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The effect of epibrassinolide on senescence in horizontal sunflower (*Helianthus annuus* L.) seedlings

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Abstract

This study examined the effect of epibrassinolide (eBL) on senescence occurring in cotyledons of sunflower (*Helianthus annuus* L.) seedlings, which were kept in vertical and horizontal positions. 10^{-11} M and 10^{-9} M eBL and 2,3,5-triiodobenzoic acid (TIBA), an inhibitor of auxin transport, were sprayed to the 14 days old seedlings. From the 15th day on, some of the seedlings from the control and the experimental groups were kept in a horizontal position, and senescence process of the cotyledons of these seedlings was observed. Applications of eBL (especially 10^{-9} M) were found to have induced senescence both in horizontally and vertically positioned plants. When it was applied with TIBA, a marked delay of senescence was noted in the lower cotyledons of the horizontally positioned plants. Total chlorophyll and protein amounts and peroxidase activity were determined. In case brassinosteroids are applied with TIBA, they do not affect senescence, implying that they accelerate senescence in the presence of auxin. Since auxin is distributed asymmetrically, eBL and auxin accumulated in the lower cotyledons may lead to accelerated senescence. This study showed that eBL and auxin may act as a senescence signal in the vertically and horizontally positioned seedlings, and earlier death of the lower cotyledons than the upper ones may be a result of asymmetrical auxin distribution. In this study, it was revealed that promoting effect of eBL on senescence occured only in the presence of auxin.

Keywords: eBL – Epibrassinolide, TIBA - 2,3,5-triiodobensoic acid, Auxin, Senescence, Sunflower (*Helianthus annuus* L.).

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Yatay konumdaki *Helianthus annuus* L. fidelerinde epibrassinolidin senesens üzerine etkisi

Özet

Bu çalışmada vertikal ve horizontal konumdaki *Helianthus annuus* L. fidelerinin kotiledonlarında meydana gelen senesens üzerine epibrassinolid (eBL) in etkisi incelendi. 14 günlük fidelere 10^{-9} M ile 10^{-11} M eBL ve oksin taşınma inhibitörü olan 2,3,5- triiyodobenzoik asit (TIBA) çözeltileri püskürtüldü. 15. günde kontrol ve deney grubu bitkilerinin bir kısmı yan yatırılıp horizontal konuma getirilerek bu bitkilerin kotiledonlarının senesens süreci takip edildi. eBL uygulamalarının (özellikle 10^{-9} M) vertikal ve horizontal konumdaki bitkilerde senesensi teşvik ettiği, TIBA ile birlikte uygulanması halinde ise özellikle horizontal konumdaki bitkilerin alt kotiledonlarında senesensin geciktiği gözlendi. Bu gözlemler total klorofil ve protein içerikleri ile peroksidaz aktivitesi ölçülerek desteklendi. Brassinosteroidler, TIBA (oksin taşınma inhibitörü) ile beraber uygulanması durumunda senesens üzerine tek başına etki etmeyip ancak oksin varlığında senesensi hızlandırdı. Yatay durumda bırakılan fidelerin gövdelerinde asimetrik dağılım gösteren ve alt kotiledonda daha fazla biriken oksin eBL ile birlikte senesensi hızlandırabilmektedir. Bu araştırmada, vertikal ve horizontal durumda bırakılan fidelerde, eBL ve oksinin birlikte senesens sinyali gibi davranabileceği, alt kotiledonun üst kotiledondan önce ölmesinin asimetrik oksin dağılımından kaynaklanabileceği gösterildi. Bu çalışmada,eBL'nin senesens üzerine teşvik edici etkisinin ancak oksin varlığında gerçekleştiği ortaya konmuştur.

Anahtar Kelimeler: eBL-Epibrassinolide, TIBA-2,3,5-triiyodobenzoik asit, Oksin, Senesens, Ayçiçeği (*Helianthus annuus* L.).

