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THE SELECTION OF SOME TISSUE LINES PRODUCERS OFANTHOCYANINS IN BILBERRY (*Vaccinium mytillus* L.) CALLUSCULTURE

Dorica BOTAU, Vanda BOLDA

Faculty of Horticulture and Forestry, University of Agricultural Sciences
and Veterinary Medicine of Banat, 119 Calea Aradului, 300645, Timisoara, Romania

Corresponding author email: dbotau@yahoo.com

Abstract

Our research aimed the obtaining a proliferative tissue at 7 bilberry (Vaccinium mytillus L.) local population, to select some anthocyanins producing tissue lines by in vitro culture. The tissue lines selected from Arieşeni, Semenic, Retezat, Valea Sebeşului, Buces-Vulcan, Vadul Dobrii and Cornereva local population, with good results of callus growth capacity in subculture, produced anthocyanins (646 mgC3GE/100g). These results demonstrated the possibility to elaborate a production system of these metabolites in controlled conditions.

Keywords: *bilberry (Vaccinium mytillus L.) local population, callus, anthocyanins.*

INTRODUCTION

Among the plants that provide raw material for obtaining a wide range of natural medications (*phytotherapeutical* products), food supplements, natural colorants and preservatives, there is also included the species *Vaccinium myrtillus* L. In our country, but also worldwide, a sustaining preference for the consumption of *phytotherapeutical* preparations can be recognized, to the disadvantage of medicines prepared by chemical synthesis. This is due to the fact that herbal therapeutic products are better tolerated by the human body (on the background of a better compatibility at a metabolic level). The curative medicinal properties of bilberry are known since antiquity and they were exploited under various forms, from fruit and leaf teas, poultices, tinctures, powders, to complex drugs obtained nowadays (Maillefert, 2010). The bilberry plants have therapeutic importance both in human medicine and in veterinary medicine. Among the many effects of the active principles of bilberries there we can find: hypoglycemic, hypotensive, antiseptic, cholagogue, vasoprotectives of capillaries (eye, brain, peripheral), antidiarrheal, antioxidant (Martz et. al, 2010).

MATERIALS AND METHODS

We used 7 local populations of bilberry collected from the central-west part of Transilvania: *Arieşeni, Semenic, Retezat, Valea Sebeşului, Buces-Vulcan, Vadul Dobrii and Cornereva*. For callus inducing three types of explants were used: meristem, leaf and stem, grown *in vitro* on three culture media (Lloyd and McCown Woody plant medium-WPM, Anderson′s Rhododendron medium-AND and Mourashige-Skoog medium-MS), under the influence of ANA (naphtalen-acetic acid) two hormonal variants (V_1, V_2, table 1). Undifferentiated tissues obtained were sub-cultured on media supplemented with the same hormonal balances. Callus was sub-cultured in three culture cycle, on WPM medium supplemented with 1,5 mg/l ANA,

Table 1. Hormonal balances tested for callus induction on culture media (WPM, AND, MS)

Variant	Hormons mg/ml		
	ANA	BAP	AS
1	1,5	1	-
2	1,5	1,5	40

The total antioxidant capacity of the selected tissue lines from the Arieşeni, Retezat and

Valea Sebesului populations has been studied, achieving good results concerning the ability of callus growth in subculture and obtaining plant biomass, with rich anthocyanin pigments (those calluses were visually selected that showed red cells, signaling the presence of anthocyanins). To determine the total antioxidant capacity we use extracts obtained from callus and foliar tissue of bilberry mother plant, using a rapid method, elaborated by Benzie and Strain (1996), modified by Varga et al. (2000); Szollosi and Varga (2002). The total anthocyanin content was expressed in cianidin 3-glucoside equivalents (C3GE) /100g fresh vegetal material-FW.

RESULTS AND DISCUSSIONS

1. Experimental results regarding the callus induction at bilberry local population

Abundant and proliferative callus was obtained from stem and foliar explants, grown on WPM media, supplemented with 1.5 mg/l ANA + 1.5 mg/l BAP + 40 mg/l AS, demonstrating that the culture media, explant type and association of citokinine with auxine, in equal proportions and in the presence of adenine sulphate-AS (40 mg/l) are key factors in inducing callus at spontaneous bilberry.

