

Article

Physiological Response of the Pea Crop (*Pisum sativum* L.) Variety ‘San Isidro’ to Water Deficit and the Application of Brassinosteroids

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Abstract

The pea is a crop of great economic and nutritional importance due to its protein contribution. However, its productivity is affected by water deficit, which is an increasingly frequent phenomenon due to rising climate variability. Brassinosteroids (BRs) are plant hormones that regulate multiple and diverse physiological processes and can mitigate the effects of water stress. The objective was to evaluate the physiological response of the ‘San Isidro’ pea crop variety to water deficit and the application of BRs during flowering. A completely randomized design was used with five treatments: well-watered control (CK) and four treatments with water stress (imposed by withholding irrigation until volumetric water content reached 5–9%, maintained for 21 days) and BR doses of 0, 0.5, 1.0, and 1.5 mg per plant. On average, water deficit reduced shoot dry biomass and pod biomass by 82% and 77%, respectively, and decreased the number of pods per plant (49%) and per branch (41%) compared to the well-watered control. It also significantly decreased water potential, net photosynthetic rate (A_n), stomatal conductance (g_s), transpiration (E), and intercellular CO_2 concentration, while foliar temperature increased. The application of BRs (especially the 0.5 mg per plant dose) increased 100-seed mass by 91.4% compared with the water-stressed 0-BR treatment. Although no statistically significant differences were detected, this dose also increased pod biomass by 130% and the number of pods by 73% compared with the water-stressed 0-BR treatment. BR application alleviated the impact of water stress by increasing q_L in leaves (at 85 DAP) and Φ_{PSII} in stipules (at 95 DAP) and by enhancing ATP synthase activity (g_H^+) in both organs and promoting recovery of gas exchange (A_n , g_s , E) and water potential. The stipules showed significant differences in more physiological variables than the leaves, suggesting potentially higher sensitivity; however, formal organ $\times$ treatment interaction analyses are needed to confirm this. It should be noted that the experimental design included only one well-watered control and did not include a factorial combination of water status $\times$ BR dose; consequently, the scope of our conclusions is restricted to the evaluation of BR effects under water-stress conditions. Further studies with a complete factorial design are required to fully disentangle the interactions between water status and BR dose.

Keywords: chlorophyll fluorescence; electrochromic change; gas exchange; stomatal conductance; water potential



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1. Introduction

The pea belongs to the legume family and is considered one of the oldest crops cultivated by humans, with archaeological references dating its domestication to more than 10,000 years ago [1]. It is an important source of protein (between 22% and 25%), carbohydrates, phosphorus, iron, magnesium, calcium, riboflavin, niacin, thiamine, and ascorbic acid; from an agricultural perspective, it plays a significant role in nitrogen fixation and improving soil fertility [2].

In Colombia, the pea is of great economic importance for small and medium farmers in the Andean regions, with production concentrated in Cundinamarca, Boyacá, Nariño and Tolima. It is a crop of great importance in the country because it generates employment in rural areas and favorable income for farmers [3]. Ninety-five percent of the national pea production is destined for human and animal consumption as a protein-rich grain, with the remaining 5% used for dry seed pea production [2]. In 2024, the cultivated area in Colombia was 21,341 hectares, with a total production of 37,142 tons and a yield of 1.92 tons per hectare, which has been decreasing since 2020, when values of 2.84 tons per hectare were recorded [4].

Despite the economic and nutritional importance of peas, their productivity has been affected by various biotic and abiotic factors that limit crop efficiency. Climate change, in particular, is a tangible natural phenomenon and a major concern due to its effects on agriculture, water availability, and natural resources. The increase in temperatures, alterations in precipitation patterns, and rising atmospheric CO₂ concentrations disrupt the climate balance, which is considered the main determinant of agricultural production. As a result, water stress intensifies because, as temperatures rise, photosynthesis efficiency improves up to an optimal point and then declines, while respiration rates continue to increase, causing plant death [5]. This leads to a reduction in planting areas, which in Colombia has decreased by 34.4% from 2019 (32,549 hectares) to 2024, as well as a 56.8% decrease in production [4], significantly impacting the country's food security. Therefore, developing strategies to strengthen crop tolerance to prolonged drought periods and mitigate the physiological effects caused by water deficits during plant development and growth is essential.