Introduction

Brassinosteroids (BRs) are a group of steroidal plant hormones with significant growth promoting activity (Zhao and Li 2012). They are required for normal plant growth and development, and are polyhydroxylated sterol derivatives with structural similarity to animal steroid hormones (Yokota 1997; Clouse 2001; Mussig and Altmann 2001; Clouse 2002; Verma et al. 2012). The brassinosteroids are not necessarily biologically active. Brassinolide, 24-epibrassinolide and 24-homobrassinolide are biologically active brassinosteroids and commonly employed in physiological studies (Rao et al. 2002). BRs occur at very low concentrations (nanogram) in plant parts including pollens, seeds, leaves, stem, root and flowers (Fujioka 1999). In addition to inducing plant growth, they play a role in several developmental processes like seed germination, rhizogenesis, flowering, senescence, abscission and maturation, and enhance resistance against different abiotic stresses (Mussig and Altmann 1999; Rao et al. 2002; Michelini et al. 2004; Sharma et al. 2011; Jiang et al. 2012; Vardhini et al. 2012; Vleesschauwer et al. 2012).

Senescence, which is a normal phase of plant development like growth and reproduction, is a destructive process leading to death of cells, organs or the whole plant during the lifecycle (Noodėn and Penney 2001; Breeze et al. 2011) and is regulated by a genetic program (Srivastava 2002). Senescence process is a cascade of metabolic pathways controlled by several endogenous and exogenous factors (Nam 1997). Age, reproductive development and phytohormones level are endogenous factors which affect senescence (Gan and Amasino 1997; Ansari et al. 2005). However, environmental conditions induce senescence, by resulting in its early occurrence. Although it is considered as a genetically controlled process, initiation and rate of senescence was reported to be affected by environmental and endogenous factors in a sunflower study (Sadras et al. 2000).

Gravity substantially influences physiological processes in a plant lifecycle. Gravitropism is the directional movement of a plant in response to the stimulus of gravity. Also, many researchers reported that BRs play an important crucial role in regulating the processes of senescence (Khripach et al. 2000; He et al. 2001; Rao et al. 2002; Srivastava 2002; Nemhauser and Chory 2004; Zhu et al. 2012). Gravitropic responses induced by BRs seem to be similar to those induced by auxin. Some auxin-induced responses are synergistically enhanced by BRs, indicating that both hormones are interactive in some aspects of plant growth and development as in gravitropism (Yopp et al. 1981; Takeno and Pharis 1982; Cohen and Meudt 1983; Katsumi 1985; Eun et al. 1989; Kim et al. 1990; Fujioka et al. 1998; Sasse 1999). As plants have acquired the ability to use gravity for regulating their growth and development, physiological processes in their lifecycle are highly influenced by gravity. Role of gravity in physiological pathway of senescence is not fully understood. Sağlam and Okatan (1990) observed that lower cotyledons of the seedlings grown in a horizontal position underwent senescence earlier than upper cotyledons did. Another research group also reported that cotyledon senescence was delayed in sunflower seedlings which were grown in horizontal clinostat (Ünal and Okatan 1996). Positive and negative gravitropism are believed to be mediated by an auxin gradient caused by a redistribution of auxin across the organs (Chen et al. 1999). Indeed, Ünal and Okatan (1996) put forward that delayed senescence is due to slow auxin transport.

The aim of this study is to investigate the effects of epibrassinolide on senescence, to get insight about the mechanism. This research has been revealed that eBL reported by many researchers working synergistically with auxin, promotes senescence in both vertical and horizontal cotyledons of *Helianthus annuus* seedlings; this action occurs only in the presence of auxin. The data obtained are important with regard to serve as a source for studies related senescence. In addition, increasing of yield of the fruit provided that delaying senescence is very important, for use of data in agriculture to benefit economically.

