International Annual Conference on Basic and Applied Science (IACBAS 2022)

Towards

"Advanced Science & Sustainable Development"

Effects of artificial light spectra on anthocyanin accumulation in blueberry callus cultures

1139-IACBAS

Gamil Rayan Abou El-Dis [1,2], Khusnetdinova Landysh Zavdetovna[1], Akulov Anton Nikolaevich[3], Walla Mohamed Abdelmaksood Abdelazeez[1,4], Timofeeva Olga Arnoldovna[1]

420008, Kazan Federal University, Kazan, Russia[1], 11753, Desert Research Center, Cairo, Egypt[2], 420111, Kazan Institute of Biochemistry and Biophysics, FRC Kazan Scientific Center of RAS[3], 11754, Faculty of Science, Al-Azhar University, Cairo,

Abstract

Blueberry is considered a health-promoting food with a high content of anthocyanins. The extraction of anthocyanins from callus has been used to solve some problems of anthocyanins production, being more effective for commercial application and high production of anthocyanins. One of the most important aims of our study is to enhance anthocyanin synthesis in callus cultures. The results showed that callus induction from the highbush blueberry *V. corymbosum* L. cv. Sunt Blue Giant depends on the type of explant and culture medium. Maximum callus induction was established on Woody Plant medium (WPM) containing 2.0 mg/L α–naphthalene acetic acid (NAA) with 1.0 mg/L 6-benzylaminopurine/benzyl adenine (BAP) from all used explants and the maximum fresh weight (for leaf, 1.03; root, 1.0; and stem, 0.8 g/jar) was recorded. After four subcultures on the same medium composition, we studied the effect of different light types on the accumulation of anthocyanins in callus culture.

https://us.modiushealth.com/blogs/news/the-benefits-of-blueberries-and-their-nutrition-facts

Goal of the work

Improving anthocyanin production by the influence of different light wavelengths on the accumulation of anthocyanins in callus culture of *V. corymbosum* L. cv. Sunt Blue Giant as an attractive alternative method to the use of whole plants for the production of anthocyanins.

https://www.simplygourmand.com/blueberry-essence-from-grasse/

https://ru.made-in-china.com/co_hefeijuly/product_Blueberry-Extract-99-Blueberry-Powder_uosiihuiiy.html

Accumulation of anthocyanins in callus *V. corymbosum* L. cv. Sunt Blue Giant

INTRODUCTION

Anthocyanins are major pigments found in many plants, combined with carotenoids or chlorophylls, being responsible for the red, purple, and blue coloration of some fruits, leaves, and seeds [1]. Blueberry is considered as one of the richest sources of anthocyanins among common fruits [2], which are responsible for a variety of health promoting values [3], these properties of blueberry and its unique set of anthocyanins content, make it especially attractive for food and pharmaceutical preparations. In this regard, the demand for blueberry and its sales have increased significantly over the last 30 years [4]. Pigments extraction from the fresh plant tissues still has some problems such as low metabolite yield, seasonal availability, fast deterioration, inconsistent product quality, pigment degradation due to the extraction and storage process [5,6]

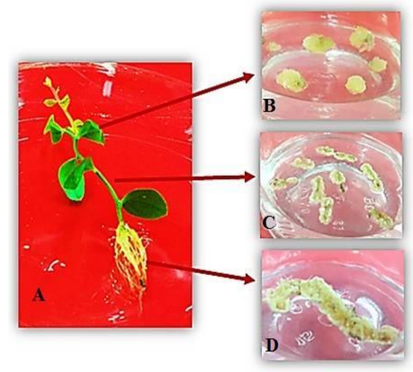
Callus culture offers a strategy to minimize all these problems for the continuous production of potentially valuable compounds for human use. Moreover, callus culture could be employed as an effective substitute for the production of anthocyanins [7]. Several studies have shown the importance *in vitro* anthocyanin production from genus vaccinium such as [8,9]. Anthocyanins biosynthesis is regulated by some environmental factors, particularly light [7]

