



The use of Isubgol and Sequestrene 138 for the *in vitro* propagation of the highbush blueberry (*Vaccinium corymbosum* L.)

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Abstract

At Fruit Research Station Cluj different cultivars of blueberry (*Vaccinium corymbosum* L.) were cultured *in vitro* for production purposes, using a classical blueberry micropropagation medium. Powder agar was used as gelling agent and FeNaEDTA as iron source. For cultivar Blue Crop, the classical micropropagation protocol proved to be totally ineffective, the plants becoming chlorotic and their bases becoming necrotic in 3-5 weeks after transfer to fresh medium. The multiplication rate was also very low. By using Isubgol as a gelling agent and Sequestrene 138 as iron source, the vigour of the plants increased spectacularly, as well as the length and number of shoots. Multiplication rate increased significantly and the phenomenon of chlorosis was totally eliminated, the shoots becoming dark green. Having in view these results, the use of Isubgol and Sequestrene 138 is recommended for the *in vitro* propagation of the blueberry.

Key words: Isubgol, Isabgol, Psyllium, Sequestrene, blueberry.

Introduction

The highbush blueberry (*Vaccinium corymbosum* L.) is a fruit shrub cultured on small surfaces in Romania. In recent years more and more farmers show their interest in this species. For this reason at SCDP Cluj the micropropagation of this species was begun in order to produce planting material free from pathogens. Usually, for blueberry tissue culture the following basal media were used: Anderson medium, Woody Plant Medium, Zimmermann & Broome medium, Economou & Read medium, etc. ^{1, 3, 7}. As growth regulators, 2-isopentenyladenine, zeatin and thidiazuron were used, zeatin proving to be very effective in inducing massive shoot proliferation ^{1-6, 10}.

In the media used for blueberry tissue culture the amount of chelated iron (FeNaEDTA) is double than in Murashige-Skoog medium or in the usual Woody Plant Medium, in order to reduce iron chlorosis and increase the survival rate of the explants. Sequestrene 138 (FeNaEDDHA) has also been used successfully, the iron in this compound being more readily available to the plants.

Isubgol (psyllium husk) is a viscous husk obtained from around the seeds of *Plantago ovata*. It is used in the Middle East in nature medicine, against digestive disorders. It has been used successfully in the micropropagation of the orchid *Dendrobium chrysotoxum*, the growth rate being three times greater than on agar-gelled media.

Among the varieties propagated at SCDP Cluj, Blue Crop grew very badly on the classical medium used, forming thin, chlorotic shoots, which develop necrosis at their bases 3-4 weeks after inoculation. These aspects determined the use of Isubgol as gelling agent and Sequestrene 138 as iron source for the micropropagation

of the blueberry variety Blue Crop. The use of Isubgol on a large scale was encouraged by very good preliminary results on Isubgol-gelled media as compared to media gelled with agar, especially for variety Blue Crop. Thus the use of Isubgol made possible the rescue of a whole batch of tissue-cultured blueberry plants belonging to variety Blue Crop.

Material and Methods

The work was done on species *Vaccinium corymbosum* L., cultivar Blue Crop. The plant material used consisted of shoot fragments 1.5-2 cm in length, containing 3-6 nodes, originating from *in vitro* cultures on agar-gelled media. Initially, the nutritive medium used had the following composition: Woody Plant Medium salts (after Lloyd and McCown including iron) in the form of prepackaged powder, stock-solutions of vitamins B1, B6 and nicotinic acid and stock-solution of 2-isopentenyladenine, the carbon source being commercial castor sugar. Powder agar was used for gelling the media (Caisson Laboratories, Inc., catalog A038, lot 6308). The pH of the media was adjusted to 5 (Table 1).

All the components were added to the medium before media autoclaving. The media were autoclaved at 121°C for 25 minutes. On this medium the plants grow very weakly, resulting short, thin, chlorotic shoots with necrosis at their bases. This aspect determined the transfer of this variety on two variants of media (Table 2).

Culture incubation was done in the growth chamber in artificial light provided by fluorescent tubes. Light intensity was of ca. 4500 lux and the temperature 24-26°C. Magenta GA₇ polycarbonate culture vessels were used with polypropylene caps. Fifty ml of

medium was poured into each vessel and 16 microcuttings were planted into each vessel. The vessels were sealed with Folpack plastic wrap.

Table 1. Modified Woody Plant medium.

Component	Concentration
WPM salts (including iron)	2.3 g/l
FeNaEDTA (as supplement)	36.7 mg/l
Myo-inositol	100 mg/l
Vitamin B ₁	2 mg/l
Vitamin B ₆	1 mg/l
Nicotinic acid	1 mg/l
2-Isopentenyladenine	5 mg/l
Sugar	30 g/l
Agar	5 g/l
pH=5	

Table 2. The experimental variants.