Materials and methods

Plant material and hormone treatments

Epibrassinolide (eBL) (Sigma-E 1641) (dissolved in ethanol) was used in this study. Sunflower (*Helianthus annuus* L. AS 6310) seedlings were grown in a growth chamber (12 h light period) under white fluorescent light providing a light intensity of 300 μmol m^{-2} s^{-1}, under day/night temperature of 25±2°C and 65±5% relative humidity. Each treatment was analyzed with at least three replicate. Cotyledons were bulked as at least 20 plants. A part of seedlings (experimental groups) were applied eBL treatments from the 14th day. After hypocotyl elongation was ceased (15th day), some of the seedlings both in the control (untreated) and the experimental (treated) groups were located in the horizontal position while others were placed vertically. Seedlings of sunflower were applied eBL (10^{-11} and 10^{-9} M) consisting of biologically active brassinosteroid and 2,3,5-triiodobenzoic acid (TIBA) (Sigma) (100 mg/l) (Okatan and Ünal 1996), which is an inhibitor of polar auxin transport. 10^{-11} M eBL and 10^{-9} M eBL alone were sprayed to seedlings of both horizontal and vertical experimental groups 14th from the day. eBL concentrations were sprayed to aboveground parts of the seedlings every other day. 10^{-9} M eBL+TIBA and 10^{-11} M eBL+TIBA experimental series were sprayed alternately, with TIBA one day, and 10^{-9} M or 10^{-11} M the other day. eBL and eBL together with TIBA (eBL+TIBA) treatments were continued for 9 days. When the avareage green areas of cotyledons of vertical control seedlings reached 50% (23rd day) cotyledons of control and experimental groups in vertical and horizontal position were harvested, and biochemical analyses were conducted.

Measurement of total chlorophyll amount

Total chlorophyll amount was determined according to Arnon (1949). Cotyledons were extracted in 80% acetone. The samples were then centrifuged (Heraeus Labofuge 400 R) at 3000 *g* (4°C) for 10 min. Supernatants were spectrophotometrically measured (Shimadzu 1601) and were put in their places in the Arnon formula and total chlorophyll amount was calculated as mg chlorophyll per cotyledon.

Measurement of soluble protein amount

For the quantitative determination of total soluble protein amount Bradford's (1976) Dye-binding method was employed. The obtained absorption values were calculated according to bovine serum albumin (BSA) protein standard which has been previously prepared and the amount of total protein was estimated as μg/cotyledon.

Determination of peroxidase activity

Peroxidase activity (POD) was determined by employing the method of Birecka et al. (1973). The reaction mixture consisted of 0.1 M sodium phosphate buffer (pH 7.0), 0.25% guaiacol, 0.1% H_2O_2 and enzyme extract. The absorbance of the colored product in the extract was recorded every 10 seconds for 2 min. at 470 nm in spectrophotometer (Shimadzu 1601), and the peroxidase activity was quantitatively provided as ΔA/g fresh weight minutes with the spectral method.

Statistical analysis

Each treatment was analyzed with at least three replicate tissue samples bulked at least 10 plants. Data were analyzed by the analysis of Student's t-Tests. Bars and "±" represented the standard errors. Comparison with $P<0.05$ and $P<0.0001$ were considered significantly different.

Results

Figure 1 shows the total chlorophyll amounts of the cotyledons of vertically placed seedlings. While total chlorophyll amounts of the cotyledons of seedlings applied with 10^{-9} M eBL decreased by 34% compared to the control ($p<0.0001$), decrease in the cotyledons applied with 10^{-11} M eBL was 28% ($p<0.0001$). TIBA was sprayed to seedlings along with eBL in order to reveal action of eBL which is known to work with IAA synergistically. We were determined that 10^{-11} M and 10^{-9} M eBL+TIBA application prevented chlorophyll destruction compared to the control ($p>0.05$ and $p<0.05$, respectively).

