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Research Article/Araştırma Makalesi

The Effect of 24–Epibrassinolide on vegetative growth of Sweet Ann strawberry seedling under lime stress conditions

24–Epibrassinolide'in Kireç Stresi Koşullarında Sweet Ann Çilek Fidelerinin Vejetatif Büyümesi Üzerine Etkisi

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ABSTRACT

The research was conducted in 2022-2023 in Yozgat. The study was set up to determine the responses of the Sweet Ann strawberry variety to different lime levels and the effects of 24-Epibrassinolide (24-eBL) applications on vegetative growth. It was observed that plant growth and development were negatively affected by the increase in lime doses. It was found that 24-eBL applications (BR) increased leaf and root fresh weight, iron and zinc uptake in calcareous conditions. In terms of leaf fresh weight, it was found to be higher in 0% Lime x 0 mg l^{-1} BR, 0% Lime x 1 mg l^{-1} BR, 0% Lime x 2 mg l^{-1} BR and 5% Lime x 0 mg l^{-1} BR applications compared to other applications, and it was determined that they were statistically in the same group. Regarding leaf area, the highest leaf area in the Lime x BR interaction was determined as 32.13 cm^2 in the 0% lime x 0 mg l^{-1} BR combination and 33.60 cm^2 in the 0% lime x 1 mg l^{-1} BR combination. Leaf chlorophyll content (SPAD) was statistically highest in 0% lime x 1 mg l^{-1} BR, 5% lime x 0 mg l^{-1} BR and 10% lime x 0 mg l^{-1} BR combinations. The highest stoma conductivity values were observed from 0% lime x 0 mg l^{-1} BR and 0% lime x 1 mg l^{-1} BR applications. Considering the lipid peroxidation (MDA) gave statistically significant the highest values 10% lime x 2 mg l^{-1} BR, 5% lime x 1 mg l^{-1} BR and 5% lime x 0 mg l^{-1} BR applications. While the highest N (%) and P (%) contents in the leaves were measured in the 0% lime x 0 mg l^{-1} BR application, the highest K (%) content was detected in the 5% lime x 1 mg l^{-1} BR application.

Key Words: Strawberry, lime, Brassinosteroid, vegetative growth, nutrient elements

ÖZ

Araştırma 2022-2023 yıllarında Yozgat'ta yürütülmüştür. Çalışma, Sweet Ann çilek çeşidinin farklı kireç seviyelerine ve 24-Epibrassinolide (BR) uygulamalarının vejetatif büyüme üzerine etkilerini belirlemek amacıyla kurulmuştur. Bitki büyüme ve gelişmesinin kireç dozlarındaki artıştan olumsuz etkilendiği görülmüştür. Kireçli koşullarda BR uygulamalarının yaprak ve kök taze ağırlığını, demir ve çinko alımını artırdığı tespit edilmiştir. Yaprak yaş ağırlığı en yüksek %0 Kireç x 1 mg l^{-1} BR, %0 Kireç x 0 mg l^{-1} BR, %0 Kireç x 2 mg l^{-1} BR ve %5 Kireç x 0 mg l^{-1} BR uygulamalarında belirlenmiştir. Yaprak alanı bakımından, Kireç x BR etkileşiminde en yüksek yaprak alanı 32,13 cm^2 ile %0 kireç x 0 mg l^{-1} BR ve 33,60 cm^2 ile %0 kireç x 1 mg l^{-1} BR kombinasyonunda belirlenmiştir. Yaprak klorofil içeriği SPAD değeri açısından incelendiğinde, Kireç x BR etkileşimi %0 kireç x 1 mg l^{-1} BR, %5 kireç x 0 mg l^{-1} BR ve %10 kireç x 0 mg l^{-1} BR kombinasyonlarında istatistiksel olarak en yüksek bulunmuştur. Stoma iletkenliği en yüksek değerleri %0 kireç x 0 mg l^{-1} BR ve %0 kireç x 1 mg l^{-1} BR uygulamalarından elde edilmiştir. Kireç x BR interaksiyonu bakımından lipid peroksidasyon (MDA) değerine bakıldığında, istatistiksel olarak en yüksek değerleri %10 kireç x 2 mg l^{-1} BR, %5 kireç x 1 mg l^{-1} BR ve %5 kireç x 0 mg l^{-1} BR uygulamalarında tespit edilmiştir. Yapraklarda N (%) ve P (%) içeriği en yüksek 0% kireç x 0 mg l^{-1} BR uygulamasında ölçülürken en yüksek K (%) içeriği 5% Lime x 1 mg l^{-1} BR uygulamasında tespit edilmiştir.