https://www.thetreecenter.com/emerald-blueberry-bush/

MATERIALS AND METHODS

1. Plant material

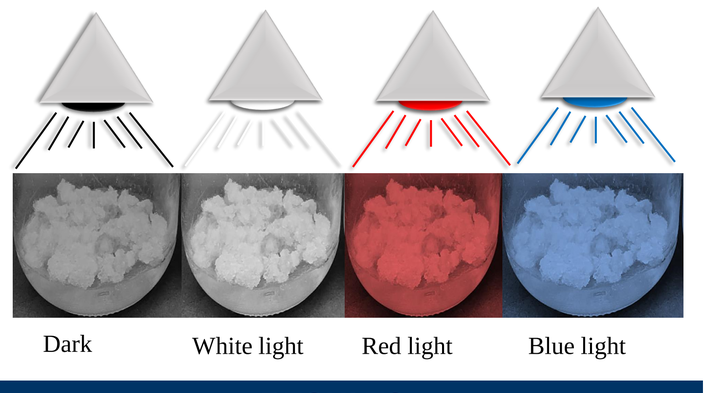
Different explants were used for induction of callus cultures (leaves, stems and roots) isolated from plantlets germinated under sterile conditions



The initial stage of callus formation from different types of explants after 12 weeks in the dark: A – source material, B – leaf explant, C – stem explant, D – root explant after 12 weeks of culture/exposure in the dark

2. Light treatments

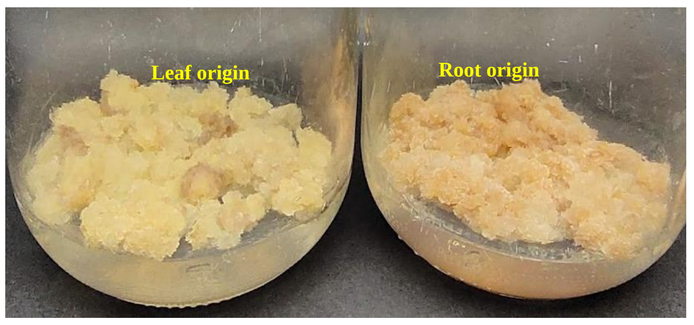
In order to study the effect of different light wavelengths on the accumulation of anthocyanins in callus culture of *V. corymbosum* L. cv. Sunt Blue Giant, callus were grown in dark for 20 days on WPM medium supplemented with 2.0 mg/L NAA + 1.0 mg/L BAP, then placed in the light for 12 days (white light, 150 µmol m2/s (light source – fluorescent lamp luminescent down shifting (LDS)-40); blue light 120 µmol m2/s (source – light emitting diode (LED)-10-Blue 10 W, wavelength 420-460 nm); red light 180 µmol m2/s (source – (LED) LL-271 red 10 W, wavelength 620-640 nm); photoperiod:16-hours). Anthocyanins contents were recorded in all samples (light and dark grown callus).



RESULTS

Establishment of callus cultures

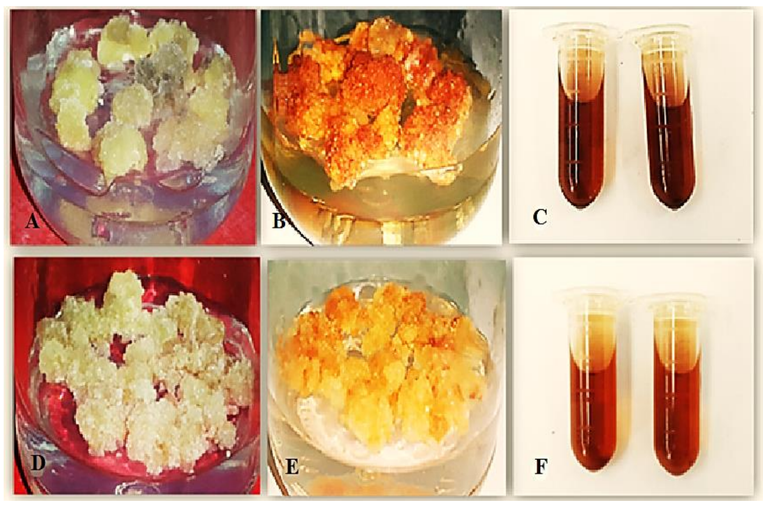
The results showed that callus induction from the highbush blueberry *V. corymbosum* L. cv. Sunt Blue Giant depends on the type of explant (leaf, root, and stem segments) and culture medium. Maximum callus induction was established on Woody Plant nutrient medium (WPM) containing 2.0 mg/L α–naphthalene acetic acid (NAA) in combination with 1.0 mg/L 6-benzylaminopurine/benzyl adenine (BAP) from all used explants and the maximum fresh weight (for leaf, 1.03; root, 1.0; and stem, 0.8 g/jar) was recorded