Total chlorophyll amounts of the control and experimental cotyledons of the seedlings in horizontal position are indicated in Figure 2. No marked difference was observed between the chlorophyll amounts of the cotyledons of the vertical seedlings and the lower or upper cotyledons of horizontal control seedlings ($p>0.05$). However, the difference was more pronounced in the lower cotyledons of the 10^{-9} M and 10^{-11} M eBL-treated seedlings in comparison to the cotyledons of the vertical seedlings (15% and 12%, respectively) ($p<0,05$ and $p<0.05$, respectively). 10^{-11} M or 10^{-9} M eBL+TIBA application did not result in a difference between the upper cotyledons of the horizontal control and the experimental seedlings ($p>0.05$), but it led to a markedly delayed senescence in the lower cotyledons. The highest delay (37%) was seen in the lower cotyledons of the seedlings applied with 10^{-9} M eBL+TIBA ($p<0.0001$).

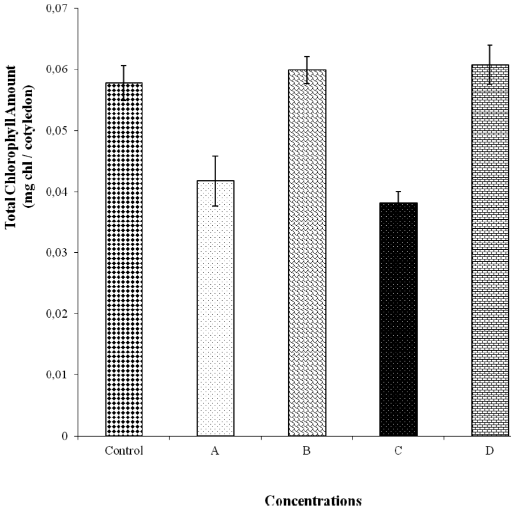

Figure 1. Total chlorophyll amounts of the cotyledons of vertically placed seedlings. Control, **A:** 10^{-11} M eBL, **B:** 10^{-11} M eBL+TIBA, **C:** 10^{-9} M eBL, **D:** 10^{-9} M eBL+TIBA. Bars represent the standart errors.

Figure 3 represents the amounts of total proteins in the cotyledons from the vertically grown seedlings. Total protein amount decreased by 14% in the cotyledons of the 10^{-9} M eBL-applied seedlings ($p<0.0001$), whereas it did not show a significant difference (5%) in the cotyledons treated with 10^{-11} M eBL compared to the controls ($p>0.05$). Additionally, 10^{-11} M or 10^{-9} M eBL+TIBA application did not give rise to any difference compared to the control ($p>0.05$).

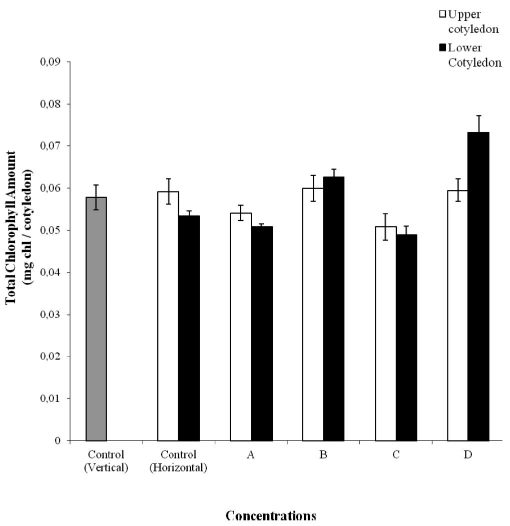

Figure 2. Total chlorophyll amounts of the cotyledons of horizontally placed seedlings. **A:** 10^{-11} M eBL, **B:** 10^{-11} M eBL+TIBA, **C:** 10^{-9} M eBL, **D:** 10^{-9} M eBL+TIBA. Bars represent the standart errors.

