

Effect of plant growth regulators and drought duration on physiology, growth and yield of soybeans (*Glycine max* (L.) Merrill) at the pod growth stage

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ARTICLE INFO	ABSTRACT
Research Paper Received: December 06, 2023 Revised: February 19, 2024 Accepted: February 29, 2024 Keywords Brassinolide Drought stress Gibberellic acid Salicylic acid Soybeans *Corresponding author Phan Hai Van Email: phvan@hcmuaf.edu.vn	During the pod growth stage, soybeans (<i>Glycine max</i> (L.) Merrill) are very susceptible to drought. The research was carried out to investigate the extent of damages by different durations of drought on soybean plants during pod growth, and the capabilities of different plant growth regulators (PGRs) to mitigate these damages. The two-factor experiment was conducted in a completely randomized design, including 12 treatments with 3 replications. Factor A was the drought durations: 0 days (D0), 4 days without irrigation (D4), 8 days without irrigation (D8); and factor R was the PGR treatments: water (W), salicylic acid (SA), brassinolide (BR), and gibberellic acid GA₃ (GA). Results showed that no differences in plant growth and development due to drought durations or PGRs were observed. However, longer drought durations severely reduced the yield by about 83.8% compared with control. Among the three PGRs used, brassinolide was most effective in mitigating drought stress effects on soybean plants, improving the total number of firm pods (35.0 pods), firm seeds (70.8 seeds), the weight of 100 seeds (16.6 g), and productivity (195.9 g). However, spraying brassinolide did not affect the protein, proline, and pigment contents.

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1. Introduction

Soybeans (*Glycine max* (L.) Merrill) are a major food source with high nutritional and economic value, and an essential short-term industrial crop, the second most important source of cooking oil globally, and accounting for more than two third of global vegetable protein

meal (OECD-FAO, 2020). In addition, soybeans are also a major source of food for livestock and raw materials for the processing industry (Ngo et al., 1999), as well as being important for soil improvement.

However, soybeans are also very susceptible to drought (Dede et al., 2022). Moderate to

severe drought could cause loss in yield of soybean from 10.4% to 21.8%, 1.56 to 3.25 times higher than that of maize (Wang et al., 2020). Especially, if drought occurs during the pod growth stage, which is an important stage that determines seeds formation and growth, it can result in major loss in yield and seed quality, which could be up to 72 - 82% (Wei et al., 2018).

The use of plant growth regulators (PGRs) to induce drought tolerance in soybeans is receiving attention because many recently discovered PGRs show the capabilities to induce stress tolerance and adaptation responses (Dede et al., 2022). However, the effectiveness of different PGRs may vary depending on the duration of drought, but studies on this topic are still very limited. Therefore, the study “Effect of plant growth regulators and duration of drought on physiology, growth and yield of soybeans (*Glycine max* (L.) Merrill) at the pod growth stage” was conducted.

2. Materials and Methods

2.1. Time and location

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

2.2. Materials

The soybean variety HLDN 910 was selected from the cross combination of HL 203 × OMDN 1.

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

Salicylic acid (SA): Merck, Germany

Brassinolide (BR): Merck, Germany

Gibberellic acid (GA₃): Merck, Germany

Fertilizer formula: 40 - 60 - 60 (N - P₂O₅ - K₂O)

Soil was taken from Xuan Thoi Thuong commune, Hoc Mon, Ho Chi Minh City, and analyzed at the Research Institute for Biotechnology and Environment, Nong Lam University, Ho Chi Minh City (Table 1).

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

Texture (%)				pH	Organic matter (%)	Total N (%)	Total P ₂ O ₅ (%)	Total K ₂ O (%)
Clay	Silt	Fine sand	Coarse sand					
11.47	18.68	31.89	37.96	8.04	2.62	0.10	0.24	0.23

2.3. Experimental design

The two-factor experiment was arranged in a completely randomized design (CRD), with 3 replications, each with 12 pots, 36 pots in total (3 plants/pot). The factors included type of PGR (R factor) with four types: W (water), SA (salicylic acid, 69 ppm), BR (brassinolide, 0.1 ppm), GA (GA₃, 75 ppm); and duration of drought stress

(D factor) with 3 levels: D0 (no drought), D4 (drought in 4 days), D8 (drought in 8 days).

2.4. Cultivation and treatment application methods

Seeds were sown in pots containing 10 kg soil/pot. Pots were watered until leaking every day. The fertilizer formula (kg/ha) of 40 - 60 - 60

(N - P_2O_5 - K_2O ; Nguyen et al., 2018) 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 rest was applied when the plants reached 4 - 5 true leaves.

