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INDUCTION OF DROUGHT RESISTANCE IN MELON (*Cucumis melo* L.) M15 WITH HORMOPRIMING BRASSINOSTEROID BASED ON MORPHOLOGY, ANATOMY, AND PHYSIOLOGY ASPECTS

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ABSTRACT

Melon productivity in Indonesia has been declining and is affected by global climate change. Prolonged drought has reduced plant growth and development. Hormopriming is an alternative to increasing the ability of melon plants to germinate and grow by soaking the seeds in a solution of plant growth regulators. Brassinosteroids can enhance germination and plant tolerance to arid conditions. This research aims to study the effect of brassinosteroid hormopriming treatment on the germination and growth of melon plants at different levels of aridity. The study utilized a two-factor, fully randomized design. The brassinosteroid concentrations were 0, 0.05, 0.10, and 0.15 ppm. Drought stress resistance was tested using media with 75%, 50%, and 25% space capacity. Water capacities were examined using the gravimetric method, a technique that measures the change in weight of the soil or growing medium to determine the water content. This study suggests that treating melon plants with brassinosteroid variations can induce drought resistance in M15 melons. A brassinosteroid concentration of 0.15 ppm was the best concentration, as it could increase plant growth and adaptation parameters under each variation of water availability provided. The findings of this study can serve as a reference for melon cultivators constrained by dry land conditions to increase the efficiency of cultivation.

INTRODUCTION

Melon (*Cucumis melo* L.) is a valuable fruit and a primary horticultural product with bright prospects. The demand for melons is increasing due to growing public awareness of nutritional intake. Melon has abundant dietary content, including fiber, minerals, beta-carotene, vitamin A, and vitamin C (Daryono & Maryanto, 2016). National production of melon increased from 186,914 quintals in 2018 to 1,318,770 quintals in 2020. However, in 2022, melon production decreased to 1,186,958 quintals (Irjayanti et al., 2022).

Erratic weather, such as a long dry season, results in watering constraints on melon cultivation, which has been a significant factor contributing to the decrease in melon production in recent years. This decrease is particularly concerning given the increasing demand for melons and their nutritional value. Understanding and mitigating the effects of drought on melon production is therefore crucial. Long periods of drought affect the condition of the soil. The water content of the soil decreases. If the level of water in the soil is low or non-existent, plants will not be able to absorb water, resulting in a water deficit. Reduced water potential and turgor, stomatal closure, and reduced photosynthesis are physiological reactions of plants to water deficiency. These reactions impact plant development and growth, lowering yield (Khumaero et al., 2014; Szejgis et al., 2024). To address these problems, efforts are made to increase melon germination and growth to increase productivity under drought-stress conditions. Seed priming treatment is one such effort.

Seed priming is a germination stimulation technique. Seeds given priming treatment will germinate better and withstand abnormal environmental conditions such as drought pressure (Lutts et al., 2016). Hormopriming is one of several seed priming methods available. It uses growth regulators that may improve plant development and seed germination in stressful environmental situations (Huang et al., 2020).

Brassinosteroid can enhance growth and resilience in plants subjected to environmental stressors, including drought. When plants are exposed to drought stress, this hormone regulates gene expression and promotes plant growth. This includes increasing root and crown growth and proline and chlorophyll levels. These adaptations are typical responses to aridity in plants (Gillani et al., 2022). Brassinosteroids can increase germination because they work antagonistically with the hormone ABA (abscisic

acid) and synergistically with GA (gibberellic acid) (Ali & Elozeiri, 2017). Seeds treated with priming can memorize responses by regulating other hormones to increase plant tolerance under aridity (Huang et al., 2020).

The use of brassinosteroid hormopriming to induce drought stress resistance has never been done on melon commodities. This application in melon commodities is new information in research, so studies on hormopriming are needed, especially for melon. This study was undertaken to ascertain the response of melons after brassinosteroid hormopriming treatment to drought stress by reviewing the characteristics of germination and growth in melon plants.