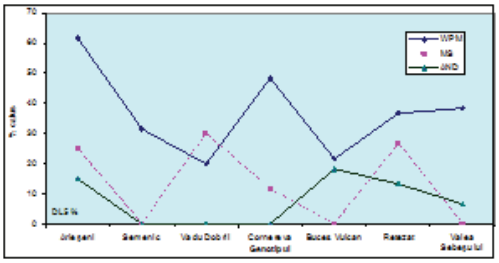

Figure 1. Graphical representation of bilberry callus percentage for different genotypes and culture medium on bilberry meristem explants.

AND medium determine the lowest variability between populations in terms of callus production. The best values registered WPM medium when it used meristem explants (fig.1).

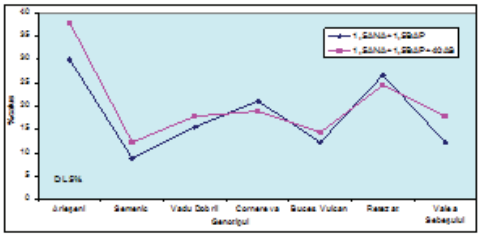

Figure 2. Graphical representation of bilberry callus percentage for different genotypes and hormonal balances on bilberry meristem explants.

However, meristem explants produced less callus (fig. 2).

The genotype is another factor which influences callus production at bilberry. Only at the populations from Arieseni, Cornereva, Retezat and Valea Sebesului, callus was obtained in greater quantities comparing to the other studied genotypes, over 90% callus from leaf and stem explants (fig.1, 2, 3, 4, 5 and 6).

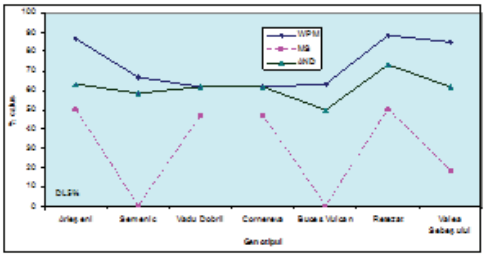

Figure 3. Graphical representation of bilberry callus percentage for different genotypes and culture mediums on bilberry leaf explants.

Under WPM medium influence the leaf explant produced callus at all bilberry local population, with values between 61,67% and 88,33% (fig.3).

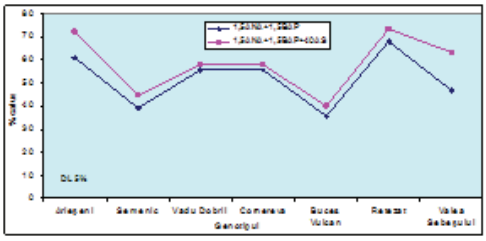

Figure 4. Graphical representation of bilberry callus percentage for different genoyupes and hormonal balances on bilberry leaf explants.

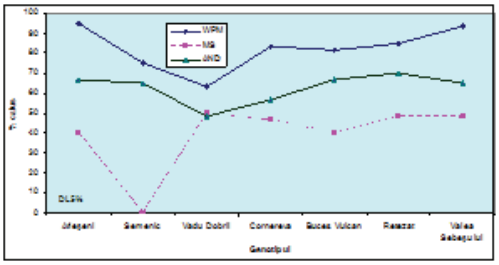

Figure 5. Graphical representation of bilberry callus percentage for different genotypes and culture media on bilberry stem explants.

The stem explant produced callus in the largest amount on WPM medium, with values between 63,33% at Vadul Dobrii population and 95% at Arieseni population.

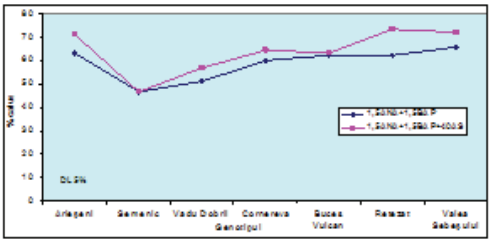

Figure 6. Graphical representation of bilberry callus percentage for different genotypes and hormonal balances on bilberry stem explants.

The two populations *Buces-Vulcan* and *Vadul Dobrii* present lower results in callus growth. The best medium which provided a good callus growth was WPM and it was used in callus subculture. The callus growth in subculture is significantly influenced by the genotype and the presence of AS. Our results are in concordance with those obtained by Litwinczuk and Wadas (2008).

The growth bilberry callus in subculture on WPM medium was influenced by genotype, callus type and AS concentration (table 2).