When 50% of plants had pods longer than 0.5 cm (49 days after sowing (DAS)), the PGRs were applied as foliar spray (100 mL per experimental plot). The next day, drought treatments were started by withholding irrigation for the durations according to each treatment. After the drought periods, plants were watered normally until harvest time.

2.5. Measured traits

Five plants of five different pots in each plot were selected randomly for the measurement of growth, yield components, and physiological traits. The plant height and number of leaves were measured on 54 DAS, and the root length, fresh and dry biomass was recorded on 60 DAS. The weight of 100 seeds (g) was calculated as the average of 3 samples, each with 100 seeds. The actual yield was calculated as the total weight of all the seeds in the whole experimental plot. Chlorophyll and carotenoid were extracted from 1 g of mature leaves with ethanol 95% for 24 h, then the absorbance at 470 nm, 664.2 nm and 664.8 nm were measured, and the chlorophyll a, b and total carotenoid contents were calculated

according to Lichtenthaler (1987). Proline content of the leaves was measured according to Paquin & Lechasseur (1979). Perform a colour reaction of 0.5 mL of the extract with 1 mL of 1% ninhydrin mixture, 60% acetic acid, 20% ethanol, boil in a water bath at 95°C for 20 min, then cool. The absorbance at 520 nm was measured, and the proline content was calculated based on a standard curve. Leaf protein content was measured with the Bradford method (Bradford, 1976). Leaf ethanol extract was reacted with Bradford reagent (Coomassie Brilliant Blue 0.01%, ethanol 4.7%, and phosphoric acid 8.5%). The absorbance at 595 nm was measured, and the protein content was calculated based on a standard curve.

2.6. Data analysis

Data were analyzed using R (version 4.2.3). After analysis of variance (ANOVA) gave significant results, the treatments were ranked using the least significant difference (LSD) test at a 5% level of probability.

3. Results and Discussion

Neither drought durations nor type of PGRs affected growth parameters such as plant height and number of leaves (Table 2). This is probably because by the time of PGR application and drought (49 and 50 DAS, respectively), the plants had already completed the vegetative growth cycle. Therefore, no significant effects of drought or PGRs were observed in these measurements.

Table 2. Effects of plant growth regulators (PGRs) and drought durations on height, number of leaves and root length of soybean plants

Parameters	PGRs (R) ¹	Drought durations (D)			Average R
		D0	D4	D8	
Plant height (cm)	W	89.8	79.0	91.4	86.7
	SA	80.2	84.3	76.2	80.2
	BR	75.7	83.2	84.8	81.2
	GA	86.4	83.0	84.1	84.5
	Average D	83.0	82.4	84.1	
CV (%) = 11.3; F _D = 0.1 ^{ns} ; F _R = 0.8 ^{ns} ; F _{DR} = 0.9 ^{ns}					
Number of leaves (leaves/ plant)	W	8.8	6.3	6.6	7.2
	SA	9.6	8.2	7.7	8.5
	BR	8.8	9.7	7.8	8.8
	GA	7.3	7.6	5.8	6.9
	Average D	8.6	8.0	7.0	
CV (%) = 25.5; F _D = 2.0 ^{ns} ; F _R = 1.9 ^{ns} ; F _{DR} = 0.4 ^{ns}					
Root length (cm)	W	20.1	31.8	30.1	27.3 ^a
	SA	31.2	32.3	32.6	32.0 ^{bc}
	BR	40.7	36.0	40.7	39.1 ^a
	GA	29.7	37.5	33.3	33.5 ^a
	Average D	30.4	34.4	34.2	
CV (%) = 12.8; F _D = 3.5 ^{ns} ; F _R = 11.8 ^{**} ; F _{DR} = 2.4 ^{ns}					

Within a group of means, values followed by the same letter are not significantly different at 5% level.

^{ns}: non significant; ^{**}: significant at 1% level.

¹W: water; SA: salicylic acid; BR: brassinolide; GA: gibberellic acid.

Figure 1 shows the comparison of root lengths of soybean among the treatments. Only types of PGRs had effects on root length of soybean, with brassinolide giving the highest root length (39.1 cm), significantly higher than the root length of soybean

plants water and salicylic acid treatment, but were not significantly different from root length of soybean plants GA₃ treatment. Elongation of roots is beneficial for plants under drought (Comas et al., 2013) as the roots can access water in more areas of the soil.

