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# Effects of concentration and time of brassinosteroid treatment on growth and yield of soybean under drought stress conditions

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## Abstract

Soybean (*Glycine max* (L.) Merrill.) is an essential food and industrial crop, but it can be heavily affected by drought, especially during the pod growth stage. Brassinosteroids (BRs) are reported to alleviate drought stress, but the effectiveness may depend strongly on BR concentration and timing of the application, and no research has investigated this issue. This study aimed to determine the suitable concentration of BRs and time of treatment to help soybean plants withstand drought conditions during pod growth. The experiment was conducted with a completely randomized design, with four concentrations of BRs: 0 ppm (water) (B0), 0.1 ppm (B1), 0.2 ppm (B2), 0.3 ppm (B3), and three-time points for the application of BR treatment: soaking before sowing (T1), leaf spray when 50% of plants flowered (T2), leaf spray when 50% of the plants had pod at least 0.5 cm in length (T3). The results showed that BRs 0.2 ppm gave the highest plant fresh and dry biomass, as well as root length and stem diameter. Treatment $B_2T_2$ had the highest total number of firm pods and the highest number of firm seeds at 22.2 and 44.6, respectively, with the weight of 100 seeds reaching 17.9 g, leading to the highest actual yield of 97.0 g/plot. These results indicate that with appropriate concentration and timing (0.2 ppm during flowering), the application of BRs can significantly alleviate drought stress effects on soybean plants during pod growth, improving seed yield and quality.

## Keywords

brassinosteroid; drought; soybean; seed quality; yield

## Introduction

Soybean (*Glycine max (L.) Merrill.)* plays an important role in global agriculture as both an important industrial crop and an essential food source, accounting for 27.7% of global cooking oil (only after palm tree), and 69.8% of global protein meal (1). However, its production is always threatened by drought stress, which soybean plants are particularly susceptible to (2). With moderate to severe drought, the loss in yield of soybean could reach 1.56 to 3.25 times higher than that of maize (3). Drought during the reproductive stage is especially damaging to soybeans and can cause up to 74% loss in yield compared to 24% for drought during vegetative growth (4). Due to the increasingly complicated situation of climate change, droughts are becoming more and more serious and widespread, threatening soybean production and food security in many regions (2).

To minimize the adverse effects of drought on soybean cultivation, researchers have explored a variety of strategies, including the application of plant growth regulators. Among these, brassinosteroids (BRs) are steroid hormones that play a key role in many physiological processes, including cell elongation, division, differentiation, photosynthesis, and stress tolerance mechanisms (5). Regarding drought stress in particular, BRs have been shown to mitigate the negative effects on the gas exchange of *Brassica juncea* plants, increasing the photosynthetic rate, stomatal conductance, and water-use efficiency (6).

The use of BRs in alleviating the adverse effects of drought stress on soybeans has been the subject of several studies (7, 8). However, choosing the appropriate concentration and timing of BR application is important to achieve optimal effectiveness in improving the growth and yield of soybeans under drought conditions, yet no research has been done on this topic. Therefore, this study was conducted to investigate the optimal BR concentration and timing of application for drought mitigation in soybeans, aiming to provide practical recommendations for farmers and agronomists to optimize soybean cultivation practices, minimize the impact of drought stress, and ensure food security in the face of changing climate conditions.

## Materials and Methods

### Time and location

The experiment was conducted at the Experimental Farm of the Faculty of Agronomy at Nong Lam University Ho Chi Minh City, Vietnam from December 2022 to May 2023.

### Materials

The soybean variety HLDN 910 was selected from a hybrid combination (HL 203 × OMDN 1). Variety characteristics: protein content 34.7%; lipids 19%; concentrated pod ripening; low pod dehiscence; resistant to rust, bacterial leaf blight, and pod rot.

Black plastic pots were 28 × 23 × 23 cm (top diameter × bottom diameter × height) in size.

Soil is taken from Xuan Thoi Thuong commune, Hoc Mon, Ho Chi Minh City, with 10 kg soil/pot. Soil samples were analyzed at the Research Institute for Biotechnology and Environment, Nong Lam University Ho Chi Minh City in 2022 (Table 1).

