FOLIAR APPLICATION OF BRASSINOSTEROIDS IMPROVES THE YIELD AND MORPHO-PHYSIOLOGICAL CHARACTERISTICS OF *ARACHIS HYPOGAEA* L., *GLYCINE MAX* (L.), AND *PHASEOLUS VULGARIS* L.

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Abstract. This study aimed to determine the application methods of brassinosteroids (BR) as an alternative production system to improve plant growth and morpho-physiological characteristics of dry bean (*Phaseolus vulgaris* L.), soya bean (*Glycine max* (L.)), and groundnut (*Arachis hypogaea* L.). Trials were laid out in a complete randomized block design, arranged in a 4 x 2 factorial design. Brassinosteroids (100 g) treatments followed preventative and corrective treatments, where the dry bean, soya bean, and groundnut (three legumes) seeds were treated with BR; BR through the foliar application, soil drenching BR, and the control (with no BR treatment). The effect time of BR treatment was two and four weeks. Results showed that BR significantly improved the yield and morpho-physiological characteristics of all legume crops in this study. The improvement of the morpho-physiological characteristics was attributed to the morphological and physiological parameters, where the increase in plant height, seedling fresh mass, seedling dry mass, root fresh mass, and root dry mass were ascribed to morphological parameters; photosynthetic and chlorophyll content parameters were ascribed to physiological parameters. Interestingly, the foliar application method was significantly ($P \leq 0.05$) better than the control in all parameters. Thus, the results confirmed that foliar BR has the potential to improve plant growth in general.
Keywords: *application method, leguminous, phytohormone, seed treatment, soil drench*

Introduction

There are over 19000 species of legumes that are cultivated globally (Garg, 2022). Among these species, the most commonly known grain legumes include peas (*Pisum sativum* L.), dry beans (*Phaseolus vulgaris* L.), groundnuts (*Arachis hypogaea* L.), and soya beans (*Glycine max* (L.)) (Hughes et al., 2022). Legumes are among the vital commercial crops cultivated extensively in most countries (Aghora et al., 2023). In South Africa, soya bean, dry bean, and groundnut are the most common legumes planted by both smallholder and commercial farmers as a primary source of income, an alternative source of protein for human consumption, livestock feed, and also as a soil ameliorant (de Bruyn et al., 2022).

Legumes are highly nutritious and rich in protein, Fe, K, Mg, Zn, Vitamin B, and Se (Messina et al., 2022). Alternatively, legumes are globally used to replace cattle milk with soymilk, animal oil with plant based-oil, and animal meat as a plant-based protein-rich source (Kaartinen et al., 2023; Păucean et al., 2023; Messina et al., 2022). Besides human consumption, legumes are used as a source of animal feed after grain harvest,

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and they are known to have high-quality forages for livestock in cultivated pastures (Ovani et al., 2022). Legumes are used to improve soil fertility by fixing atmospheric nitrogen in the soil, utilizing Rhizobium bacteria in the root hairs of the plant to form nodules in which nitrogen is stored (Hassan et al., 2023). The nitrogen absorbed from the atmosphere is used for plant growth and is kept in the nodules of the root. With farmers, it is a common cultivation practice that the roots are left in the ground to decompose and release nitrogen into the soil, making a rich source of nutrient-rich organic matter or mulches that supply nitrogen for the following crops, especially during intercropping (Van Dung et al., 2022). Therefore, growing legumes as a rotating crop or in an intercropping system it is essential and advisable because the next crop will always benefit from it. This technique reduces environmental pollution and eutrophication and can also help poor-resourced farmers, as they do not need to purchase large amounts of expensive chemical fertilizers, and it also reduces environmental pollution (Francaviglia et al., 2022).

The successful production of these crops can be affected by abiotic and biotic stress (Khetsha et al., 2022a); however, this can always be mitigated by the use of brassinosteroids (BRs) as described by Yao et al. (2023) and Chaudhuri et al. (2022). Brassinosteroids are extracted from *Brassica napus* (L.) pollen, which are growth promoter steroid hormones and are categorized depending on the number of carbons present in their structures, namely C27, C28 and C29 (Gogna et al., 2023). Brassinosteroids are polyhydroxy steroid plant hormones that are accountable for many plants physiological processes such as cell expansion, division, seed germination, xylem differentiation, reproductive development, pollen elongation, formation of the pollen tube, disease, and abiotic stress resistance (Chaudhuri et al., 2022; Sharma et al., 2022). For example, during cell elongation, BRs affect the mechanical properties of the cell walls 26 through genomic and non-genomic pathways that allow turgor-driven cell enlargement to proceed (Pereira-Netto, 2011).

To improve plant growth and metabolism BRs interact with other plant hormones such as ethylene, auxins, cytokinins, and gibberellins (Gruszka et al., 2023; Khetsha et al., 2023b) synergistically. Brassinosteroids have been extensively reported to improve plant health, plant nutrient assimilation, vitamins, antioxidants, and carbohydrates (Han et al., 2023; Piacentini et al., 2023; Yao et al., 2023; Chaudhuri et al., 2022). Studies on BRs started 36 years ago, and the top well-known BRs extracted from plants are brassinolide (BL), castasterone (CS), teasterone (TE), and 6-deoxycastaterone (6-deoxy CS), the C28-BRs with a 24α-methyl group (Van der Watt, 2005). These C28-BRs with a 24α-methyl group have shown an enormous application potential in the agricultural industry and might play a significant role in the optimization of plant growth and plant protection against biotic and abiotic stress (Khetsha et al., 2022a; Van der Watt, 2005). Therefore, the main objective of this study was to determine the most effective application method of BRs in three legume crops: dry bean, soya bean, and groundnut.