Water deficit induces a wide range of morphological, physiological, and chemical responses in pea cultivation, such as a decrease in the rate of nutrient transfer from the soil to the roots and their absorption efficiency, stimulation of stomatal closure and subsequent reduction in cell growth and photosynthesis, and a decrease in nodulation and the capacity for symbiotic nitrogen fixation, which limits crop yield [6]. Although water deficit triggers numerous physiological responses, strategies such as the use of growth regulators help mitigate its effects and increase plant tolerance. These compounds also stimulate the production of other phytohormones, allowing for the maintenance of proper development and growth, thus ensuring good crop performance. In this regard, brassinosteroids (BRs) have gained prominence in recent years, as they regulate various physiological processes in plants [7]. Their role is especially important under drought conditions, as BRs increase proline accumulation, a key amino acid that contributes to plant resistance to adverse conditions. Additionally, it has been proposed that BRs can regulate the expression of proteins induced by water stress, as well as induce *de novo* synthesis of specific polypeptides, which promotes the repair of damaged cell membranes and the maintenance of homeostasis. These effects result in better control of relative water content (RWC) and a higher membrane stability index [8].

Brassinosteroids are also involved in processes such as root elongation and development, meristem maintenance, lateral root initiation, and root hair formation [9]. Additionally, they regulate key functions such as elongation and cell division, photomorphogenesis, xylem differentiation, and reproduction; they also influence seed development, flowering

time, and pollen formation [10]. According to Pradhan et al. [8], antioxidant activity in cells increases when they adopt protective mechanisms against stress conditions, which affects yield-related parameters. However, further research is needed to demonstrate the importance of brassinosteroids as growth regulators, their interaction with other hormones, and their relationship with stress-related traits, which could contribute to developing drought-tolerant pea cultivars. Considering the above, the objective of this research was to evaluate the physiological response of pea (*Pisum sativum* L.) variety San Isidro to water stress through the application of brassinosteroids during flowering.

2. Results

2.1. Effect of Brassinosteroids on Performance, Biomass, and Assimilate Partitioning

Significant differences were observed between treatments (Table 1) in the dry mass of the aerial part (SDM) and in the dry mass of the seed pods (PDM). The well-watered control treatment (CK) showed the highest values, while the water-stressed treatments experienced an average reduction of 82% and 77% in SDM and PDM, respectively, compared to CK. Additionally, although not statistically significant, BR application numerically resulted in greater accumulation of SDM and PDM than the water-stressed 0-BR treatment, with the 1.0 mg dose showing the highest SDM (27.15 g) and the 0.5 mg dose showing the highest PDM (43.27 g). However, these differences were not statistically significant (Table 1).

Table 1. Partition of assimilates and yield components in ‘San Isidro’ pea plants subjected to water deficit and the application of brassinosteroids.

Treatment		SDM (g)	PDM (g)	NBB	RNP	RNB	PPP	PPB	SPP	SPB	HSM (g)
Water Stress	Dose of BRs (mg)										
No	0	127.26 a	151.75 a	1.60 a	20.40 a	12.80 a	34.60 a	22.80 a	173.20 a	111.80 a	58.16 a
Yes	0	14.95 b	18.78 b	1.40 a	11.80 a	9.10 a	12.20 b	9.50 b	44.60 b	31.80 c	18.07 c
Yes	0.5	26.51 b	43.27 b	1.40 a	16.00 a	11.70 a	21.20 b	15.80 ab	73.60 b	58.70 bc	34.59 b
Yes	1.0	27.15 b	40.79 b	1.20 a	15.60 a	12.90 a	18.40 b	15.00 ab	84.80 b	69.80 b	31.22 bc
Yes	1.5	21.35 b	35.00 b	1.60 a	17.00 a	11.40 a	18.80 b	13.30 b	73.20 b	50.20 bc	30.66 bc

Shoot dry mass (SDM), Pod dry mass with seeds (PDM), Number of basal branches (NBB), Reproductive nodes per plant (RNP), Reproductive nodes per branch (RNB), Pods per plant (PPP), Pods per branch (PPB), Seeds per plant (SPP), Seeds per branch (SPB), 100-seed mass (HSM). Different letters per column indicate significant differences between treatments according to the Tukey test ($p < 0.05$). Values are means of eight replicates per treatment.