Concerning the horizontal seedlings, data are presented in Figure 4 While there was no difference between the cotyledons of the vertical control seedlings and the upper cotyledons of horizontal control seedlings ($p>0.05$), a decrease of 10% was seen in the protein amounts of the lower cotyledons ($p<0.05$). However, when the cotyledons of the vertical seedlings were compared with the cotyledons of the 10^{-11} M or 10^{-9} M eBL-treated horizontal seedlings it was seen that the lower cotyledons had less protein content ($p<0.05$). No difference was seen between the protein amount in the upper cotyledons of the horizontal seedlings applied with 10^{-11} M or 10^{-9} M eBL+TIBA the upper cotyledons of the horizontal control seedlings ($p>0.05$). However, it is quite remarkable that the protein amount of the lower cotyledons of the horizontal seedlings applied with 10^{-9} M eBL+TIBA was 21% higher than the lower cotyledons of the horizontal control seedlings ($p<0.05$).

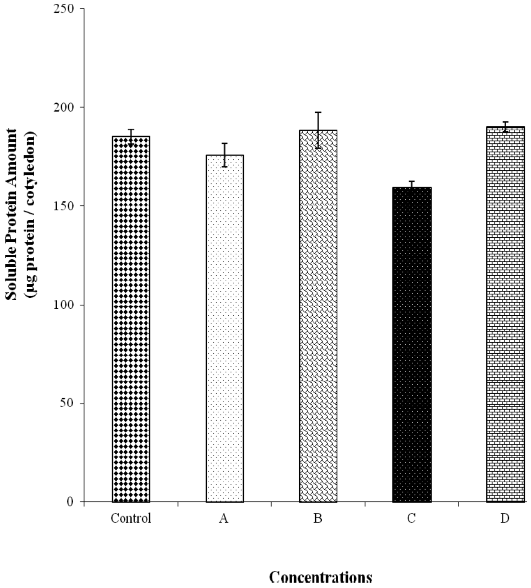

Figure 3. Total protein amounts of the cotyledons of vertically placed seedlings. **A:** 10^{-11} M eBL, **B:** 10^{-11} M eBL+TIBA, **C:** 10^{-9} M eBL, **D:** 10^{-9} M eBL+TIBA. Bars represent the standart errors.

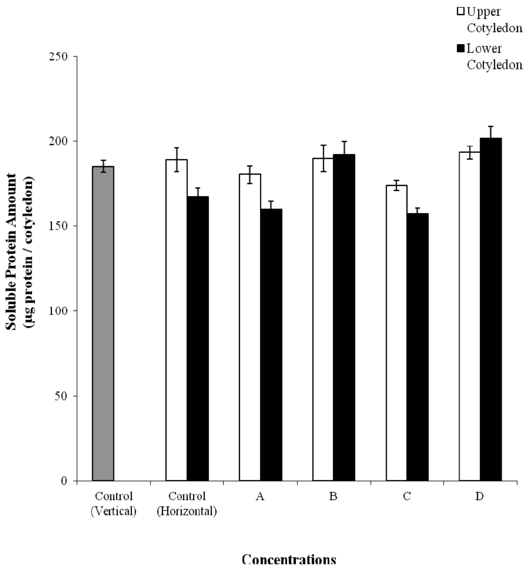

Figure 4. Total protein amounts of the cotyledons of horizontally placed seedlings. **A:** 10^{-11} M eBL, **B:** 10^{-11} M eBL+TIBA, **C:** 10^{-9} M eBL, **D:** 10^{-9} M eBL+TIBA. Bars represent the standart errors.

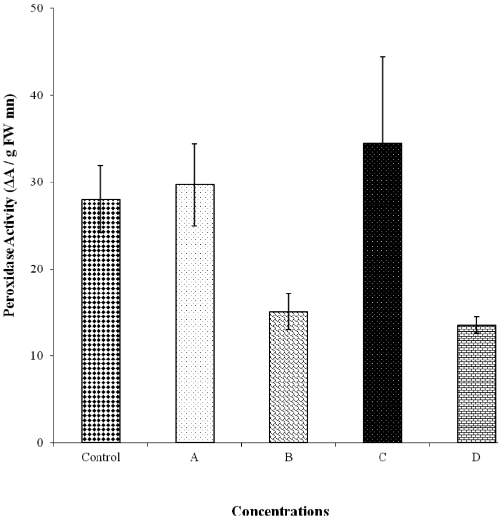

Figure 5. Peroxidase (POD) activity of the cotyledons of vertically placed seedlings. **A:** 10^{-11} M eBL, **B:** 10^{-11} M eBL+TIBA, **C:** 10^{-9} M eBL, **D:** 10^{-9} M eBL+TIBA. Bars represent the standart errors.