In light of the previously stated conditions, the problem formulation that needs to be resolved is as follows: a. Does hormopriming with brassinosteroid impact the mean number of days required for germination of melon (*Cucumis melo* L.) M15 seeds? b. Does the application of brassinosteroid via hormopriming affect the growth of melon (*Cucumis melo* L.) M15 under aridity? c. Which concentration of hormopriming treatment with brassinosteroid is optimal to induce resistance in melon (*Cucumis melo* L.) M15 plants under aridity? The goals of this research are: a. To assess the effects of hormopriming with brassinosteroid on the average days to germination of melon (*Cucumis melo* L.), b. To assess the effect of hormopriming with brassinosteroid on the growth of melon (*Cucumis melo* L.) M15 under aridity, c. To identify the optimal concentration of hormopriming treatment with brassinosteroid to induce resistance in melon (*Cucumis melo* L.) M15 under aridity.

MATERIALS AND METHODS

Location and Time of Research

The research was conducted from December 2023 to April 2024 at the Biology Laboratory, the Integrated FMIPA Laboratory, and the Greenhouse Integrated Laboratory Unit of Universitas Sebelas Maret, Central Java, Indonesia.

Research Design

This study used an entirely random design with two sources of variation: water availability (75%, 50%, and 25% of space capacity) and brassinosteroid hormopriming

(0, 0.05, 0.10, and 0.15 ppm). Based on this design, 12 treatment combinations were obtained, each with three replications.

Research Tools and Materials

Melon seeds (*Cucumis melo* L.) variety Metando 15 (M15) used in this study were obtained from the seed company CV Multi Global Agrindo Karanganyar, with a release certificate number 474/Kpts/LB.240/8/2004. The chemicals used included brassinosteroid hormone solutions (0, 0.05, 0.10, and 0.15 ppm) in distilled water, 80% acetone in distilled water, clear cuttings, standard proline (Merck), 3% (w/v) sulphosalicylic acid in distilled water, 0.14 M ninhydrin acid in distilled water, AAG (Galactic Acetic Acid), sodium hypochlorite in distilled water, toluene 99.5% pro-analysis Merck, and phosphate buffer with pH 7.2. The planting medium consisted of a 1:1 mixture of regosol and manure. The tools used included germination cups, scissors, petri dishes, beakers, soil testers, vial bottles, stirring rods, pH paper, micropipettes and tips (P1000), tweezers, mortar and pestle, glass funnel, test tubes, polybags, digital balance, water bath, Whatman filter paper, vortex mixer, cuvettes, light microscope (Nikon), oven, and UV-Vis spectrophotometer (Hitachi).

Research Procedure

Preparation of Melon Seeds

Melon seeds harvested in October 2023 were obtained from the seed bank of CV Multi Global Agrindo Karanganyar. Melon seeds were stored in aluminum foil to protect them from the external environment. Seeds of uniform quality were selected based on their physical attributes, including morphology, color, and size.

Hormopriming treatment

Brassinosteroid solutions were prepared in four concentration variations (0, 0.05, 0.10, and 0.15 ppm). Before soaking the melon seeds in the hormopriming solution, they were weighed using an analytical balance to determine their weight. Seeds were carefully sterilized with 1% sodium hypochlorite for 15 minutes, ensuring the safety and hygiene of our experiment. After surface sterilization, the seeds were soaked in various brassinosteroid hormone solutions for 12 hours at room temperature. Following soaking, the seeds were placed in a dry plastic box with tissue paper for 48 hours. Dried seeds

were subsequently weighed using an analytical balance to ascertain their weight post-soaking (Huang et al., 2020).

Test for Germination

The treated seeds were sprouted on filtered paper media. The seeds were soaked in water for 12 hours. The seeds soaked with water were then tested for germination. Every 24 hours, the seeds were watered with 15 mL of distilled water until the end of the germination period. Percentage germination and average days to germinate were calculated. Germination incubation was two weeks at room temperature. The formulas for calculating the percentage of germination and average days to germinate were as follows (Fatikhasari et.al., 2022; Lasut et. al, 2022):

$$Germination\ (\%) = \frac{number\ of\ germinated\ seeds}{number\ of\ seeds\ germinated} x\ 100$$

$$Average\ days\ to\ germinate = \frac{N1T1+N2T2+\cdots.NxTx}{Number\ of\ germinated\ seeds}$$

Description:
N= Number of seeds sprouting in the specified time.
T= Duration of the test at a certain observation interval from start to finish.