**Figure 1.** Root length in different treatments of drought durations and plant growth regulators.

Plant biomass is an indicator of the impact of drought on crops. The results in Table 3 show that both factors and their interaction had significant effects on both fresh and dry biomass of the plants. Prolonged drought up to 8 days significantly reduced fresh biomass of soybeans

compared to non-drought condition. Research has also shown that the main effect of drought stress on plant morphology is a reduction in plant size while a reduction in photosynthetic rate is also an important factor leading to a reduction in plant size and biomass production.

Table 3. Effects of plant growth regulators (PGRs) and drought durations on biomass of soybean plants

Biomass	PGRs (R) ¹	Drought durations (D)			Average R
		D0	D4	D8	
Fresh (g/ plant)	W	15.5 ^{cde}	13.1 ^{de}	5.9 ^e	11.5 ^C
	SA	31.7 ^{a-d}	15.5 ^{cde}	25.6 ^{b-e}	24.3 ^B
	BR	45.0 ^{ab}	40.3 ^{ab}	32.8 ^{abc}	39.4 ^A
	GA	26.2 ^{a-e}	47.2 ^a	11.1 ^{de}	28.1 ^B
	Average D	29.6 ^A	29.0 ^A	18.8 ^B	
CV (%) = 25.8 ; F _D = 9.8 ^{**} ; F _R = 26.7 ^{**} ; F _{DR} = 6.9 ^{**}					
Dry (g/plant)	W	2.5 ^d	4.2 ^{cd}	2.8 ^d	3.2 ^C
	SA	10.3 ^{abc}	3.7 ^d	3.4 ^d	5.8 ^{BC}
	BR	14.0 ^a	14.8 ^a	15.2 ^a	14.6 ^A
	GA	6.3 ^{bcd}	12.5 ^{ab}	3.9 ^{cd}	7.6 ^B
	Average D	8.3 ^{AB}	8.8 ^A	6.3 ^B	
CV (%) = 26.3; F _D = 4.7 [*] ; F _R = 50.9 ^{**} ; F _{DR} = 6.9 ^{**}					

Within a group of means, values followed by the same letter are not significantly different at 5% level

. *: significant at 5% level; ** significant at 1% level.

¹W: water; SA: salicylic acid; BR: brassinolide; GA: gibberellic acid.

Among the PGRs, brassinolide treatment gave the highest results for both fresh and dry biomass. Regarding the interaction between two factors, only brassinolide application showed consistent improvement of soybean plant biomass in all drought durations, while GA₃ application only improved plant biomass in case of 4 days without irrigation, and salicylic acid did not show any effects. This highlights the difference in the effectiveness of these PGRs under different drought durations. According to Zhang et al. (2008), brassinolide participates in many developmental processes including flowering, leaf senescence, chloroplast development, and root growth and development. Brassinolide also

regulates biotic and abiotic stress responses, helps plants limit water loss and increases the biomass accumulation ability of soybeans.

Chlorophyll and carotenoid contents are parameters that are strongly impacted by drought stress. Chlorophyll contents typically decrease under drought, which could be a result of pigment photo-oxidation and chlorophyll degradation as a symptom of oxidative stress (Anjum et al., 2011). Data in Table 4 show that under a short duration of drought, chlorophyll b and carotenoid contents remained unchanged statistically compared to non-drought condition, while chlorophyll a content increased to 26.6 mg/g. This increase could be due to

overcompensation during the recovering period (Dong et al., 2019), showing that soybean plants could recover from short-term drought in terms of leaf physiology. However, under more prolonged drought of 8 days, both chlorophyll a and b contents decreased significantly (23.4 mg/g

and 13.0 mg/g, respectively), while carotenoid content increased, indicating that the plants were under severe stress beyond recovery, and substantial amount of chlorophyll was lost due to oxidative damage while carotenoid accumulated to combat the oxidative stress.