### Experiment design

The two-factor experiment was arranged in a completely randomized design (CRD), with 3 replications, each with 12 pots (3 plants per pot). The experiment consisted of the following experimental factors: concentration of BRs (B factor) with 4 levels: B0: 0 ppm (water), B1 (0.1 ppm), B2 (0.2 ppm), B3 (0.3 ppm); and time of BR application (T factor) with 3 levels: T1 (before sowing, by seed soaking), T2 (when 50% of the plants flowered, by leaf spray), T3 (when 50% of the plants had pods longer than 0.5 cm, by leaf spray).

### Methods

The fertilizer formula (kg/ha) of 40-40-60 (N - $P_2O_5$ - $K_2O$) was used for all treatments, equivalent to 0.84 g urea, 3.6 g superphosphate, and 0.96 g potassium chloride per pot. All the phosphate fertilizer was mixed with the soil before potting. Half the amount of nitrogen and potassium was applied by top-dressing when the plants had 1–2 true leaves. The remaining fertilizer was applied when the plants had 4–5 true leaves.

Before the drought, plants were watered every day to full soil capacity (indicated by leaking). Drought condition was applied by withholding water for 8 days, starting from when 50% of the plants had pods longer than 0.5 cm, corresponding to 49 days after sowing (DAS). Soil water content (SWC) was monitored every day during the drought period. Pots were rewatered to full soil capacity when the drought period was over and received normal irrigation from then until harvest. For T1 treatments, before sowing, the seeds were soaked for 8 hours in BR solutions (Merck, Germany) of different concentrations depending on treatments. In the remaining treatments, BR solutions of different concentrations were sprayed evenly on the upper side of the leaves (100 mL per experimental plot) at the times designed.

### Measurements

Growth, yield components, and physiological traits were measured randomly on five plants in five different pots in each plot. The plant height, stem diameter, and number of primary branches were measured 14 days after the onset of the drought period. On 60 DAS, the fresh and dry biomass was obtained. The weight of 100 seeds (g) was measured by weighing 3 samples, each with 100 seeds, and taking average. The actual yield was calculated as the total weight of all the seeds in the whole experimental plot. Proline content of the leaves was determined according to Paquin and Lechasseur (9).

### Statistical analysis

All data were calculated using Microsoft Excel 2010, and analyses of variance (ANOVA) were performed using RStudio 4.1.0 software (Posit, PBC, USA). The treatments were ranked using the least significance difference (LSD) test at the probability of 0.05.

**Table 1**. Physical and chemical properties of soil used in the experiment

| Texture (%) | | | | pH | Organic matter (%) | Total N (%) | Total $P_2O_5$ (%) | Total $K_2O$ (%) |
|---|---|---|---|---|---|---|---|---|
| Clay | Silt | Fine sand | Coarse sand | | | | | |
| 11.47 | 18.68 | 31.89 | 37.96 | 8.04 | 2.62 | 0.10 | 0.24 | 0.23 |

## Results and Discussion

### Soil water content during drought period

The suitable SWC for soybean plants at the pod growth stage is 70–80%. However, as shown in Figure 1, on the fourth day since water withdrawal, the SWC already dropped below 70%, which affected pod growth. At the end of the drought period (8 days of water withdrawal), the SWCs in all treatments dropped below 50% (Table 2). Interestingly, the application of BRs, regardless of concentration, led to statistically higher SWCs compared to treatments with no BR application. This result shows that the application of BRs induced the plants to reduce water consumption, which could help them withstand drought conditions better. However, there was no statistical difference in SWC among treatments with different times of BR application, indicating that this effect of BRs in inducing water conservation under drought in soybean is long-lasting, and could be retained from the time the seeds were primed with BRs until pod growth stage. Perez-Borroto et al. (7), while investigating the effects of a functional BR analog DI-31 on soybean drought tolerance, also showed that DI-31 prevented the decline in canopy growth and enhanced the water efficiency of soybean plants since the early stages of water deprivation.