Treatment of drought stress

The drought stress treatments, which included 75%, 50%, and 25% space capacity for 45 days, were conducted with meticulous care. The gravimetric approach was used to calculate space capacity, ensuring the accuracy of our measurements. Seeds were planted at 100% space capacity for seven days, and only the most uniform seedlings, in terms of size and growth, were transferred to polybag media with a predetermined moisture content. The determination of space capacity follows the method conducted by Sinamo et al. (2018) with our own modifications. The initial weight of the media (W0) was weighed and then saturated with water until all voids were filled to determine the volume capacity. The saturated medium was left to stand for a full day or until the water stopped flowing out of the polybag. Once the water no longer dripped from the polybag, the final weight (Wa) was determined by reweighing the media. To determine the moisture content at 100% space capacity (Wt), the final weight of the press (Wa) was subtracted from the initial weight of the media (W0).

Observation of melon growth

Growth parameters such as height, dry weight, root-to-shoot ratio, and the number of leaves were measured at the end of the drought stress treatment, specifically on day 45. The plant height measurements and number of leaves were taken from the bottom of the stem above the soil surface to the apical growth point. Plant weight and root-to-crown ratio were determined with the plants in a dry condition. Our thorough drying process involved 48 hours in a 70°C oven to ensure complete dryness. Then, the plants were weighed as a whole, and the crown and roots were weighed separately (Rusmana, 2017). Tamin (2020) provides a formula for calculating the root-crown ratio.

$$root\ crown\ ratio\ (gram) = \frac{crown\ dry\ weight}{root\ dry\ weight}$$

Physiological parameters were thoroughly quantified, including proline content, total chlorophyll, and carotenoid content. The measurement of physiological parameters was conducted on the last day of the drought stress treatment, which was on day 45. Proline content was measured according to the method described by Bates et al. (1973) in Hendrati dkk. (2016). Fresh leaf samples (0.1 grams) were meticulously analyzed for proline content. Fresh leaves were crushed and dissolved with 5 mL of sulphosalicylic acid (3% by volume) in a centrifuge at 5,000 x g for 15 minutes. The resulting products of homogenization with a centrifuge were the supernatant and pellet. Two mL of the supernatant were pipetted into 2 mL of 0.14 M ninhydrin acid (C6H6O4) solution, followed by adding 2 mL of glacial acetic acid (GAA). The supernatant was treated in a 100°C water bath for one hour. The supernatant was transferred to a beaker containing ice for five minutes. Subsequently, the supernatant was mixed with 4 mL of toluene (99.5%). The mixture was vortexed for 15 to 20 seconds until two distinct layers of different colors were formed. A UV-Vis spectrophotometer was used to measure the absorbance of the sample at a wavelength of 520 nm, ensuring the thoroughness of our research process.

Total chlorophyll and carotenoid content were quantified using the fifth fresh leaf from the shoot. Fresh leaves (0.1 grams) were crushed with a mortar and pestle. Eighty percent acetone (10 mL) was used to extract the sample. The extract was filtered using Whatman filter paper No 47, producing a filtrate. The filtrate was analyzed using a UV-Vis spectrophotometer at 480nm, 645nm, and 663nm, which are the specific wavelengths for chlorophyll and carotenoid absorption. A blank containing 3 mL of 80%

acetone was also used (Kurniawan et al., 2010). The following equation determined the chlorophyll and carotenoid content with Easton solvent (Sumiati, 2021).

$$\text{Chlorophyl a} = (OD663 \times 12.7) - (OD645 \times 2.69) \times 10^{-1}$$
$$\text{Chlorophyl b} = (OD645 \times 22.90) - (OD663 \times 4.68) \times 10^{-1}$$
$$\text{Total chlorophyl} = (OD\ 663 \times 8.02) + (OD645 \times 20{,}20) \times 10^{-1}$$
$$\text{Carotenoids} = OD480 + (OD663 \times 0.114) - (OD645 \times 0.638)$$

Description:
OD480 = Optical density value at 480 nanometers.
OD645 = Optical density value at 645 nanometers.
OD663 = Optical density value at 480 nanometers.
Unit = mg/L