Anahtar Kelimeler: Çilek, kireç, Brassinosteroid, vejetatif büyüme, besin elementleri

Introduction

The cultivated strawberry (Fragaria x ananassa L.) is a member to the Rosaceae family of the Rosales order and is included in the genus Fragaria. It is a fruit consumed with pleasure by many people in the world and its cultivation is carried out in very different ecologies. Strawberries are cultivated in northern European countries, countries close to the equator and countries with temperate and subtropical climates between these two areas (Pio et al., 2019). The areas with the highest strawberry production in our country and in the world have a temperate and subtropical climate between 28° and 60° latitude (Ağaoğlu, 2013; Türemiş and Ağaoğlu, 2013).

Strawberries can be grown in areas with an annual rainfall of 250 mm, in cold areas with an altitude of up to 3500 m, in subtropical climates, even in the Arctic, where it is constantly bright during the summer months, provided that it is irrigated (Türemiş and Ağaoğlu, 2013). Although strawberry plants can be grown in a wide range of temperatures, cultivated species are damaged at temperatures below -5°C. When the temperature drops further, serious damage occurs (Warmund, 1993). Optimum temperatures for strawberries are between 20 and 26°C. Growth and development slow down at temperatures below 20°C and stop at temperatures higher than 35°C (Galletta and Bringhurst, 1990). The effect of climatic characteristics on plant growth and development is very important. Fall and winter climatic conditions have a great influence on their development in spring (Kronenberg et al., 1976). When strawberries are exposed to low temperatures in winter, deficiency in flower formation or fewer flowers are observed in the spring (Guttridge, 1958). Covered cultivation with or without heating is practiced to reduce cold damage. In this way, earliness is also ensured (Hancock and Simpson, 1995).

According to FAOSTAT 2022 data, world strawberry production is 9.569.865 tons and this production is carried out on approximately 3.976.030 decares of land. Strawberry production in our country increased from 669.195 tons in the 2021 production season to 728.112 tons in 2022. China ranked first in the world strawberry cultivation by producing 3.354.804 tons on an area of 1.267.770 da, while the USA ranked 2nd by producing 1.261.890 tons of strawberries on an area of 212.870 da. Türkiye ranked 3rd by producing 728.112 tons of strawberries on an area of 222.720 da. Egypt ranked fourth with 637.842 tons of production and Mexico ranked fifth with 568.272 tons of production (FAOSTAT, 2024).

Half of the cultivated agricultural areas in the world are located in arid and semi-arid regions and ¼ of these are composed of calcareous soils (Bates, 1982). It is known that the lime content of the soils of our country is high except for the Eastern Black Sea Region. High lime and pH cause damages by preventing the uptake of some nutrients such as phosphorus, iron and zinc by the plant. It is not possible to remove this stress caused by lime. As a solution, it is tried to reduce the pH of the soil by using sulfur preparations, but this requires a period of time and is not applicable in large areas. Therefore, there is a need for alternatives, simple-to-use, transferable to practice, and non-harmful to human health alternatives and approaches to provide stress resistance in plants (Koç, 2022; Çetin and Koç, 2023).

Until the first half of the 1900s, it was believed that plant hormones consisted of five classes including auxin, cytokinin, gibberellin, ethylene and abscisic acid. However, in recent studies, some compounds such as brassinosteroids, jasmonates, nitric oxide and salicylic acid, which are synthesized by plants and are determined to play very important vital roles in the plant structure, have been added to the category of plant hormones (Koç, 2022; Çetin and Koç, 2023). It is recognized that plants develop some specific mechanisms to survive under stress conditions and that internal hormones play an extremely important role during this period by increasing or decreasing. Especially these substances, called 'new generation hormones', stand out. Some of these include plant steroids, which comprise more than 70 compounds with structural similarities to insect, animal and human steroid hormones. Brassinosteroids (BRs), a new class of plant hormones, are a specific group of plant steroids commonly found in plants. At very low concentrations, BRs have been found to regulate plant growth and development such as cell division, elongation and expansion, photomorphogenesis, reproductive organ development, leaf senescence, total biomass and yield increase, as well as adaptation to environmental stresses (Surgun et al. 2012).

Brassinosteroids are involved in root growth and development together with several auxin signaling genes. Brassinosteroids increase primary root outgrowth at small doses and supress it at higher doses (Mussig et al., 2003). They control lateral root growth by auxin (Nemhauser et al., 2004; Bao et al., 2004). External application of BRs at the root formation stage increased root elongation in wild plants (Mussig et al., 2003). High lime levels cause severe chlorosis in strawberries and significantly reduce plant growth and yield (Balci, 2021).

