RESEARCH ON THE COMPOSITION OF MACROELEMENTS, MICROELEMENTS AND HEAVY METALS OF SOME WILD BILBERRY (*VACCINIUM MYRTILLUS* L.) CALLUSES OBTAINED BY *IN VITRO* CULTURES

Vanda VERES BOLDA[1], Dorica BOTĂU[1]
E-mail: vvmadalina@yahoo.com

Abstract

Calluses derived from 3 different bilberry populations from Romania were analyzed for macroelements, microelements and heavy metals content using atomic absorption spectrophotometry (AAS). The calluses used in this study were analyzed after three *in vitro* subcultures on a WPM medium culture supplemented with ANA, BAP and different concentration of adenine sulphate(AS). The content of macro and microelements from calluses have varied in correlations with the AS concentration from the medium culture and genotype. We found the iron level was much higher in the calluses composition than in the mother plants and zinc levels were 5-9-times higher than those of the control(range 0,06-1,8 µmol/DM).

Key words: *Vaccinium myrtillus* L., calluses, macroelements , microelements, AAS

In many different plant species the obtaining cell mass (callus) can be used as a source of secondary metabolic products and specific induction of the morphogenetic processes (Botău, 2006; Lloyd and McCown, 1980). Callus formation is a particular meal consisting of a holding cell a uniform histological structure. Genetic instability of its cells and their easy polyploidization under certain conditions of culture, callus culture make a valuable biological material that enables the selection, obtaining cell lines with special traits. *In vitro* propagated callus cultures can become an alternative to plants grown in their native environment due to the fact that under controlled conditions, plant tissues can produce significant amounts of metabolites of interest. Moreover, the content of macro- and microelements may represent parameters that indicate the occurrence of somatic variability in the callus, this fact being important in selecting the cell lines of interest.

The lowbush bilberry (*Vaccinium myrtillus* L.) belongs to Ericaceae family and is a very important medicinal plant for human nutrition and in the treatment of many disease, because of its high nutritional value and therapeutic properties. The fruits and leaves are rich in phenolic compounds, especially in anthocyanins and other antioxidants (Martz et al. 2010). It was found that the antioxidant capacity of blueberry (*Vaccinium corymbosum* L.) cultivars could be influenced by the genotype but other factors (e.g. growing season, location, the age of plants, storage condition of samples) can also affect these parameters (Piljac-Žegarac et al. 2009). Over the years, a series of chemical analyses have revealed these health-beneficial compounds in bilberry fruits, however, the underlying genetic diversity and the variation in biochemical composition between populations and *in vitro* callus cultures, remain to be thoroughly investigated.

In this study we investigated the influence of genotype and *in vitro* conditions on callus proliferative capacity and also the macro and microelements composition of callus lowbush bilberry.

MATERIAL AND METHOD

Plant material representing native populations of bilberry was collected from three sites of Carpathian mountains in western Romania, in Arieseni, Retezat, Sebes Valley districts. Because of the difficulties of the representative sampling for elemental analysis the fully expanded leaves of naturally growing plants were collected in June 2011. Five plants were selected each time and were transported to laboratory in an icebox. Then the leaves were detached and after washing with double distilled water, they were stored at -80 °C until analysis (Kovacheva et al. 2000). To obtain calluses, different types of explants were

[1] [1]University of Agricultural Sciences and Veterinary Medicine of Banat Timisoara

taken from various tissues of bilberry plants, originated from the three different mountain locations. After surface sterilization the explants were inoculated on the Woody Plant Medium (WPM) (Lloyd&McCown's Woody Plant Medium, *Phyto*Technology Laboratories, Lenexa, Kansas, USA), supplemented with 1,5 mg/L *1*-naphthylacetic acid **(**NAA) and 1 mg/L benzylaminopurine (BAP) and with different concentrations of AS (Sigma, 40, 60, 80 mg/L). The calluses used in this study were analyzed after three in vitro subcultures on the same type of culture medium and under the same hormonal influence.

The content of macro- (K, Ca, Mg) and microelements (Cu, Zn, Fe, Ni) as well as some heavy metals (Ni, Cr, Cd, Pb) in *Vaccinium myrtillus* calluses and mother plants leaves was determined by atomic absorption spectrometry (AAS) with a Hitachi Z-8200 spectrophotometer (Tokiy Japan). For each sample, 100 milligrams of dried plant or dried callus tissues where used. Plant material was homogenized and placed in test tubes containing 5 ml of concentrated HNO_3 and 4 ml of 30% H_2O_2 at 200^{o}C for 2 hours. For the determination of metal content, 3 replicas were taken of each concentration and time of exposure. Values of concentrations in the samples are given in µmol g^{-1} dry mass (DM).

