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Influence of Brassinosteroids on Pigment Content of *Glycine max* L. (Soybean) Grown in Dark and Light

Gül CEVAHIR[1,*] Semahat YENTÜR[2] Fadime ERYILMAZ[3] Nadim YILMAZER[3]

[1,2] Istanbul University, Faculty of Science, Department of Botany, 34134, Süleymaniye-İstanbul/TURKEY

[3] Istanbul University, Hasan Ali Yücel Faculty of Education, Department of Primary Education, Beyazıt-Istanbul/TURKEY

[*] Corresponding Author
e-mail: cevahirg@istanbul.edu.tr

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Abstract

Brassinosteroids (BRs) are potent plant growth regulators of steroidal nature, of which first compound isolated from a natural source is brassinolide. They are involved in regulatory processes, which are more specific to plant growth, including photomorphogenesis and skotomorphogenesis. In this study the effect of a plant growth regulator (24-epiBL) on pigment biosynthesis was investigated in light and dark grown soybean seedlings. 10^{-5}, 10^{-7} and 10^{-9} M concentrations of 24-epiBL were sprayed onto the aboveground parts of the seedlings which were kept both in light and dark in the growth chamber for 12 days. Then the seedlings were examined in terms of chlorophyll, carotenoid and anthocyanin contents in the hypocotyl, cotyledon and 1st internode. The data showed that exogenously applied epi-BL affects chlorophyll, carotenoid and anthocyanin production, and varied concentrations of epi-BL result in different responds in plant parts, depending on light and dark factors.

Key words: Brassinosteroid, 24-epiBL, Chlorophyll, Carotenoid, Anthocyanin

INTRODUCTION

Brassinosteroids (BRs) are one of the natural plant regulators, which possess pleiotropic effects, and occur in many types throughout the kingdom of plants. They were at first recognized from the fact that pollen extracts from several species of genus *Brassica* resulted in overexpansion of internodes in dotted bean [1, 2], and termed brassins due to their action on inducement of growth. Brassinolid (24-epiBL) and castasteron (CS) are two important BRs often occurring in higher plants [3]. The former is of additional importance in terms of being first plant steroid which is of hormone activity. BRs are widely spread in plants including dicotyls, monocotyls, gymnosperms, ferns and algae, and exist in all parts of the plant [2, 3]. Mainly produced in pollens, they are also present in seeds, stems, young leaves and buds in less amounts [4-8]. Furthermore, BRs which are known to be produced in roots have been detected also in crown tumors resulted from *Agrobacterium tumeficiens*, commonly present in soil, in relatively higher amounts. Additionally, they function to develop resistance against several abiotic stress conditions in plants [9].

BRs are known to induce elongation of stem, petiole and pedicel in dicotyls, and elongation of coleoptyles in monocotyls. Beside, exogenously applied BRs give rise to elongation of young growth zones in intact and excised seedlings. They function in the process of cell elongation due to their action on gene expression and enzyme activity [10]. Another important feature of these steroidal hormones is that they trigger growth respond even in quite lower concentrations [3]. Plant growth and development is regulated by coordinated relations between light and phytohormones. Applying BL and CS exogenously to mutant plants grown both in dark and light converts these plants to wild phenotypes [11-19]. Like gibberellins and cytokinins, BRs possess light-dependent physiological effects, in that BR-deficient mutants grown in dark cause changes in structural and physiological responds when compared with the seedlings grown in light. Lacking formation of apical hook, BR-deficient mutants of certain plants such as *Arabidopsis* and tomato possess broad cotyledons and primary leaves, and their genes related to anthocyanin production and photosynthesis are expressed [20]. In addition, BRs inhibit root formation and growth. It is known that exogenous BRs induce differentiation of xylem, growth of pollen tube, development of male flowers, and seed germination in *Arabidopsis*. BRs are also known to accelerate senescence and abscission by increasing ethylene biosynthesis, and to increase gravitropism [21-23].

In our day, studies on changes of pigment levels are of considerable importance in plant physiology. The presence of plant pigments chlorophyll, carotenoid and anthocyanin which undertake utmost significant roles is closely associated with plant growth regulators [24-27].

The purpose of this study is to explore possible effects of various concentrations of epi-BL on pigment contents in *Glycina max* L. (Leguminosae) grown both in light and dark.

