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The effect of brassinosteroid on cucumber (*Cucumis sativus*) growth under varying water availability

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Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Sebelas Maret. Jl. Ir. Sutami 36A, Surakarta 57126, Central Java, Indonesia. Tel./fax.: +62-271-663375, ♥email: solichatun@staff.uns.ac.id

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Abstract. *Nurrohmah A, Solichatun, Pitoyo A. 2026. The effect of brassinosteroid on cucumber (*Cucumis sativus*) growth under varying water availability. Asian J Agric 10 (1): g100144. https://doi.org/10.13057/asianjagric/g100144.* Cucumber (*Cucumis sativus*) is a horticultural crop belonging the family Cucurbitaceae and is widely consumed in Indonesia. Cucumber production in the country tends to fluctuate due to extreme climate conditions, which cause variations in water availability and consequently disrupt plant growth. One approach to enhance cucumber tolerance to fluctuating water conditions is the application of brassinosteroid. This study aimed to evaluate the effects and determine the most effective concentration of brassinosteroid on cucumber growth under different level of water availability. A Completely Randomized Design (CRD) was employed with two factors and four replications. The first factor was field capacity (100%, 60%, and 40%), and the second was brassinosteroid (0, 0.5, 1, and 2 ppm). Cucumbers were grown for 40 days after transplanting and brassinosteroid was applied weekly as a foliar spray to the leaf surface. Data were analyzed using two-way Analysis of Variance (ANOVA) and followed by one-way unstucked ANOVA at a significance level of $P < 0.05$. Then tested by Duncan's Multiple Range Test (DMRT) at a 95% confidence level. The Correlation analysis used Spearman's rank correlation coefficient. The results showed that brassinosteroid significantly improved cucumber growth parameters under different water availabilities. A brassinosteroid concentration of 0.5 ppm was most effective in improving vegetative growth parameters and plant biomass at 60% field capacity, while also increasing chlorophyll content under well-watered conditions (100% field capacity). In contrast, a higher concentration of 2 ppm promoted proline accumulation at 40% field capacity. The research suggest that lower brassinosteroid promote cucumber growth under moderate, whereas higher concentrations facilitate osmotic adjustment under severe drought conditions.

Keywords: Brassinosteroid, cucumber, foliar spray, growth, water availability

INTRODUCTION

The consumption of cucumber (*Cucumis sativus*) in Indonesia has increased it could be consumed as food, medicine, and cosmetics (Fajri et al. 2022). According to the Ministry of Agriculture (2023), cucumber consumption in Indonesia remained relatively stable, showing a marginal decline of less than 0.1% between 2022 and 2023. However, national production decreased by approximately 10% over the same period (BPS 2024). This decline indicates a widening gap between cucumber production and consumer demand.

One of the major factors contributing to the decline in cucumber production is climate change, which intensifies fluctuations in water availability. Both water deficit and excess impose physiological constraints on plants. Drought conditions increase evapotranspiration, thereby restricting plant growth (Khodadadi et al. 2020; Nurwenda et al. 2024). Excess water causes soil saturation and anaerobic soil conditions, which reduced infiltration (Rupngam and Messiga 2024). This imbalance disrupts stomatal conductance and reduces carbon dioxide uptake, thus affecting photosynthesis (Ploschuk et al. 2022; Akhoundnejad and Dasgan 2020).

Reduced stomatal conductance alters chloroplast energy balance and promotes the accumulation of Reactive Oxygen Species (ROS), which disrupt plant metabolism (Castañeda-Murillo et al. 2022). Moderate ROS levels stimulate proline accumulation, which functions as an osmolyte to regulate cytoplasmic osmotic potential and mitigate oxidative stress (Hayat et al. 2012). However, excessive ROS accumulation damages chloroplast structures, reduces sugar and carbohydrate production, decreases total chlorophyll content, and intensifies oxidative stress (Salah et al. 2022; Yang et al. 2021), ultimately inhibiting cucumber growth under varying water availability.

