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Short communication

TESTING OF DIFFERENT IRON SOURCES AND CONCENTRATIONS ON SHOOT MULTIPLICATION OF BLACKBERRY (*Rubus fruticosus* L.)

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The aim of this work was to evaluate shoot multiplication of two blackberry (*Rubus fruticosus* L.) cultivars 'Black Satin' and 'Loch Ness' on two culture media: Murashige & Skoog (MS) and its modification Murashige & Skoog Van der Salm medium (MS VDS) which differ only in iron source (FeNaEDTA, FeEDDHA, respectively). Statistical analyses showed no significant difference in shoot multiplication between different iron sources for both tested cultivars. For 'Black Satin' it was shown that double concentration of chelates FeNaEDTA and FeEDDHA in culture media negatively affected on shoot growth and multiplication. These results can be very useful for further optimization of micropropagation process for different *Rubus* cultivars.

Key words: Rubus, cultivar, shoot proliferation, iron, chelate

INTRODUCTION

Rubus spp. is a large genus of flowering plants of the family Rosaceae. It occurs predominantly in Europe, North America and west Asia and includes hundreds of species and hybrids. To the most widely grown fruit plants in this species belong to raspberries (*R. idaeus*), blackberries (*R. fruticosus*) and black raspberries (*R. occidentalis*) (JENNINGS *et al*., 1991). Berries are valuable source of vitamin C, dietary fiber, minerals, and contain high levels of

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natural polyphenols acting as potent antioxidants (BATTINO *et al*., 2009). Both raspberries and blackberries can be commercially propagated by vegetative propagation using stem cuttings, tip layers or bush division. However, these methods are time-consuming and can support spread of pathogens (NAJAF-ABADI and HAMIDOGHLI, 2009). Biotechnological methods like micropropagation open up new solutions for breeding and rapid propagation of valuable, virus-free and genetically stable cultivars under controlled *in vitro* conditions (ILIEV *et al.*, 2010). Numerous protocols for micropropagation of blackberry and raspberry cultivars were described (RUŽIĆ and LAZIĆ, 2006; NAJAF-ABADI and HAMIDOGHLI, 2009; ZAWADZKA and ORLIKOWSKA, 2006; POOTHONG and REED, 2014). Apical or axillary buds were mostly used as primary explants, followed by *in vitro* shoot initiation and proliferation using 6-benzylaminopurine (BAP) in concentration 0.5–3 mg l^{-1} usually with auxin indole-3-butyric acid (IBA) in concentration range 0.01–0.5 mg l^{-1}. It was often recorded that individual cultivar's responses differ under *in vitro* conditions, thus there arises a need of further optimalization of micropropagation protocols for different cultivars of *Rubus* spp.

The aim of this study was to evaluate shoot multiplication of two *Rubus fruticosus* cultivars by testing two culture media which differ only in iron source (FeNaEDTA and FeEDDHA). The effect of these iron sources and their concentrations on shoot proliferation was determined to optimize conditions for more effective propagation under *in vitro* conditions.

