.

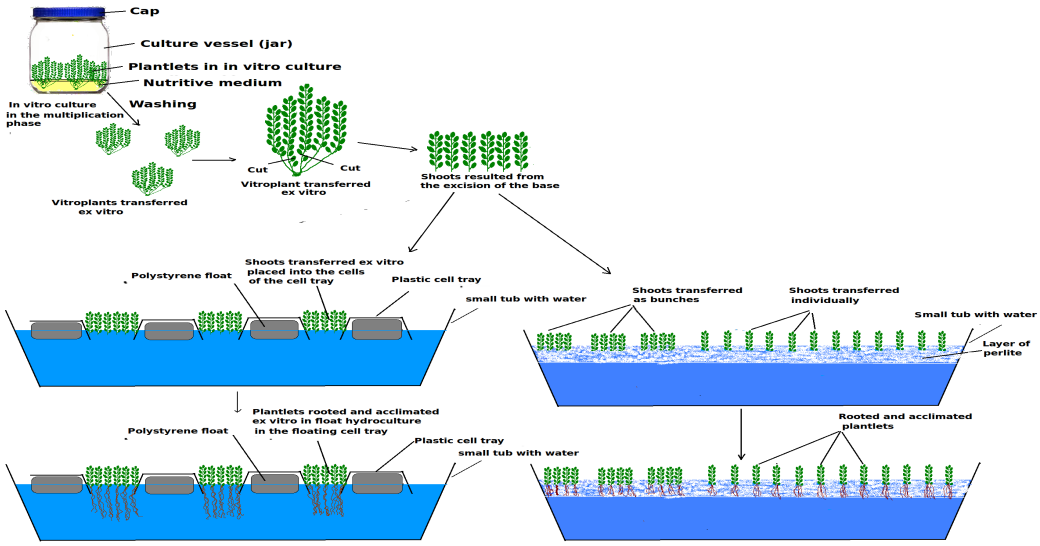

Fig. 1. Alternative methods for the *ex vitro* rooting and acclimation of blackberry (*Rubus fruticosus*) in float hydroculture

4. Non-conventional substrates were tested for *ex vitro* rooting and acclimation:

a) rockwool, sponge and wooden chips. Shoots 3-5 cm in length were planted into a rockwool block, perpendicular to the layers of fibers. On the surface of the rockwool block, more shoots were placed in horizontal position. The block was moistened with water and cultured in a plastic tray covered with a transparent plastic lid. The tray was incubated in the growth room at 23±3°C. This variant was repeated without using a lid. Rockwool was also tested in a cell tray containing 48 cells, into which rockwool cubes were introduced, with the fibers oriented vertically. Shoots of 3-5 cm in length were inserted among the layers of fibers. The cultures were incubated in a plastic tray covered with a transparent plastic lid, in the growth room.

b) cubes of autoclavable plastic sponge ('Genius' brand) were made. A vertical incision was made into each cube to the middle of the cubes and 3-5 cm long shoots were inserted into the incisions (one shoot/cube). The cubes were introduced in cell trays equipped with polystyrene floats and set to float in the water in a small tub in the greenhouse. No lid was used for maintaining air humidity. The tub was maintained in the greenhouse in the conditions presented at Point 1.

c) wooden chips were tested in the conditions described for the potting mixes, in plastic cell trays in Multi Purpose Trays, but in greenhouse conditions.

5. Several experiments were carried out for *ex vitro* rooting and acclimation of blackberry shoots in aeroponics as well as by being suspended in air saturated with humidity, without inserting the bases in water or rooting mix.

RESULTS AND DISCUSSIONS

The standard protocol for blackberry *in vitro* propagation used at the Fruit Research Station Cluj proved to be very effective for the 'Loch Ness cultivar, also. Average multipication rate, calculated by gathering data from 7 vessels was 61.31, whereas the average number of shoots resulted/plantlet was 46.89. Most of the shoots could yield a single microcutting 2-3 cm in length and some of the shoots could be cut into 2 microcuttings. The use of starch as gelling agent resulted in superior multiplication rates, of around 80 times /two month culture cycle. The shoots obtained in these cultures were used in these experimental variants and they reacted in the following way:

1. Combining the method of *ex vitro* rooting and acclimation in liquid substrate with the method of *ex vitro* rooting and acclimation in perlite, by using floating perlite (Fig. 1) proved to be very efficient but more difficult in terms of labour as compared to the method of rooting and acclimation in floating cell trays. Both in greenhouse conditions and in the growth room in the laboratory, in the individual shoots higher rooting rates were obtained than in the shoots planted in bunches.