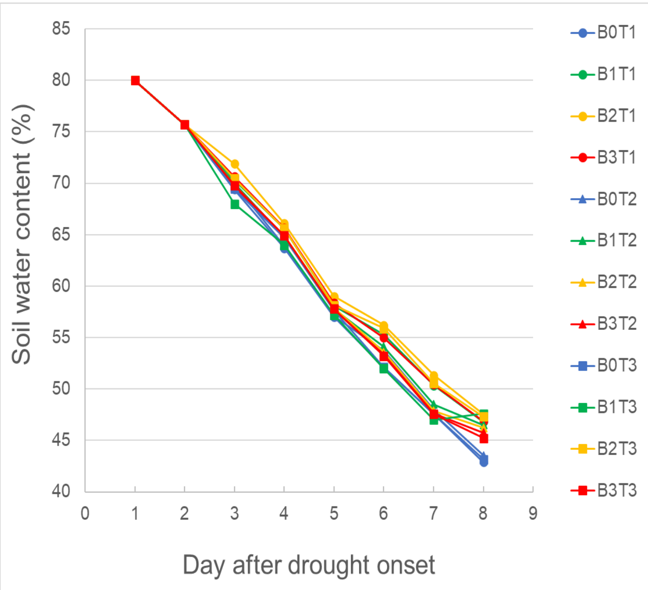


**Fig. 1.** Soil water contents in all treatments during drought period

**Table 2.** Soil water content of the treatments at the end of the drought period

| Time of application (T) | BR concentration (B) | | | | Average T |
|---|---|---|---|---|---|
| | $B_0$ | $B_1$ | $B_2$ | $B_3$ | |
| $T_1$ | 42.9 | 46.8 | 47.5 | 46.8 | 46.0 |
| $T_2$ | 43.5 | 46.5 | 46.2 | 45.7 | 45.5 |
| $T_3$ | 43.1 | 47.6 | 47.3 | 45.2 | 45.8 |
| Average B | 43.2 B(1) | 46.9 A | 47.0 A | 45.9 A | |
| CV (%) = 2.4; $F_B$ = 22.3***(2); $F_T$ = 0.7 ns; $F_{BT}$ = 1.2 ns | | | | | |

(2): ns, *** denote nonsignificant or significant difference at α = 0.001, respectively, according to two-way ANOVA;

(1): Numbers with the same alphabet are not statistically different according to the LSD test at α = 0.05.

### Effect of concentration and time of brassinosteroid treatment on height, stem diameter, and number of primary branches of soybean plants

The application of BRs did not affect plant height and number of primary branches under drought conditions, regardless of the BR concentration and time of application (Table 3). The plant height ranged from 82.1 cm to 114.1 cm. The number of primary branches ranged from 2.1 branches/plant to 3.0 branches/plant. This could be because, by the time the plants experienced drought, they had already completed the vegetative growth cycle. Therefore, no significant adverse effects of drought, and consequently no improving effects of BRs, were observed in these measurements.

However, the concentration of BRs was shown to have statistically significant effects on stem diameter. Unlike stem elongation and branch development, stem horizontal expansion is more related to secondary growth that occurs after elongation through the development of the vascular cambium layer (10, 11), and could still be happening during the drought period in this experiment, which could explain the differences among treatments. The initiation and differentiation of vascular cambium are strongly affected by environmental factors, particularly water availability (12,13), thus the drought period could harm stem expansion and development of soybean plants in this experiment. BR application was shown to help mitigate these adverse effects. The lowest stem diameter

**Table 3.** Effect of concentration and time of BR treatment on height, stem diameter, and number of primary branches of soybean plants under drought