This experiment was conducted to determine the responses of Sweet Ann strawberry cultivar to different lime levels and the effects of 24-epibrassinolide (24-eBL) applications on vegetative growth.

Materials and Methods

Material

This research was conducted between 2022-2023 in the research application greenhouse of Yozgat Bozok University, Faculty of Agriculture, and Department of Horticulture. Frigo seedlings of Sweet Ann variety were used in the experiment. Sweet Ann strawberry variety is a neutral day plant and has round, conical shaped, large, hard, bright red and sweet fruits (Noğay, 2017). These seedlings were planted in 2 liter pots filled with peat:perlite mixed at a ratio of 1:1 and slacked lime (CaO) was added at different rates (0, 5, and 10%). When the seedlings had 4 leaves and 15 days after the first application, three different doses (0, 1 and 2 mg l^{-1}) of 24-eBL were applied.

Seedlings in pots were watered with ½ Hoagland solution. The content of Hoagland solution used in plant nutrition is given in Table 1 (Gül, 2019).

Table 1. Content of fertilizer used in plant nutrition.

Nutrient	mg l^{-1}
N	210
P	40
K	250
Ca	150
Mg	50
Fe	2
Mn	0.75
Cu	0.50
Zn	0.40
B	0.10
Mo	0.05

Method

A 1:1 mixture of peat:perlite was used as a growing medium. Lime was added to this medium at 0%, 5%, and 10% by weight ($w.w^{-1}$). Frigo seedlings of Sweet Ann variety were planted in 2 liter pots on 28.05.2022. Four lime doses and three 24-eBL concentrations were used in the study. Strawberry plants were irrigated three times a week for 10 weeks after planting and once a week with ½ Hoagland solution. In addition, the normal irrigation program was continued and cultural maintenance of the plants was maintained throughout the study.

Approximately 25 days after transplanting, when the first 4 leaves of the seedlings reached full size, three different concentrations (0, 1 and 2 mg l^{-1}) of 24-eBL were sprayed on the above-ground organs of the plants. The second application of 24-eBL was made 15 days after the first application by spraying the leaves at the same rate and in the same way.

This research was completed 10 weeks after planting in order to determine the effects of BR applications on vegetative growth of strawberry seedlings in calcareous environments. Leaf, stem and root fresh and dry weight (g), number of leaves per plant (pcs), leaf area (ADC BioScientific Area Meter AM300, cm^2), leaf color (L, a, b value Minolta CR 400), leaf chlorophyll value were determined in three replications with 3 leaves in each replicate (Konica Minolta SPAD-502 Plus, Chlorophyll Meter, SPAD), anthocyanin content of the leaves was measured with three replicates and 3 leaves in each replicate (Opti Science ACM-200 Plus, Anthocyanin Meter, ACI), stoma conductivity was measured with "Leaf Porometer" device (Decagon Leaf Porometer Model SC^{-1}, mmol m-2s-1).