These physiological disturbances are also reflected in plant morphological responses during the vegetative stage. Morphological changes may include alterations in leaf number and leaf area, leaf yellowing, and inhibition of plant height (Yang et al. 2021). Reductions in leaf number and area occur as an adaptive mechanism to decrease the rate of plant transpiration. This response is closely associated with stomatal closure, which functions to prevent excessive water loss from plant cells (He et al. 2020). In addition, plant height inhibition may occur due to structural changes in leaves and roots that limit cell expansion (Yang et al. 2021).

The application of exogenous phytohormones has been proposed as an effective strategy to alleviate water stress in plants (Abbas et al. 2023). Brassinosteroids are polyhydroxysteroid phytohormones present in both lower and higher plants and are known to regulate plant growth and development (Vardhini 2019). Brassinosteroids are predominantly synthesized in roots, where they play an important role in strengthening and stimulating root elongation (Fridman and Savaldi-Goldstein 2013). In addition, these hormones are actively produced in shoots and young leaves, where they support cell division and elongation, as young leaves represent metabolically active tissues that require enhanced cellular activity for rapid growth (Duran et al. 2017). Under environmental stress, the synthesis of brassinosteroids is required as part of the plant defense mechanism, strengthening plant responses to extreme conditions. For instance, brassinosteroids enhance the activity of antioxidant enzymes such as superoxide dismutase and catalase, which prevent excessive ROS accumulation and help maintain cellular metabolic functions (Vardhini 2019).

Previous research by Yadav et al. (2022) evaluated brassinosteroid application at concentrations of 0.5, 1, and 2 ppm to enhance vegetative growth in cucumber during the dry season, however physiological responses were not assessed. In contrast, studies on other plant species have demonstrated that brassinosteroids enhance antioxidant activity, increase proline accumulation, reduce reactive oxygen species under drought conditions, and maintain photosynthetic efficiency under waterlogging stress (Behnamnia et al. 2009; Sanchez et al. 2025). Nevertheless, studies evaluating the concentration-dependent effects of brassinosteroids on cucumber growth and physiological responses under graded levels of water availability remain limited. Based on these considerations, this study aimed to evaluate the interactive effects of brassinosteroid concentration and water availability on cucumber growth and to identify the most effective concentration for improving physiological traits, including chlorophyll, carotenoid, and proline contents.

MATERIALS AND METHODS

Time and place

This research was conducted from November 2024 to January 2025 at the greenhouse of the Integrated Laboratory Technical Implementation Unit and the Biology Laboratory, Universitas Sebelas Maret (UNS), Surakarta, Central Java, Indonesia.

Materials and equipment

The materials used in this study included soil, compost, cucumber (*Cucumis sativus*) 'Tina' from CV. Multi Global Agrindo, released under decree No. 514/Kpts/SR.130/D/VI/2021, distilled water, brassinosteroid, Whatman No. 42 filter paper, 80% acetone, leaf samples, 3% sulfosalicylic acid, 0.14 M ninhydrin solution, glacial acetic acid, toluene (99.5%), ice cubes, and L-Proline.

The equipment used comprised seedling trays (54 × 28 × 4.2 cm), shovels, 20 × 20 cm polybags, sprayers, buckets, measuring tapes, analytical balances, ovens, mortars and pestles, UV-Vis spectrophotometers, cuvettes, glass containers, centrifuges, vortex mixers, and water baths.

Research design

This study was conducted as an experimental research in the greenhouse and Biology Laboratory, Universitas Sebelas Maret (UNS). A Completely Randomized Design (CRD) with two factors was employed. The first factor was water availability, expressed as percentages of field capacity (FC), namely 100%, 60%, and 40% FC. The second factor was foliar application of brassinosteroid at concentrations of 0, 0.5, 1, and 2 ppm. Each treatment combination was replicated four times, resulting in a total of 48 experimental units.