Anatomical parameters, including stomatal density, were quantified. Stomatal density was determined using the stomatal printing method, a technique developed in collaboration with Febjislami and Hasibuan (2023). The fifth leaf from the shoot was taken, and the stem end was soaked in water to maintain freshness. Abaxial leaves covering an area of 2 cm² were smeared with polish to dry. After drying, clear duct tape was applied to the dry area with a small amount of rubbing and gentle pressure. The clear tape was then removed slowly and placed on a microscope slide. Stomata were observed with a light microscope (Nikon Eclipse) with a 10 x 40 or 400x magnification. Because the microscope's field of view is circular, the width of the field can be found by the formula of the circle (1/4 μ d2). Micrometers were used for diameter measurements. The formula for calculating stomatal density was as follows (Karubuy et al., 2018).

$$\textit{Stomatal density (per } mm^2) = \frac{number\ of\ stomata}{unit\ area\ of\ view}$$

Data Analysis

An analysis of variance (ANOVA) was performed. Where significant differences were found, Duncan's Multiple Range Test (DMRT) with a 95% confidence level was used.

RESULTS AND DISCUSSION

The germination test was conducted for seven days. All seeds of the control and hormopriming treatment groups germinated 100%. Brassinosteroid treatment affects the germination time of melon seeds. Figure 1 shows the average number of days for melon

seeds to germinate. The treatment of 0.15 ppm brassinosteroid produced the fastest average germination time, with all melon seeds germinating in just 1.20 days. The 0.05 and 0.10 ppm brassinosteroid treatments resulted in average germination times slower than the 0.15 ppm treatment, at 1.83 and 1.67 days, respectively. The brassinosteroid hormopriming treatment at all concentrations generally resulted in faster germination than the control, which took 3.13 days. According to Suraweera et al. (2024), hormopriming with brassinosteroids at a concentration of 0.4 ppm can improve the germination ability (germination energy and germination capacity) of *Pinus nigra* J. F. Arnold seeds.

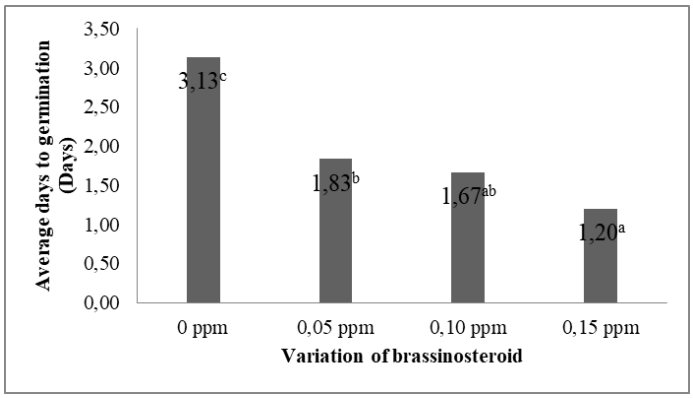

Figure 1. Average number of days for melon seeds to germinate after hormopriming treatment with brassinosteroid. Note: For same-letter values, a 5% DMRT test indicates no significant difference

Brassinosteroid treatment accelerates melon seed germination, a process that is not solely attributed to brassinosteroids but also to the collaborative efforts of GA (gibberellic acid). These two compounds work synergistically to trigger the production of REP-1 (cysteine proteinase) during seed germination. REP-1, in turn, converts nutritional reserves, such as proteins, into amino acids essential for embryo development, thereby enhancing germination.

Our research involved meticulous growth tests, conducted over a period of 45 days under varying conditions. At the end of the test, we assessed a range of plant growth parameters, including plant height, number of leaves, plant dry weight, root-crown ratio, proline content, chlorophyll and carotenoid content, and stomatal density, ensuring a comprehensive understanding of the plant's response to the treatments.

