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Effect of abscisic acid on anthocyanin production in callus culture of *Petunia hybrida* cv. Bravo Blue

Usha*, T. Janakiram, K.V. Prasad and Surender Kumar**
Division of Floriculture and Landscaping, ICAR-Indian Agricultural Research Institute, New Delhi 110 012

ABSTRACT

Development of an efficient tissue culture system for commercial production of anthocyanin requires an integrated approach combining the effects of various enhancement strategies. To develop a method which does not require the complicated operation and results in the anthocyanin induction in callus cultures of *Petunia hybrida*, we proposed a convenient culture method using Murashige and Skoog medium supplemented with different concentrations of Abscisic acid (ABA), *viz.*, 0 (control), 20, 30, 40 and 50 µM and the callus cultured on it. Supplementation of 30 µM resulted in a synergistic increase in anthocyanin production in terms of pigment content and pigment production with highest response coefficient (91.29 ± 2.07%) in shortest duration (12.87 ± 0.18) days. Least response coefficient (65.55 ± 2.94%) was recorded under control treatment. The colour value index (CV) was observed maximum with the addition of ABA 30 µM (1.58 ± 0.08 CV g^{-1} FCW) and maximum response for pigment production (3.22 ± 0.12^{b} CV test $tube^{-1}$). Significant reduction in the callus growth was observed due to addition of ABA as it was maximum in control treatment (3.15 ± 0.70 g). Anthocyanin pigmentation was observed as bluish-purple dots scattered over the callus.

Key words: Elicitors, *Petunia hybrida*, callus culture, abscisic acid, anthocyanin.

INTRODUCTION

Petunia hybrida Vilm. is one of the most popular and attractive annuals grown in the garden. It is both a genus of enormous horticultural interest and a model plant that is subject to numerous scientific research projects. It has widely used as a model plant for the study of biosynthetic pathway of anthocyanin pigments. Anthocyanins constitute a special class of plant-derived flavonoids. They are of chemotaxonomic value and recognized as genetic markers (Hrazdina, 5). Increasing concern over the use of artificial food colourants and anti-oxidative additives has resulted in a steady increase in demand for anthocyanins as a natural alternative (Deroles, 4).

Plant cell and tissue-based production of anthocyanins and other natural colorants is now being viewed with renewed interest (Lila, 6). To enhance the productivity, optimization of medium composition or addition of enhancer such as elicitor has been investigated (Cormier *et al*., 3). Elicitors interact with plant membrane receptors, generating signal compounds, which subsequently activate specific genes for enzymes involved in secondary metabolite biosynthesis (Brooks and Watson, 1). In recognition of its valuable properties, the unusual combination of bringing an attractive colour to the food as well as strongly enhancing the health-beneficial properties, this research was undertaken to determine the effect of abscisic acid (ABA) on anthocyanin biosynthesis by *in vitro* cultures of *Petunia hybrida*.

MATERIALS AND METHODS

The present study was carried out at the Division of Floriculture and Landscaping and Central Tissue Culture Laboratory, IARI, New Delhi, during 2010-2013. Petunia callus (*Petunia hybrida* cv. Bravo Blue) obtained from leaf explant was used. Callus was maintained on Murashige and Skoog (MS) Medium + double vitamin + IBA + Kin + AdS (19.60 µM + 4.65 µM + 81.45 mM resp.), 3% sucrose (w/v) pH 5.8 at 24 ± 1°C in complete darkness. Sub-culturing was performed every 20-21 days. To investigate the effect of ABA addition on anthocyanin production from *Petunia hybrida* calli, the MS medium containing ABA at 0, 20, 30, 40 and 50 µM were tested. Abscisic acid was used as elicitor.

Callus with the purple vacuole can be distinguished from non-pigmented callus by microscopic observation using a Carl Zeiss Discovery.v8 stereo microscope (Carl Zeiss Micro-Imaging GmbH, Berlin, Germany) and images were captured with a Carl Zeiss Axiovision digital camera (software version: Axiovision 4.8.2). The Colour Value (CV) index which is an indicator of total anthocyanins was calculated for pigment content {CV g^{-1} fresh cell weight (FCW)} and pigment production (CV test-$tube^{-1}$) with the following equations:

*Corresponding author's E-mail: usha17iari@gmail.com
**A.D.G. (Hort.), KAB-I, ICAR, New Delhi

Pigment content (CV g^{-1} FCW) = 0.1 × OD_{525} × dilution factor.

Pigment production (CV test-$tube^{-1}$) = Pigment content × respective mean fresh cell mass obtained at the end of each culture.

The experiment was laid out in completely randomized design with at least three independent determinates for each treatment. Data are presented as mean ± standard error and were analyzed using one-way analysis of variance (ANOVA). The differences among means were tested by the post hoc test Tukey's honestly significant difference (HSD) in the statistical software SPSS version 16.0 (SPSS Inc., USA).