The number of basal branches, reproductive nodes per plant, and reproductive nodes per branch showed no significant differences between treatments. The number of pods per plant and pods per branch differed significantly between treatments (Table 1). The CK treatment had the highest values for pods per plant and per branch, while plants subjected to water stress showed a 49% and 41% reduction in pods per plant and per branch, respectively. In the stress treatments, BR application increased the number of pods by 59% and 54% compared with the water-stressed 0-BR treatment.

Under water stress, the number of seeds per plant and per branch decreased significantly by 60% and 53%, respectively, compared with CK. In contrast, BR application under water stress increased seeds per plant and per branch by 73% and 87%, respectively, compared with the water-stressed 0-BR treatment, although water stress alone significantly reduced these parameters compared with CK. Water stress significantly reduced 100-seed mass (HSM) by an average of 50% compared with the CK treatment. The 0.5 mg BR treatment showed the highest HSM (34.59 g) among the BR-treated treatments, representing a 91.4% increase compared to the water-stressed 0-BR treatment (18.07 g) (Table 1).

2.2. Physiological, Photochemical, and ATP Synthase Activity Responses

Tables 2 and 3 present the physiological, fluorescence, and electrochromic parameters measured in leaves and stipules, respectively, for the four water-stressed treatments (BR0, BR0.5, BR1.0, and BR1.5). The well-watered control (CK) was not included in these measurements, as the study focused on the response to BR application under stress. Comparisons between leaves and stipules are presented descriptively; no formal statistical test for organ $\times$ treatment interaction was performed. The leaf inclination angle (LEFANG) showed no significant differences between treatments in any of the measurements taken for both the leaf and stipule (Tables 2 and 3). The average values were 12.4, 12.4, and 29.1 for the leaf and 13.9, 18.4, and 38 for the stipule at 85, 95, and 108 days after planting (DAP), respectively. The thickness of the leaf and stipule (StTHICK) showed no significant differences among treatments at any measurement point for leaves (Table 2). In stipules, StTHICK differed significantly at 108 DAP (Table 3), with the 1.5 mg BR treatment showing the highest numerical value; however, the Tukey test indicated that this value was not significantly different from the water-stressed 0-BR treatment (0.40 mm) or from the 1.0 mg dose. The average values were 0.69, 0.50, and 0.54 mm for the leaf and 0.6, 0.32, and 0.46 mm for the stipule at 5, 15, and 28 days after stress, respectively. The leaf temperature differential (LTD) only showed significant differences in the final measurement (108 DAP), where plants in the water-stressed 0-BR treatment showed the highest LTD values, while treatments with water stress and BR application at doses of 1.5, 1.0, and 0.5 mg were 5.45%, 5.96%, and 21.68% lower, respectively. Stipule temperature differential (STD) only showed significant differences between treatments in the first two measurements (85 and 95 DAP). At 85 DAP, BR application decreased leaf temperature by 6.5% compared with the water-stressed 0-BR treatment, while 0.5 and 1.0 mg doses had higher temperatures than the water-stressed 0-BR treatment. At 95 DAP, the plants in the water-stressed 0-BR treatment and those treated with 1.5 mg of BRs had the smallest significant temperature difference from the environment, while plants under stress and treated with 0.5 and 1 mg plant^{−1} of BRs had the greatest temperature differences from the environment.

Table 2. Physiological, fluorescence, and electrochromic parameters in pea leaves subjected to water deficit and different doses of brassinosteroids.