Table 1. Average height of melon plants after drought stress treatment and brassinosteroid hormopriming

Variation of space capacity	Variation of brassinosteroid concentration				Average height (cm)
	0 ppm	0.05 ppm	0.10 ppm	0.15 ppm	
75%KL	45.0±5.8^{ab}	48.5±17.5 ^{ab}	49.5±2.4 ^{ab}	56.0±11.8 ^{bc}	49.79
50%KL	41.0±11.3^{ab}	49.5±6.6 ^{ab}	53.0±10.2 ^{bc}	68.4±7.5^{cd}	52.98
25%KL	34.0±13.7^{a}	83.8±27.9^{d}	103.8± 10.1^{e}	108.0± 6.0^{e}	82.4
Average	40	60.63	68.79	77.47	

Note: For same-letter values, a 5% DMRT test indicates no significant difference

The study revealed that an increase in brassinosteroid concentration in each water availability model corresponded with a significant increase in average plant height (Table 2). The brassinosteroid hormopriming treatments of 0.10 and 0.15 ppm at varying water availability conditions showed substantial differences compared to the control at 25% K.L. The presence of brassinosteroids was found to play a crucial role in the hormonal dynamics of the apical system, influencing the process of cell division and elongation. This led to a maximum cell division and elongation, resulting in an increase in plant height (Castorina & Consonni, 2020). Hormopriming with brassinosteroid was observed to increase plant height under water stress conditions by enhancing auxin biosynthesis through the expression of transcription factor genes such as SAUR15 (Small auxin upregulated 15).

The interaction of brassinosteroid with gibberellic acid (GA) was found to encourage cell elongation (Huang et al., 2020). Brassinosteroids were found to activate BZR1 (Brassinazole resistant 1) and regulate the expression of the GA3ox-2 gene, which encodes an enzyme that activates GA, resulting in increased GA biosynthesis and cell elongation (Kour et al., 2021). Huang et al. (2020) found that when peanuts were treated with drought, the plant height ratio increased compared to non-primed. The 0.10 ppm treatment produced a considerable difference from the control, with a ratio of 150% against 70%.

Table 2. Average leaf number of melon plants after drought stress treatment and brassinosteroid hormopriming

Variation of space capacity	Variation of brassinosteroid concentration				Average leaf number
	0 ppm	0.05 ppm	0.10 ppm	0.15 ppm	
75%KL	9.80 ± 0.45^{b}	10.60 ± 0.55^{c}	10,80 ± 0.45^{c}	12.40 ± 0.55^{d}	10.90
50%KL	9.40 ± 0.55^{b}	10.80 ± 0.45^{c}	11.00 ± 0.71^{c}	14.20 ± 0.45^{f}	11.35
25%KL	8.60 ± 0.55^{a}	13.40 ± 0.55^{e}	16.00 ± 0.00^{g}	16.60 ± 0.55^{g}	13.65
Average	9.26	11.60	12.60	14.40	

Note: For same-letter values, a 5% DMRT test indicates no significant difference

Table 2 provides crucial information on the average number of leaves of melon plants after treatment with variations in water availability and brassinosteroid hormopriming. The significant increase in the number of leaves of melon plants, even under drought stress, due to brassinosteroid application, underscores the importance of this research. Brassinosteroid's role in promoting cell division and leaf cell differentiation, leading to more significant leaf growth, is a significant finding (Oh et al., 2020).

Table 3. Average dry weight of melon plants after drought stress treatment and brassinosteroid hormopriming

Variation of space capacity	Variation of brassinosteroid concentration				Average dry weight (g)
	0 ppm	0.05 ppm	0.10 ppm	0.15 ppm	
75%KL	0.45±0.01^{c}	0.66±0.01^{e}	0.75±0.00^{f}	1.11±0.01^{h}	0.74
50%KL	0.34±0.01^{b}	0.55±0.00^{d}	1.01±0.01^{g}	1.33±0.01^{i}	0.81
25%KL	0.16±0.01^{a}	1.70±0.01^{j}	1.92±0.01^{k}	2.73±0.01^{l}	1.63
Average	0.32	0.97	1.23	1.72	

Note: For same-letter values, a 5% DMRT test indicates no significant difference

Table 3 displays the average dry weight of plants after treatment with drought stress and brassinosteroid. Notably, plant dry weight increased with rising brassinosteroid concentrations in each water availability variation, showing significant differences. The relationship between plant dry weight and photosynthesis, which indicates carbon dioxide assimilation during growth, was observed. Optimal photosynthesis leads to higher plant dry weight. Brassinosteroid, as per Hola (2019), enhances photosynthetic efficiency under drought stress, thereby improving assimilate accumulation. Brassinosteroids also enhance photosystem efficiency and the production of NADPH, a crucial component for the Calvin cycle in photosynthesis, as demonstrated by Siddiqui et al. (2018). This comprehensive impact of brassinosteroids is further highlighted by the findings of Arentes et al. (2020), which showed that at 0.5 ppm, brassinosteroids significantly increased the dry weight of *Psidium* sp. than control by promoting both shoot and root growth.