It can observe that a significantly callus growth in subculture was registered at Retezat, Valea Sebesului and Arieseni populations (2,70-2,83) in presence of 60 mg/l AS.

The origin of callus is a factor influencing in a certain extent the callus growth in subculture. The stem callus has a good growth compared with foliar callus at bilberry local population.

Table 2. The effect of the genotype, callus type and AS concentration on the growth of the bilberry callus in subculture on the solid medium WPM (1,5 mg/l ANA+1,5 mg/l BAP)

Genotype	**Arieseni**	
Concentration	Callus type	
(AS)	**Foliar**	**Stem**
40 AS	y2,21 b	x2,54 b
60 AS	y2,50 a	x2,78 a
80 AS	x2,26 b	x2,38 b
Genotype	**Vadu Dobrii**	
Concentration	Callus type	
(AS)	**Foliar**	**Stem**
40 AS	x2,13 a	x2,25 b
60 AS	y2,10 a	x2,68 a
80 AS	y1,99 a	x2,65 a
Genotype	**Cornereva**	
Concentration	Callus type	
(AS)	**Foliar**	**Stem**
40 AS	y1,71 a	x2,07 b
60 AS	y1,60 a	x2,35 a
80 AS	y1,69 a	x2,22 ab
Genotype	**Buceş Vulcan**	
Concentration	Callus type	
(AS)	**Foliar**	**Stem**
40 AS	x1,74 a	x1,62 b
60 AS	y1,61 ab	x1,90 a
80 AS	y1,50 b	x2,04 a
Genotype	**Retezat**	
Concentration	Callus type	
(AS)	**Foliar**	**Stem**
40 AS	y1,92 b	x2,35 b
60 AS	y2,33 a	x2,70 a
80 AS	y2,20 ab	x2,51 ab
Genotype	**Valea Sebeşului**	
Concentration	Callus type	
(AS)	**Foliar**	**Stem**
40 AS	y2,35 a	x2,62 a
60 AS	y2,56 a	x2,83 a
80 AS	x2,34 a	x2,34 b

$DL_{5\%}$=0,23 g $DL_{1\%}$=0,31 g $DL_{0,1\%}$=0,40 g

2. Results regarding the evaluation of the total anthocyanin content (on solid WPM media)

The callus produced by *Retezat, Valea Sebesului* and *Arieseni* populations in subculture synthesised anthocyanins that was analysed (fig.7).

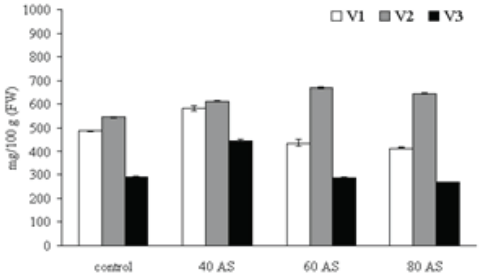

Figure 7. The variance analysis for the total anthocyanin content (mg C3GE /100 g fresh weight) of the bilberry tissue lines obtained on solid WPM medium for the Arieseni (V1), Retezat (V2) si Valea Sebesului (V3) populations

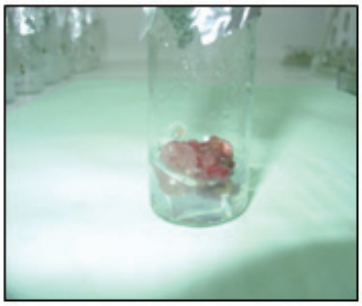

Figure 8. Callus with anthocyanins at the bilberry tissue lines from Retezat population

We selected tissue lines with higher anthocyanin content than control samples, for all three populations, due to the contribution of AS we suppose. The best antioxidant capacity was registered at *Retezat* bilberry population in presence of 60 mg/l AS (V2, 646 mg/ 100 g fresh weight).

CONCLUSIONS

Our results demonstrated the strong influence of genotype, culture medium and hormonal balance on callus induction and anthocyanin biosynthesis capacity of bilberry tissue cultivated *in vitro*. An abundant callus can be induced from leaf end stem explants at wild bilberry, on WPM medium, in presence of ANA and BAP in equal amount (1,5 mg/l) and 40 mg/l AS. In callus subculture, the genotype and amount of AS are important factors that allow selection of tissue lines with anthocyanin biosynthesis capacity.

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