MATERIAL AND METHOD

Soybean seeds, experimental material of this study, were left to germinate in humid chambers at 25ºC for two days. Germinated seeds were then planted in pots filled with common garden soil. After three days passed, 10^{-5}, 10^{-7} and 10^{-9} M concentrations of epi-BL were sprayed onto the aboveground parts of the seedlings every other day. This treatment was continued for 12 days for

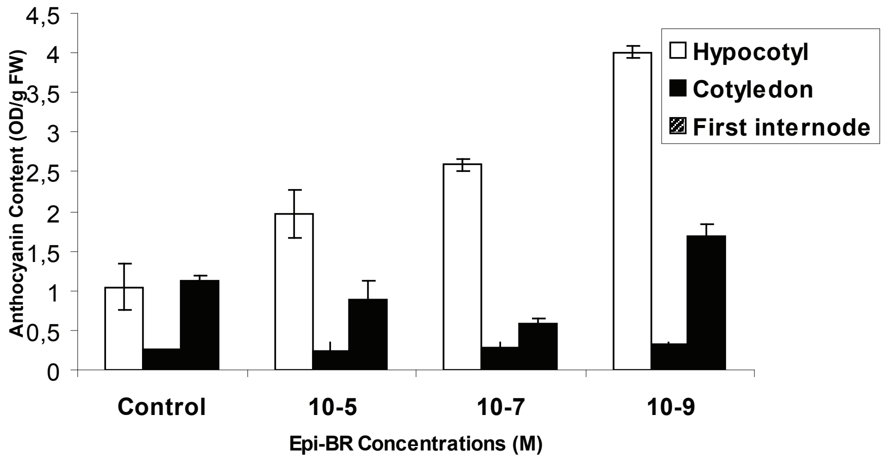

Figure 1. Changes of anthocyanin amount by epi-BL concentrations in the seedlings grown in light.

the seedlings which were kept both in light and dark in the growth chamber (65-70% humidity, 20-25ºC and 5000 lux light). Chlorophyll a and b, carotenoid and anthocyanin contents of hypocotyl, cotyledon and 1st internode of the harvested seedlings were spectrophotometrically measured.

Determination of chlorophll and carotenoid

The plant material was extracted in 90% acetone, and the obtained extract was kept in the dark at 4ºC for 24 h, centrifuged at 3000 g for 10 h. The amounts of chlorophyll and carotenoid were determined in µg/g.F.W., by employing Parson and Strickland's method [28].

Anthocyanin Determination

Mancinelli's method [29] was used in the determination of anthocyanin content in the distinct parts of the experimental plants. Anthocyanin content was quantitatively defined as OD 530/g fresh weight.

RESULTS

Changes in anthocyanin amounts by epi-BL concentrations (10^{-5}, 10^{-7} and 10^{-9} M) in the hypocotyl, cotyledon and 1st internode of 12 days old seedlings grown in light are presented in Figure 1.

As can be seen from the figure, it was the anthocyanin content that was the most affected one by epi-BL. With the decrease in the concentration, there was a marked increase in anthocyanin amounts in hypocotyls of the plants grown in light. Upon comparison of experiment concentrations with the control, there were increases of 87%, 147%, 282% at 10^{-5}, 10^{-7} and 10^{-9} M epi-BL concentrations, respectively. While anthocyanin amount in the cotyledon remained unaffected from epi-BL applications, the concentrations of 10^{-5} and 10^{-7} M epi-BL slightly inhibited anthocyanin amount in the 1st internode. However, the 1st internode had a higher value than that of the control group at 10^{-9} M epi-BL, the increment being 50%.

Figure 2 displays anthocyanin amount of the growing-in-dark seedlings as shown in the histograms. Since 1st internode was not developed in these seedlings, only data of hypocotyl and cotyledon are given. Contrary to the plants grown under light, 10^{-9} M epi-BL resulted in inhibition of anthocyanin, while anthocyanin amounts of the plants grown in dark showed an increase at 10^{-5} and 10^{-7} M epi-BL. With the cotyledons, however, there was a decrease at 10^{-5} and 10^{-7} M epi-BL, whereas a marked increase (43%) was determined at 10^{-9} M epi-BL.