Table 4. Mean dry root crown ratio of melon after drought stress treatment and brassinosteroid hormopriming

Variation of space capacity	Variation of brassinosteroid concentration				Average dry root crown ratio
	0 ppm	0.05 ppm	0.10 ppm	0.15 ppm	
75%KL	17.60±0.11^{e}	19.58±0.12^{g}	19.78±0.11^{gh}	20.79±0.15^{i}	19.44
50%KL	14.37±0.07^{b}	16.95±.12^{d}	18.31±0.17^{f}	19.92±0.06^{h}	17.39
25%KL	11.54±0.23^{a}	15.83±0,04^{c}	17.03±0.16^{d}	21.58±0.05^{j}	16.50
Average	14.50	17.45	18.38	2.76	

Note: For same-letter values, a 5% DMRT test indicates no significant difference

The average root-crown ratio of melon plants increased with higher brassinosteroid concentrations in each water availability variation (Table 4). Brassinosteroid treatment enhances crown and root growth under drought stress. Brassinosteroid synergizes with auxin in roots by stimulating BIN2 (Brassinosteroid Intensive 2) and BZR1 (Brassinazole Resistant 1), affecting the activity of A.R.F. (Auxin Response Factor), an essential transcription factor in auxin biosynthesis. Importantly, brassinosteroids optimize photosynthetic efficiency, a key factor in plant growth, contributing to crown growth (Tian et al., 2018; Kour et al., 2021; Zhang et al., 2022). Increased crown growth increases crown dry weight (Makrufah et al., 2023). Huang et al. (2020) found that treating peanuts with brassinosteroid hormone priming resulted in a minor loss in dry weight of roots and shoots after drought treatment. Among the other treatments, a concentration of 0.15 ppm results in the least biomass loss.

Table 5. Average leaf proline content of melon plants after drought stress treatment and brassinosteroid hormopriming

Variation of field capacity	Variation of brassinosteroid concentration				Average proline content (M)
	0 ppm	0.05 ppm	0.10 ppm	0.15 ppm	
75%KL	0.48±0.01[a]	1.13±0.01[c]	1.57±0.01[f]	1.80±0.01[i]	1.24
50%KL	1.05±0.01[b]	1.47±0.01[e]	1.83±0.00[j]	1.87±0.01[k]	1.56
25%KL	1.21±0.01[d]	1.63±0.01[g]	1.67±0.01[h]	2.54±0.01[l]	1.77
Average	0.92	1.41	1.69	2.07	

Note: For same-letter values, a 5% DMRT test indicates no significant difference

Proline levels generally increased with higher brassinosteroid concentrations in each field capacity variation (Table 5). This key finding suggests that brassinosteroid treatment enhances plant adaptation to drought stress by inducing genes involved in proline biosynthesis. A 0.15 ppm brassinosteroid concentration produced the highest average proline levels in each water availability treatment: 1.80 M, 1.87 M, and 2.54 M in 75%KL, 50%KL, and 25%KL, respectively. Proline acts as an osmoregulator, protecting cells from oxidative damage by R.O.S. and preventing protein denaturation (Kaur and Asthir, 2015). The same opinion was voiced by Dehghan et al. (2020), who claimed that proline accumulation in response to drought stress is regulated by brassinosteroids in wheat (Triticum aestivum). The study determined that proline accumulated at 17.29 (mmol/g F.W.) at the optimal concentration, which was found to be 0.1 ppm.