MATERIALS AND METHODS

Biological material for experiments were *in vitro* shoots of two *Rubus fruticosus* cultivars 'Black Satin' and 'Loch Ness' grown on Murashige & Skoog (MS) culture medium (MURASHIGE and SKOOG, 1962) supplemented with 30 g l^{-1} sucrose, 8 g l^{-1} plant agar and addition of 1 mg l^{-1} BAP, 0.5 mg l^{-1} IBA and 0.1 mg l^{-1} gibberellic acid (GA_3). Two types of media were tested for shoot multiplication: MS medium (MURASHIGE and SKOOG, 1962) and its modification MS VDS medium (VAN DER SALM *et al*., 1994). The composition of both media is exactly the same with exception of iron source. MS medium contains FeNaEDTA (ferric sodium ethylenediaminetetraacetate) as iron source in concentration of 36.7 mg l^{-1}. MS VDS medium contains FeEDDHA (ethylenediamine di-2-hydroxyphenyl acetate ferric) as iron source in concentration of 96 mg l^{-1}. We tested the effect of MS and VDS media on shoot multiplication on cultivar 'Black Satin' and 'Loch Ness' and double concentrations of iron chelates in MS and MS VDS culture media on shoot multiplication of cultivar 'Black Satin'. For this reason we designed four types of experimental media that were used in this study: a) **MS** (MS + 1 mg l^{-1} BAP + 0.5 mg l^{-1} IBA + 0.1 mg l^{-1} GA_3 with basic concentration of FeNaEDTA 36.7 mg l^{-1}); b) **MS VDS** (MS VDS + 1 mg l^{-1} BAP + 0.5 mg l^{-1} IBA + 0.1 mg l^{-1} GA_3 with basic concentration of FeEDDHA 96 mg l^{-1}); c) **MS 2x FeNaEDTA** (MS + 1 mg l^{-1} BAP + 0.5 mg l^{-1} IBA + 0.1 mg l^{-1} GA_3 with double concentration of FeNaEDTA 73.4 mg l^{-1}) and d) **MS VDS 2x FeEDDHA** (MS VDS + 1 mg l^{-1} BAP + 0.5 mg l^{-1} IBA + 0.1 mg l^{-1} GA_3 with double concentration of FeEDDHA 192 mg l^{-1}).

For cultivar 'Black Satin', all four types of media were tested and for cultivar 'Loch Ness', the effect of first two culture media were evaluated. For each culture media 42 explants were analyzed, the length of one subculture was 4 weeks and the experiment was repeated 3 times. All cultures were maintained in Combiness vessels in growth chamber at 22 ± 2°C using a 16/8 h photoperiod. The results were statistically evaluated by analysis of variance (ANOVA).

Data were subjected to Duncan test at P value < 0.05 and evaluated using software STATISTICA version 8.0.

RESULTS AND DISCUSSION

Results have shown that iron sources and especially their concentrations are important factors in shoot multiplication of different blackberry cultivars. For cultivar 'Black Satin', 6.50 shoots per explant were multiplied on MS medium with FeNaEDTA and 6.59 shoots per initial explant on MS VDS medium supplemented with FeEDDHA. For cultivar 'Loch Ness', 3.13 shoots per explant was achieved on MS medium with FeNaEDTA and 2.76 shoots per explant on MS VDS medium with FeEDDHA. In both cultivars, observed differences between iron sources in basic concentration were not statistically significant, so we can conclude that both tested iron sources in basic concentration can be used for successful shoot multiplication of cultivars 'Black Satin' and 'Loch Ness' (Table 1 and 2).

Table 1. The influence of different iron sources and concentrations on axillary shoot multiplication of cultivar 'Black Satin' (R. fruticosus)

Medium	n	Average number of shoots/explant (±SE)
MS	126	$6.50^{a} \pm 0.27$
MS VDS	126	$6.59^{a} \pm 0.31$
MS 2x FeNaEDTA	126	$3.84^{b} \pm 0.24$
MS VDS 2x FeEDDHA	126	$2.74^{c} \pm 0.15$

Mean values ± SE within right column followed by the same letter are not statistically significant according Duncan test ($P < 0.05$). n = number of explants

Table 2. The influence of different iron sources and concentrations on axillary shoot multiplication of cultivar 'Loch Ness' (R. fruticosus)

Medium	n	Average number of shoots/explant (±SE)
MS	126	$3.13^{a} \pm 0.18$
MS VDS	126	$2.76^{a} \pm 0.16$

Mean values ±SE within right column followed by the same letter are not statistically significant according Duncan test ($P < 0.05$). n = number of explants

There already exist several publications dealing with influence of iron source on *in vitro* responses in different species including *Rubus* sp. In consistency with our results, SOKOLOV *et al*. (2015) found that NaFeEDTA in basic concentration (36.7 mg l^{-1}) can be recommended for shoot multiplication of *Magnolia* (Magnoliaceae) and *Prunus* (Rosaceae), resulting in 6.5 and 6.3 shoots/explant, respectively. The beneficial influence of FeEDDHA on shoot length and chlorosis elimination was confirmed by VAN DER SALM *et al*. (1994) in rose. Increasing in adventitious bud regeneration using FeEDDHA was noticed by TSAO and REED (2002) at the five blackberry cultivars. Also ZAWADZKA and ORLIKOWSKA (2006) stated that 50 mg l^{-1} FeEDDHA

added in culture medium reduced chlorosis, increased content of chlorophyll and was especially effective in adventitious regeneration.