Chlorophyll a and b amounts in hypocotyls, cotyledons, and 1st internodes of soybean seedlings grown under light

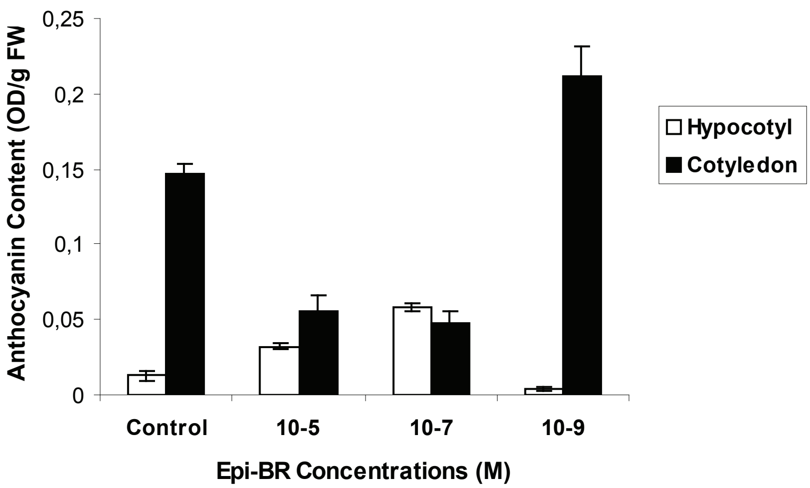

Figure 2. Changes of anthocyanin amount by epi-BL concentrations in the seedlings grown in dark.

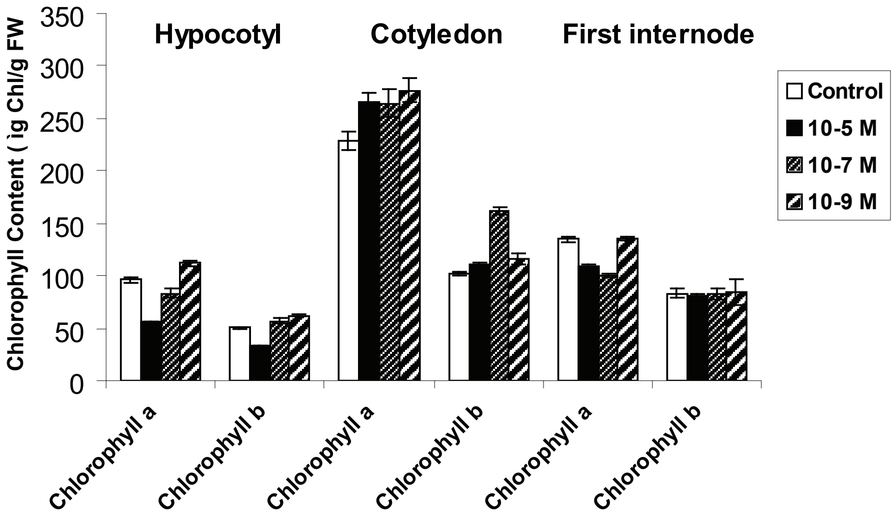

Figure 3. Amounts of chlorophyll a and b in hypocotyl, cotyledon and 1st internode of the growing-in-light seedlings treated with epi-BL concentrations.

were inspected at three different epi-BL concentrations. Measured chlorophyll contents of the test seedlings grown under light are shown in Figure 3. The amount of chlorophyll a in hypocotyl and 1st internode had a value that was below the control group at 10^{-5} and 10^{-7} M epi-BL, and above the control in all three plant parts at 10^{-9} M epi-BL. As for chlorophyll b amounts, while 10^{-5} M epi-BL resulted in inhibition in the hypocotyl, 10^{-7} M epi-BL had a stimulating effect in the cotyledons. 10^{-9} M epi-BL led to the results close to the control in all three plant parts.

Relationships between carotenoid amount and applied epi-BL concentrations in the seedlings grown in light are indicated as histograms in Figure 4. The amount of carotenoid in the hypocotyls and 1st internodes of soybean seedlings grown under light showed an increase with descending concentration. However, this increase was found to be close to or below the control group. Carotenoid amount in the cotyledon showed an increase by 18% and 25% at 10^{-7} M and 10^{-9} M epi-BL, respectively.

Contents of chlorophyll a and b in the growing-in-dark seedlings are given in Figure 5 in which data belong to hypocotyl and cotyledon because of undeveloped 1st internode.