Parameter	DAP	Dose of Brassinosteroids (mg per Plant)			
		0	0.5	1.0	1.5
LEFANG (°)	85	8.66 a	14.01 a	11.08 a	15.78 a
	95	8.24 a	10.34 a	17.59 a	13.52 a
	108	40.92 a	32.62 a	19.05 a	23.43 a
LEAFTHICK (mm)	85	0.65 a	0.57 a	0.60 a	0.98 a
	95	0.55 a	0.41 a	0.42 a	0.64 a
	108	0.40 a	0.74 a	0.56 a	0.48 a
LTD (°C)	85	−5.83 a	−4.90 a	−4.15 a	−6.08 a
	95	−5.49 a	−6.13 a	−6.02 a	−7.97 a
	108	−5.26 a	−6.40 b	−5.57 ab	−5.54 a
RCC (SPAD)	85	53.74 a	46.16 a	51.44 a	52.27 a
	95	37.92 a	37.19 a	36.32 a	37.63 a
	108	40.37 a	41.05 a	40.89 a	41.64 a
ECSt (mAU)	85	0.00057 a	0.00110 a	0.00116 a	0.00034 a
	95	0.00178 a	0.00127 a	0.00115 a	0.00176 a
	108	0.00102 a	0.00039 b	0.00065 ab	0.00063 ab

Table 2. Cont.

Parameter	DAP	Dose of Brassinosteroids (mg per Plant)			
		0	0.5	1.0	1.5
ECS τ (s)	85	0.0061 a	0.0061 a	0.0074 a	0.0039 a
	95	0.0140 a	0.0103 a	0.0075 a	0.0153 a
	108	0.0089 a	0.0041 c	0.0064 b	0.0059 bc
g_H^+ (s $^{-1}$)	85	176.94 ab	173.73 ab	142.42 b	258.39 a
	95	78.98 a	98.44 a	134.75 a	82.73 a
	108	113.30 c	224.60 a	156.80 b	170.60 b
v_H^+ (mAU s $^{-1}$)	85	0.087 a	0.171 a	0.154 a	0.085 a
	95	0.129 a	0.123 a	0.118 a	0.121 a
	108	0.118 a	0.096 a	0.099 a	0.108 a
Φ_{PSII}	85	0.31 a	0.41 a	0.41 a	0.45 a
	95	0.27 a	0.28 a	0.34 a	0.23 a
	108	0.31 a	0.40 a	0.31 a	0.40 a
Φ_{NPQ}	85	0.24 a	0.35 a	0.27 a	0.25 a
	95	0.58 a	0.55 a	0.50 a	0.61 a
	108	0.35 a	0.15 a	0.30 a	0.20 a
q_L	85	0.15 b	0.38 a	0.26 ab	0.28 ab
	95	0.41 a	0.37 a	0.50 a	0.38 a
	108	0.21 a	0.18 a	0.19 a	0.21 a
NPQt	85	0.60 a	0.98 a	0.86 a	0.47 a
	95	5.25 a	3.73 a	3.84 a	6.62 a
	108	1.12 a	0.33 a	1.03 a	0.51 a
Φ_{NO}	85	0.45 a	0.24 a	0.32 a	0.30 a
	95	0.15 a	0.16 a	0.16 a	0.15 a
	108	0.34 a	0.45 a	0.39 a	0.40 a
Fv'/Fm'	85	0.76 a	0.71 a	0.73 a	0.77 a
	95	0.48 a	0.52 a	0.52 a	0.45 a
	108	0.70 a	0.79 a	0.71 a	0.77 a
LEF ($\mu\text{mol e}^- \text{m}^{-2} \text{s}^{-1}$)	85	49.84 a	113.88 a	92.43 a	60.00 a
	95	62.68 a	53.05 a	52.72 a	69.18 a
	108	51.40 a	26.43 a	28.74 a	49.12 a