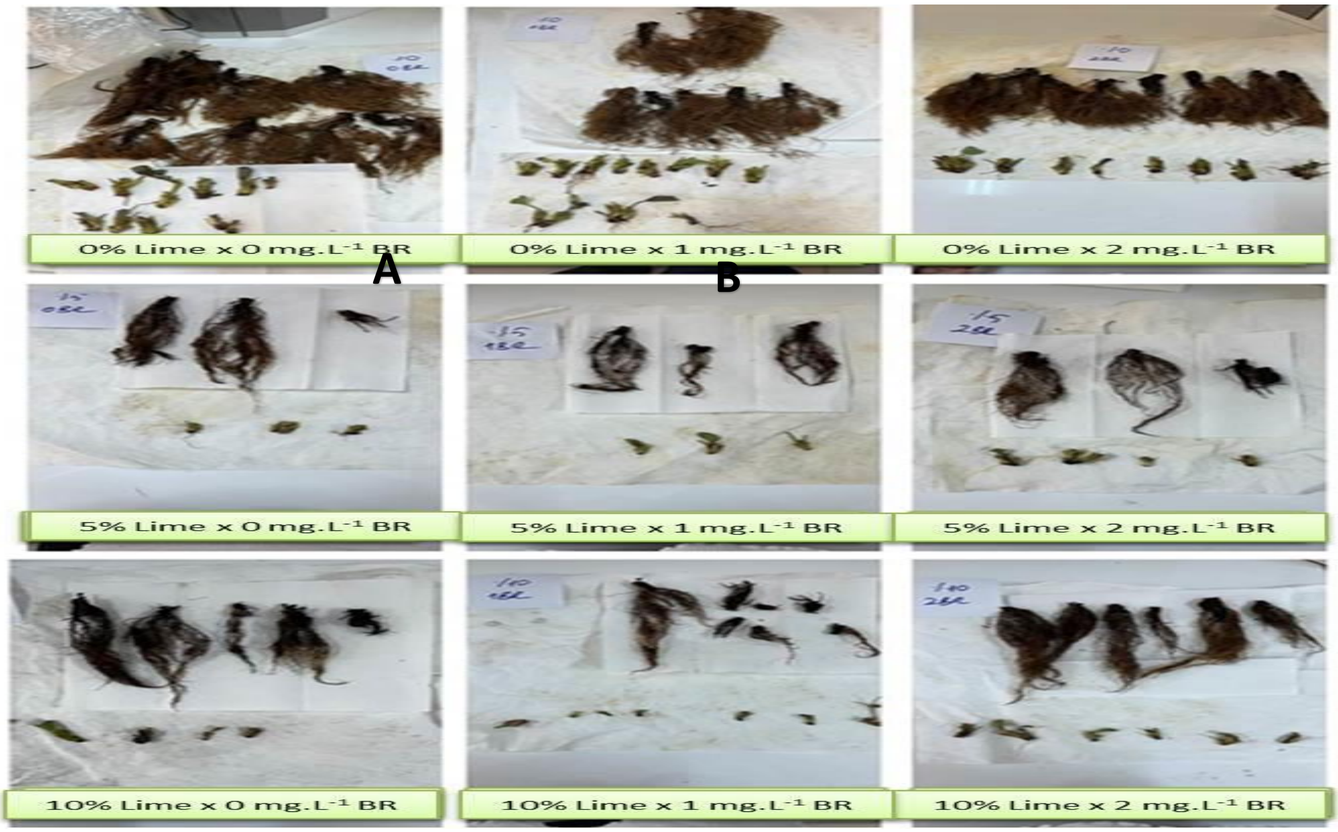

Figure 1. Effect of lime x BR combinations on root growth

Lipid peroxidation: 0.5 g of fresh leaves were taken from each replicate of each treatment and homogenized by adding 6 ml of 10% TCA and this mixture was centrifuged at 10,000 g for 15 min. After taking 2 ml of supernatant from the centrifuged samples, 2 ml of 0.6% thiobarbutric acid (TBA) containing 20% TCA was added and boiled at 100 °C for 30 min and then placed in an ice bath. Afterwards, absorbance readings were taken at 400, 532 and 600 nM in a spectrophotometer and MDA content was calculated according to the formula in Zhang et al. (2008).

MDA (µmol.g^{-1} TA)=6.45 x (A532 - A600) - 0.56 x A450

Nutrient elements in leaves: Leaf samples taken from the plants were first washed in tap water, then washed with 0.1N HCl and finally washed twice with pure water and the excess water was removed with filter paper. The leaves were dried at 70ºC for 48 hours. The dried leaf samples were ground and burned with a mixture of 5ml HNO3 and 2ml H_2O_2 in a microwave system (CEM-MarsXpress) and total macro and micro elements were determined by ICPAES (Inductively Coupled Plasma-Atomic Emission Spectrometer) (Soltanpour et al., 1979).

Statistical analysis

The experiment was established with three lime doses (0, 5, and 10%), three different concentrations of 24-eBL (0, 1 and 2 mg l^{-1}), 3 replications and 3 plants in each replicate according to the factorial design method in randomized blocks. The results obtained were evaluated using SPSS 20.0 package program. As a result of statistical analysis, 'Duncan multiple comparison test' was applied to determine the difference between the averages.

Results and Discussions

Plant Fresh and Dry Weight (g): When the interaction table (Table 2) of three different BR treatments at different lime doses was examined, it was seen that there was a statistically significant difference in leaf fresh weight, stem and root wet and dry weights, but there was no statistically significant difference only in leaf dry weight.

The highest values in terms of leaf fresh weight were found in 0% Lime x 1 mg l^{-1} BR (3.21 g), 0% Lime x 0 mg l^{-1} BR (3.20 g), 0% Lime x 2 mg l^{-1} BR (3.14 g) and 5% Lime x 0 mg l^{-1} BR (3.14g) treatments. Leaf dry weights ranged from 1.07 (10% Lime x 2 mg l^{-1} BR) to 1.21 g (5% Lime x 0 mg l^{-1} BR).

In terms of stem fresh weight, the highest values were measured in 0% Lime x 0 mg l^{-1} BR (3.14 g), 0% Lime x 1 mg l^{-1} BR (3.01 g) and 0% Lime x 2 mg l^{-1} BR (3.18 g) treatments. In terms of stem dry weight, the highest values were determined in 0% Lime x 0 mg l^{-1} BR (0.86 g), 0% Lime x 1 mg l^{-1} BR (0.76 g) and 0% Lime x 2 mg l^{-1} BR (0.85 g) treatments.