Figure 6 represents the results of carotenoid amount for the same seedlings. The chlorophyll a values for the hypocotyls of seedlings grown in dark were quite low at all three concentrations. However, chlorophyll b was above the control, and the most marked stimulating effect was observed at 10^{-5} M epi-BL. The chlorophyll a amount in the cotyledon showed an increase contrary to descending concentration; however this increase remained below the control. Chlorophyll b amount was found to be above the control with an increase of 59%, especially at 10^{-7} M epi-BL. As seen in Figure 6, carotenoid amount in the hypocotyls of the seedlings grown in dark was above the control in all three concentrations; however this was not a significant increase. As for the cotyledons, a marked increase (86%) was seen at 10^{-7} M epi-BL compared to the control.

DISCUSSION

BRs are a group of plant steroidal compounds which possess common biological activities. Recent genetic and biochemical studies focus on the roles of BRs in plant development. BRs are known to act in responds to several stress factors in the process of plant growth and development, such as cell division, elongation and differentiation, seed germination, vegetative

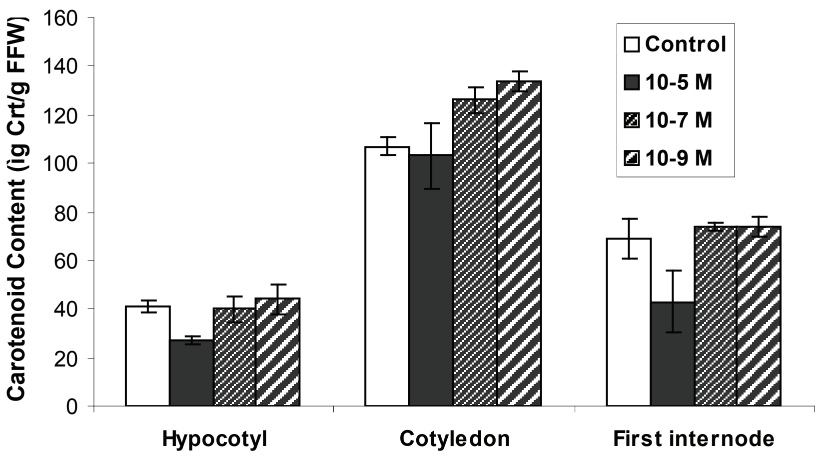

Figure 4. Carotenoid amounts by epi-BL concentrations in the seedlings grown in light.

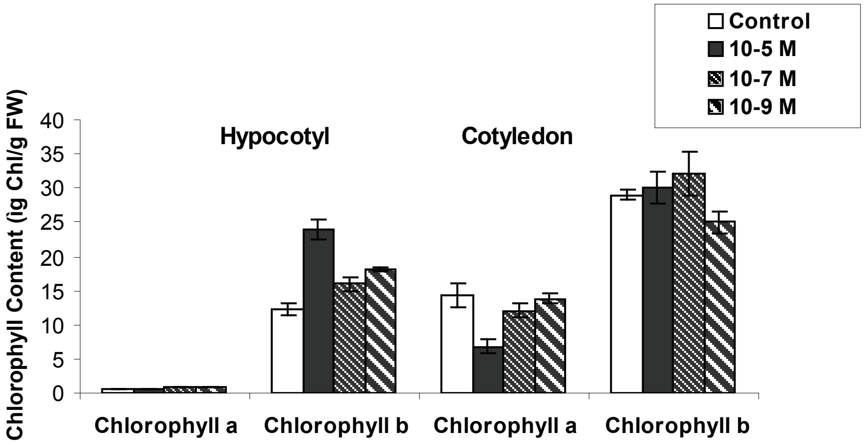

Figure 5. Contents of chlorophyll a and b by epi-BL concentrations in the seedlings grown in dark.