Figure 5 contains the values of peroxidase (POD) activity of the cotyledons of the vertical seedlings. POD activity was the highest (more than 23%) in the cotyledons of the seedlings treated with 10^{-9} M eBL when compared with the control ($p<0.05$). Application of 10^{-9} M eBL+TIBA gave rise to a significant decrease (mean: 49%) in POD activity in comparison with the control ($p<0.05$).

POD activities of the cotyledons from the horizontal seedlings were also determined and data in question were presented in Fig. 6. On comparing POD activities of the cotyledons from the vertical and horizontal control seedlings, it appeared that the activity had decreased by 43% in the upper cotyledons of the horizontal control seedlings ($p<0.05$) while it had increased by 12% in the lower cotyledons ($p<0.05$). An increase of 6% in POD activity was reported from the lower cotyledons of the horizontal seedlings applied with 10^{-9} M eBL compared to the lower cotyledons of the horizontal control seedlings ($p>0.05$). Increased POD activity was significantly higher (84%) in the upper cotyledons from the horizontal experimental group (10^{-9} M eBL) compared to the upper cotyledons of the horizontal control seedlings ($p<0.05$). In both eBL concentrations, it was noteworthy that a nearly equal decrease was observed among the lower or upper cotyledons of horizontal seedlings applied with eBL+TIBA compared to the cotyledons of the seedlings applied with eBL alone. Similarly, TIBA caused a decreased POD activity in the lower cotyledons.

Discussion

Senescence occurring in the cotyledons of the vertically positioned sunflower seedlings to which eBL (10^{-11} M and 10^{-9} M) and eBL+TIBA (10^{-11} M or 10^{-9} M+100 mg/l) were applied was time-dependent. The cotyledons of the eBL-applied vertical plants showed senescence which started and finished earlier than the vertical control plants. On the other hand, cotyledons of vertical plants which applied with 10^{-11} M or 10^{-9} M eBL+TIBA exhibited delayed senescence (Fig. 1,3,5).

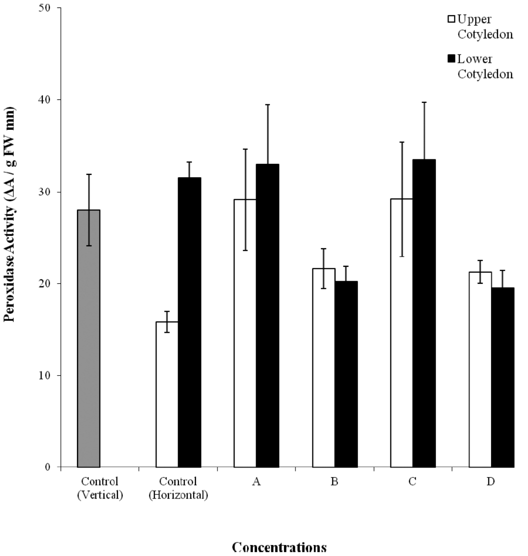

Figure 6. Peroxidase (POD) activity of the cotyledons of horizontally placed seedlings. **A:** 10^{-11} M eBL, **B:** 10^{-11} M eBL+TIBA, **C:** 10^{-9} M eBL, **D:** 10^{-9} M eBL+TIBA. Bars represent the standart errors.