In the shoots cultured in greenhouse conditions (Fig. 2) as individual shoots, from the total of 81 shoots 74 well-rooted plantlets that were suitable for planting into pots were obtained (91.35%), 3 poorly rooted plantlets–3.7% (roots 3-5 cm in length), 2 non-rooted (2.7%) and 2 dead (2.7%). In the well-rooted plantlets average root length was 8.14 cm, considering the lengths of bunches of roots in every plant. Great variation was observed regarding root lengths (Fig. 3).

In the plantlets rooted as bunches of shoots, from the total of 83 shoots 69 rooted well (83.13%), 4 did not root (4.81%), 5 rooted poorly (6.02%) and 5 died (6.02%). Average root length in the well-rooted plantlets was 7.07 cm.

Fig. 2. Blackberry cultivar ‘Loch Ness’ rooted and acclimated *ex vitro* in floating perlite bed:
a-plants in the mini-basin in the greenhouse; b–plants resulted from shoots planted individually;
c–plants rooted as bunches of shoots

2. In the experiment regarding rooting and acclimation in floating perlite in the growth room the rooting and acclimation rates were also high. In the shoots planted individually, from 39 shoots 37 got rooted and acclimated (94.87%), one (2.56%) died and one (2.56%) did not root. There was no correlation between shoot length, root length and the number of main roots, as some of the plantlets with long shoots had short roots and some with short shoots had long roots. In the variant with the shoots planted as bunches, from the total of 171 shoots from 20 bunches 85% were well rooted, 6% were alive but not rooted and 8% were dead.

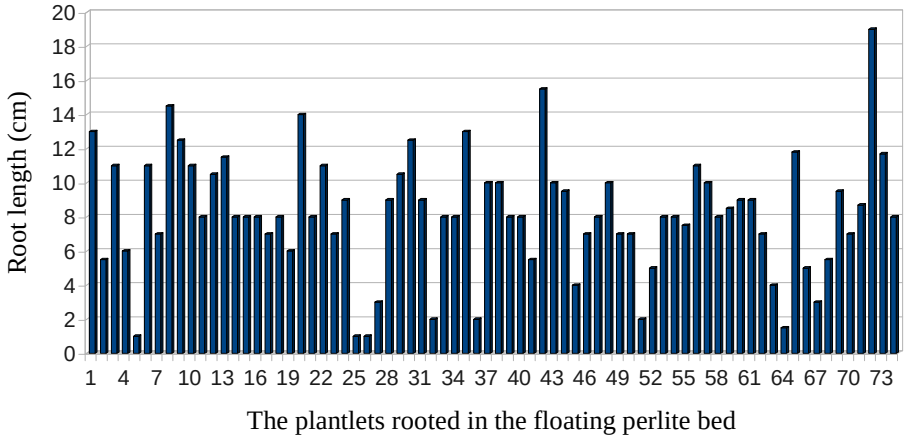

Fig. 3. The variation of root lengths in the shoots planted individually in floating perlite in greenhouse conditions

3. From the 3-5 cm long shoots planted into Jiffy7 pellets 95% were rooted and acclimated (76 out of 80). Among the potting mixes tested for blackberry direct *ex vitro* rooting and acclimation, Sol Vit G gave the highest rooting and survival rates, followed by Florasol (Fig. 4). Figure 5 presents the average numbers of main roots and average root lengths in the variants tested. In the plants rooted in Jiffy7 pellets root numbers and root lengths could not be established, as the roots could not be separated from the coatings of the Jiffy7 pellets. The acclimation rates, compared to the control variant (Sol Vit G) were statistically significant only in the Florimo variant ($p=0.007$). The differences regarding the number of main roots as well as root lengths, in the experimental variants Florasol and Florimo compared to the control (Sol Vit G) were not significant statistically.

4. When using non-conventional substrates it was observed that:

a) in the experiment of rooting in rockwool blocks, in the variant covered with plastic lid better results were obtained than in the variant without a lid. In the non-covered variant, from the total of 56 shoots 1-1.5 cm in length 22 survived (39.28% survival rate). From the total of 96 shoots 3-5 cm in length 50 survived and rooted (52.08% survival rate), 20 were alive but not rooted and 26 were dried out. The shoots, which were placed horizontally onto the rockwool block, got de-hydrated in about 90 minutes.

In the experimental variant where the culture was covered with a plastic lid, from the total number of 38 shoots placed horizontally onto the rockwool block 7 were rooted (18.42% rooting rate). The rest were alive, non-rooted. Generally, the shoots placed horizontally had most of their leaves dried out and the stems were green. For the shoots inserted in the rockwool block, from the total of 28 shoots 27 were rooted (96.42%) survival rate). The roots were very vigorous and they passed through the block and were visible on the lower side. In case of rooting in cell trays containing rockwool plugs, from the total of 48 shoots 42 were rooted and survived, (87.5% acclimation rate), 3 did not root but survived and 3 died. The 6 shoots lost in this way were 3 cm in length. Figure 6 presents images of plants rooted in rockwool.