The highest root fresh weight was determined in 10% Lime x 2 mg l^{-1} BR treatment (3.48 g), while the highest root dry weight was measured in 10% Lime x 2 mg l^{-1} BR (1.28 g) and 10% Lime x 0 mg l^{-1} BR (1.19 g) treatments.

Table 2. Effects of Lime x BR treatments on leaf, stem, root fresh and dry weight

Lime doses (%)	BR doses (mg l^{-1})	Leaf		Stem		Root	
		Fresh weight (g)*	Dry weight (g) NS	Fresh weight (g)*	Dry weight (g)*	Fresh weight (g)*	Dry weight (g)*
0	0	3.20 ab	1.09	3.14 a	0.86 a	3.24 b	0.80 d
	1	3.21 a	1.11	3.01 a	0.76 ab	3.26 b	0.98 cd
	2	3.14 ac	1.17	3.18 a	0.85 a	3.01 c	0.99 cd
5	0	3.14 ac	1.21	0.65 e	0.20 f	1.65 g	0.92 cd
	1	3.03 cd	1.18	0.67 e	0.23 ef	1.60 g	0.85 d
	2	2.91 d	1.15	1.18 c	0.60 bc	2.20 f	0.89 cd
10	0	2.90 d	1.16	1.22 c	0.40 de	2.73 d	1.19 ab
	1	3.04 bd	1.10	0.90 d	0.30 ef	2.46 e	1.05 bc
	2	3.01 cd	1.09	1.52 b	0.51 cd	3.48 a	1.28 a

* The differences between the means shown with different letters in the same column are statistically significant ($p \leq 0.05$)
NS Non-significant

Karlıdag (2011) determined that when strawberry were grown under salt stress conditions and BR was applied through leaves, their fresh and dry weights increased. Balcı (2018) reported that 24-eBL application at different doses had no statistically significant effect on leaf fresh and dry weights of strawberry under cadmium (Cd) stress conditions. In terms of root weights, foliar 24-eBL applications had no effect on dry weights under Cd stress conditions, while the effect on root fresh weights was statistically significant. It was reported that 24-eBL increased the fresh and dry weights of stems and roots when applied to short-day strawberry cultivars.

Number of Leaves per Plant, Leaf Area (cm^2) and Leaf Color (L, a*, b*):

In the lime x BR interaction, the number of leaves per plant and b value expressing yellow color were found to be insignificant, while leaf area, a* value expressing red color and L values were found to be statistically significant (Table 3).

Table 3. Effects of lime x BR interaction on leaf number, leaf area and leaf color

Lime doses (%)	BR doses (mg l^{-1})	Number of Leaves (pcs)^{NS}	Leaf Area (cm^2)*	Leaf Color L*	Leaf Color a*	Leaf Color b^{NS}
0	0	5.44	32.13 ab	32.00 b	2.94 b	11.01
	1	5.78	33.60 a	32.55 b	9.75 a	11.60
	2	7.56	26.87 b	32.59 b	9.63 a	11.63
5	0	6.67	14.47 cd	40.88 a	9.71 a	12.51
	1	7.00	12.51 cd	36.55 ab	10.45 a	17.59
	2	6.50	11.93 d	33.73 ab	9.92 a	14.25
10	0	5.50	10.68 d	35.90 ab	10.88 a	19.79
	1	4.67	10.42 d	37.93 ab	12.31 a	16.23
	2	5.06	19.05 c	34.87 ab	10.22 a	16.86

* The differences between the means shown with different letters in the same column are statistically significant ($p \leq 0.05$)
NS Non-significant

Leaf area was statistically highest with 32.13 cm2 (0% lime x 0 mg l^{-1} BR) and 33.60 cm2 (0% lime x 1 mg l^{-1} BR). L value was statistically highest at 5% and 10% lime doses and 0 mg l^{-1}, 1 mg l^{-1} and 2 mg l^{-1} BR doses. The a* value of all treatments (9.63-12.31) except 0% lime x 0 mg l^{-1} BR dose (2.94) were statistically in the same group. The b* value varied between 11.01 (0% lime x 0 mg l-1 BR) and 19.79 (10% lime x 0 mg l^{-1} BR).