Statistics

The statistical analysis of results was carried out using STATISTICA 9.0 software. Non-parametric test (Kruskal-Wallis ANOVA) was used to test the differences of means. In order to determine the relationship between the biochemical parameters, a non-parametric analysis of correlation (Spearman's Rank Order Correlation) was used. Data are given in mean values±standard deviation (SD) and calculated for fresh mass. Level of significance was generally $p<0.05$.

RESULTS AND DISCUSSIONS

The amount of macroelements of the *Vaccinium myrtillus L.* calluses derived from several populations had a high diversity and ranged from 73.6 to 346 µmol g^{-1} DM for potassium, 6.8-35 µmol g^{-1} DM for calcium and 11.6-47 µmol g^{-1} DM for magnesium (Fig. 1A). The potassium level was usually higher in the calluses than in the mother plants that were regarded as control (84.1 and 119.5 µmol g^{-1} DM), while the calcium and magnesium content of the mother plants was generally higher than those of the calluses. Addition of 40-80 mg l^{-1} AS to the culture medium did not enhance the macroelement content of callus tissues, in contrast, increasing AS concentrations may result in a decline in K content in certain cell lines.

Copper level of the calluses were similar to those of the mother plants and very low (0-0.25 µmol g^{-1} DM), but zinc levels of the calluses were 5-9-times higher than those of the control (range 0.06-1.8 µmol g^{-1} DM). At the same time, calluses showed very high iron levels compared to control (range 0.6-5 µmol g^{-1} DM, Fig. 1B).

The amounts of Ni (essential element) and Cr were very low and the concentrations of two other non-essential heavy metals, Cd and Pb were close to zero in all samples (Fig. 1C).

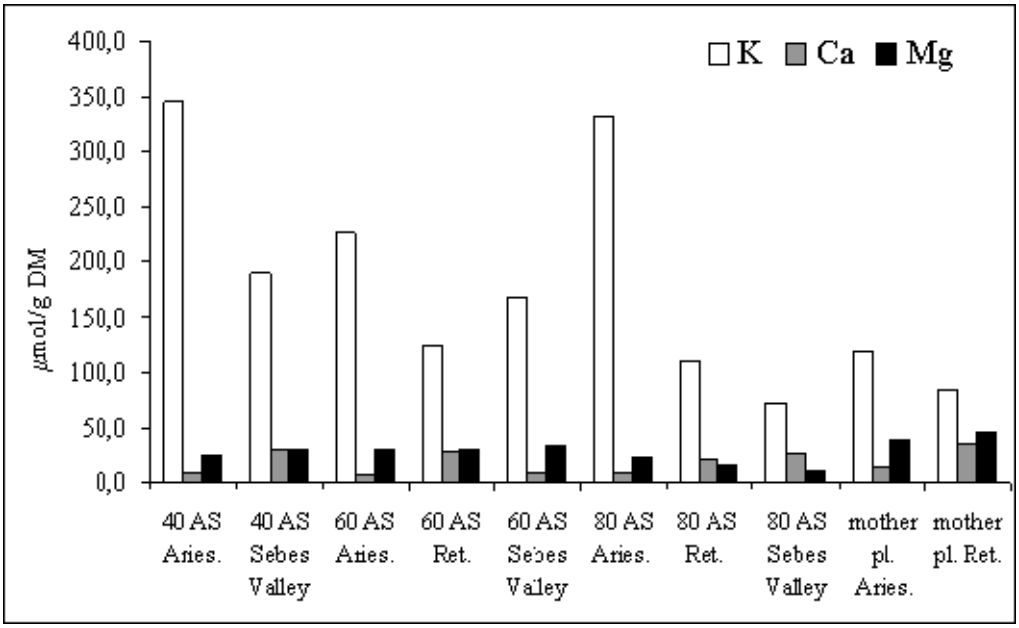

Fig. 1A **The macroelement (K, Ca, Mg) content of the calluses and the mother plants of *Vaccinium myrtillus* L. Data are given in µmol/g DM (dry matter).**