Procedure

Cucumber seed germination

The planting medium consisted of garden soil from Karangpandan mixed with compost in a 1:1 ratio. A total of 10 grams of the soil-compost mixture was placed into seedling trays (54 × 28 × 4.2 cm). Cucumber seeds were sown into holes filled with the mixture at a pH of 7. The trays were placed in a greenhouse with a light intensity of 2,390 lux and an average temperature of 31.3°C. Seedlings were irrigated daily with 50 mL of tap water for 14 days.

Planting and treatment cucumber plants

A total of 1 kg of garden soil mixed with compost was placed into each 20 × 20 cm polybags. Fourteen-day-old cucumber seedlings were transplanted into the polybags and grown for 40 days until the plants entered the generative phase. The cucumber plants were maintained in a greenhouse at a temperature of 28-30°C and relative humidity of 68-82%, and arranged according to the two treatment factors: field capacity and brassinosteroid application.

Water availability treatments were designated as K0, K1, and K2, corresponding to 100%, 60%, and 40% field capacity. Irrigation was applied every five days using 385 mL, 231 mL, and 154 mL of water for 100%, 60%, and 40% field capacity, respectively. The selection of these field capacity levels was based on previous studies in cucumber that established a soil moisture gradient ranging from well-watered (100% FC) to moderate (60% FC) and severe drought stress conditions (40% FC) under decreasing water availability (Alam et al. 2023). Field capacity was determined gravimetrically as the water content retained by 1 kg of growing medium after saturation and 24 h of free drainage in a 20 × 20 cm polybag (Arsyadmunir 2016). The weight of the medium at field capacity was used as a reference value. During the experiment, polybags were re-weighed every five days, and water was added to restore the growing medium to the designated percentage of FC (100%, 60%, or 40%) at the beginning of each irrigation cycle.

A commercial brassinosteroid containing 0.15% active brassinolide (CAS No. 72962-43-7) was applied as a foliar spray on leaf surface at contentration 0, 0.5, 1, and 2 ppm at 7, 14, 21, 28, and 35 days after transplanting (DAT). The brassinosteroids concentrations (0.5, 1, and 2 ppm) were selected based on the findings of Yadav et al. (2022), who reported improved vegetative growth of cucumber plants treated with these concentrations during the dry season. Accordingly, this concentration range was adopted in the present study to assess its physiological effects under varying field capacity conditions. Plants aged 1-25 DAT were sprayed with 2 mL of solution per plant, while plants aged 30-40 DAT received 4 mL per plant.

Vegetative growth parameters

Plant height was measured from the base of stem in soil surface to tip of apical growing point. The total number of leaves was determined by counting all fully opened leaves. Leaf area was measured at 40 DAT using the gravimetric method and calculated according to Irwan and Wicaksono (2017)

Biomass parameters

Fresh weight was measured by weighed cucumber plant using an analytical balance. The plants were then oven-dried at 50°C for 5 × 24 hours until a constant weight was achieved, following the method of Abouelsaad et al. (2026) with minor modification. Dry weight was measured using an analytical balance and shoot-root ratio calculated according to Tamin and Puri (2020).

Physiological parameters

Total chlorophyll and carotenoid content in cucumber leaf

Fresh leaf samples were cleaned and weighed to an accuracy of 0.1 g using an analytical balance. The sample were ground using a mortar and pestle until finely homogenized. Each sample was extracted with 10 mL of 80% acetone and filtered using Whatman No. 42 filter paper. A 3 mL aliquot of the filtrate was transferred into a cuvette and analyzed using a UV–Vis spectrophotometer at wavelengths of 480, 645, and 663 nm. A 3 mL aliquot of 80% acetone was used as the blank (Kurniawan et al. 2010). The formula for calculating total chlorophyll is as follows:

Total Chlorophyll ($mg.g^{-1}$ FW) = $[(8.02\ x\ OD663) + (20.2\ x\ OD645)]\ x\ \frac{V}{1000\ FW}$ (Arnon 1949)

Carotenoid content was calculated according to Rifki et al. (2024), is as follows:

Carotenoid Content (mg. g^{-1} FW) = $OD480 + (OD663\ x\ 0.114) - (OD645\ x\ 0.638)$

Where:
OD663: Optical density of wavelength 663 nm
OD645: Optical density of wavelength 645 nm
OD480: Optical density of wavelength 480 nm
V: Extract volume
FW: Fresh weight of leaf

Proline content in cucumber leaf

Proline content was determined following the method of Bates et al. (1973) and Prellia et al. (2023). Cucumber leaf from the fourth node were cleaned, finely ground, and 0.1 g of the sample was weighed. Leaves from the fourth node were collected, cleaned, finely ground, and 0.1 g of the sample was weighed. The sample was homogenized with 5 mL of 3% sulfosalicylic acid and centrifuged at 5,000× g for 15 minutes. A 2 mL aliquot of the supernatant was mixed with 2 mL of 0.14 M ninhydrin solution and 2 mL of glacial acetic acid, then heated in a water bath at 100°C for 60 minutes. The mixture was immediately cooled in an ice bath for 5 minutes. Subsequently, 4 mL of 99.5% toluene was added and vortexed for 15-20 seconds until phase separation occurred. The red-colored toluene layer containing proline was collected, and absorbance was measured at 520 nm using a UV–Vis spectrophotometer. A standard curve was prepared using L-proline (Sigma) at concentrations of 0, 0.625, 1.25, 2.5, and 5 ppm. Proline content was calculated using the following formula:

Proline content (µmol g^{-1}FW) = $[(\mu g proline/ml\ x\ ml\ toluena)/115.5\ \mu mol]/(g\ sample)/5]$

Where, 115.5 is the molecular weight of proline (Bates et al. 1973; Mwadzingeni et al. 2016)

Data analysis

The quantitative data were analyzed using two-way analysis of variance (ANOVA) and followed by one-way unstucked ANOVA at a significance level of $P < 0.05$. Then tested by Duncan's Multiple Range Test (DMRT) at a 95% confidence level. The Correlation analysis used Spearman's rank correlation coefficient to evaluate the monotonic relationship between variables. The Spearman correlation coefficient ranges from -1 (perfect negative correlation) to +1 (perfect positive correlation), and 0 indicates no correlation between variables.

RESULTS AND DISCUSSION

Vegetative growth parameters

The results of the analysis showed that the combination of brassinosteroids concentration and field capacity significantly affected plant height and leaf area, but had no significant effect on the number of leaves (Table 1). In general, plants grown under 60% field capacity exhibited better vegetative growth compared to those grown under 100% and 40% field capacity. At 60% field capacity, the application of 0.5 ppm brassinosteroids produced the tallest plants, reaching 249.5 cm, and the largest leaf area, reaching 21.87 cm^2. A similar pattern was also observed at 100% and 40% field capacity, where a brassinosteroids concentration of 0.5 ppm was more effective in increasing vegetative plant parameters compared to the 2 ppm concentration.

The analysis of the number of leaves did not show significant differences among treatments. However, based on the numerical trend, the increase in leaf number was in line with plant height growth, where the application of 0.5 ppm brassinosteroids at 60% field capacity resulted in the highest number of leaves, reaching 16.5 leaves.

Biomass parameters

The results showed that the combination of brassinosteroids and field capacity significantly affected the fresh weight, dry weight, and shoot-to-root ratio of cucumber plants (Table 2). Biomass accumulation of plants grown under 60% field capacity was higher than that of plants grown under 100% and 40% field capacity. The application of 0.5 ppm brassinosteroid at 60% field capacity produced the highest fresh and dry weights, reaching 57.69 g and 4.93 g, respectively. These values were higher than those obtained from other treatments at the same field capacity. A different pattern was observed in the shoot-to-root ratio parameter, where the highest value was recorded at 100% field capacity with a brassinosteroid concentration of 1 ppm. In general, cucumber plants treated with brassinosteroids showed higher biomass values compared with the control treatment.