Chlorophyll (SPAD), Anthocyanin (ACI), Lipid Peroxidation (MDA) and Stoma Conductivity:

The differences between chlorophyll value, anthocyanin content, stoma conductance and MDA values of Lime x BR interaction were found statistically significant ($p<0.05$). The highest leaf chlorophyll content was obtained in the interaction of 0% lime x 1 mg l^{-1} BR, 5% lime x 0 mg l^{-1} BR, 10% lime x 0 mg l^{-1} BR (Table 4). High lime content in the growing medium causes a decrease in the amount of active Fe in the leaf and consequently a decrease in the amount of chlorophyll (Byrne and Rouse, 1995; Pestana et al., 2001). An increase in soil pH causes a decrease in the amount of Fe in the leaf, negatively affecting the synthesis and amount of chlorophyll. It has been reported in many studies that leaf chlorophyll content SPAD value is related to chlorophyll concentration (Schaper & Chocko, 1991; Daşgan, 1999; Eker, 2001). Karlıdağ et al. (2011) reported that foliar application of BR significantly increased leaf chlorophyll content in strawberries under salt stress. Çoban (2014), in his study on salt stress, determined that the effect of salt applications on chlorophyll content was negative. In the study, it was determined that 0.5 and 1.5 mg l^{-1} 24-eBL applied at 0 and 100 mM salt concentrations had a positive effect by increasing the amount of chlorophyll in plants. Altaş (2016) stated that maximum light yield and total chlorophyll content of two maize varieties decreased statistically significantly under salinity stress. Kara (2018) found a very close relationship between the chlorophyll concentrations and SPAD values of the varieties due to Fe deficiency and stated that the SPAD value decreased as the lime dose increased. Balcı (2018) reported a significant effect on chlorophyll content in Albion strawberry variety as a result of statistical analysis of the data obtained from 24-eBL applications against different doses of cadmium. It is known that BR applications improve the chlorophyll content of plants under Cd stress conditions (Hayat et al., 2010).

The highest leaf anthocyanin content (ACI) was obtained in the interaction of 5% lime x 0 mg l^{-1} BR (6.94) and 5% lime x 2 mg l^{-1} BR (8.36) in Table 4. There are research reports that BRs synthesized in plants increase the accumulation of secondary metabolites with antioxidant properties, which are extremely important for human health. As a matter of fact; Farooq et al. (2009) found that 24-eBL and 28-hBL application increased soluble phenolic compounds and anthocyanin content in plants under drought stress in paddy plants. Again, Farooq et al. (2010) determined that exogenous 24-eBL application increased anthocyanin amounts in their drought stress study in paddy plants. Balcı (2018) evaluated the effects of 24-eBL applications on Cd stress on Albion variety and determined that it had no effect on leaf anthocyanin and membrane permeability.

Considering the lime x BR interaction, 0% lime x 0 mg l^{-1} BR and 0% lime x 1 mg l^{-1} BR, applications gave the highest stoma conductance values. The combinations of 5% and 10% lime doses with BR were not effective in stoma conductance. Yu et al. (2004) reported that 24-eBL application promoted photosynthesis in cucurbits and was associated with V-ATPase, which is thought to have effects on hypocotyl elongation. Acharya and Assmann (2009) reported that BRs are effective in the management of stomatal apertures such as auxin, cytokinin and ethylene. Kara (2018) applied 0%, 5%, 10%, 15%, 15% and 20% lime doses to seven different strawberry cultivars and reported that the difference between the averages of stomatal permeability in terms of lime doses was significant, the highest was measured in Hilal77 cultivar and the lowest in Bolverim77 cultivar. In his study, he determined that while higher values were determined in the control, the values decreased due to the increase in lime doses.

When lipid peroxidation (MDA) value was considered, 10% lime x 2 mg l-1 BR, 5% lime x 1 mg l^{-1} BR and 5% lime x 0 mg l^{-1} BR treatments gave the highest values statistically (Table 4). Yan et al. (2013) reported that the amount of MDA increased with increasing amount of methyl jasmonate as a result of Cd toxicity in red peppers. It was reported that MDA concentration decreased and cell damage decreased with the application of MeJA to diseased plants (Sun et al., 2013). It was stated that the application of giberellic acid (GA3) in pepper increased CAT and SOD activity and decreased the amount of MDA; GA3 application had no statistical effect on cell damage (Uzal, 2017). Zhang et al. (2008) reported that the amount of MDA in strawberry varied between 3.12- 4.87 $\mu mol.g^{-1}FW$. Gündoğdu et al. (2019) reported that the amount of MDA varied in strawberry varieties as a result of the application of giberellic acid and methyl jasmonate, Honeoye variety was determined to have a higher amount of MDA, while Sweet Ann variety was determined to have a lower amount of MDA. The highest MDA value was measured from Honeoye variety with MDA content of 29.42 µmol g-1FW with 0.25 mM dose application of methyl jasmonate and 30.88 µmol $g^{-1}FW$ with 100 ppm dose of giberellic acid.