Table 4. Effects of plant growth regulators (PGRs) and drought durations on chlorophyll a, chlorophyll b and carotenoid content of soybean plants

Parameters	PGRs (R) ¹	Drought durations (D)			Average R
		D0	D4	D8	
Chlorophyll a content (mg/g)	W	25.6 ^{abc}	27.8 ^a	24.1 ^{cd}	25.8 ^A
	SA	24.2 ^{cd}	26.2 ^{abc}	23.2 ^d	24.6 ^B
	BR	24.5 ^{bcd}	26.1 ^{abc}	27.5 ^a	26.0 ^A
	GA	27.3 ^a	26.5 ^{ab}	18.9 ^e	24.2 ^B
	Average D	25.4 ^B	26.6 ^A	23.4 ^C	
CV (%) = 2.7; F _D = 64.2 ^{**} ; F _R = 14.4 ^{**} ; F _{DR} = 38.3 ^{**}					
Chlorophyll b content (mg/g)	W	12.7 ^{de}	24.2 ^{ab}	11.5 ^{de}	16.1 ^{AB}
	SA	11.6 ^{de}	16.5 ^{cd}	11.4 ^{de}	13.2 ^B
	BR	11.8 ^{de}	13.3 ^{de}	20.9 ^{bc}	15.3 ^{AB}
	GA	29.1 ^a	15.1 ^{cde}	8.3 ^e	17.5 ^A
	Average D	16.3 ^A	17.3 ^A	13.0 ^B	
CV (%) = 14.7; F _D = 11.3 ^{**} ; F _R = 5.6 ^{**} ; F _{DR} = 32.8 ^{**}					
Carotenoid content (mg/g)	W	5.7 ^{ab}	4.4 ^b	5.8 ^{ab}	5.3 ^A
	SA	5.5 ^{ab}	5.2 ^{ab}	6.9 ^a	5.9 ^A
	BR	6.1 ^{ab}	5.6 ^{ab}	5.2 ^{ab}	5.7 ^A
	GA	2.2 ^c	5.9 ^{ab}	5.0 ^{ab}	4.3 ^B
	Average D	4.9 ^B	5.3 ^{AB}	5.7 ^A	
CV (%) = 11.9; F _D = 4.9 [*] ; F _R = 10.2 ^{**} ; F _{DR} = 11.1 ^{**}					

Within a group of means, values followed by the same letter are not significantly different at 5% level.

*: significant at 5% level; **: significant at 1% level.

¹W: water; SA: salicylic acid; BR: brassinolide; GA: gibberellic acid.

Among the PGRs, only brassinolide was capable of improving leaf chlorophyll a and b contents (27.5 mg/g and 20.9 mg/g, respectively) compared to treatment with water (24.1 mg/g and 11.5 mg/g, respectively) under 8 days with no irrigation. This once again shows the

potential of brassinolide in mitigating the effects of prolonged drought in soybeans. However, carotenoid content did not show any changes statistically among treatments of different PGRs after 8 days without irrigation.

The improvement effects of brassinolide on chlorophyll contents of soybean leaves could help soybean plants maintain their photosynthetic ability, especially during the crucial period of seed setting and filling, which could determine the weight of seeds and yield of the crop. According to Nagasuga (2019), there is a strong association between photosynthesis and yield during the seed filling stage. The study of Rahdari et al. (2012) showed that drought can damage the photosynthetic apparatus including thylakoid membranes, photosynthetic pigments and enzymes, leading to a decrease in chlorophyll content in leaves. These damages depend on the duration and severity of the drought and result in reduced photosynthesis.

Soybean plants that were not watered for 4 and 8 days had a significantly reduced total number of pods and total number of firm seeds compared to normal irrigation, and the reduction was more severe under longer drought (Table 5). Specifically, the total number of firm pods on plants under 4 and 8 days of drought decreased by 17.8% (21.7 pods/plant) and 33.3% (17.6 pods/plant), respectively, compared to non-drought condition (26.4 pods/plant), and the total number of firm seeds decreased by 17.1% (42.7 seeds/plant) and 30.1% (36.0 seeds/plant), respectively, compared to non-drought condition (51.5 seeds/plant). Among the PGRs used, brassinolide had the most positive effects on the total number of firm pods and seeds of soybeans under drought condition, reaching 28.8 (pods/plant) and 56.9 (seeds/plant), respectively.

Table 5. Effects of plant growth regulators (PGRs) and drought durations on pod and seed parameters of soybean plants