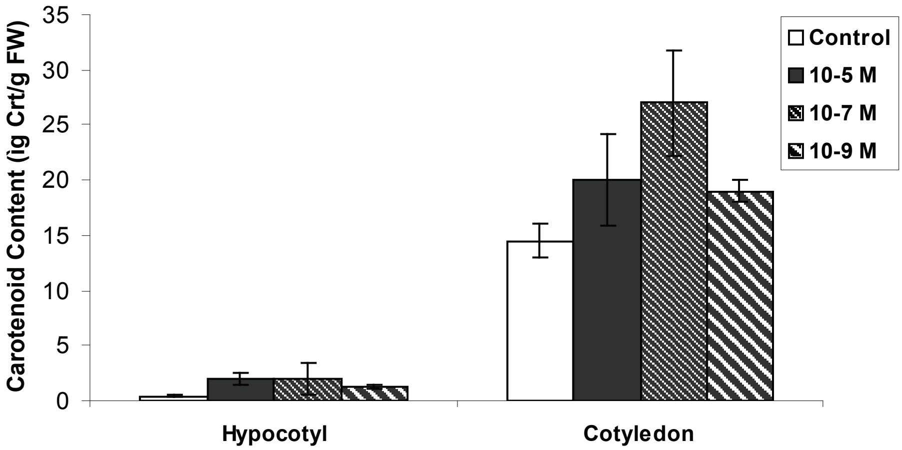

Figure 6. Carotenoid amounts by epi-BL concentrations in the seedlings grown in dark.

growth, apical dominancy, reproductive growth and senescence [5]. Light plays a role in some physiological events involving BRs [30]. Although light and BRs both influence on pigment biosynthesis and accumulation, the molecular mechanisms of their interactions are not understood. Thus, relationships between light, pigments and BRs are needed to be investigated since studies on this topic are quite few in current literature.

From the results of the present study, it is obvious that descending epi-BL concentrations result in marked changes in pigment contents of different parts of the soybean seedlings grown in dark and light. Anthocyanin amount was found to remain unchanged by descending epi-BL concentrations in the control cotyledons of the seedlings grown in light while it increased adversely in hypocotyl with applied concentrations (Figure 1). As to internode, a decrease was observed for the concentrations 10^{-5} and 10^{-7} M whereas the concentration 10^{-9} M resulted in an increase by 40% when compared to the control. Taking into consideration epi-BL as the only factor concerning anthocyanin synthesis in hypocotyl and cotyledon of the growing-in-dark seedlings, an increase in cotyledon and a decrease in hypocotyl for 10^{-9} M are quite intriguing (Figure 2). One may say from this finding that concentration influences anthocyanin synthesis and inhibition, and that epi-BL causes different responds in distinct plant parts, depending on light and dark conditions. Furthermore, it is reasonable to think that epi-BL replaces light in the process of anthocyanin biosynthesis under dark conditions.

Similar results were obtained for hypocotyl and internode as a conclusion of interaction of photosynthetic pigments chlorophyll a and b and accessory pigment carotenoid with epi-BL in light, whereas elevated amounts of those pigments were observed in cotyledon, 10^{-9} M epi-BL being the most effective concentration (Figure 3). On interpreting carotenoid results of the seedlings in light, the highest carotenoid amount was found in cotyledons, whereas it was close to the control in hypocotyl and 1st internode, and increased in cotyledon at 10^{-9} M after epi-BL treatment (Figure 4). Bajgus and Asami [31] also found epi-BL had a stimulatory effect on the photosynthetic pigments in *W. arrhiza* cultures, and BR at a concentration of 10^{-9} M increased the content of chlorophyll a and b and carotenoids. In addition, they have observed the greatest inhibitory effect on the pigment content at 10^{-4} M. However, we found the highest inhibitory effect on chlorophyll a and b content at 10^{-5} M. These results are parallel with the data which we obtained from this study.

With regard to dark condition, it was quite interesting that chlorophyll a content was very low in the control hypocotyl, epi-BL did not cause an increase, and chlorophyll b was elevated with descending concentrations. Concerning cotyledons of the seedlings grown in dark, both chlorophyll a and b contents of the control were higher in comparison with hypocotyl (Figure 5). Following epi-BL applications, chlorophyll a was gradually decreased to control levels, while chlorophyll b content was slightly increased at 10^{-7} M, and then decreased in the cotyledon. Our findings reveal that the presence of chlorophyll a is completely light-dependent [20]. When we look at the results of the growing-in-dark seedlings, carotenoid amount which was very low in the control hypocotyl appeared not to be affected by epi-BL, while it was markedly increased by 10^{-7} M epi-BL in the cotyledons (Figure 6).

In conclusion, one may put forward that exogenously applied epi-BL affects anthocyanin, chlorophyll and caroteniod production, and that varied concentrations of epi-BL result in different responds in plant parts, depending on light and dark factors. We confirm that the levels of anthocyanin, chlorophylls a and b and carotenoids are stimulated by epi-BL in soybean seedlings grown in light and dark, depending on low concentrations, especially.

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