DAP: days after planting; leaf angle (LEFANG); leaf thickness (LEAFTHICK); leaf temperature differential (LTD); relative chlorophyll content (RCC); Total electrochromic shift magnitude (ECSt); electrochromic shift relaxation time constant (ECS τ); proton conductance of ATP synthase (g_H^+); steady-state proton flux (v_H^+); effective quantum yield of Photosystem II (Φ_{PSII}); quantum yield of regulated energy dissipation (Φ_{NPQ}); fraction of open PSII reaction centers (q_L); non-photochemical quenching coefficient (NPQt); quantum yield of non-regulated energy dissipation (Φ_{NO}); maximum efficiency of open PSII reaction centers in light conditions (Fv'/Fm'); and linear electron flow (LEF). Different letters per row indicate significant differences according to Tukey's test ($p < 0.05$). CK (well-watered control) was not measured for these parameters. Values are means of eight replicates per treatment.

No statistical differences were observed between treatments for leaf SPAD values; however, in the last measurement (108 days after planting), stipule SPAD values differed significantly (Table 3). The 1.0 mg BR treatment showed the highest value, significantly different from 0.5 mg; however, the 1.5 mg dose was not significantly different from the water-stressed 0-BR treatment. The averages recorded for the measurements taken at 85, 95, and 108 days after planting were 50.9, 37.2, and 40.9 for the leaves and 47, 45.3, and 27.7 for the stipules, respectively.

The total electrochromic shift (ECSt) of the leaves only showed significant differences between treatments in the last measurement (108 DAP), where treatments with

BRs exhibited an average decrease of 45% in ECSt values compared to the water-stressed 0-BR treatment. Conversely, ECSt in the stipules showed significant differences in the first two measurements at 85 and 95 DAP, with the highest values in the treatments with 0.5 mg of BRs and the water-stressed 0-BR; at 95 DAP, the water-stressed 0-BR treatment and the 1.5 mg BR treatment showed the highest values and were not significantly different from each other, while the 0.5 and 1.0 mg doses had significantly lower values. The relaxation time constant (ECS τ) of the leaves showed significant differences only in the third measurement (108 DAP), where plants treated with BRs had average ECS τ values 38.8% lower than the water-stressed 0-BR treatment; similarly, the ECS τ of the stipules from plants treated with BRs was on average 60% lower than the water-stressed 0-BR treatment.

Table 3. Physiological, fluorescence, and electrochromic parameters in pea stipules subjected to water deficit and different doses of brassinosteroids.

Parameter	DAP	Dose of Brassinosteroids (mg per Plant)			
		0	0.5	1.0	1.5
LEFANG (°)	85	14.63 a	10.19 a	15.11 a	15.96 a
	95	13.82 a	16.93 a	21.32 a	21.66 a
	108	32.91 a	40.18 a	57.32 a	21.58 a
StTHICK (mm)	85	0.58 a	0.50 a	0.65 a	0.67 a
	95	0.23 a	0.41 a	0.29 a	0.36 a
	108	0.40 ab	0.26 b	0.59 ab	0.61 a
STD (°C)	85	−5.87 ab	−4.82 ab	−4.56 a	−6.26 b
	95	−1.41 a	−6.16 b	−7.39 b	−2.82 a
	108	−7.43 a	−8.64 a	−8.09 a	−8.39 a
RCC (SPAD)	85	44.74 a	45.69 a	46.78 a	50.80 a
	95	42.16 a	41.43 a	51.76 a	46.07 a
	108	22.25 ab	20.93 b	40.06 a	27.74 ab
ECSt (mAU)	85	0.00062 a	0.00070 a	0.00032 b	0.00027 b
	95	0.00220 a	0.00087 b	0.00098 b	0.00199 a
	108	0.00057 a	0.00075 a	0.00031 a	0.00096 a
ECS τ (s)	85	0.0061 a	0.0057 a	0.0040 a	0.0059 a
	95	0.0259 a	0.0074 b	0.0070 b	0.0166 ab
	108	0.0090 a	0.0107 a	0.0063 a	0.0152 a
g_H^+ (s ^{−1})	85	165.32 a	175.98 a	226.75 a	183.99 a
	95	26.00 b	143.55 a	144.25 a	67.16 b
	108	119.70 a	78.25 a	171.19 a	80.64 a
v_H^+ (mAU s ^{−1})	85	0.082 ab	0.122 a	0.082 ab	0.059 b
	95	0.098 a	0.123 a	0.111 a	0.125 a
	108	0.062 a	0.091 a	0.045 a	0.069 a
Φ_{PSII}	85	0.34 a	0.41 a	0.43 a	0.36 a
	95	0.16 b	0.38 a	0.38 a	0.17 b
	108	0.27 a	0.22 a	0.33 a	0.21 a
Φ_{NPQ}	85	0.19 ab	0.29 a	0.12 b	0.26 ab
	95	0.71 a	0.32 b	0.31 b	0.72 a
	108	0.47 a	0.52 a	0.15 a	0.49 a
q_L	85	0.15 a	0.28 a	0.19 a	0.21 a
	95	0.24 a	0.27 a	0.30 a	0.35 a
	108	0.18 a	0.18 a	0.13 a	0.11 a