Physiological parameters

The results showed that the combination of brassinosteroids concentration and field capacity significantly affected chlorophyll and proline contents in cucumber leaves (Table 3). Based on the observed trend, the application of brassinosteroids tended to increase chlorophyll content at each level of field capacity. The highest chlorophyll content was recorded at 100% field capacity with the application of 0.5 ppm brassinosteroids, reaching 8.59 mg g^{-1} FW.

In contrast to chlorophyll, the application of brassinosteroids did not increase the carotenoid content of cucumber leaves, although differences in carotenoid values were observed among treatments. The highest carotenoid content was found in the control treatment at 60% field capacity. This result indicates that increasing brassinosteroids concentration did not directly enhance carotenoid accumulation in cucumber plants.

For the proline parameter, an increasing trend in proline content was observed under lower water availability and higher brassinosteroids concentrations. The highest proline content was recorded at 40% field capacity with 2 ppm brassinosteroid, reaching 0.646 μmol g^{-1} FW.

Correlation between vegetative growth parameters, biomass parameters, and physiological parameters

The correlation coefficient values among the parameters in cucumber plants are presented in Table 4. Several parameters showed significant relationships. Fresh weight showed a positive correlation with dry weight (r = 0.814**), shoot to root ratio (r = 0.337*), plant height (r = 0.540**), and leaf area (r = 0.313*). Dry weight was also significantly and positively correlated with shoot to root ratio (r = 0.448**), chlorophyll (r = 0.305*), plant height (r = 0.448**), and leaf area (r = 0.417**). Leaf area showed a positive correlation with plant height (r = 0.445**) and number of leaves (r = 0.726**). A negative correlation was only observed between chlorophyll and carotenoid (r = -0.607**). Meanwhile, proline did not show a significant correlation with other parameters in this study.

Table 1. Plant height, number of leaves, and leaf area of cucumber after application of brassinosteroid

Field Capacity (% FC)	Concentration of brassinosteroid (ppm)	Plant height (cm)	Number of leaves	Leaf area (cm^2)
100	0	204.25±13.96^{bc}	14.75±2.36	20.68±0.4^{a}
	0.5	220±31.6^{abc}	15.5±3.1	20.85±2.29^{a}
	1	220±22.9^{abc}	15±2.94	20.53±2.43^{a}
	2	189±18.79^{c}	13.25±2.62	19.61±1.6^{a}
60	0	249.25±5.37^{a}	16±1.41	21.71±1.35^{a}
	0.5	249.5±11.73^{a}	16.5±1.29	21.87±1^{a}
	1	226.5±27.06^{ab}	14±1.82	21.22±1.53^{a}
	2	217.25±17.89^{abc}	13.75±1.91	19.79±1.37^{a}
40	0	203±28.08^{bc}	13.5±2.38	19.69±1.74^{a}
	0.5	208.25±25.14^{bc}	14±2.44	20.08±1.6^{a}
	1	203.5±25.68^{bc}	13.5±1.73	19.62±0.61^{a}
	2	196±9.38^{bc}	13±0.81	16.05±0.22^{b}

Note: Values followed by different letters within the same column indicate significant differences based on DMRT at the 95% confidence level

Table 2. Fresh weight, dry weight, and shoot-root ratio of cucumber after application of brassinosteroid