| Measurements | Time of application (T) | BR concentration (B) | | | | Average T |
|---|---|---|---|---|---|---|
| | | $B_0$ | $B_1$ | $B_2$ | $B_3$ | |
| Plant height (cm) | $T_1$ | 82.1 | 103.4 | 112.1 | 106.8 | 101.1 |
| | $T_2$ | 97.6 | 101.4 | 114.1 | 95.6 | 102.2 |
| | $T_3$ | 90.2 | 97.6 | 90.7 | 94.4 | 93.2 |
| | Average B | 89.9 | 100.8 | 105.6 | 98.9 | |
| CV (%) = 17.4; $F_B$ = 1.3 ns(1); $F_T$ = 0.9 ns; $F_{BT}$ = 0.6 ns | | | | | | |
| Number of primary branches (branches/ plant) | $T_1$ | 2.3 | 2.7 | 2.4 | 2.5 | 2.5 |
| | $T_2$ | 2.5 | 2.6 | 3.0 | 2.5 | 2.7 |
| | $T_3$ | 2.1 | 2.4 | 2.7 | 2.6 | 2.4 |
| | Average B | 2.3 | 2.5 | 2.7 | 2.5 | |
| CV (%) = 13.3; $F_B$ = 2.7 ns; $F_T$ = 1.7 ns; $F_{BT}$ = 0.9 ns | | | | | | |
| Stem diameter (mm/plant) | $T_1$ | 3.5 | 4.8 | 4.7 | 4.6 | 4.4 |
| | $T_2$ | 4.2 | 4.1 | 4.9 | 4.2 | 4.3 |
| | $T_3$ | 3.8 | 4.8 | 4.2 | 4.3 | 4.2 |
| | Average B | 3.8 B(2) | 4.6 A | 4.6 A | 4.3 AB | |
| CV (%) = 13.6; $F_B$ = 3.3*; $F_T$ = 0.1ns; $F_{BT}$ = 0.3 ns | | | | | | |
| Root length (cm/plant) | $T_1$ | 21.0 | 22.8 | 27.2 | 22.2 | 23.2 |
| | $T_2$ | 20.0 | 23.1 | 28.1 | 25.9 | 24.3 |
| | $T_3$ | 21.2 | 22.7 | 25.5 | 22.1 | 22.8 |
| | Average B | 20.7 B | 22.5 B | 26.9 A | 23.4 AB | |
| CV (%) = 11.3; $F_B$ = 8.5***; $F_T$ = 1.0 ns; $F_{BT}$ = 0.6 ns | | | | | | |

(1): ns, *, *** denote nonsignificant or significant difference at α = 0.05 or 0.001, respectively, according to two-way ANOVA;

(2): in the same row, numbers with the same alphabets are not statistically different according to the LSD test at α = 0.05.

was in soybean plants under drought conditions without BRs (3.8 mm/plant) and was significantly different from that of soybean plants sprayed with BRs at two concentrations of 0.1 ppm (4.6 mm/plant) and 0.2 ppm (4.6 mm/plant). In turn, more developed vascular tissues allowed better water and nutrient transportation, which could help with plant tolerance to drought and subsequent recovery, increasing yield. These results were similar to (14), who showed that chickpeas (*Cicer arietinum*) exposed to water stress and treated with BRs had significant increases in stem thickness as well as fresh and dry weight, number of tillers, root activity, and nitrate reductase activity.

Similarly, root length was found to be highest when BR solution at the concentration of 0.2 ppm was applied, reaching 26.9 cm (Table 2 and Fig. 2), although the difference was not statistically significant compared to the treatment with 0.3 ppm BRs. The time of BR application and the interaction between the two factors did not produce significant effects. Reduction in root length is one of the major adverse effects of drought (15). Chen et al. have shown that mutant cotton plants (*Gossypium hirsutum* L.) with BR deficiency had shorter primary roots and fewer lateral roots, and were consequently more sensitive to drought stress (16). As shown in our study, a suitable concentration of BRs could help mitigate the negative impact of drought on root length, which, in turn, could help plants access more water in the soil, allowing for better tolerance to drought.

## Effect of concentration and time of brassinosteroid treatment on the biomass of soybean plants

Application of BRs at the concentration of 0.2 ppm resulted in the highest fresh and dry biomass of soybean plants, significantly different from other BR concentrations. In particular, plants treated with 0.2 ppm BR had three folds higher fresh and dry weights compared to those treated with only water (Fig. 3). This indicates that photosynthesis and biomass accumulation were maintained well in these plants under drought conditions, and highlighting the effectiveness of BRs in mitigating drought damage in soybean plants. According to Krishna (17), BRs help increase plant tolerance to drought by increasing water uptake and membrane stability and maintaining higher carbon dioxide and nitrogen assimilation rates. A study on tomatoes by Yuan et al. (18) showed that the photosynthetic rate significantly decreased under water stress due to a reduction in stomatal conductance, and BR could alleviate water stress and increase photosynthesis. Similarly, Huang et al. (19) showed that BRs could eliminate the drought-induced down regulation of genes involved in photosynthesis and plant growth regulator signaling, maintaining photosynthetic rate, growth, and yield under moderate drought. However, no significant differences were found as a result of different BR application times across treatments of the same BR concentrations in our study, except at a BR concentration of 0.2 ppm between BR application at flowering time (T2) and pod growth time (T3).