Table 3. Cont.

Parameter	DAP	Dose of Brassinosteroids (mg per Plant)			
		0	0.5	1.0	1.5
NPQt	85	0.41 a	0.96 a	0.26 a	0.82 a
	95	6.84 a	1.08 a	1.24 a	7.12 a
	108	2.28 a	2.15 a	0.30 a	1.15 a
Φ_{NO}	85	0.47 a	0.30 b	0.45 ab	0.38 ab
	95	0.13 ab	0.30 a	0.30 a	0.10 b
	108	0.26 a	0.26 a	0.52 a	0.30 a
Fv'/Fm'	85	0.78 a	0.71 a	0.79 a	0.73 a
	95	0.42 b	0.70 a	0.69 a	0.38 b
	108	0.61 a	0.61 a	0.79 a	0.58 a
LEF ($\mu\text{mol e}^- \text{ m}^{-2} \text{ s}^{-1}$)	85	51.96 a	91.29 a	33.54 a	56.96 a
	95	47.37 a	65.58 a	90.12 a	78.08 a
	108	24.48 a	15.20 ab	9.36 b	21.29 ab

DAP: days after planting; Leaf angle (LEFANG); stipule thickness (StTHICK); stipule temperature differential (STD); relative chlorophyll content (RCC); Total electrochromic shift magnitude (ECSt); electrochromic shift relaxation time constant (ECSt τ); proton conductance of ATP synthase (g_H^+); steady-state proton flux (v_H^+); effective quantum yield of Photosystem II (Φ_{PSII}); quantum yield of regulated energy dissipation (Φ_{NPQ}); fraction of open PSII reaction centers (q_L); non-photochemical quenching coefficient (NPQt); quantum yield of non-regulated energy dissipation (Φ_{NO}); maximum efficiency of open PSII reaction centers in light conditions (Fv'/Fm'); and linear electron flow (LEF). Different letters per row indicate significant differences according to Tukey's test ($p < 0.05$). CK (well-watered control) was not measured for these parameters. Values are means of eight replicates per treatment.

The proton conductivity of ATP synthase (g_H^+) in the leaves showed significant differences in the first and third measurements. At 85 DAP, the 1.5 mg BR treatment showed a 46% higher g_H^+ value (258.39 s^{-1}) compared to the water-stressed 0-BR treatment (176.94 s^{-1}), while the 0.5 and 1.0 mg doses did not differ significantly from the water-stressed 0-BR treatment. At 108 DAP, BR treatments had, on average, 62% higher g_H^+ values compared to the water-stressed 0-BR treatment. In stipules, at 95 DAP, the 0.5 and 1.0 mg BR treatments showed a 453% increase in g_H^+ compared to the water-stressed 0-BR treatment (143.55 and 144.25 s^{-1} vs. 26.00 s^{-1}), whereas the 1.5 mg dose was not significantly different from the water-stressed 0-BR treatment. The steady-state proton flux (v_H^+) in the leaves did not show significant differences. In stipules, v_H^+ showed a significant overall treatment effect at 85 DAP according to the ANOVA ($p = 0.0322$). However, the post-hoc Tukey test indicated that the 0.5 mg BR treatment (0.122 mAU s^{-1}) was not significantly different from the water-stressed 0-BR treatment (0.082 mAU s^{-1}) (Table 3). The 0.5 mg dose was, however, significantly higher than the 1.5 mg dose (0.059 mAU s^{-1}).