Field Capacity (% FC)	Concentration of brassinosteroid (ppm)	Fresh weight (gram)	Dry weight (gram)	Shoot-root ratio (gram)
100	0	34.56±3.21^{de}	3.2±0.44^{de}	19.37±6.4^{cde}
	0.5	40.31±4.55^{cde}	4.05±0.33^{bcd}	23.55±3.1^{cd}
	1	38.15±12.56^{cde}	3.80±0.8^{bcde}	48.07±15.22^{a}
	2	37.51±6.44^{cde}	3.51±0.26^{bcde}	31.39±5.5^{bc}
60	0	41.53±3.92^{bcd}	3.48±0.44^{bcde}	14.28±5.12^{de}
	0.5	57.69±5.29^{a}	4.93±0.83^{a}	44.36±20.43^{ab}
	1	50.48±10.2^{ab}	4.14±0.61^{abc}	29.13±7.5^{cd}
	2	45.46±1.85^{bc}	4.36±0.47^{ab}	25.58±6.92^{cd}
40	0	30.31±2.92^{e}	3.02±0.22^{e}	4.28±1.12^{e}
	0.5	37.01±5.02^{cde}	3.39±0.43^{cde}	28.41±9.38^{cd}
	1	34.44±4.76^{de}	3.25±0.76^{cde}	28±9.01^{cd}
	2	32.92±7.14^{de}	3.03±0.64^{e}	17.16±10.28^{cde}

Note: Values followed by different letters within the same column indicate significant differences based on DMRT at the 95% confidence level

Table 3. Total chlorophyll, carotenoid, and proline in cucumber leaf after application of brassinosteroid

Field Capacity (% FC)	Concentration of brassinosteroid (ppm)	Chlorophyll (mg.g^{-1} FW)	Carotenoid (mg.g^{-1} FW)	Proline (µmol g^{-1}FW)
100	0	7.42±0.14^{d}	2.43±0.4^{a}	0.229±0.01^{d}
	0.5	8.59±0.32^{a}	2.13±0.16^{c}	0.287±0.02^{cd}
	1	7.97±0.17^{c}	2.32±0.06^{ab}	0.324±0.04^{bcd}
	2	7.79±0.32^{cd}	2.35±0.07^{ab}	0.54±0.14^{ab}
60	0	6.94±0.21^{e}	2.44±0.009^{a}	0.324±0.04^{bcd}
	0.5	8.05±0.13^{bc}	2.34±0.08^{ab}	0.396±0.02^{bcd}
	1	8.41±0.4^{ab}	2.27±0.09^{b}	0.386±0.34^{bcd}
	2	7.88±0.17^{c}	2.38±0.28^{ab}	0.5±0.14^{abc}
40	0	6.6±0.29^{e}	2.38±0.13^{ab}	0.391±0.02^{bcd}
	0.5	7.83±0.14^{cd}	2.28±0.08^{ab}	0.436±0.28^{abcd}
	1	7.76±0.49^{cd}	2.41±0.14^{ab}	0.633±0.01^{a}
	2	8.48±0.06^{a}	2.38±0.03^{ab}	0.646±0.04^{a}

Note: Values followed by different letters within the same column indicate significant differences based on DMRT at the 95% confidence level

Table 4. Spearman correlation matrix between vegetative growth parameters, biomass parameters, and physiological parameters

Parameters	Fresh weight	Dry weight	Shoot to root ratio	Chlorophyll	Carotenoid	Proline	Plant height	Number of leaves	Leaf area
Fresh Weight	1								
Dry Weight	0.814**	1							
Shoot to Root Ratio	0.337*	0.448**	1						
Chlorophyll	0.251	0.305*	0.260	1					
Carotenoid	-0.155	-0.236	-0.180	-0.607**	1				
Proline	-0.102	-.053	-0.127	0.053	0.177	1			
Plant Height	0.540**	0.448**	0.136	-0.096	0.039	-0.175	1		
Number of Leaves	0.116	0.240	0.157	0.023	-0.090	-0.203	0.360*	1	
Leaf Area	0.313*	0.417**	0.112	-0.106	-0.082	-0.219	0.445**	0.726**	1
N	48	48	48	48	48	48	48	48	48

Note: *: Correlation is significant at the 0.05 (2-tailed) and **Correlation is significant at the 0.01 level (2-tailed)