Materials and methods

Study site and general crop management practices

The experiment was carried out as a carry-over study over two growing seasons (2015/16 and 2016/17) in the glasshouses of the Department of Soil, Crop, and Climate Sciences, under natural daylight conditions with a day length varying from 9.1 to 13 h during the growing season at the University of the Free State, Bloemfontein, South

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Africa (29°07’S, 26°11’E), at 1390 m asl. The day/night temperatures during the growing seasons were 28°C/18°C. Dry bean (var. Kranskop), soya bean (var. PAN 1522 R), and groundnut (var. Tufa choice) seeds were used and obtained from a local supplier SENWES (RSA).

The pots were filled with reddish brown, fine sandy loam soil, classified as Bainsvlei Amalia soil containing 8% clay with a pH of 5 and bulk density of 1500 $kg.m^{-3}$, collected from Kenilworth Experimental Research Farm of the Department of Soil, Crop and Climate Sciences (Soil Classification Working Group, 1991). Fertilizer was applied based on soil analysis and expected yield outcome according to the prescriptions by Agricultural Research Council-SGI, Bethlehem, South Africa for three legume crops (ARC, 2015), as detailed in *Table 1*, with a yield potential of 2 tons/ha for all legume crops and obtained from a local supplier SENWES (RSA). No phytophagous pests were documented during the experiment, however, Malasol (an organophosphate; Efekto, RSA) was preventatively sprayed at 1.75 ml/L throughout the cropping seasons. These applications were repeated for three to six days, at four-week intervals. Field water capacity (FWC) was determined gravimetrically to calculate the amount of water applied to bring the soil to 70% FWC. Pots were weighed daily, and distilled water was added when necessary to bring the water content of the pot back to 70% of the water available at field capacity.

Table 1. *Glasshouse trial specifics and fertilizer requirements for dry bean, soya bean, and groundnut cultivated under irrigation conditions, as recommended by the Agricultural Research Council - SGI, Bethlehem, South Africa (ARC Technical datasheet 2015)*

Crop	Dry bean	Soya bean	Groundnut
Fertilizer	3:2:1 [a](25)	3:2:1 (25)	3:2:1 (25)
LAN	4.64 mg/kg	-	-
N	10 mg/kg	13.33 mg/kg	6.66 mg/kg
P	8.88 mg/kg	8.88 mg/kg	13.33 mg/kg
K	4.44 mg/kg	4.66 mg/kg	2.22 mg/kg

[a]25 in fertilizer = The balanced blend of equal portions of N, P, and K

Treatments

Trials were laid out in a complete randomized block design, arranged in a 4 × 2 factorial design. Treatments consisted of a preventative and corrective treatment, where the three legume crops were treated with BR at 100 g/ha (2 mg/kg soil) (Agraforum, GMBH Germany) in all three treatment methods (seed treatment, foliar application, and soil drenching) and the control (no BR treatment). First, seed treatment was conducted by treating seeds with 100 g BR/ha (5 ml water/kg seed); secondly, as a foliar application it was sprayed on the leaves one week after emergence; thirdly, it was also applied on the soil by drenching it using a hand pump sprayer; there was zero application of BR for the control. The BR time-effect (weeks) treatments consisted of two- and four-weeks BR-effects factors.

Parameters

The trial was conducted in two different ways using different pot sizes; small pots (17.3 x 15 cm) were filled with 2.5 kg soil and used for two weeks’ interval destructive

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sampling, replicated eight times, with each pot containing two plants for each legume crop. Large pots (24.3 × 19.9 cm) were filled with 6 kg soil and replicated four times, with each pot containing two plants for each legume crop. Plants in large pots were allowed to grow up to maturity and used for non-destructive sampling. Before filling with soil, all pots were lined with polyethylene bags to prevent leaching and contamination from the sides of the pot.

Destructive measurements

In this study, the following destructive measurements were taken; carotenoid content, chlorophyll content ((chlorophyll (a); chlorophyll (b); chlorophyll (a + b)), root fresh mass, dry mass, seed emergence, seedling fresh mass, and dry mass from the small pots (17.3 × 15 cm filled with 2.5 kg soil). After two weeks of crop emergence, four replications were harvested, and the remaining four replications were harvested two weeks later for the chlorophyll parameters. A total of 0.2 g leaf material (third leaves from the top of each plant) from seedlings were cut into small pieces, and 3 ml acetone was added (1:15; m/v) to determine the chlorophyll parameters (chlorophyll (a); chlorophyll (b); chlorophyll (a + b); and carotenoids content). The leave materials were crushed using a mortar and pestle until the supernatant was dark green. The extract was then centrifuged at 6000 rpm, and an aliquot of 1 ml was used to determine absorbance spectra using a Shimadzu UV spectrophotometer (Shimadzu, USA). The chlorophyll a, b, total chlorophyll, and carotenoid content were read at three different wavelengths (661.6 nm, 644.8 nm, and 470 nm) and calculated following the methods described by Wellburn (1994). Plant seedlings were harvested every two weeks from the day of transplant and separated into roots and aerial parts of the crop. This was done by cutting the plant just above the soil surface to separate the aerial and root parts. Seed emergence (%), seedling fresh mass (g), dry mass (g), root fresh mass (g), and dry mass (g) were measured following the description by Perez-Harguindeguy et al. (2016).

The stem diameter (mm) was measured about 1 cm above the soil every two weeks up to week six using an electronic Vernier Calliper (RS Pro, RSA). Plants were measured from the soil surface to the highest point of the arch of the uppermost leaf whose tip is pointing down. The natural plant height (cm) was measured every two weeks before harvesting using a ruler as the distance from the soil surface excluding the first 2 cm which represented the planting depth. The extended plant height (cm) described as the the highest point on the plant was measured to the highest point every two weeks before harvesting using a ruler as the distance from the soil surface excluding the first 2 cm which represented the planting depth (Perez-Harguindeguy et al., 2016).