RESULTS AND DISCUSSION

All the cultures initiated on the tested abscisic acid levels were able to biosynthesize anthocyanins, but they varied in response coefficient, number of days required for pigment initiation and intensification, fresh weight and colour value index for pigment content (PC) and pigment production (PP). The response coefficient (%) was observed maximum (91.29 ± 2.07**%**) when MS medium was supplemented with 30 µM ABA. On the other hand, this particular level did not differ significantly with 20 µM ABA with regard to response coefficient (84.07 ± 2.59%). Least response coefficient (65.55 ± 2.94%) was recorded under control treatment.

It took minimum number of days for calli for pigment induction (12.87 ± 0.18) and intensification (20.73 ± 0.52) when MS medium was supplemented with ABA 30 µM and it was examined found statistically at par with control treatment. However, calli cultured on the ABA 50 µM took maximum days (15.07 ± 0.13) to pigment initiation and intensification (23.33 ± 0.29). Maximum gain in callus growth was observed under the control treatment (3.15 ± 0.70 g). It is evident from the Table 1 that addition of abscisic acid reduced the growth of callus significantly. Anthocyanin pigmentation was observed as bluish-purple dots over the callus which was clearly microscopically observed (Fig. 1). From the results of colour value index (CV), it was observed that the addition of ABA 30 µM (Fig. 2) to MS medium gave maximum pigment content (1.58 ± 0.08CV g^{-1} FCW) and maximum response for pigment production (3.22 ± 0.12^{b} CV test $tube^{-1}$). However, no anthocyanin biosynthesis was observed when calli was cultured on control treatments. Cultures initiated in media without hormonal supplementation presented an expressive reduction in anthocyanin content. Several lines of evidence support the induction of anthocyanin biosynthesis by ABA.

Abscisic acid have been reported to show enhancing effects on the synthesis of anthocyanin in many plant species like tomato hypocotyl (Carvalho *et al*., 2), grapevine leaves (Pirie and Mullins, 8) and torenia shoots (Nagira *et al*., 7) and our results are in accordance with that. This is supported by an increase in the expression of the *UFGT* (UDP- glucose: flavonoid-3-0-glycosyl tranferase coding for a specific to the anthocyanin pathway) and *VvMYBAI* genes (coding for a transcriptional regulator controlling anthocyanin biosynthesis), as well as other genes coding upstream-located enzymes (PAL-phenylalanine ammonia-lyase, CHI-chalcone isomerase, CHS- chalcone synthase etc.). These findings established that abscisic acid is a promotive regulator of anthocyanin biosynthesis.

Moreover, it was also postulated that ABA induces phenylalanine ammonia-lyase (PAL), a key enzyme for the biosynthesis of anthocyanin and other phenolic compounds. Further work is needed to enhance the anthocyanin production for biotechnological exploitation of this system to produce natural food colourants.

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Table 1. Standardisation of ABA concentrations for anthocyanin pigment induction.

Treatment (µM)	Response coefficient (%)	Days taken for		Callus FW (g)
		Pigment induction	Pigment intensification	
0 Control	65.55 ± 2.94^{a}	12.33 ± 0.18^{b}	20.13 ± 0.75^{a}	3.15 ± 0.70^{d}
20 ABA	84.07 ± 2.59^{bc}	11.21 ± 0.21^{a}	18.87 ± 0.18^{a}	2.66 ± 0.12^{c}
30 ABA	91.29 ± 2.07^{c}	12.87 ± 0.18^{b}	20.73 ± 0.52^{ab}	2.05 ± 0.90^{b}
40 ABA	78.33 ± 1.67^{b}	14.27 ± 0.18^{c}	22.80 ± 0.20^{bc}	1.67 ± 0.56^{ab}
50 ABA	75.13 ± 1.51^{ab}	15.07 ± 0.13^{c}	23.33 ± 0.29^{c}	1.56 ± 0.10^{a}
HSD (P ≤ 0.05)	7.01	0.55	1.40	0.29