Discussion

Water availability is a critical factor influencing the growth and physiological responses of cucumber plants. In this study, field capacity levels were determined following the method described by Alam et al. (2023), using 100%, 60%, and 40% field capacity. These levels were used to represent different water availability conditions in the field, where 100% field capacity represents well-watered conditions, 60% field capacity represents moderate water availability, and 40% field capacity represents severe drought stress. Drought stress can increase evapotranspiration and inhibit plant growth, whereas soil saturation may create anaerobic conditions that limit root respiration and reduce the efficiency of water uptake by plants. These conditions may lead to physiological imbalances that disrupt stomatal conductance and reduce carbon dioxide uptake, thereby limiting photosynthetic activity (Akhoundnejad and Dasgan 2020; Khodadadi et al. 2020; Ploschuk et al. 2022; Nurwenda et al. 2024; Rupngam and Messiga 2024). Foliar application of brassinosteroids has been reported to enhance the vegetative growth of cucumber plants under dry season (Yadav et al. 2022). In addition, foliar spraying of plant hormones can enhance photosynthetic activity and facilitate more efficient nutrient translocation, thereby supporting improved plant growth and physiological performance (Shah and Patel 2023).

The results showed that the application of 0.5 ppm brassinosteroid under 60% field capacity was the most effective treatment for increasing plant height, number of leaves, leaf area, fresh weight, and dry weight (Tables 1 and 2). The increase in plant height is likely associated with the synergistic interaction between brassinosteroid and auxin, which regulates auxin distribution and promotes cell elongation (Devi et al. 2022; Yin et al. 2025). This hormonal interaction activates ATPase in the plasma membrane, which pumps protons into the apoplast (the space between the plasma membrane and the cell wall), thereby lowering the pH of the cell wall and loosening its structure. This condition facilitates cell elongation and expansion, ultimately enhancing plant growth (Oh et al. 2020). The increase in plant height observed in this study was also accompanied by increases in the number of leaves and leaf area. Exogenously applied brassinosteroids can interact with auxin and cytokinin to stimulate cell division. This process generates undifferentiated cells that are subsequently directed by auxin (IAA) to develop into provascular tissues. These provascular cells then differentiate into xylem and phloem, facilitating nutrient transport and hormonal signaling that support the formation and development of new leaves (Oh et al. 2020). In addition, the continuously active shoot apical meristem produces nodes bearing leaves, hereby contributing to a positive linear relationship between plant height and leaf number (Ando et al 2025). The improvement in vegetative growth parameters in cucumber plants also contributed to increased fresh and dry biomass. Exogenous application of brassinosteroids are known to stimulate the development of roots, stems, and leaves. This hormone enhances root development, resulting in a stronger and more efficient root system for water absorption while also improving the maintenance of plant water status under varying water availability (Lone et al. 2022).

Brassinosteroids also influenced biomass allocation between shoots and roots, which is reflected in the shoot–root ratio. In this study, the highest shoot root ratio was observed in plants treated with 1 ppm brassinosteroid under 100% field capacity (Table 2). An increase in the shoot root ratio indicates a shift in photosynthate allocation toward shoot growth due to enhanced metabolic activity and photosynthetic capacity. Brassinosteroids are known to increase chlorophyll content, Rubisco enzyme activity, and CO_2 assimilation rate, thereby supporting greater shoot biomass accumulation (Yu et al. 2004). This response may also be related to the hormonal effects of brassinosteroids, which stimulate stem and leaf growth more strongly than root growth. In contrast, the 40% field capacity treatment resulted in the lowest average of shoot to root ratio parameters in cucumber plants. This condition reflects a water deficit that triggers physiological root adaptations to enhance water uptake efficiency. Under drought stress, plants tend to elongate their root systems and increase root biomass in deeper soil layers (Qiao et al. 2024). Consequently, photosynthate allocation is preferentially directed toward root growth rather than shoot development (Effendi 2008). This allocation supports the formation of longer, denser, and stronger roots, thereby optimizing soil water absorption (Comas et al. 2013).