Non-destructive measurements

Crops were grown up to harvest and the photosynthetic rate, stomatal conductance, transpiration rate, sub-stomatal conductance CO_2 concentration, stem diameter, natural plant height, and extended plant height; and yield (pod mass, number of pods per pot; seed number per pot, and mass of seeds per pot) parameters were measured on the larger pots (24.3 x 19.9 cm filled with 6 kg soil) as non-destructive measurements. Photosynthesis was measured to determine physiological responses of gas exchange parameters such as the CO_2 assimilation rate, stomatal conductance, transpiration, and

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fluorescence. Photosynthesis was determined using a LiCor 6400XT portable analyzing meter (LiCor, USA), measuring the effect of the treatments every two weeks under glasshouse conditions of every third leaf from the top of the plant. The system determines the photosynthesis rate by establishing the rate at which CO_2 is used every second for each sample measured. Photosynthesis rate was captured in the presence of white light (2000 ímol photons m^{-2}/s) in the glass house using a light source (LED light) and CO_2 supplied (400 ppm) by the CO_2 tank of the system. Measurements were performed every two weeks between 11 h and 13 h under optimal growth conditions with a temperature of 25°C and 6 cm^2 of exposed leaf surface.

All pods per pot were harvested after reaching physiological maturity, counted, and weighed, and the data was recorded as average mass and number per pot to determine the pod mass and number per pot. To determine the seed number per pot and mass of the seed per pot, all pods were shelled, and the number of seeds per pot was counted, weighed, and recorded as the average seed number and seed mass per pot. The final yield was taken as seed mass per pot.

Statistical analysis

Data were subjected to analysis of variance using the statistical analysis system (SAS) program 9.2 packages for Windows V8 (SAS Institute Inc, 1999-2010). Treatment means were compared using the least significant difference (LSD) at a 5% level of significance to determine significant differences between treatment means.

Results

Effects of BRs application method on morphological parameters

A 100% germination (emergence) was recorded in all three legumes (groundnut, soya bean, and dry bean) crop seeds planted. Therefore, this study did not consider the species factor as a treatment factor. In all legume species, the BR application methods and the BR time effect significantly affected the morphological parameters as measured every two to four weeks (*Fig. 1*).

Morphological parameters of dry bean showed that the seedling fresh mass (SFM) and seedling dry mass (SDM) were significantly ($P \leq 0.05$) increased when BR was applied using foliar application compared to the control. Interestingly, BR’s seed treatment and soil drenching recorded similar results and were still better than the control (*Fig. 1A, B*). The root fresh mass (RMS) and the root dry mass (RDM) were also recorded significantly ($P \leq 0.05$) high when the foliar application method was used compared to the control; however, this was not significantly different compared to the soil drenching method (*Fig. 1C, D*). The foliar application observed the highest increase in plant growth (SFM, SDM, RMS, and RDM) parameters, followed by the soil drench and seed treatment.

As illustrated in *Figure 1E-H*, significant ($P \leq 0.05$) effects of three application methods of BR and the BR time effects on the morphological parameters were observed on soya beans. All the applied methods of BR significantly increased plant growth compared to the control. However, no significant difference occurred between the seed treatment and the soil drenching of BR. The foliar application method was still recorded as the best method to increase plant growth compared to soil drenching and seed treatment with BR.

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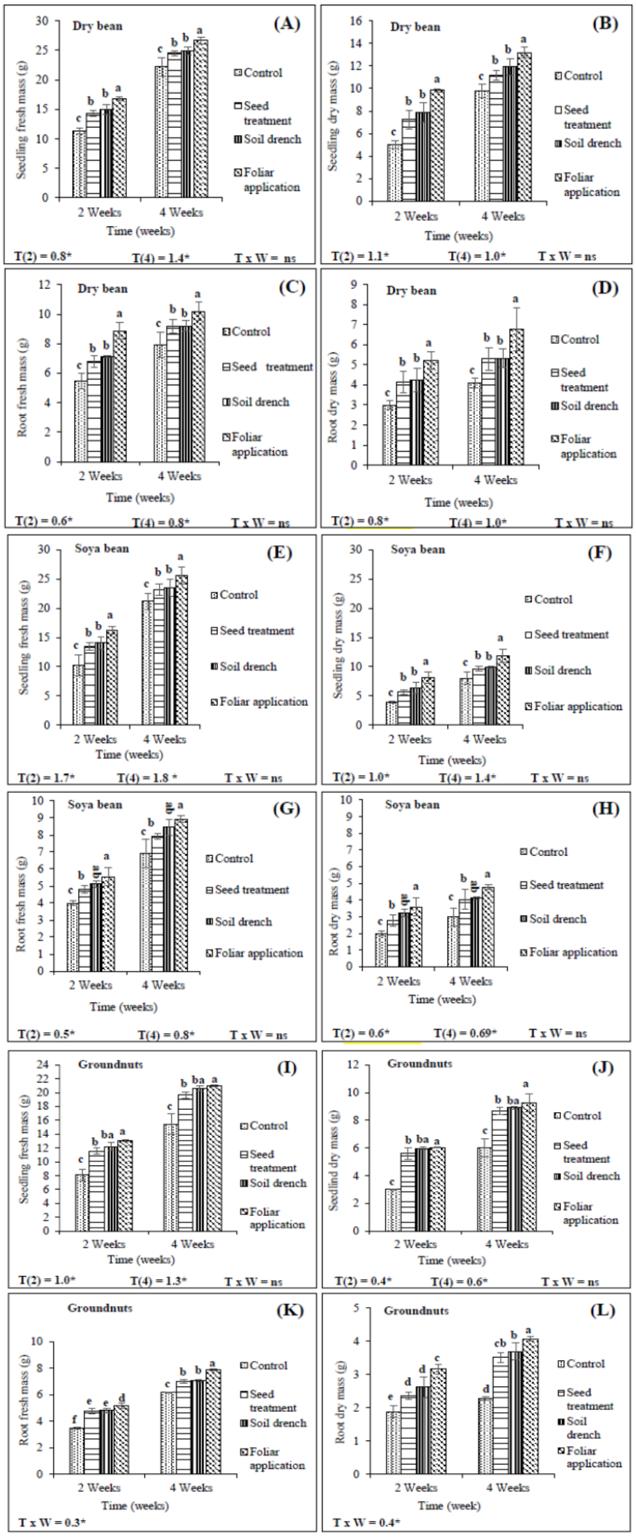