## Effect of concentration and time of brassinosteroid treatment on yield components of soybean plants

Damage due to drought stress during the vegetative growth stage can be compensated to some extent with sufficient watering during the reproductive growth stage. However, drought stress during the reproductive growth stage tends to reduce yield irreversibly (20). Reduction in seed filling which leads to flat seeds, seed abortion, and flat pods is a major adverse effect of drought on legume plants, particularly soybeans. Du et al. (21) reported that drought stress impedes the activation of sucrose metabolism and the plant's capacity to unload sucrose into seeds during early seed development in soybeans. At the middle and late stage of seed-filling, sucrose flow from leaves to seeds was diminished, and the balance of sucrose metabolism was impaired in seeds, resulting in seed mass reduction. The introduction of drought at the seed growth stage not only reduces seed growth but (can also decrease the protein and lipid contents of the seeds, leading to diminished seed quality (22).


**Fig. 2**. Root length in all treatments on 60 DAS

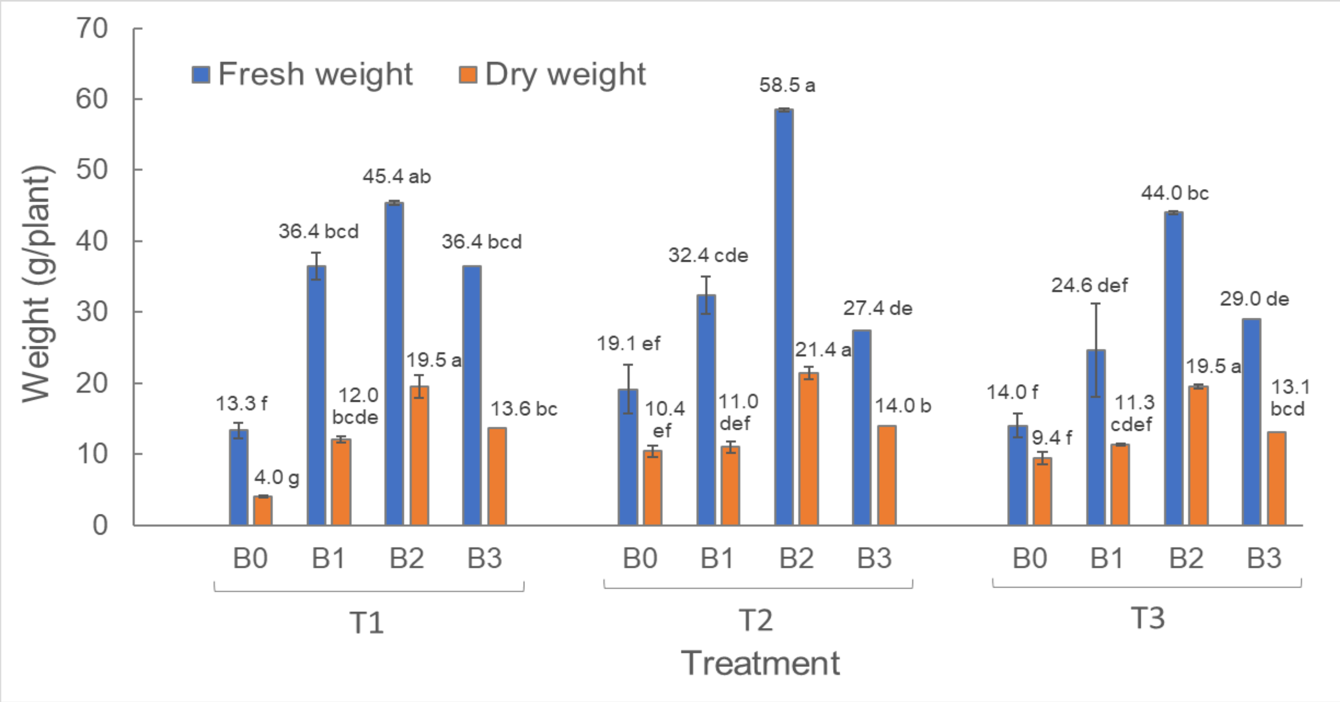


**Fig. 3**. Effect of concentration and time of BR treatment on the biomass of soybean plants under drought.

(1): in the same measurement, numbers with the same alphabet are not statistically different according to the LSD test at α = 0.05; Bars represent standard errors.

