

REPORT FROM SHORT-TERM SCIENTIFIC MISSION COST 863 ACTION

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TOPIC OF STSM: Establishment of reliable protocols for micropropagation *in vitro* of various blueberry cultivars (*Vaccinium corymbosum* L.)

PURPOSE OF VISIT:

Fully equipped laboratory for micropropagation of the Fruit Research Institute in Čačak (former Fruit and Grape Research Centre) was established in 1980. It also includes highly trained staff in the respective field.

However, many years ago, in early stages of introduction of micropropagation, no success in micropropagation of blueberry was made. In addition, recently, the interest in growing of this fruit variety, particularly highbush blueberry, has been greatly increased as the soil of our region favours growing of this culture (pH 4.2 – 4.8).

I am well acquainted with achievements in propagation of blueberry of a group of scientists of the Institute of Plant Genetics and Biotechnology of the Slovak Academy of Sciences (Dr Alena Gajdosova, Head of the team). Therefore, I have decided to ask for help through the programme provided by the STSM of COST 863 Action.

DESCRIPTION OF THE WORK CARRIED OUT DURING THE VISIT:

The principle part of this STSM was to improve my knowledge of some critical factors in the method of blueberry micropropagation *in vitro*. This primarily referred to the following steps:

1. Terms of explants taking;
2. Establishment of the aseptic culture (isolation of explants; surface sterilization; type and size of explants);
3. Protocols for the inductive media, media for multiplication and rooting (composition and pH value of media).

1. We collected explants, i.e. twigs of highbush blueberry (*Vaccinium corymbosum* L.) from the trial planting of the Research Station Krivá (Orave) which includes a rich collection of 30 blueberry cultivars grown at an area of ½ ha. This Station is situated at an altitude of 800-900 m, with average annual air temperature of +6°C, and 3.8 pH value of soil.

We collected twigs of the following 16 highbush blueberry cultivars: Brigitta, Sunrise, Sierra, Blue Crop, Nui, Darrow, Berkeley, Ozarkblue, Blueray, Duke, Herbert, Chandler, Patriot, Spartan, Early Blue and Nelson. The twigs were safely packed, transported and placed at +4°C for the period of 3-6 weeks, in order to terminate dormancy.

Naturally, the twigs for establishing aseptic culture may also be collected in February or March.

2. The procedure of establishing aseptic culture in this laboratory includes the following activities: one nodal cuttings are rinsed in the running water (1 hour), following by 2 min in 70% ethanol, and 4–6 min in 0,1% mercuric chloride. The final step refers to the conventional triple rinsing in the sterile water. Explants, i.e. one nodal cuttings are placed horizontally, touching medium, basal part being more immersed in the medium.

Recommendation – prior to rinsing in running water the skin of the cutting should be removed (peeled off). Further on, part of the cutting at the opposite end of axial bud which is placed in such a manner as to touch the medium physically, should be removed by a vertical cut (along the entire length of the cutting).

3. Anderson's Rhododendron medium (1980) with zeatin in concentration of 0.5 mg l⁻¹ added by the filter sterilization, and with 4.8 – 5 pH value of the medium was used for both the establishment of the aseptic culture and shoot induction.

Experiences gained in this laboratory suggest that zeatin is the best cytokinin for successful regeneration and further multiplication *in vitro* of highbush blueberry.

DESCRIPTION OF MAIN RESULTS OBTAINED:

As the aforementioned is placed within the initial phase of micropropagation, i.e. establishment of aseptic culture and shoot induction, it is too early to discuss the first results of establishment of the aseptic culture.

Apart from already mentioned samples of 16 highbush blueberry cultivars, we have been working on establishing aseptic culture of two cultivars, Sunrise and Duke, for the purpose of acquisition of the protocol.

By kindness of my hosts, I have also obtained 4 highbush blueberry cultivars in multiplication phase (cvs Blueray, Blue Crop, Berkeley and Gold Traube) in order to ensure continuation of the investigation and development of the protocol for their rapid propagation with the use of different types and concentration of cytokinins and cytokinin-like substance.

FUTURE CO-OPERATION WITH HOST INSTITUTION:

With the help of the COST 863 Action our two institutions have applied for the INTERGOVERNMENTAL PROGRAMME OF THE SCIENTIFIC-TECHNOLOGICAL COOPERATION BETWEEN SERBIA AND MONTENEGRO AND THE SLOVAK REPUBLIC 2006 - 2008 with the project entitled: *In vitro* regeneration of highbush blueberry (*Vaccinium corymbosum*), determination of genetic variability and development of the *in vitro* transformation protocols.

This STSM was used in the initial stages of our co-operation and joint project which has already been evaluated, although no decisions have been made as yet on both sides.

Regardless of the success in obtaining of this joint project we were discussing the continuation of our joint work that will include not only development of the protocol for the most rapid and the most economical method of blueberry propagation - obtainment of healthy and geneticaly stable plants for introduction into production (the work on application of chemotherapy *in vitro* is essential), but also adventitious regeneration and development of the genetic transformation protocol for obtainment of plants with improved properties.

OTHER COMMENTS:

Closing this report I wish to emphasize that my stay was very beneficial not only for me personally but for the institution I work in as well. It will also contribute to rapid introduction into production of this fruit variety for which there is an ever growing interest on the Serbian market. Finally, it adds up greatly to further co-operation between our two institutions.

I also wish to thank the persons in charge of managing STSM within the COST 863 Action who provided the grant. Finally, I express my deepest appreciation to my hosts, Dr Alena Gajdosova for kindness, hospitality and generosity in passing knowledge in this scientific field.

Čačak, October 19, 2006

Dr Djurdjina Ružić