Figure 1. *The morphological (seedling fresh mass (A; E; I), seedling dry mass (B; F; J), root fresh mass (C; G; K), and root dry mass (D; H; L)) response of dry bean, soya bean, and groundnut to three BR application methods (T) measured every two weeks over a period of four weeks. Where T (2) = BR effect after two weeks; T (4) = BR effect after 4 weeks; T x W is the interaction between BR and BR time-effects. Statistical significance is indicated as LSD = * significant (P ≤ 0.05) and different letters, and ns = not significant at 5% using Tukey's LSD test. Vertical bars with horizontal caps indicate standard deviation.*

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In groundnut, all the applied methods of BR significantly ($P \leq 0.05$) increased the SFM and SDM compared to the control (*Fig. 1I-L*). Plant growth was recorded to be significantly high on groundnuts when foliar application was used to apply BR, followed by soil drench and seed treatment. On the other hand, the RMS and RDM were significantly ($P \leq 0.05$) increased by the interaction between the BR application method and the BR time effects. The highest RMS and RDM were recorded when plants were treated with BR using the foliar application method in the fourth week compared to the control. The foliar application method had the highest effects on the morphological parameters in all three legume crops, as illustrated in *Figure 1*.

Table 2 and *Figure 2* show the effect of the three application methods of BRs on the morphological parameters of all three legumes measured over 12 weeks. In all three legume crops, administering BR using the foliar application method increased the natural plant height, extended plant height, and stem diameter significantly throughout the cultivation period, 12 weeks, when compared to the control. With dry beans, soil drenching was the second-best method compared to the seed treatment and the control for the first four weeks; however, there were no significant differences in plant height and extended plant height from the sixth week until harvest, the 12th week. Interestingly, drenching BR in the soil on soya beans was the second-best method compared to the seed treatment and the control throughout the 12 weeks. This was not the case with groundnut, soil drenching BR was similar compared to the seed treatment throughout the growing period, except for the first two weeks.

Figure 2. *Schematic representation of the three leguminous dry bean (A), soya bean (B), and groundnut (C) plants as affected by the application methods of the brassinosteroids two weeks after emergence*

As illustrated in *Table 2*, no significant differences were observed between soil drenching BR and seed treatment on dry bean and groundnut stem diameter throughout the growing period. Interestingly, drenching BR in the soil was the second-best method to increase the stem diameter of soya beans compared to the seed treatment throughout the 12 weeks. In all three legume plants, as shown in *Table* 2, it can be resolved that all the applied methods of BR caused an increase in the morphological and plant growth parameters compared to the control; however, the foliar application method showed to be the most effective method (*Table 2; Fig. 2*).

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Table 2. *The effect of BR application methods on morphological parameters (natural plant height, extended plant height, and stem diameter) of dry bean, soya bean, and groundnut measured over a period of 12 weeks*