Table 4. Effects of Lime x BR interaction on Chlorophyll, Anthocyanin, Stoma conductance and MDA

Lime doses (%)	BR doses (mg l^{-1})	Chlorophyll (SPAD)*	Anthocyanin (ACI)*	Stoma conductance (mmol $m^{-2}s^{-1}$)*	MDA (µmol g^{-1} FW)*
0	0	34.82 bc	5.44 bd	211.31 a	4.85 bc
	1	36.99 ab	5.64 bd	178.50 ab	3.14 de
	2	36.01 bc	5.61 bd	161.86 bc	3.09 de
5	0	39.14 a	6.94 ab	117.36 cd	6.08 ab
	1	33.56 c	4.79 cd	164.14 bc	6.31 a
	2	33.57 c	8.36 a	85.82 d	3.91 cd
10	0	40.02 a	6.34 bc	130.15 cd	2.19 e
	1	27.48 d	4.09 d	161.79 bc	4.25 cd
	2	33.90 bc	4.91 bd	88.11 d	6.49 a

* The differences between the means shown with different letters in the same column are statistically significant (p≤ 0.05)

Nutrient Elements in Leaves

Nitrogen (N%) and phosphorus (P%) contents in the leaves of Sweet Ann strawberry cultivar were found to be statistically significant ($p<0.05$) in terms of lime x BR interaction (Table 5). The highest amounts of N and P were determined as 1.63% and 0.22% in 0% lime x 0 mg l^{-1} BR interaction, respectively. As lime and BR doses increased, %N and %P in leaves decreased. In his study, Kara (2018) determined that the differences between the averages were not significant when leaf nitrogen (%N) content was examined in terms of varieties and lime doses. Yağmur et al. (2021) reported that the effect of powdered lime and slurry applications on the macro and micro element amounts of pepper plant leaves was statistically significant, these applications significantly increased the N and P contents of the leaves compared to the control application and powdered lime application provided the highest total nitrogen amount. In a study conducted to determine the effect of liming materials applied to acidic soil on yield and mineral matter content in maize plant, it was found that these applications to maize plant increased total N, P, K, Ca and Mg contents (Kant et al., 2006). Kaçar and Katkat (2006) stated that increasing the pH of the growing medium decreased phosphorus uptake. Kara (2018) found that different lime doses applied to the soil significantly affected the amount of leaf phosphorus in all varieties, the highest amount of phosphorus was determined in the control and 5% lime dose, while this amount decreased with increasing lime dose. Balcı (2022), in his study in which he examined the P contents in leaves, stems and roots of strawberry seedlings uprooted at three different periods by MEL applied in a calcareous environment, stated that P uptake in leaves decreased as the amount of lime in the growing medium increased.

Leaf potassium content (% K) was found to be the highest (1.07%) in 5% lime x 1 mg l^{-1} BR combination. As a result of the study conducted by Kara (2018), when the amount of leaf potassium was analyzed in terms of lime doses, the differences between lime doses were found to be statistically significant. The highest and lowest values differed according to lime dose and strawberry varieties. The effect of lime applications was found to be insignificant in Doruk77, Hilal77, Erenoğlu77, Bolverim77 varieties and significant in Dorukhan77 and Ata77 strawberry varieties.

The highest values of leaf magnesium content (% Mg) were found in 10% lime x 2 mg l^{-1} BR combination (0.62%) and 10% lime x 0 mg l^{-1} BR combination (0.61%). Kara (2018) reported that leaf magnesium content did not show significant differences in varieties and lime applications. Balcı (2022) determined that the effect of MEL applications on Mg contents in strawberries was significant in all three removals. The Mg content was found to be in the range of 0.84-0.60% in the first harvest one month after planting, 0.63-0.31% in the second harvest and 0.50-0.30% in the third harvest (fruiting period).

Leaf calcium (Ca) content was highest at 5% and 10% lime doses. In the lime x BR interaction, 1.90 ppm Ca was found to be the highest in the 5% lime x 2 mg l^{-1} BR combination. Kara (2018) reported that the amount of leaf calcium (Ca) was not statistically significant when analyzed in terms of lime doses in his study, but there was an increase in the amount of Ca in the varieties due to the increase in lime doses. Balcı (2022) determined the highest Ca content in the leaves of Albion strawberry cultivar in 1% lime/0% MEL application in all removals. In his study, the Ca content of the plants in the control group applied 5 µM MEL was higher than the plants in the same group, while MEL applications decreased the Ca content of the plants applied 1% lime.