Our results demonstrate that BR application could mitigate these negative impacts of drought, and the effectiveness depended on BR concentration. Table 4 and Fig. 4 show the total number of firm pods and firm seeds of soybeans reached the highest of 22.2 pods/plant and 44.6 seeds/plant, respectively, when the plants were treated with 0.2 ppm BRs at the time of flowering (T2), which was statistically higher than all other treatments except for treatment B3T1. While differences in terms of the weight of 100 seeds were less clear, treatment B2T2 was still ranked among the best treatments. These results culminated in the highest actual yield in this treatment (97.0 g), representing 62–83% increases compared to treatments with no BR applications, and statistically higher than all other treatments. Other studies have reported similar improvement effects of BRs or their analogs on the yield of soybeans under drought (7) (8).

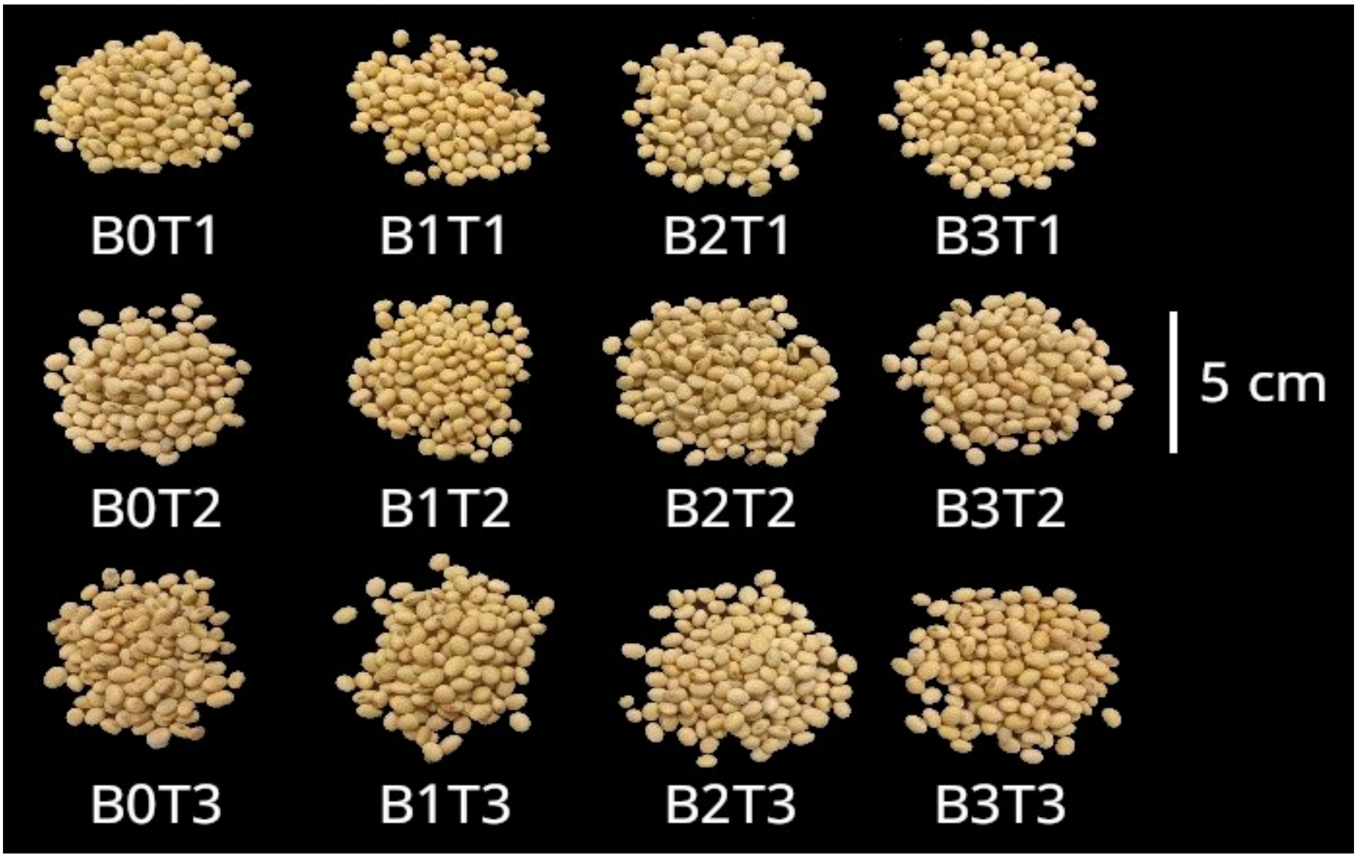


**Fig. 4**. Seeds of soybean plants under drought stress with different BR concentrations and times of application on 60 DAS.

**Table 4**. Effect of concentration and time of BR treatment on yield components of soybean plants under drought

| Measurements | Time of application (T) | BR concentration (B) | | | | Average T |
|---|---|---|---|---|---|---|
| | | $B_0$ | $B_1$ | $B_2$ | $B_3$ | |
| Number of firm pods (pods/plant) | $T_1$ | 10.7 ef[1] | 15.5 bc | 15.2 bc | 18.6 ab | 15.0 A |
| | $T_2$ | 9.4 f | 13.3 cde | 22.2 a | 11.8 def | 14.2 A |
| | $T_3$ | 8.4 f | 11.6 def | 11.0 ef | 11.2 ef | 10.5 B |
| | Average B | 9.5 C | 13.4 B | 16.1 A | 13.9 B | |
| | | CV (%) = 8.6; $F_B$ = 49.8***[2]; $F_T$ = 49.3”*”; $F_{BT}$ = 23.3*** | | | | |
| Number of firm seeds (seeds/plant) | $T_1$ | 18.7 e | 29.7 bc | 30.6 b | 39.3 a | 29.5 A |
| | $T_2$ | 17.0 e | 26.8 bcd | 44.6 a | 23.2 cde | 27.9 A |
| | $T_3$ | 16.7 e | 22.0 de | 21.8 de | 23.0 cde | 20.9 B |
| | Average B | 17.4 C | 26.1 B | 32.3 A | 28.5 B | |