Parameters	**Weeks**						
	Dry bean						
	Treatments	**2 weeks**	**4 weeks**	**6 weeks**	**8 weeks**	**10 weeks**	**12 weeks**
Natural plant height (cm)	Control	12.9^{d}	17.9^{d}	23.9^{c}	30.3^{c}	34.4^{c}	38.8^{c}
	Seed treatment	14.6^{c}	18.8^{c}	26.9^{b}	32.9^{b}	38.8^{b}	43.9^{b}
	Soil drench	16.7^{b}	21.8^{b}	27.3^{b}	33.5^{b}	39.1^{b}	44.1^{b}
	Foliar application	18.9^{a}	24.8^{a}	30.5^{a}	36.7^{a}	41.5^{a}	47.2^{a}
	$LSD_{T0.05}$	0.7*	0.5*	0.7*	0.7*	0.8*	0.6*
Extended plant height (cm)	Control	18.1^{d}	25.2^{c}	32.7^{c}	39.8^{c}	47.8^{c}	55.8^{c}
	Seed treatment	21.1^{c}	27.8^{b}	35.9^{b}	43.9^{b}	51.8^{b}	57.8^{b}
	Soil drench	22.1^{b}	28.1^{b}	36.0^{b}	44.2^{b}	52.1^{b}	58.1^{b}
	Foliar application	25.2^{a}	31.5^{a}	38.3^{a}	46.9^{a}	54. ^{a}	61.6^{a}
	$LSD_{T0.05}$	1.0*	0.6*	0.5*	0.9*	0.7*	0.9*
Stem diameter (mm)	Control	3.4^{c}	3.7^{c}	4.2^{c}	4.6^{c}	4.8^{c}	5.0^{c}
	Seed treatment	3.7^{b}	3.9^{b}	4.4^{b}	4.8^{b}	5.0^{b}	5.2^{b}
	Soil drench	3.7^{b}	4.0^{b}	4.4^{b}	4.8^{b}	5.0^{b}	5.3^{b}
	Foliar application	4.0^{a}	4.4^{a}	4.8^{a}	5.3^{a}	5.4^{a}	5.7^{a}
	$LSD_{T0.05}$	0.1*	0.1*	0.1*	0.04*	0.04*	0.1*
	Soya bean						
Natural plant height (cm)	Control	9.7^{d}	14.8^{d}	22.7^{d}	28.8^{d}	36.6^{d}	42.6^{d}
	Seed treatment	11.0^{c}	18.0^{c}	25.0^{c}	34.6^{c}	42.6^{c}	48.4^{c}
	Soil drench	12.4^{b}	19.4^{b}	27.0^{b}	35.9^{b}	44.3^{b}	52.0^{b}
	Foliar application	14.0^{a}	21.9^{a}	29.1^{a}	38.5^{a}	47.6^{a}	55.4^{a}
	$LSD_{T0.05}$	1.2*	0.9*	1.8*	1.2*	1.1*	1.1*
Extended plant height (cm)	Control	17.4^{d}	23.2^{d}	33.4^{d}	41.4^{d}	51.2^{d}	56.2^{d}
	Seed treatment	19.5^{c}	27.4^{c}	37.2^{c}	45.1^{c}	54.6^{c}	59.1^{c}
	Soil drench	21.9^{b}	30.8^{b}	39.5^{b}	47.3^{b}	58.3^{b}	65.1^{b}
	Foliar application	23.9^{a}	33.6^{a}	44.9^{a}	54.0^{a}	64.1^{a}	69.3^{a}
	$LSD_{T0.05}$	1.5*	1.1*	1.2*	1.4*	2.7*	1.0*
Stem diameter (mm)	Control	3.4^{d}	3.7^{d}	4.1^{d}	4.6^{d}	4.9^{d}	5.0^{d}
	Seed treatment	3.7^{c}	3.8^{c}	4.7^{c}	4.9^{c}	5.5^{c}	5.7^{c}
	Soil drench	3.9^{b}	4.1^{b}	4.8^{b}	5.1^{b}	5.7^{b}	6.0^{b}
	Foliar application	4.3^{a}	4.4^{a}	4.9^{a}	5.3^{a}	5.9^{a}	6.1^{a}
	$LSD_{T0.05}$	0.1*	0.1*	0.1*	0.1*	0.1*	0.1*
	Groundnuts						
Natural plant height (cm)	Control	8.9^{d}	14.9^{c}	20.8^{c}	27.0^{c}	32.8^{c}	37.5^{c}
	Seed treatment	9.5^{c}	16.9^{b}	23.4^{b}	28.9^{b}	35.8^{b}	39.5^{b}
	Soil drench	10.0^{b}	17.1^{b}	24.6^{b}	30.7^{b}	36.3^{b}	40.6^{b}
	Foliar application	11.0^{a}	18.8^{a}	27.2^{a}	34.2^{a}	39.0^{a}	45.6^{a}
	$LSD_{T0.05}$	0.4*	1.4*	1.3*	1.9*	1.9*	1.3*
Extended plant height (cm)	Control	12.2^{d}	19.1^{c}	25.8^{c}	32.1^{c}	37.1^{c}	41.0^{c}
	Seed treatment	13.9^{c}	21.4^{b}	28.0^{b}	33.8^{b}	39.0^{b}	43.0^{b}
	Soil drench	16.3^{b}	22.3^{b}	28.2^{b}	34.1^{b}	39.3^{b}	44.1^{b}
	Foliar application	18.8^{a}	24.7^{a}	32.0^{a}	38.0^{a}	42.5^{a}	47.4^{a}
	$LSD_{T0.05}$	1.2*	1.2*	0.7*	0.8	0.8*	1.4*
Stem diameter (mm)	Control	3.4^{c}	3.7^{c}	3.9^{c}	4.4^{c}	4.7^{c}	4.8^{c}
	Seed treatment	3.5^{b}	3.8^{b}	4.2^{b}	4.6^{b}	4.9^{b}	5.0^{b}
	Soil drench	3.5^{b}	3.8^{b}	4.3^{b}	4.7^{b}	4.9^{b}	5.1^{b}
	Foliar application	3.8^{a}	3.9^{a}	4.4^{a}	4.8^{a}	5.1^{a}	5.2^{a}
	$LSD_{T0.05}$	0.1*	0.1*	0.1*	0.04*	0.1*	0.2*

Statistical significance is indicated as LSD = * significant ($P \leq 0.05$) and different letters, and ns = not significant at 5% using Tukey's LSD test

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Effects of BRs application method on physiological parameters

The results presented in *Figure 3* show the physiological responses of dry bean, soya bean, and groundnut to the three BR application methods measured every two to four weeks. Results in this study revealed that there is a significant ($P \leq 0.05$) interaction between the BR application method and the BR time effects on chlorophyll (a) for dry beans (*Fig. 3A*). The highest chlorophyll (a) content was recorded when BR was applied using the foliar application method on the fourth week compared to the control on dry beans. However, this was not the case for soya bean and groundnuts; on these two legume crops, the highest chlorophyll (a) content was recorded when the foliar application method was used on both separate periods, two and four weeks compared to the control (*Fig. 3E, I*). As illustrated in *Figure 3B, F, J,* chlorophyll (b) was significantly ($P \leq 0.05$) increased by administering BR through the foliar application method on both separate periods compared to the control in all legume plants. The total chlorophyll (a + b) and carotenoids content as illustrated in *Figure 3C, G* and *D, H*, respectively, were also significantly ($P \leq 0.05$) increased by applying BR using the foliar application method on both separate periods compared to the control in dry bean and soya bean. Interestingly, significant interactions were observed between the BR application method and the BR time effects on the total chlorophyll (a + b) and carotenoid content of groundnut. The highest total chlorophyll (a + b) and carotenoid content were recorded when BR was applied using the foliar application method on the fourth week compared to the control for groundnut (*Fig. 3K*). The chlorophyll parameters results confirmed that the foliar application differed from seed treatment, soil drench, and control, respectively.