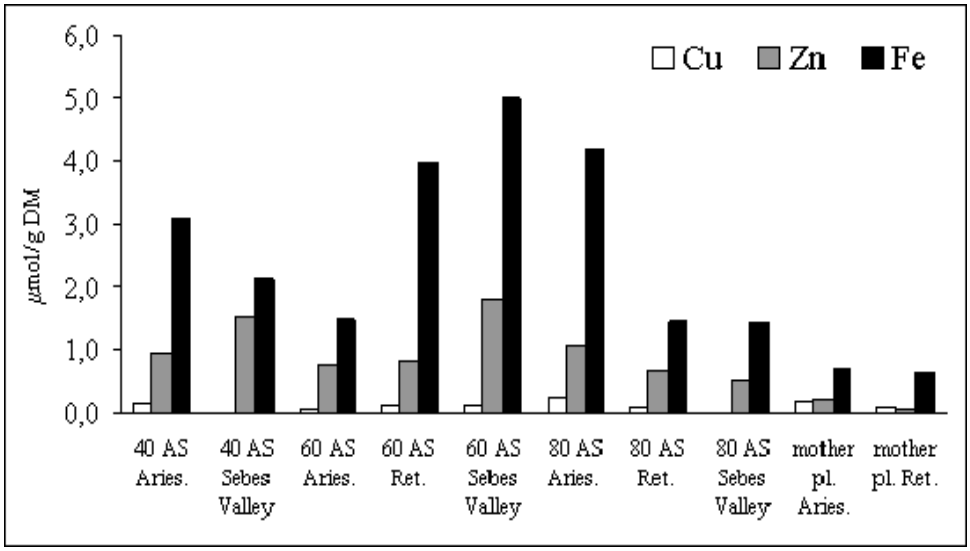

Fig. 1B. **The microelement (Cu, Zn, Fe) content of the calluses and the mother plants of *Vaccinium myrtillus* L. Data are given in µmol/g DM (dry matter).**

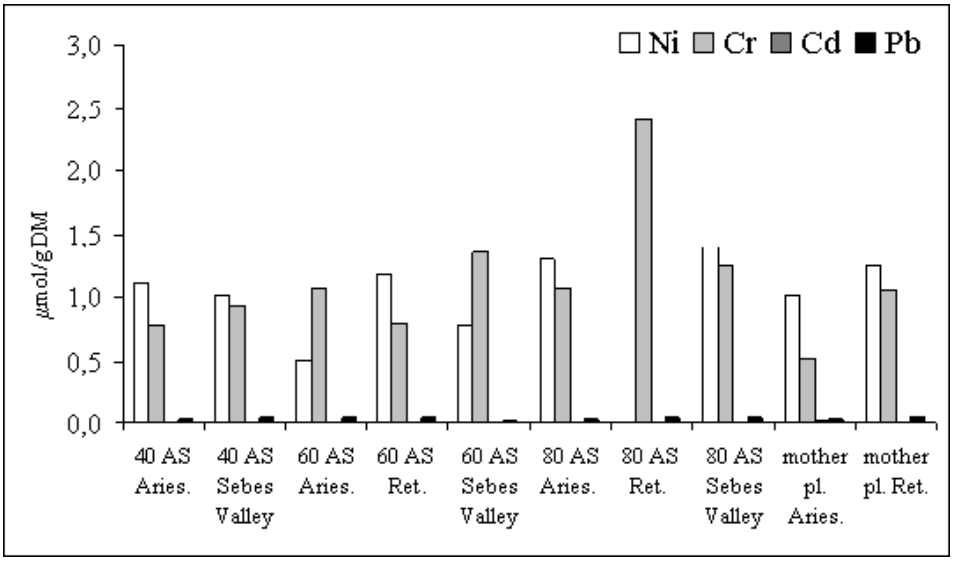

Fig. 1C **The heavy metal (Ni, Cr, Cd, Pb) content of the calluses and the mother plants of *Vaccinium myrtillus* L. Data are given in µmol/g DM (dry mass).**

In our experiments it was revealed that elemental composition of callus cultures can show significant differences from the tissues of mother plants and in some cases there were significant differences between the tissues originated from different populations. Increasing the amount of adenine sulphate (AS) (60-80 mg/l) in the culture medium causes the accumulation of iron in bilberry callus. The tissues also accumulate zinc in smaller amounts, and the zinc content in callus cultures is higher than in the mother plant. This phenomenon makes the callus tissue usable in treatments to combat the deficit in iron and zinc of food, in form of food or feed dietary supplements (Maksimiec, 2007; Panou-Filotheou et al. 2001).

Regarding the heavy metals, chromium content reaches slightly higher values in the callus tissues than in the mother plants, but this level does not exceed the European standard limits for foods (Szőllősi et al., 2011; Prasad and Strzalka, 2002). Growing callus on culture medium which is supplemented with 40 mg / l AS and 60 mg / l AS does not change the content of these non-essential heavy metals compared to the mother plants.

CONCLUSIONS

The *Vaccinium myrtillus* calluses cultivated in presence of adenine sulphate (60-80 mg / l) can be successfully used in treatments, as a more convenient alternative (from the point of view of the macro and microelement content) than the normal plants. These are more rich in iron and zinc.

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Abbreviations: ANA, naftil acetic acid; BAP, 6 -benzyl amino purine; AS, adenine sulphate.

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