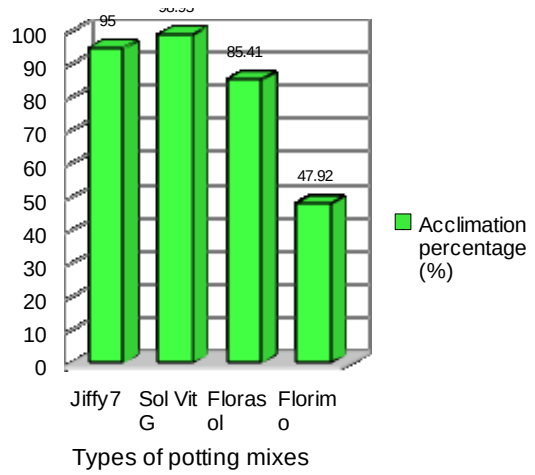

Fig. 4. Survival rates in the various solid substrates

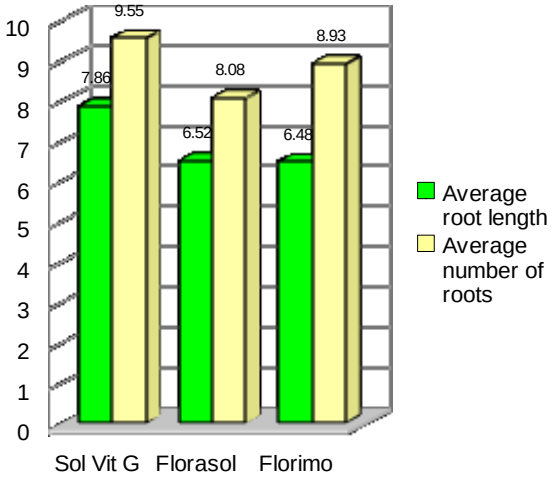

Fig. 5. Root lengths and root numbers obtained in the various solid substrates

Fig. 6. 'Loch Ness' blackberry shoots rooted in rockwool blocks and cubes

b) 100 % of the 3-5 cm long shoots planted into the sponge cubes survived and normal plants resulted. The roots got anchored into the cubes and grew sideways as well as downwards into the mass of water in the rooting tub (Fig. 7).

c) 50 % of the shoots planted in wooden chips in the cell trays rooted and survived. Average root length was 4.9 cm and the average number of main roots/plant was 4.95. There was lesser growth and development than in the other substrates.

5. The aeroponics experiments as well as the ones consisting of culturing shoots suspended in humid air yielded negative results. The first series of aeroponics yielded more than 90 % dead shoots, whereas the second series 75% dead shoots. The shoots suspended in humid air exhibited a slight tendency to root but eventually 100 % of them died.

Fig. 7. *Ex vitro* rooting and acclimation in sponge cubes in floating cell trays in blackberry cultivar 'Loch Ness'

CONCLUSION

Among the methods used for the *ex vitro* rooting and acclimation of the blackberry, acclimation in float hydroculture by the use of floating cell trays proved to be the easiest and fastest, followed by the method of rooting in float hydroculture in floating perlite in the case of planting bunches of shoots into the layer of floating perlite. The use of Jiffy7 pellets, although it was very efficient, was surprisingly slow and difficult in terms of manual work, the shoots being difficult to separate and plant separately, one by one into the Jiffy7 pellets. Planting into peat + perlite mixture or into the commercial potting mixes was easy compared to planting into Jiffy pellets, nevertheless it was slower than planting into float hydroculture and it needed, comparatively, a higher amount of labour. From the commercial potting mixes that we tested we recommend Sol Vit G and, alternatively, Florasol. We do not recommend Florimo, as it gave inferior results regarding *ex vitro* rooting and acclimation.

The most ineffective and difficult variants were rockwool and wooden chips.

In the Jiffy7 pellets as well as in the commercial potting mixes the plants grew spectacularly in height, acquiring rich foliage. In float hydroculture the plants also grew in height, up to twice the initial size. In rockwool as well as sponge there was negligeable growth. In the floating perlite beds the shoots rooted very well but the aerial parts grew very little, in the greenhouse as well as in the growth room.

We recommend, especially, the *ex vitro* acclimation method in float hydroculture by the use of plastic cell trays equipped with polystyrene floats, as this method is simple and effective and it can be applied in the greenhouse as well as in the growth chamber or in a shelter improvised in open air (Fira *et al.*, 2011). Another possibility recommended is the use of floating perlite beds, as it is simple and easy to apply, ensuring superior efficiency according to our results. Both the method of *ex vitro* rooting and acclimation in floating cell trays as well as acclimation in floating perlite beds eliminates the need for sophisticated installations for ensuring air humidity.

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