The effect of different application methods of BR on the photosynthetic rate, stomatal conductance, transpiration rate, and sub-stomatal CO_2 concentration was measured fortnight until the twelfth week for the three legume crops (*Fig. 4*). For this study, the photosynthetic rate numbers were taken as absolute values. As illustrated in *Figure 4A, E, I,* a significant ($P \leq 0.05$) interaction between the BR application method and weeks was recorded for soya bean and groundnut. *Figure 4E, I* further shows that there were no significant differences between seed treatment and soil drenching of BR; however, a significant increase in photosynthetic rate was observed when the foliar application method was used between week 11 and 13 compared to the seed treatment, soil drench, and control, respectively, for soya bean and groundnut.

A significant ($P \leq 0.05$) interaction was observed between the BR application method and weeks recorded on the stomatal conductance for the dry bean (*Fig. 4B*). Interestingly when the foliar application method was used between weeks 9 to 13 compared to the seed treatment, soil drench, and control, the stomatal conductance was higher (*Fig. 4B*). However, this was not the case for soya bean and groundnut, where the main effects showed significant ($P \leq 0.05$) differences between the three BR application methods compared to the control for soya bean and groundnut (*Fig. 4F, J*).

The transpirational rate and the sub-stomatal CO_2 concentration were significantly ($P \leq 0.05$) increased by the interaction between the BR application method and weeks for dry bean and soya bean; however, this was not the case for groundnut. Observation from the interactions observed on the dry bean and soya bean and the main effect of BR application demonstrated that with foliar application of BR from week 3 to week 9, the transpirational rate and the sub-stomatal CO_2 concentration increased. However, during physiological maturity at weeks 11 and 13, a huge decline in all physiological activities, including transpirational rate and the sub-stomatal CO_2 concentration, could be observed; nonetheless, foliar application outperformed the other BR treatment methods.

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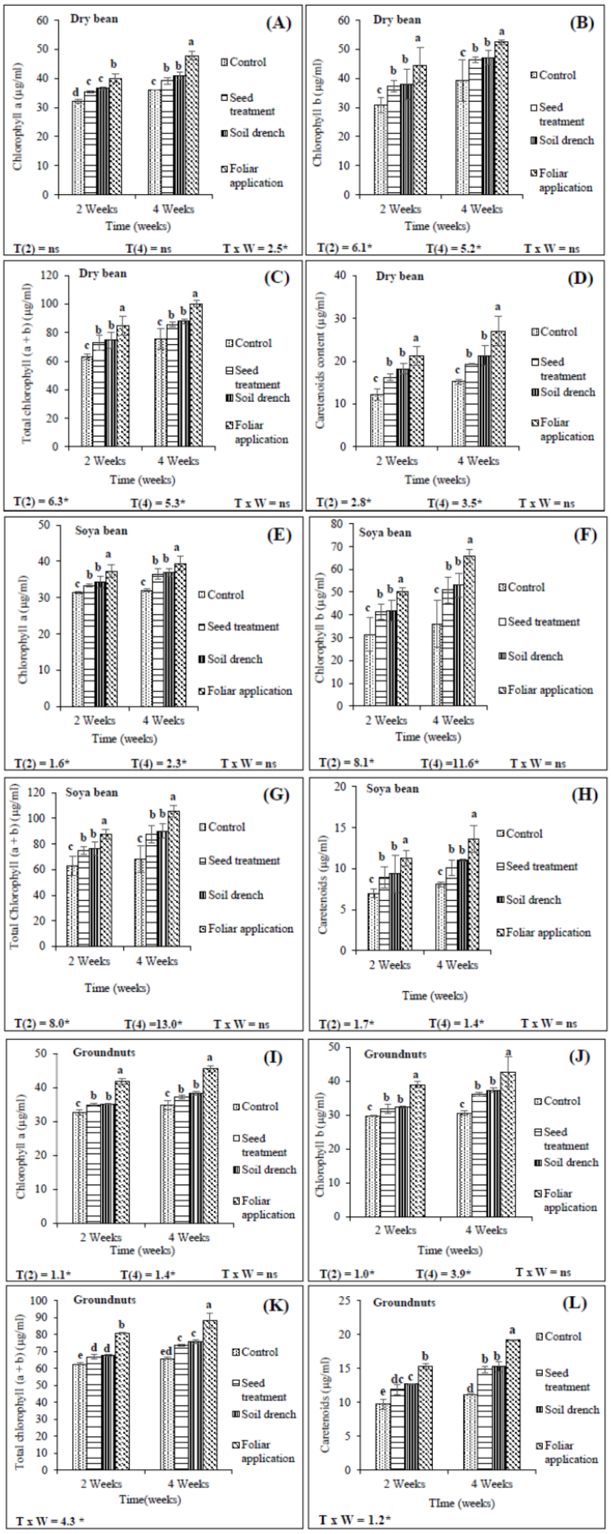

Figure 3. *The physiological (chlorophyll a (A; E; I), chlorophyll b (B; F; J), total chlorophyll (a + b) (C; G; K) and carotenoids content (D; H; L)) response of dry bean, soya bean, and groundnut to three BR application methods (T) measured every two weeks over a period of four weeks. Where T (2) = BR effect after two weeks; T (4) = BR effect after 4 weeks; T x W is the interaction between BR and BR time effects. Statistical significance is indicated as LSD = * significant (P ≤ 0.05) and different letters, and ns = not significant at 5% using Tukey's LSD test. Vertical bars with horizontal caps indicate standard deviation*