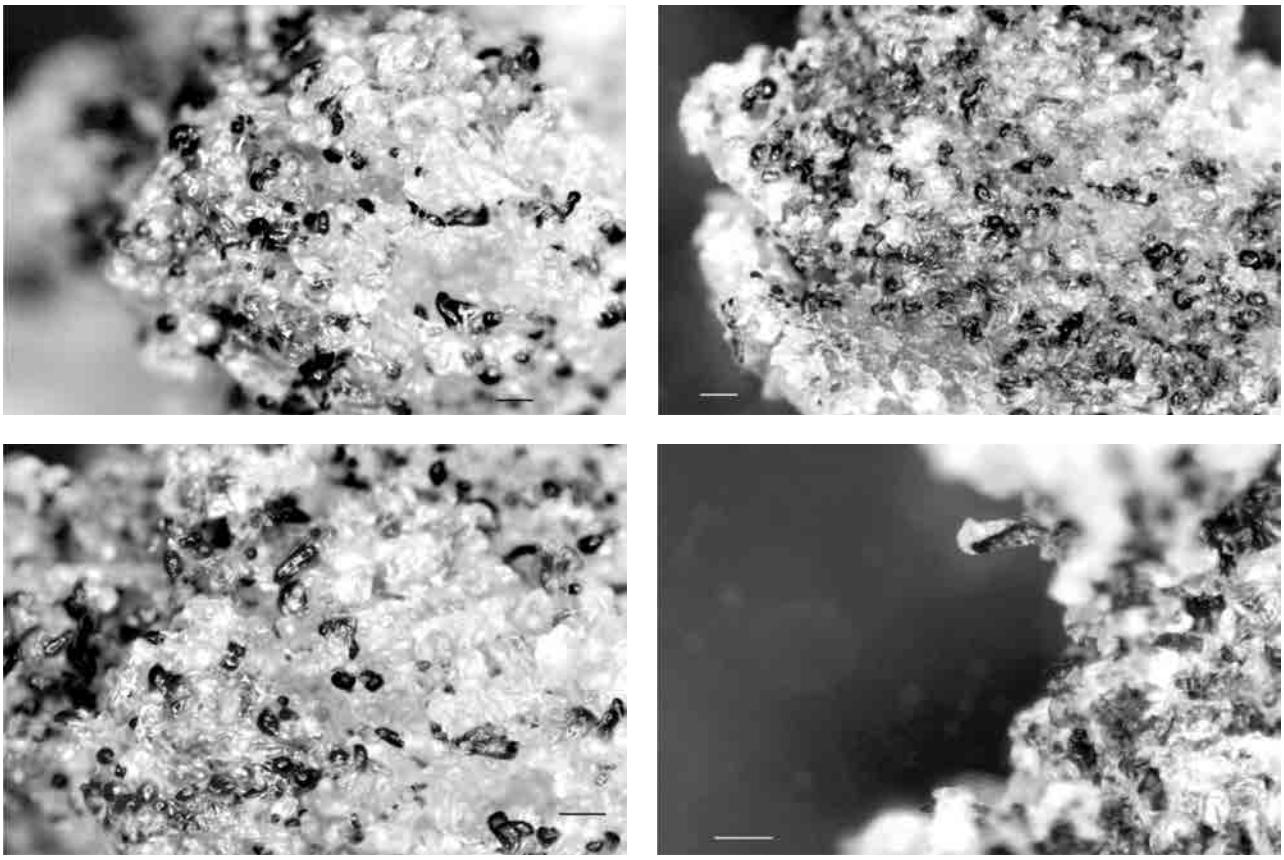

Fig. 1. ABA induced anthocyanin in callus cultures of *Petunia hybrida*. The explants were treated with different concentrations of ABA (a) 20 µM; (b) 30 µM; and (c) 40 µM.

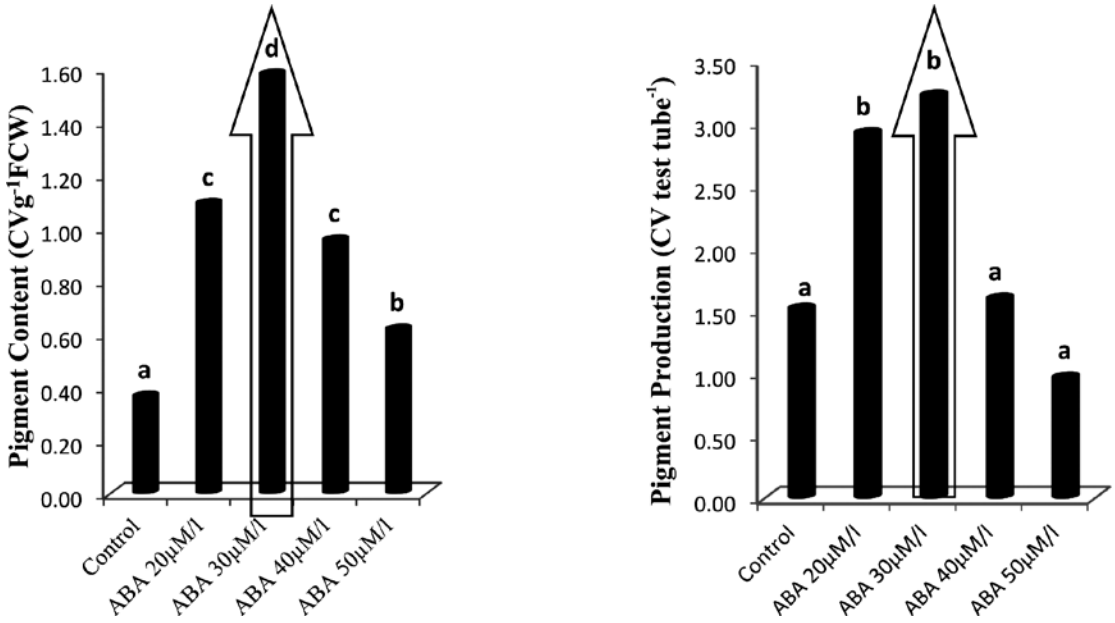

Fig. 2. Effect of different concentration of ABA on pigment content and pigment production. Data represents the means of three replicates. Same letters on the bar graph did not differ significantly at 5% level of significance when compared by Tukey's HSD test.

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