The operational quantum yield of Photosystem II (Φ_{PSII}) in leaves did not show significant differences among treatments at any measurement point (Table 2). However, in stipules, significant differences were observed in the second measurement (95 DAP), where the 0.5 and 1.0 mg BR treatments showed 144.6% higher Φ_{PSII} values (0.38) than the water-stressed 0-BR treatment (0.16), while the 1.5 mg dose (0.17) did not differ significantly from BR0 (Table 3). The quantum yield of regulated non-photochemical energy loss in Photosystem II (Φ_{NPQ}) and the quantum yield of non-regulated energy dissipation (Φ_{NO}) in leaves did not show significant differences, while for stipules there were differences between treatments in the first (85 DAP) and second measurement (95 DAP) for Φ_{NPQ} and Φ_{NO} . However, no clear trend emerged among treatments for Φ_{NPQ} , although Φ_{NPQ} values in stipules were 5% higher than in leaves. BR applications resulted in 36% less Φ_{NO} than the water-stressed 0-BR treatment at 85 DAP.

The fraction of open reaction centers of PSII (q_L) in leaves differed significantly only at the first measurement (85 DAP). Only the 0.5 mg BR treatment (0.38) showed a significant increase (145.3%) compared to the water-stressed 0-BR treatment (0.15), while the 1.0 and 1.5 mg doses (0.26 and 0.28) did not differ significantly from the water-stressed 0-BR treatment (Table 2). For stipules, q_L showed no statistical differences between treatments. The non-photochemical quenching coefficient (NPQt) in the leaves showed no significant differences, while in the stipules, differences were only observed in the second measurement at 95 DAP ($p = 0.0202$); however, Tukey's post-hoc test did not detect significant pairwise differences between individual doses, indicating that the overall treatment effect was not strong enough to differentiate one specific BR dose as being better than the others. The maximum efficiency of open PSII reaction centers in light conditions (Fv'/Fm') in leaves did not show significant differences in any measurement, but in stipules, significant differences were only observed in the second measurement, where treatments with 0.5 and 1.0 mg of BRs had the highest Fv'/Fm' values. Linear electron flow (LEF) in leaves was similar across treatments; however, in stipules, significant differences were observed in the third measurement (108 DAP), where the water-stressed 0-BR treatment showed the highest values, while BR-treated plants had, on average, 37.6% lower LEF compared with the water-stressed 0-BR treatment.

2.3. Gas Exchange and Regulation of the Water Status in Leaves and Stipules

The net CO_2 assimilation rate (A_n) of the leaves and stipules of pea plants showed significant differences between treatments (Table 4). Pea plants subjected to water stress significantly decreased the A_n of stipules and leaves by 65% and 70%, respectively, compared to the well-watered control treatment. Additionally, among the BR-treated treatments, the 1.5 mg dose showed the highest A_n values in both leaves ($23.09 \mu\text{mol } CO_2 \text{ m}^{-2} \text{ s}^{-1}$) and stipules ($21.45 \mu\text{mol } CO_2 \text{ m}^{-2} \text{ s}^{-1}$), representing increases of 130% and 154%, respectively, compared to the water-stressed 0-BR treatment (Table 4).

Table 4. Gas exchange parameters in leaves and stipules of pea (*Pisum sativum* L.) subjected to water deficit and different doses of brassinosteroids.