The application of brassinosteroids in cucumber plants under water availability also improved photosynthetic responses (Table 3). In this study, brassinosteroid treatments increased leaf chlorophyll content across different levels of field capacity. This interaction suggests that brassinosteroids mitigate the adverse effects of water deficit on chlorophyll biosynthesis. According to Ha et al. (2018), brassinosteroids can reduce the accumulation of abscisic acid (ABA) in leaves, thereby maintaining stomatal opening and facilitating the diffusion of CO_2 into mesophyll tissues. This condition enables plants to sustain photosynthetic activity even under suboptimal water availability. In constract, brassinosteroid application did not significantly enhance carotenoid content (Table 3). This response suggests that water deficit induces oxidative stress, which stimulates carotenoid accumulation as a protective mechanism. Brassinosteroids enhance the activity of antioxidant enzymes, including superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD), which reduce the accumulation of reactive oxygen species (ROS) and protect plant tissues from oxidative damage (Anwar et al. 2018). Accordingly, cucumber plants exposed to drought stress without brassinosteroids application exhibited higher carotenoid content, as carotenoids function as antioxidants that protect photosynthetic organs, such as chloroplasts, from oxidative damage (Oskouiee et al. 2025). Since carotenoid and brassinosteroid share similar protective roles against oxidative damage, brassinosteroid application may reduce the plant's reliance on carotenoid for protection. This finding is consistent with Rifki et al. (2024), who reported that brassinosteroids increased chlorophyll content while reducing carotenoid levels compared to the control.

The physiological response of plants to water stress conditions was also reflected in the accumulation of proline in plant tissues. In this study, the highest proline accumulation was observed in the treatment with 40% field capacity combined with the application of 2 ppm brassinosteroids. This response may be attributed to brassinosteroids induced upregulation of the proline biosynthetic gene Δ^1-pyrroline-5-carboxylate synthase (P5CS), which enhances proline synthesis (Zhang et al. 2024). Proline functions as an osmotic regulator in plant cells to maintain osmotic balance (Jin et al. 2024). In addition, Kaur and Asthir (2015) report that proline also acts as an antioxidant and signaling molecule under environmental stress. Plants grown under 40% field capacity accumulated higher proline levels than those under 60% and 100% field capacity, reflecting a typical drought stress response. Under water deficit conditions, proline contributes to cellular stability, regulation of reactive oxygen species (ROS), maintenance of redox balance, and osmotic adjustment, thereby enhancing plant tolerance to limited water availability (Júnior et al. 2025).

The results of this study indicate that the response of cucumber plants to brassinosteroids application is influenced by the level of water availability. At 60% field capacity, brassinosteroids application played a role in enhancing vegetative growth and the photosynthetic capacity of the plants. Cucumber growth is strongly influenced by water availability in the field and soil moisture (Aguyoh and Odhiambo 2022). Similar findings were reported by Alam et al. (2023), who showed that 60% field capacity combined with salicylic acid application increased cucumber yield compared to 100% and 40% field capacity. At 60% field capacity, the soil was neither saturated nor subjected to extreme drought conditions. In agricultural practice, soil moisture around this level often occurs under moderate irrigation regimes or temporary water deficits, making this condition relevant to typical cucumber cultivation. In contrast, under 40% field capacity conditions (drought stress), higher brassinosteroid concentrations tended to promote stress tolerance mechanisms through increased accumulation of proline. These findings suggest that brassinosteroids perform different physiological roles depending on the level of water stress experienced by the plants.

This study demonstrated that the effectiveness of brassinosteroids application in cucumber plants depends on the level of field capacity. A brassinosteroids concentration of 0.5 ppm was most effective in improving vegetative growth parameters and plant biomass at 60% field capacity, while also increasing chlorophyll content under well-watered conditions (100% field capacity). In contrast, a higher concentration of 2 ppm promoted proline accumulation at 40% field capacity, indicating enhanced osmotic adjustment and membrane stability under drought stress. Further research should focus on field-scale trials to evaluate the effectiveness of brassinosteroids application on cucumber yield, water use efficiency, and irrigation management under practical cultivation conditions.

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