| | | CV (%) = 8.9; $F_B$ = 65.1***; $F_B$ = 46.1***; $F_{BT}$ = 27.4*** | | | | |
| Weight of 100 seeds (g) | T1 | 12.9 cd | 14.5 bcd | 13.3 cd | 18.0 a | 14.7 B |
| | T2 | 12.2 d | 16.1 abc | 17.9 a | 17.3 ab | 15.8 A |
| | T3 | 12.9 cd | 18.1 a | 15.1 abcd | 16.8 ab | 15.7 A |
| | Average B | 12.7 C | 16.2 AB | 15.5 B | 17.4 A | |
| | | CV (%) = 6.4; $F_B$ = 35.5**; $F_T$ = 5.1*; $F_{BT}$ = 7.3*** | | | | |
| Actual yield (g) | T1 | 52.9 e | 74.8 bc | 68.9 bcd | 68.2 bcde | 66.0 B |
| | T2 | 58.1 de | 62.1 bcde | 97.0 a | 73.2 bcd | 72.8 A |
| | T3 | 59.9 cde | 68.7 bcd | 67.1 bcde | 79.8 b | 68.9 AB |
| | Average B | 57.0 C | 68.5 B | 77.7 A | 73.7 AB | |
| | | CV (%) = 7.2; $F_B$ = 29.1***; $F_T$ = 5.6**; $F_{BT}$ = 12.5*** | | | | |

(2): ns, *, **, *** denote nonsignificant or significant differences at α = 0.05, 0.01, or 0.001, respectively, according to two-way ANOVA;

(1): In the same matrix, numbers with the same alphabet are not statistically different according to the LSD test at α = 0.05.

The yield data were largely in agreement with the data of plant biomass in terms of BR concentration, i.e., 0.02 ppm BRs was the most effective at mitigating drought effects in soybeans. However, they also highlight the importance of proper application time for the maximum effect of BRs. Application of 0.02 ppm BRs at the flowering time significantly improved the effectiveness of BRs, giving an actual yield (97.0 g) that was 22% higher than the second-best treatment (B3T3, with the actual yield of 79.8 g). To our knowledge, this study is the first to show the influence of application time on the efficacy of BRs in facilitating drought tolerance in soybeans. However, more detailed studies are needed to determine whether this influence is more associated with the relativity between the application time and the drought period (i.e., pre-emptive application 7 days before the drought), or is due to the application at the correct developmental stage of the plants (during flowering) to induce pod growth and improve yield.