Leaf iron content was statistically significant ($p<0.05$) in terms of lime x BR interaction (Table 5). In the lime x BR interaction, the highest iron value was measured in 10% lime x 2 mg l^{-1} BR combination (119.50 ppm). Unlike our results, Kara (2018) found the highest leaf iron value in the control (0%) dose, while the amount of leaf iron decreased with increasing lime doses. Balcı

(2018) reported that the highest Fe content in strawberry leaves was obtained from 0 lime/10 µM MEL and 1% lime/5 µM MEL applications at the first uprooting. The Fe content in the leaves was found to be between 24.40-19.37 ppm at the flowering stage and 29.38-20.36 ppm at the fruiting stage.

Leaf copper (Cu) content was found to be statistically significant ($p<0.05$) in terms of lime x BR interaction. The highest copper content in the leaves of Sweet Ann strawberry cultivar was determined in 0% lime x 0 mg l^{-1} BR interaction (11.56 ppm). Cu content in leaves decreased as lime and BR doses increased.

Leaf zinc (Zn) content of Sweet Ann strawberry cultivar was found to be statistically significant ($p<0.05$) in terms of lime x BR interaction. The highest Zn content was obtained from 2 mg l^{-1} BR application with 46.29 ppm and from 10% lime x 2 mg l-1 BR interaction with 59.26 ppm. As the lime and BR doses decreased, the % zinc content in the leaves also decreased.

Tablo 5. Effects of lime x BR applications on some nutrients in leaves

Lime doses (%)	BR doses (mg l^{-1})	N (%)*	P (%)*	K (%)*	Mg (%)*	Ca (ppm)*	Fe (ppm)*	Cu (ppm)*	Zn (ppm)*
0	0	1.63 a	0.22 a	0.77 f	0.37 g	1.52 h	81.78 g	11.56 a	41.86 f
	1	1.49 b	0.11 b	0.83 d	0.43 f	1.65 g	84.62 f	11.48 b	35.58 h
	2	1.42 c	0.09 c	0.80 e	0.43 f	1.66 g	80.30 i	10.14 f	31.74 i
5	0	1.37 d	0.07 d	0.80 e	0.46 e	1.75 d	97.66 e	9.36 h	43.36 d
	1	1.33 e	0.07 d	1.07 a	0.59 c	1.68 f	109.02 b	9.20 i	47.86 c
	2	1.34 e	0.08 cd	0.83 d	0.53 d	1.90 a	81.62 h	9.56 g	47.88 b
10	0	1.42 c	0.05 e	0.89 c	0.61 ab	1.82 b	106.50 c	10.30 e	38.78 g
	1	1.48 b	0.08 cd	0.95 b	0.60 bc	1.74 e	103.54 d	11.04 c	42.72 e
	2	1.28 f	0.04 e	0.53 g	0.62 a	1.76 c	119.50 a	10.50 d	59.26 a

* The differences between the means shown with different letters in the same column are statistically significant ($p\leq 0.05$)

Conclusions

Considering the rapid increase in population and the decrease in agricultural lands day by day, it is of great importance to ensure that the highest yield is obtained from agricultural lands. Water and nutrient needs of plants should be met at optimum level. There are many factors that limit the usefulness of nutrients and agricultural production. Plants try to survive by developing many responses to all biotic and abiotic factors that they perceive as stress factors. These responses vary according to the effectiveness of the stressor and the genetic characteristics of the plant. Thanks to these stress responses, plants are able to adapt to the stress factors they experience in order to survive.

Calcareous soils are directly linked to plant development, such as soil-water relations, fertility and nutrient availability. Excess CaCO3 in the soil affects soil pH, and high pH decreases the availability of plant nutrients and leads to nitrogen losses in the form of ammonia. There is a decrease in the solubility of phosphorus, and the usefulness of microelements such as Fe, Cu, Zn and Mn decreases with increasing pH levels (Grattan & Grieve, 1999).

In order to prevent all these unfavorable soil conditions from limiting plant growth and increasing productivity, various applications are made. Brassinosteroids, defined as the sixth group of hormones, can be listed as cell division and expansion, cellular differentiation, lateral root development, maintenance of apical dominance, flowering, senescence and increasing stress tolerance (Rao et al., 2002; Savaldi-Goldstein & Chory, 2006). BRs promote growth by accelerating cell elongation and division. The positive effects of BRs in plant response to stress have been confirmed by many studies (Divi et al., 2010). At the end of the study, it was concluded that foliar application of 24-eBL had a positive effect on vegetative growth and nutrient uptake under calcareous soil conditions.

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