In addition, for cultivar 'Black Satin' we evaluated the effect of double concentration of iron sources in culture media on further shoot multiplication. We observed strong inhibition of shoot multiplication on media supplemented with doubled concentration of both types of iron source in culture media (Table 1, MS 2x FeNaEDTA and MS VDS 2x FeEDDHA). Shoot multiplication was 3.48 shoots per explant on MS medium with double concentration of FeNaEDTA and 2.74 on MS VDS medium with double concentration of FeEDDHA. Shoot cultures of cultivar 'Black Satin' are more sensitive to increased concentration of FeEDDHA than FeNaEDTA since significantly higher inhibition of shoot multiplication was observed when cultures were grown on MS VDS 2x FeEDDHA medium (Table 1).

Analogous to our experiment, POOTHONG and REED (2014) examined the effect of 0.5 – 4.0 - fold increased concentration of FeEDTA on morphological parameters of the five raspberry cultivars. Except for cultivar 'Willamette', increasing iron concentration above MS level significantly decreased the number of shoots in all other cultivars similarly like double concentration of both iron sources in our study. Similar results were found also by LICEA-MORENO *et al*. (2015) who focused on walnut (*Juglans major*) micropropagation. Increasing FeEDTA levels up to 1.5 times (10.21 mg l^{-1} Fe^{2+}) did not improve microshoot performance. Significant reductions were recorded for average shoot length and higher levels of FeEDTA completely stopped growth. TREJGELL *et al*. (2012) tested the effect of iron chelates FeEDTA and FeEDDHA and their concentrations on shoot multiplication in *Carlina onopordifolia* (Asteraceae*).* Both chelate and iron concentration had no effect on axillary shoot multiplication but shoot growth was significantly inhibited by a double and triple FeEDDHA concentration in MS culture medium. Contrary, EVENOR *et al*. (2001) found out that addition of 36.7 mg l^{-1} FeNaEDTA to the MS culture medium significantly enhanced the proliferation rate of *in vitro* shoots of *Alchemilla mollis.*

Our study clearly showed that individual species and cultivars can respond differently to the changes of mineral nutrition. Additional experiments will be required to define optimum conditions for *Rubus* spp. micropropagation from mineral nutrition point of view.

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UTICAJ RAZLIČITIH IZVORA I KONCENTRACIJA GVOŽĐA NA MULTIPLIKACIJU IZDANAKA KUPINE (*Rubus fruticosus* L.)

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Izvod

Cilj ovoga rada bio je da se ispita kapacitet za multiplikaciju izdanaka dve sorte kupine (*Rubus fruticosus* L.), Black Satin i Loch Ness, gajenih na dve hranljive podloge – Murashige & Skoog (MS) i modifikovana podloga Murashige & Skoog Van der Salm (MS VDS) – koje se razlikuju u izvoru gvožđa, odnosno helatnom obliku gvožđa (FeNaEDTA i FeEDDHA, po redosledu). Statističkom analizom, kod obe sorte, nije utvrđen značajan uticaj izvora gvožđa na kapacitet za multiplikaciju. Kod sorte Black Satin dvostruka koncentracija helatnih oblika gvožđa (FeNaEDTA i FeEDDHA) u hranljivoj podlozi je imala negativan efekat kako na rast, tako i na indeks multiplikacije izdanaka. Dobijeni rezultati mogu imati značaja za dalju optimizaciju protokola za multiplikaciju različitih sorti u okviru roda *Rubus*.

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