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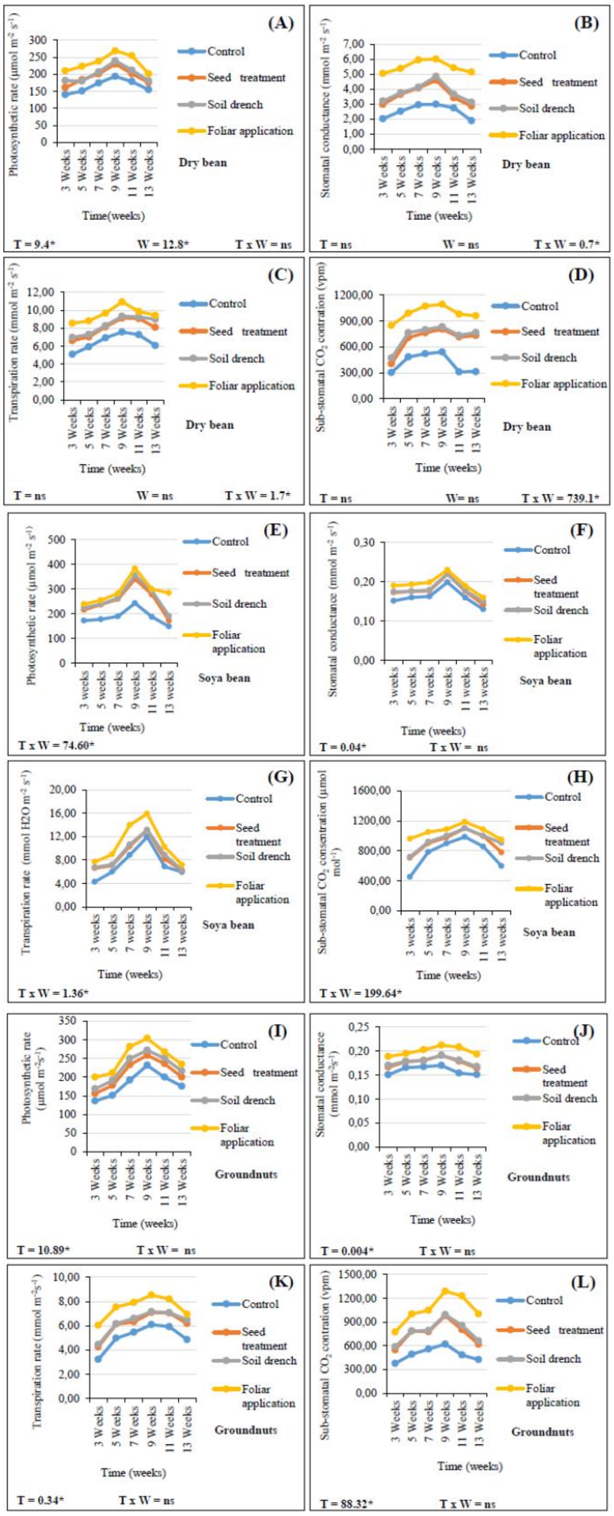

Figure 4. *The physiological (photosynthetic rate (A; E; I), stomatal conductance (B; F; J), transpiration rate (C; G; K) and sub-stomatal* CO_2 *concentration (D; H; L)) response of dry bean, soya bean, and groundnut to three BR application methods (T) measured every two weeks over a period of four weeks. Where T (2) = BR effect after two weeks; T (4) = BR effect after 4 weeks; T x W is the interaction between BR and BR time-effects. Statistical significance is indicated as LSD = * significant (P ≤ 0.05) and different letters, and ns = not significant at 5% using Tukey's LSD test. Vertical bars with horizontal caps indicate standard deviation*

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From this study, as illustrated in *Figure 4*, it can be seen from the results that all the applied methods of BR caused an increase in all physiological parameters compared to the control. At most, the highest rates were observed at nine weeks, which is the most active vegetative growth stage by all the three methods of BR application, with foliar application as the highest, followed by soil drench and seed treatment, respectively.

Effects of BRs application method on yield parameters

Table 3 illustrates the effects of the three BR application methods on the yield and yield components (number of pods per pot, mass of pods per pot, and mass of seeds per pot) of the three legume crops’. The results showed that the highest yield and yield components were recorded where BR was administered using foliar application spray compared to the control. BR’s seed treatment and soil drenching were significantly better than the control; however, there were no significant differences between the two BR application methods in all legume crops. Therefore, this part of the study demonstrated that the yield and yield components could be increased significantly by administering BR using the foliar application method in the three legume crops.

Table 3. *The effect of BR application methods on yield components (number of pods per pot, the mass of pods per pot, number of seeds per pot, and mass of seeds per pot (final yield per pot) of dry bean, soya bean, and groundnut*

Treatments	**Parameters**			
	Dry bean			
	Number of pods	**Mass of pods (g)**	**Number of seeds**	**Mass of seeds (g)**
Control	20.0^{c}	15.6^{c}	30.0^{c}	13.5^{c}
Seed treatment	23.8^{b}	19.7^{b}	43.3^{b}	16.2^{b}
Soil drench	24.3^{b}	21.9^{b}	44.8^{b}	17.6^{b}
Foliar application	30.0^{a}	28.3^{a}	48.5^{a}	23.0^{a}
$LSD_{T0.05}$	2.4*	2.8*	3.4*	2.5*
	Soya bean			
Control	45.8^{c}	21.6^{c}	89.3^{c}	15.9^{c}
Seed treatment	56.5^{b}	29.5^{b}	114.0^{b}	31.0^{b}
Soil drench	56.8^{b}	30.0^{b}	118.0^{b}	31.2^{b}
Foliar application	78.5^{a}	38.1^{a}	134.8^{a}	41.0^{a}
$LSD_{T0.05}$	10.1*	7.4*	14.6*	7.7*
	Groundnut			
Control	47.5^{c}	22.6^{c}	72.8^{c}	18.4^{c}
Seed treatment	55.8^{b}	27.8^{b}	115.8^{b}	24.5^{b}
Soil drench	56.8^{b}	28.2^{b}	119.0^{b}	24.8^{b}
Foliar application	65.3^{a}	31.3^{a}	128.0^{a}	27.4^{a}
$LSD_{T0.05}$	8.1*	2.2*	7.3*	2.1*

Statistical significance is indicated as LSD = * significant ($P \leq 0.05$) and different letters, and ns = not significant at 5% using Tukey’s LSD test

Discussion

Agriculture is a sector of the worldwide economy that will remain critical to the global community and the stability of the general global economy (OECD FAO, 2022).