Parameters	PGRs (R) ¹	Drought durations (D)			Average R
		D0	D4	D8	
Number of firm pods (pods/plant)	W	32.0 ^{ab}	18.9 ^{de}	10.6 ^{fg}	20.5 ^B
	SA	27.8 ^{bc}	18.6 ^{de}	15.4 ^{ef}	20.6 ^B
	BR	23.0 ^{cd}	28.3 ^{bc}	35.0 ^a	28.8 ^A
	GA	22.8 ^{cd}	21.0 ^{ed}	9.4 ^g	17.8 ^C
	Average D	26.4 ^A	21.7 ^B	17.6 ^C	
CV (%) = 8.3; F _D = 70.0 ^{**} ; F _R = 61.3 ^{**} ; F _{DR} = 51.0 ^{**}					
Number of firm seeds (seeds/plant)	W	64.0 ^{ab}	35.5 ^{cd}	23.3 ^{ef}	40.9 ^B
	SA	55.5 ^b	36.4 ^{cd}	30.7 ^{de}	40.9 ^B
	BR	43.8 ^c	56.2 ^b	70.8 ^a	56.9 ^A
	GA	42.7 ^c	42.7 ^c	19.1 ^f	34.8 ^C
	Average D	51.5 ^A	42.7 ^B	36.0 ^C	
CV (%) = 8.3; F _D = 55.9 ^{**} ; F _R = 62.1 ^{**} ; F _{DR} = 56.2 ^{**}					
Weight of 100 seeds (g)	W	15.6 ^{ab}	11.1 ^{def}	6.8 ^g	11.1 ^C
	SA	17.5 ^a	14.5 ^{abc}	9.4 ^{efg}	13.8 ^B
	BR	17.4 ^a	13.9 ^{bcd}	16.6 ^{ab}	15.9 ^A
	GA	14.0 ^{bcd}	12.0 ^{cde}	8.4 ^{fg}	11.5 ^C
	Average D	16.1 ^A	12.9 ^B	10.3 ^C	
CV (%) = 7.6; F _D = 102.6 ^{**} ; F _R = 45.2 ^{**} ; F _{DR} = 13.6 ^{**}					

Within a group of means, values followed by the same letter are not significantly different at 5% level. **, significant at 1% level.

¹W: water; SA: salicylic acid; BR: brassinolide; GA: gibberellic acid.



Figure 2. Seeds and pods of soybean plants in different treatments of drought durations and plant growth regulators.

When considering the interaction between the two factors, it can be observed that brassinolide actually reduced the number of firm pods and seeds of soybeans under non-drought condition (23.0 pods/plant and 43.8 seeds/plant, respectively) compared to treatment with water (32.0 pods/plant and 64.0 seeds/plant, respectively). However, under drought condition of both durations (4 and 8 days), brassinolide significantly improved the number of firm pods (28.3 and 32.0 pods/plant, respectively) and seeds (56.2 and 70.8 seeds/plant, respectively) compared to all other treatments. Interestingly, GA_3 failed to provide any improvement in these two parameters in any drought or non-drought conditions, despite improving plant biomass in some of these treatments. These results highlight the role of brassinolide as a stress PGRs, and the role of GA_3 as a growth inducing substance rather than a stress regulator.

Similarly, decrease in seed weight was observed to be more severe the longer the drought lasted (Table 5 and Figure 2), dropping from 16.1 g/100 seeds with normal irrigation to 12.9 g/100 seeds under 4 days without irrigation (20% reduction), and 10.3 g/100 seeds under 8 days without irrigation (36% reduction). When considering the interaction of the factors, GA_3 applications showed no statistical improvement compared to

water treatments under both drought durations. Interestingly, salicylic acid treatment proved to be able to improve seed weight (14.5 g/100 seeds) under short drought duration, despite previously showing no improvement in other parameters. This once again implies the different mechanisms by which salicylic acid, brassinolide and GA_3 induce stress response in plants, that focus on different organs and physiological processes of the plants. However, when the drought lasted to 8 days, only brassinolide treatment showed to be able to rescue the seed weight consistently, while salicylic acid and GA_3 failed to have any significant improvement compared to water treatment.

The combination results of the yield components led to superior performance of plants in treatments with brassinolide in terms of actual yield (Table 6). Both under short-term (4 days) and long-term drought (8 days), treatments with brassinolide had the highest actual yield compared to all other treatments at the same drought durations. At 8 days of drought, treatment with brassinolide showed no reduction in yield (195.9 g) compared to non-drought treatment. However, at 4 days of drought, treatment with brassinolide showed some reduction in yield (150.6 g) compared to non-drought treatment, suggesting that this PGR

may be slow acting, and may be more effective for prolonged drought. Besides brassinolide, salicylic acid also showed improvement effects, but to a lesser extent, on actual yield of soybeans

under drought condition, mostly through improving seed weight as mentioned above. On the other hand, GA₃ also exhibited no mitigation effects on the damage to yield of drought stress.