Table 6. Average total chlorophyll content of melon leaves after drought stress and brassinosteroid treatment

Variation of space capacity	Variation of brassinosteroid concentration				Average total chlorophyll content (mg/g)
	0 ppm	0.05 ppm	0.10 ppm	0.15 ppm	
75%KL	23.59±0.42^{g}	25.55±0.52^{h}	25.55±0.43^{h}	32.14±0.69^{i}	26.70
50%KL	7.96±0.45^{b}	8.12±0.11^{b}	10.44±0.20^{c}	12.98±0.51^{d}	9.87
25%KL	6.24±0.22^{a}	12.52±0.20^{d}	13.84±0.26^{e}	15.43±0.16^{f}	12.00
Average	12.59	15.39	16.61	20.18	

Note: For same-letter values, a 5% DMRT test indicates no significant difference

Table 7. Average leaf carotenoid content of melon plants after drought stress and brassinosteroid treatment (mg/g wet weight)

Variation of space capacity	Variation of brassinosteroid concentration				Average carotenoid content (mg/g)
	0 ppm	0.05 ppm	0.10 ppm	0.15 ppm	
75%KL	1.88±0.32^{cd}	1.70±0.08^{bcd}	1.70±0.18^{bcd}	1.69±0.19^{bcd}	1.74
50%KL	1.90±0.08^{cd}	1.29±0.23^{ab}	1.24±0.14^{ab}	1.22±0.12^{a}	1.41
25%KL	2.09±0.56^{c}	1.61±0.17^{abc}	1.33±0.31^{ab}	1.88±0.01^{ab}	1.72
Average	1.95	1.53	1.42	1.59	

Note: For same-letter values, a 5% DMRT test indicates no significant difference

Table 6 and Table 7 demonstrate a significant increase in total chlorophyll with brassinosteroid treatment under various water availability conditions (75%KL, 50%KL, and 25%KL), while carotenoid levels decreased but were not substantially different from the control. The potential of brassinosteroids to maintain chlorophyll levels under water stress by stimulating the transcription and translation of enzymes is an encouraging finding (Siddiqui et al., 2018). Similar studies have reported increased chlorophyll content in peanut plants (*Arachis hypogaea* L.) treated with brassinosteroids under drought stress. The study found that the doses of 0.10 and 0.15 ppm produced the highest levels of chlorophyll content, measuring 53 and 55 SPAD. These results were statistically significant when compared to the control. Based on the chlorophyll meter SPAD-502 used in the study, the SPAD unit represents the amount of chlorophyll in leaves(Huang et al., 2020).

Table 8. Average stomatal density of melon leaves after drought stress and brassinosteroid treatment

Variation of space capacity	Variation of brassinosteroid concentration				Average stomatal density (/mm^2)
	0 ppm	0.05 ppm	0.10 ppm	0.15 ppm	
75%KL	116.25±14.29^{c}	124.60±18.75^{c}	181.12±9.14^{e}	231.21±7.65^{f}	163.29
50%KL	104.05±7.08^{b}	146.44±12.26^{d}	224.79±10.98^{f}	245.34±8.73^{g}	180.15
25%KL	90.56±7.08^{a}	122.67±9.49^{c}	223.50±7.65^{f}	257.55±7.71^{h}	173.57
Average	103.62	131.24	209.81	244.7	

Note: For same-letter values, a 5% DMRT test indicates no significant difference

The number of stomata generally increased with higher brassinosteroid concentrations in each water availability variation (Table 8). A 0.15 ppm brassinosteroid concentration yielded the highest stomatal density in each water availability treatment, with significant differences from the control. Stomatal formation is initiated by the activity of S.P.C.H. (Speechless transcription factor), which regulates protodermal differentiation to form stomatal modifications. Drought suppresses S.P.C.H. expression in C3 plants, reducing stomatal numbers. Brassinosteroids enhance stomatal development by increasing S.P.C.H. activity through BIN2 inhibition (Siddiqui et al., 2018; Zoulias et al., 2018; Song et al., 2023). It's worth noting that no study has measured stomatal density in response to brassinosteroid hormone priming therapy coupled with drought, making our research a significant contribution to the field. Huang et al. (2020) have reported measuring stomatal conductance on peanuts. Stomatal conductance reduces considerably during a drought.

CONCLUSION

The research on hormopriming with brassinosteroid and its effects on melon (*Cucumis melo* L.) growth holds great potential. The application of a brassinosteroid concentration of 0.15 ppm induces drought resistance in melon plants M15, enhancing plant height, leaf number, plant dry weight, and root/crown ratio under varying field capacity water availability. This concentration emerges as the most effective, offering a promising avenue for enhancing plant growth and adaptation under varying water availability conditions.

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