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To achieve this, farmers are required to be more efficient, productive, and agile to produce sufficient food for a rising and starving world, but at the same time, crops continue to be exposed to various stresses such as weeds competition, drought stress, over application of herbicides, pests, and diseases, causing a significant decline in crop production. The application of BR as a novel biostimulant has been extensively reported as a possible cheaper alternative option in the production systems, to alleviate stresses on crops (Vaishnav and Chowdhury, 2023; Khetsha et al., 2023b).

As reported in the results, it has been demonstrated that the three methods of BR application in all three legume crops improved the morpho-physiological parameters and yield parameters compared to the control. However, the foliar application method was the most effective, followed by soil drenching BR and seed treatment. From the results, it is clear that there is an increase in all growth characters, and the results further showed an increase in chlorophyll and photosynthetic parameters, which contributed to an increase in yield. The significant changes in morpho-physiological parameters in this study could be attributed to the method of BR administration, and this was associated with previous reports on crops such as wheat (*Triticum aestivum* L.), beetroot bulbs (*Beta vulgaris* L.), lettuce heads (*Lactuca sativa* L.) and carrots (*Daucus carota* (Hoffm.) Schübl. & G. Martens) (Alam, 2004; Van der Watt, 2005).

In recent studies, Tomar et al. (2021) revealed that a foliar application of BRs significantly increased the root length, plant dry mass, and leaf area of *Brassica juncea* (L.) Czern. Moreover, these results are in accordance with Verma et al. (2011), who reported that the application of BR improved groundnut growth, chlorophyll, and shoot elongation and consequently improved the photosynthetic rate of the crop. Tomar et al. (2021) and Liu et al. (2019) also reported that the net rate of photosynthesis and chlorophyll content can be increased by administering 24-epibrassinolide, another type of BR on *Brassica juncea* (L.) Czern and maize (*Zea mays* L.), respectively. Basit et al. (2022) reported that foliar administration of BR improved the seed germination ratio, photosynthetic attributes, plant growth, and biomass of two soya bean varieties. Pacholczak et al. (2021) reported that the BR foliar administration improved the degree of rooting, the root length, and the chlorophyll content of barberry (*Berberis thunbergii* L.). In another study, BR administration increased the total dry matter of *Kharif* groundnut compared to the control, another indicator that BR administration may increase crops' morpho-physiological parameters (Meena et al., 2019). Verma et al. (2011) also reported that the application of BR significantly improved plant growth and increased chlorophyll content, positively affecting the photosynthetic rate. Additionally, the results are similar to that of Wu et al. (2014), who reported that the application of BR increased the accumulation of biomass and chlorophyll content of eggplant (*Solanum melongena* L.) seedlings, resulting in a high photosynthetic rate. It was further indicated by Meena (2023) that BR positively affects overall plant growth because these plant hormones play an increasing role in seed germination, cell elongation, and cell division.

On the other hand, BR was recorded to be more effective after four weeks than two weeks. Interestingly, BR's effectiveness peaked in the ninth week, followed by a significant decline in all physio-morphological parameters. However, it was interesting to note that where foliar application of BR was administered using foliar spray, a notable increase was recorded between weeks 11 and 13. This was corroborated by Lin (2020), who reported that an application of BR by foliar spraying at a specific developmental stage can enhance crop development and yield, which was the case in

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this study. Khatoon et al. (2021) also reported that the timing of BR administration significantly improved the growth and yield of strawberries (*Fragaria* × *Ananassa* Duch.). Therefore, the changes in these parameters could be attributed to BR, as it accounted for many plant processes such as cell expansion and division, xylem differentiation, reproductive development, disease resistance, and abiotic stress, as described by Meena (2023) and Khetsha et al. (2022c).

On the yield and yield components, El-Bassiony et al. (2012) reported that administering BRs through foliar spraying increases the pod weight and total pod yield significantly; this was compared to untreated plants for two consecutive seasons. In the same study, El-Bassiony et al. (2012) indicated that the foliar administration of BR also increased the total pod yield; thus, this further corroborated that foliar spraying of different crops with BRs has a positive effect on overall plant growth and, therefore, validates that BRs application successfully improves the growth performance of plants under various abiotic stresses. The yield and yield components of groundnuts were significantly improved by foliar administration of BR under moisture stress; however, the author further corroborates that even under normal conditions, BR may still improve these parameters (Menpadi et al., 2022). Meena et al. (2019) reported that the number of pods and yield of *Kharif* groundnut were increased by foliar administration of BR. In this study, similar results were observed; therefore, it could be demonstrated that the yield and yield components can be increased significantly by administering BR using the foliar application method in the three legume crops.

Conclusion

In conclusion, all application methods of BRs improved plant growth in general, where foliar application showed the most remarkable improvement in all the measured parameters, followed by soil drench and seed treatment, respectively. The use of BRs will be vital to farmers to optimize and increase the production yield and quality of the three legume plants. BRs are cheap and readily available to farmers in South Africa; therefore, applying this biostimulant is recommended, and its effectiveness throughout the production system in four weeks.

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