In the our previous studies, it was thought that senescence signal is a substance which may be of IAA nature or acts like IAA (Sağlam-Çağ 1997; Gören and Çağ 2007). Although we know that steroidal eBL acts together with IAA (Cohen and Meudt 1983; Katsumi 1985; Eun et al. 1989; Fujioka et al. 1998; Sasse 1999; Mussig and Altmann 2003; Minorsky 2004), there are some researchers who suggest actions of BRs which are independent of auxin (Clouse 2002; Tanaka et al. 2003). In the cotyledons of the vertical seedlings applied with 10^{-11} M or 10^{-9} M eBL, total chlorophyll and protein amounts were observed to be decreased (Fig. 1 and 3) together with accelerated senescence, while POD activity was observed to be increased (Fig. 5). These results are confirmed by some studies in which chlorophyll and protein amounts were seen to be decreased during senescence (Sağlam-Çağ 2007; Gören and Çağ 2007; Zhang et al. 2012). It was claimed from these studies that decreased protein may be related to proteins in senescence signaling or elevated enzyme synthesis (Thayer et al. 1987; Sağlam-Çağ 2007). There are several reports showing that POD activity increases during the senescence of excised leaves and whole plants (He et al. 1996; Kanazawa et al. 2000; Hung and Kao 2004). POD activity significantly increased in the cotyledons of the eBL-treated vertical seedlings compared to the control and the eBL+TIBA-treated vertical seedlings. In this study, in the vertical seedlings applied with eBL, auxin transport was inhibited by TIBA application, total chlorophyll and protein amounts were observed to be increased together with delayed senescence, while POD activity was observed to be decreased. These results reveal that eBL possesses no action when auxin transport is prevented. Moreover, these results showed that there was a relation between chlorophyll amount and POD activity. Parish (1968) suggested that the increase in the activity of POD is one of the most reliable indicators of maturity and senescence. It seems possible that POD has functional significance during the senescence process of cotyledons of seedlings. The stems of horizontal seedlings exert negative gravitropism since auxin is transported to the lower side of the seedlings under unidirectional gravity (Van Overbeek et al. 1945; Filner and Hertel 1970; Parker and Briggs 1990; Park 1998; Leyser and Day 2003). In addition, BL induces gravitropic blend, an IAA-leading response (Kim et al. 2000). The present study reveals that eBL did not induce senescence in the case of auxin deficiency since auxin which was transported to the lower side accelerated senescence in the lower cotyledons in the presence of 10^{-9} M eBL, while application of 10^{-9} M eBL+TIBA delayed it (Fig. 2,4,6). In other words, eBL alone does not induce senescence, and auxin is needed for senescence activation. This conclusion is in compliance with some studies (Cohen and Meudt 1983; Katsumi 1985; Eun et al. 1989; Fujioka et al. 1998; Sasse 1999; Mussig and Altmann 2003; Minorsky 2004), although there are some opposite studies (Bara 1977; Clouse 2002). Based on these data, the earlier senescence of the lower cotyledons of the horizontal seedlings compared to the upper cotyledons and the cotyledons of the control seedlings can be explained by interaction of eBL with accumulated auxin in that cotyledon. Yet, application of eBL one day before the seedlings were horizontally positioned, especially senescence occurring in the upper cotyledons of the 10^{-9} M eBL-applied experimental seedlings earlier than the upper cotyledons of the horizontal controls, strengthens the possibility of interaction of eBL, also influencing the upper cotyledon, with auxin. Chlorophyll loss was found to increase during senescence occurring in the cotyledons of vertical and horizontal experimental seedlings applied with eBL (10^{-11} M or 10^{-9} M) compared to the vertical and horizontal control seedlings, respectively (Fig. 1 and 2). It seems possible that eBL causes increased chlorophyll destruction in the presence of auxin. Studies by Li et al. (1996) and Vardhini and Rao (2002) indicate that eBL induces chlorophyll destruction. Also, 10 mM eBL was shown to accelerate senescence, and to induce chlorophyll destruction in wheat leaf segments by Sağlam-Çağ (2007). On the other hand, in several investigations it was also observed that EBL had an increasing effect on the chlorophyll content of plants grown in different stress conditions (Ali et al. 2008; Yusuf et al. 2012).