Callus formation on WPM containing 2.0 mg/L α–naphthalene acetic acid (NAA) in combination with 1.0 mg/L 6-benzylaminopurine/benzyl adenine (BAP) after fourth subculture. (best treatment)

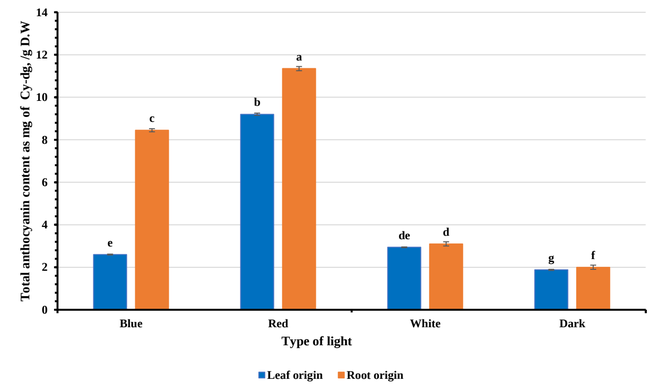
Effect of light treatments on total anthocyanin content in callus obtained from leaf and root origin

Initial identification of anthocyanins was implemented by placing callus in an atmosphere saturated with the vapor of concentrated hydrochloric acid (acidic pH) for 15-20 minutes [10], callus coloration can be seen in next Fig



Changes in the color of callus *V. corymbosum* L. cv. Sunt Blue Giant grown in the red light after transfer to the atmosphere saturated with the vapor of hydrochloric acid for 15-20 minutes: A – callus of root origin before treatment, B – callus of root origin after treatment, C – methanol extract of callus of root origin, D – callus of leaf origin before treatment, E – callus of leaf origin after treatment, F – methanol extract of callus of leaf origin

The effect of three types of light (white, red and blue) on the accumulation of anthocyanins in callus culture obtained from leaf and root of *V. corymbosum* L. cv. Sunt Blue Giant in comparison with callus cultures growing in the dark, has been studied. (Fig. 6). Total anthocyanin content showed significant differences in callus cultures, where the highest level of anthocyanin was observed in callus obtained from root origin growing in red light (the anthocyanins content was 11.35 mg/g dry weight), which represented about 5.7 fold comparing with anthocyanins content in callus obtained from root origin grown in dark (control). Moreover, blue and white light also increased the content of anthocyanins (4.23 and 1.55 fold respectively) comparing with anthocyanins content in callus obtained from root origin grown in dark, but to a lesser extent than that obtained with red light (5.7 fold). Red light also affected the content of anthocyanins in callus obtained from leaf origin, which increased by 4.9 folds in comparison to control. Blue and white light also increased the content of anthocyanins (1.38 and 1.56 fold respectively) comparing with anthocyanin content in callus obtained from root grown in dark), also, a lesser extent than that obtained with red light (4.9 fold).