Variant	V ₀	V ₁	V ₂
Basal medium + growth regulators	WPM*	WPM*	WPM*
Iron supplement	FeNaEDTA 36.7 mg/l	FeNaEDTA 36.7 mg/l	Sequestrene 138 100 mg/l
Gelling agent	Agar 5 g/l	Isubgol 15 g/l	Isubgol 15 g/l

*According to Table 1

Results and Discussion

On the culture medium presented in Table 1, used for the *in vitro* culture of numerous varieties, cultivar Blue Crop grew very badly, forming thin, chlorotic shoots, which develop necrosis at their bases 3-4 weeks after inoculation.

Multiplication rate was also very low. Because of the apparent phenomena of iron deficit found in the plants cultured *in vitro*, the media were supplemented with FeNaEDTA (36.7 mg/l) and Sequestrene 138 (FeNaEDDHA) (100 mg/l) (variants V₁ and V₂ in Table 2). Agar was also replaced with another gelling agent, Isubgol (15 g/l).

Isubgol is a mucilage extracted from around the seeds of *Plantago ovata*. It is also named Isabgol or Psyllium. It is produced in the Middle East, being used in nature medicine. It was successfully used as gelling agent for Chrysanthemum and orchids⁸.

In some research papers the use of some media with high iron content was presented^{9,11} as well as their beneficial effect on blueberry *in vitro* culture. Iron is essential for chlorophyll synthesis, being a vital component for ensuring plant health. Because iron has a low mobility in the plant, the newly formed plant organs often present signs of iron deficiency. In incipient phases, the leaves present chlorosis among the veins, in these zones the leaves being light green to yellow.

Sequestrene 138 (FeNaEDDHA) is a chelatic iron source easily accessible to plants, usable as iron source in tissue culture and in the field as well. For the plants in the field it can be applied on the leaves or in the soil.

As a measure of emergency, the plants were transferred to the two variants of media, V₁ and V₂, which rapidly led to a good evolution of the plants. The morphological characteristics of the plants in variants V₁ and V₂ were very different from those in the plants cultured on agar-gelled medium: long, robust shoots, with big leaves; the absence of necrosis at the base of the plants;

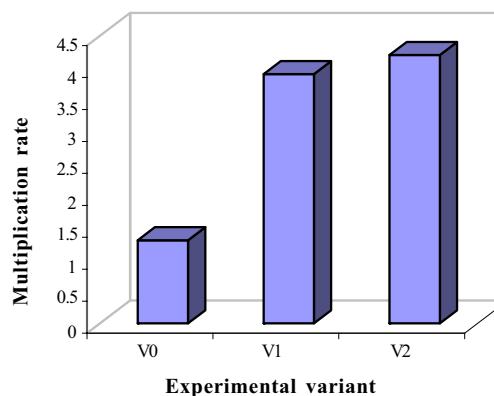


Figure 1. Multiplication rate on the 3 variants of media in cultivar Blue Crop.

abundant sprouting of shoots in some inoculi; the plants in variant V₂ were dark-green as compared to those in variant V₁, which were greenish in color.

From the cultures corresponding to the 3 variants, after 12 weeks of culture, 15 vessels were randomly taken from each variant and the plants were multiplied in order to establish multiplication rate. The multiplication rate of the plants in variant V₀ (agar-gelled medium) was of 1.3, and it was 3.9 for variant V₁ and 4.2 for V₂ (Fig. 1).

The results demonstrate the fact that for cultivar Blue Crop the product Isubgol is more adequate as gelling agent than the agar initially used, greatly increasing multiplication rate, shoot length and thickness and implicitly the quality of the plant material resulting from *in vitro* culture. The compound Sequestrene 138 is also beneficial in blueberry tissue culture, the plants being greener, with a healthy appearance.

We recommend the concomitant use of Isubgol and Sequestrene 138 for increasing multiplication rate and vigour of the plants. Although the propagation rates on media gelled with Isubgol are not very spectacular in the case of variety Blue Crop, the method has great practical importance, yielding robust plants. Also, Isubgol is far cheaper than agar.

Conclusions

Isubgol can be successfully used as gelling agent for blueberry *in vitro* culture, especially for cultivar Blue Crop, as it greatly increases plant vigour. The compound Sequestrene 138 yields better results than FeNaEDTA in blueberry *in vitro* culture, conferring dark green colour to the plants. The concomitant use of the two products increased multiplication rate and plant vigour in cultivar Blue Crop.

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