This study also investigated changes in protein amounts, one of the important senescence parameters. As seen in Fig. 3 and Fig. 4, total protein amounts were seen to be decreased in eBL treated seedlings during senescence while eBL+TIBA application resulted in increased protein amount together with delayed senescence. The lower cotyledons, which underwent early senescence, of the horizontal experimental plants applied with eBL (10^{-11} M or 10^{-9} M) contain less protein amount than the cotyledons of the vertical control plants and the lower cotyledons of the horizontal control plants (Fig. 4). Effect of gravitropism on protein amount was examined by several authors (Bara 1977; Oputa and Mazelis 1977). For example, Bara (1977) reported no increase in total protein amount. While some proteins are continually synthesized during senescence, some are destroyed. The balance between these two processes varies depending on the growth phase of the plant such as senescence, and internal and external factors. It is also a known fact that proteins decrease by age in tissues (Anderson and Rowan 1965). The plant initiates synthesis of proteolytic enzymes before it enters the senescence phase (Martin and Thimann 1972; Drivdahl and Thimann 1977). However, protein synthesis is needed for senescence signal to occur (Thayer et al. 1987). This study indicates that POD activities increase during senescence process. POD activities of the lower cotyledons undergoing early senescence of the horizontal experimental plants applied with eBL are higher than those of the vertical control plants and the upper cotyledons of the horizontal control plants. eBL combined with TIBA eliminates eBL action, and decreases POD activity. There is a negative correlation between POD activity and total protein and chlorophyll amounts. These results are in compliance with some studies reporting increased POD activities during senescence (He et al. 1996; Kanazawa et al. 2000; Sağlam-Çağ 2007). A system which perceives gravity and converts it to physiological changes exists in plants (Ünal 1991). From the studies investigating hormonal regulation of gravitropism, it is known that auxin increases in the bottom side of the stem of horizontally positioned plants, while it decreases in the top side, and the stem grows blending upward (Leyser and Day 2003). Similarly, Sağlam and Okatan (1990) reported earlier and quicker senescence in lower cotyledons in their study with horizontal sunflower seedlings. They explained their finding with asymmetric auxin dispersal due to gravity. Park (1998) also showed increased auxin in the bottom side of horizontal seedlings, while it is observed to be decreased in the top side, and the stem grows blending upward. We know that BR synergistically enhances some of the auxin-induced responses are, and these two hormones interact with each other during the growth phase of plants (Katsumi 1985; Eun et al. 1989; Kim et al. 1990; Fujioka et al. 1998; Minorsky 2004; Mussig and Altmann 2003; Sasse 1999, Walcher and Nemhauser 2012). In fact, Meudt (1987) concluded that BR application improves gravitropic response of bean hypocotyls. Also, the fact that gravitropic response of tomato hypocotyls increases by BR (Park 1998) supports the possibility that BRs play a role in the regulation of stem gravitropism. Gravitropic blending induced by BL is suggested to occur because of IAA which interacts with BL (Kim et al. 2000). Gravitropic response is also inhibited by TIBA, an inhibitor of polar auxin transport.

In this research, the early senescence occurring in the lower cotyledons of eBL-applied horizontal experimental seedlings compared to the upper cotyledons and the quite delayed senescence occurring in the lower cotyledons in contrast to the case in which eBL applied with TIBA and the earlier senescence occurring in the upper cotyledons imply that BRs are involved in an auxin-mediated process for gravitropic response. In fact, BR levels were found to be increased in gravitropic response resulted from IAA (Park 1998). Application of TIBA to the horizontal seedlings delayed senescence in the lower cotyledons in the absence of auxin, and these results are contrary to the literature suggesting that auxin cause delayed senescence (Baker 1983; Srivastava 2002). At the same time, delaying of senescence in lower cotyledons in this plants taking into account the asymmetrical distribution of eBL has been revealed that epibrassinolide alone does not promote senescence in the absence of auxin, on the contrary it delays senescence. The data obtained are in agreement with studies on delaying senescence by eBL (Srivastava 2002).

In this study, it was revealed that promoting effect of eBL on senescence occured only in the presence of auxin.

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