Values followed by the same lower-case letter do not different significant at p≤ 0.05

Effect of light quality on accumulation of anthocyanin in callus culture of *V. corymbosum* L. cv. Sunt Blue Giant on WPM medium + 2 mg/L NAA + 1 mg/L BAP after 12 days of exposure

CONCLUSION

Based on the above data, it can be concluded that, WPM supplemented with 1.0 mg/L BAP in combination with 2.0 mg/L NAA was the best for callus induction and multiplication up to the fourth subculture in *V. corymbosum* L. cv. Sunt Blue Giant. Callus must be transferred to fresh medium every 20-25 days. Root explant was the best for *in vitro* production and accumulation of total anthocyanins, the callus formed from leaf origin was characterized by special qualitative composition of anthocyanins largely than in callus obtained from root origin. The application of light, especially red light, has been proven to be a promising approach for enhancing the production of anthocyanins in the callus culture of V. corymbosum L. cv. Sunt Blue Giant. Further studies could be performed on a large-scale for the production of anthocyanin by cell suspension culture of blueberry *V. corymbosum*.

REFERENCES

1. Miao L, Zhang Y, Yang X, Xiao J, Zhang H, Zhang Z, Wang Y, Jiang G (2016) Colored light-quality selective plastic films affect anthocyanin content, enzyme activities, and the expression of flavonoid genes in strawberry (*Fragaria× ananassa*) fruit. Food chemistry 207:93–100. https://doi.org/10.1016/j.foodchem.2016.02.077
2. Wu X, Beecher GR, Holden JM, Haytowitz DB, Gebhardt SE, Prior RL (2006) Concentrations of anthocyanins in common foods in the United States and estimation of normal consumption. Journal of agricultural and food chemistry 54(11):4069–4075. https://doi.org/10.1021/jf060300l
3. Li C, Feng J, Huang W-Y, An X-T (2013) Composition of polyphenols and antioxidant activity of rabbiteye blueberry (*Vaccinium* ashei) in Nanjing. Journal of Agricultural and Food Chemistry 61(3):523–531. https://doi.org/10.1021/jf3046158
4. Rowland LJ, Hammerschlag FA. 8.1 *Vaccinium* spp. Blueberry (2005). In: Litz R (ed) Biotechnology of Fruit and Nut Crops. Biotechnology in Agriculture CABI Publishing, Wallingford, UK Series no 29 :222–246. (Accessed 29 October 2020).
5. Lila MA (2004) Anthocyanins and human health: an *in vitro* investigative approach. BioMed Research International (5):306–313. https://doi.org/10.1155/S111072430440401X
6. Ram M, Prasad K, Kaur C, Singh S, Arora A, Kumar S (2011) Induction of anthocyanin pigments in callus cultures of Rosa hybrida L. in response to sucrose and ammonical nitrogen levels. Plant Cell Tissue and Organ Culture (PCTOC) 104(2):171–179. https://doi.org/10.1007/s11240-010-9814-5
7. Debnath SC, Goyali JC (2020) *In Vitro* Propagation and Variation of Antioxidant Properties in Micropropagated *Vaccinium* Berry Plants—A Review. Molecules 25(4):788. https://doi.org/10.3390/molecules25040788
8. Migas P, Cisowski W, Dembińska-Migas W (2005) Isoprene derivatives from the leaves and callus cultures of *Vaccinium corymbosum* var. Bluecrop. Acta Pol Pharm 62: 45–51.
9. Migas P, Luczkiewicz M, Cisowski W (2006) The influence of auxins on the biosynthesis of isoprene derivatives in callus cultures of *Vaccinium corymbosum* var. bluecrop. Z Naturforsch C 61:565–570. https://doi.org/10.1515/znc-2006-7-816
10. Simões C, Bizarri CHB, da Silva Cordeiro L, de Castro TC, Coutada LCM, da Silva AJR, Albarello N, Mansur E (2009) Anthocyanin production in callus cultures of *Cleome rosea*: modulation by culture conditions and characterization of pigments by means of HPLC-DAD/ESIMS. Plant Physiology and Biochemistry 47(10):895–903. https://doi.org/10.1016/j.plaphy.2009.06.005

Dr. Gamil Rayan Abou El-Dis Mobile: (+2015)52330482 Email: Gamil.rayan306@gmail.com Desert Research Center, Cairo, Egypt