It is also important to note that seed soaking with BR solutions was still capable of significantly mitigating drought damage and improving yield. A previous study by Huang et al. (19) also demonstrated the possibility of applying seed priming with BRs to improve the growth and yield of peanuts under drought conditions. These results show the long-lasting effect of BRs in inducing drought tolerance in plants, allowing for more options in the practical application of BRs.

### Effect of concentration and time of brassinosteroid treatment on proline content of soybean plants

Table 5 shows that when analyzed for single-factor effects, the proline content in leaves tended to decrease as the BR concentration increased. While the time of BR application alone did not influence proline accumulation, the interaction between this factor and BR concentration also showed statistically significant effects on leaf proline content, but no clear trends were observed.

Proline is an amino acid usually accumulated when plants experience stresses, and has multiple roles in plant response to stresses, particularly drought, such as stabilizing protein and preventing protein aggregation, acting as osmolyte, scavenging ROS and preventing oxidative damage, stabilizing cellular homeostasis, etc. (23). Reduction in proline content as the BR concentration increased could be an indication that the negative effects of drought were alleviated more with higher BR concentration, which is largely in agreement with growth and yield data. These results show that BRs mitigate drought stress in soybeans not by inducing accumulation of proline to combat drought damage but by a different mechanism independent of proline. However, it is interesting to note that this trend of proline content reduction continued when the BR concentration increased to 0.3 ppm, while plant growth and yield at this BR concentration were lower than at the BR concentration of 0.2 ppm. This suggests that BRs have other direct influences on proline synthesis and accumulation besides the indirect effects through alleviating plant stress. More detailed studies are needed to confirm and explain these observations.

## Conclusion

Application of BRs at the concentration of 0.2 ppm significantly improved growth measurements, such as stem diameter, root length, and fresh and dry weight, of soybean plants subjected to drought stress during the pot growing stage. The application of this BR concentration at the correct time, i.e., during the flowering stage of the plants, increased the effectiveness of BRs in drought stress mitigation, and greatly improved all yield components, giving the highest actual yield of 97.0 g, which was 62–83% higher than that of plants with no BR application. However, the effects of BRs in mitigating drought stress were not dependent on the induction of proline accumulation in the leaves.


## Acknowledgements

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## Authors' contributions

VHP designed the study and participated in the growth and biochemical measurements and analyses. TTHL conducted the cultivation, stress, and BR application, and participated in growth measurement. DMP participated in the statistical analysis and writing manuscript. LTTN participated in biochemical analysis. KCN participated in the cultivation and growth analyses. TMB participated in the designing and coordination of the study, and writing manuscript. All authors read and approved the final manuscript.

## Compliance with ethical standards

**Conflict of interest:** Authors do not have any conflict of interest to declare.

**Ethical issues:** None.

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**Table 5**. Effect of concentration and time of BR treatment on proline content of soybean plants under drought

| Time of application (T) | BR concentration (B) | | | | Average T |
|---|---|---|---|---|---|
| | $B_0$ | $B_1$ | $B_2$ | $B_3$ | |
| $T_1$ | 18.6 bc[(1)] | 23.6 a | 16.0 cd | 12.0 de | 17.5 |
| $T_2$ | 24.3 a | 10.0 e | 20.6 abc | 11.3 de | 16.5 |
| $T_3$ | 19.6 abc | 22.6 ab | 10.0 e | 12.0 de | 16.0 |
| Average B | 20.8 A | 18.7 AB | 15.5 B | 11.7 C | |
| CV (%) = 15.9; $F_B$ = 19.9***[(2)]; $F_T$ = 0.9 ns; $F_{BT}$ = 13.1*** | | | | | |

(2): ns, *** denote nonsignificant or significant difference at α = 0.001, respectively, according to two-way ANOVA;

(1): In the same matrix, numbers with the same alphabet are not statistically different according to the LSD test at α = 0.05.

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