Table 6. Effects of plant growth regulators (PGRs) and drought durations on actual yield of soybeans

Parameters	PGRs (R) ¹	Drought durations (D)			PGRs (R)
		D0	D4	D8	
Actual yield (g)	W	193.0 ^a	65.8 ^{de}	31.2 ^f	96.6 ^B
	SA	161.1 ^b	83.6 ^{cd}	71.1 ^{de}	105.3 ^B
	BR	201.5 ^a	150.6 ^b	195.9 ^a	182.7 ^A
	GA	106.2 ^c	58.5 ^e	47.3 ^{ef}	70.7 ^C
	Average D	165.4 ^A	89.6 ^B	86.3 ^B	
CV (%) = 6.9; F _D = 393.2 ^{**} ; F _R = 342.6 ^{**} ; F _{DR} = 55.5 ^{**}					

Within a group of means, values followed by the same letter are not significantly different at 5% level.

^{**}: significant at 1% level.

¹W: water; SA: salicylic acid; BR: brassinolide; GA: gibberellic acid.

In order to study the mechanism of the drought mitigation effects of these PGRs in soybeans, we measured the proline and protein contents in the leaves (Table 7). Proline is known as a stress-induced amino acid, acting as an osmolyte to regulate osmotic pressure,

maintain protein and cell membrane stability, as well as helping protect the cells from oxidative stress (Wang et al., 2022). Proline biosynthesis and degradation also play an important role in the energy balance between chloroplasts and mitochondria (Lisar et al., 2010).

Table 7. Effects of plant growth regulators (PGRs) and drought durations on proline content and protein content of soybean leaves

Parameters	PGRs (R) ¹	Drought durations (D)			PGR (R)
		D0	D4	D8	
Proline content (mg/g)	W	9.7 ^{efg}	27.6 ^b	22.3 ^c	19.9 ^B
	SA	10.4 ^{efg}	9.2 ^{fg}	11.1 ^{ef}	10.2 ^D
	BR	8.0 ^g	16.3 ^d	12.2 ^e	12.1 ^C
	GA	8.9 ^{fg}	25.9 ^b	31.6 ^a	22.1 ^A
	Average D	9.2 ^B	19.8 ^A	19.3 ^A	
CV (%) = 4.8; F _D = 699.7 ^{**} ; F _R = 499.1 ^{**} ; F _{DR} = 167.6 ^{**}					
Protein content (mg/g)	W	91.1 ^{ef}	115.4 ^b	107.3 ^c	104.6 ^A
	SA	85.9 ^{fg}	83.4 ^g	121.6 ^a	96.9 ^B
	BR	84.3 ^g	97.2 ^d	93.6 ^{de}	91.7 ^C
	GA	86.7 ^{fg}	105.6 ^c	97.0 ^d	96.4 ^B
	Average D	165.4 ^A	89.6 ^B	86.3 ^B	
CV (%) = 1.8; F _D = 307.3 ^{**} ; F _R = 75.8 ^{**} ; F _{DR} = 117.9 ^{**}					

Within a group of means, values followed by the same letter are not significantly different at 5% level.

^{**}: significant at 1% level.

¹W: water; SA: salicylic acid; BR: brassinolide; GA: gibberellic acid.

The results in Table 7 show that both salicylic acid and brassinolide, the two PGRs that exhibited drought mitigation effects in soybeans, reduced the content of proline in the leaves at both 4 days and 8 days without irrigation compared to the treatments with only water. Similar results were observed for protein content, except for salicylic acid treatment at 8 days without irrigation, which was higher than water treatment at the same drought duration. These results suggested that both salicylic acid and brassinolide induce drought tolerance in soybeans through mechanisms unrelated to proline accumulation. The stress mitigation effects of salicylic acid and brassinolide might in fact reduce the stress level experienced by leaves, and consequently the leaves did not have to accumulate large amount of proline in order to cope with the stress. According to Krishna (2003), brassinolide are a group of natural plant steroid compounds that help increase crop yields by altering metabolism and protecting plants from environmental stresses. In recent years, genetic and biochemical studies have established the essential role of brassinolide in their ability to increase plant resistance to various stresses, such as low temperature, drought, salt and pathogen attack.

4. Conclusions

Drought at the stage of pod growth and seed filling in soybeans did not affect the growth and development of the vegetative parts of the plants, but greatly affected yield. Brassinolide were the most effective PGR in mitigating the effects of drought at pod growth stage on soybeans, maintaining the yield and seed quality comparable to non-drought condition. However, the proline contents in brassinolide treatments were lower than those of water treatments, suggesting that brassinolide may act through a mechanism unrelated to proline accumulation.

Conflict of interest

The authors have no conflicts of interest to declare.

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