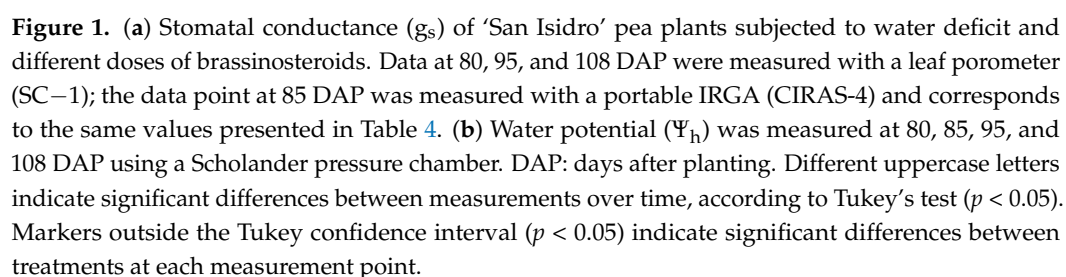
Organ	Treatment		A_n ($\mu\text{mol } CO_2 \text{ m}^{-2} \text{ s}^{-1}$)	g_s ($\text{mmol } H_2O \text{ m}^{-2} \text{ s}^{-1}$)	E ($\text{mmol } H_2O \text{ m}^{-2} \text{ s}^{-1}$)	C_i ($\mu\text{mol } CO_2 \text{ mol}^{-1}$)
	Water Stress	Dose of BRs (mg)				
Leaf	No	0	28.71 a	344.15 a	3.94 a	396.45 a
	Yes	0	10.04 d	93.90 d	1.13 d	307.06 d
	Yes	0.5	16.64 c	172.00 c	2.00 c	351.31 c
	Yes	1.0	17.37 c	178.63 c	2.09 c	357.20 bc
	Yes	1.5	23.09 b	243.38 b	2.83 b	382.48 ab
Stipule	No	0	27.80 a	333.58 a	3.76 a	396.40 a
	Yes	0	8.43 d	82.00 d	1.04 d	312.06 d
	Yes	0.5	16.61 c	170.90 c	2.05 c	346.95 b
	Yes	1.0	17.36 bc	177.45 c	2.13 c	363.56 b
	Yes	1.5	21.45 b	227.23 b	2.72 b	384.20 a

BRs: Brassinosteroids; Net CO_2 assimilation rate (A_n); Stomatal conductance (g_s); Transpiration (E); Intercellular CO_2 concentration (C_i). Different letters per column and classified by organ indicate significant differences between treatments according to Tukey's test ($p < 0.05$). Values are means of eight replicates per treatment.

Stomatal conductance (g_s) in leaves and stipules at 85 DAP differed significantly among treatments (Figure 1a). Pea plants subjected to water stress significantly decreased g_s by 72.7% in leaves and 75.4% in stipules compared with the well-watered control

Figure 1 is a line graph showing Stomatal conductance (mmol m⁻² s⁻¹) on the Y-axis (ranging from 0 to 300) versus Time (DAP) on the X-axis (ranging from 80 to 110). The graph displays four data series representing different treatments: Water stress + 0 mg BR (dashed line, open squares), Water stress + 0.5 mg BR (solid line, triangles), Water stress + 1.0 mg BR (solid line, circles), and Water stress + 1.5 mg BR (solid line, squares). A light green shaded area represents Tukey's confidence band. Letters A, AB, C, and BC indicate significant differences at various time points.

Time (DAP)	Water stress + 0 mg BR	Water stress + 0.5 mg BR	Water stress + 1.0 mg BR	Water stress + 1.5 mg BR
80	205	220	220	250
85	95	175	180	245
95	85	105	120	80
108	115	155	115	115



<https://doi.org/10.3390/stresses6030067>

increased the E of stipules and leaves in pea plants by 120% and 104%, respectively, on average, when compared to the water-stressed 0-BR treatment.

Intercellular CO₂ concentration (C_i) differed significantly between treatments in stipules and leaves. The non-stressed treatment and the water-stressed treatment with 1.5 mg of BRs had similar results, while the water-stressed 0-BR treatment had the lowest C_i values in both leaves and stipules.

Water potential (Ψ_h) differed significantly among treatments only at the first (80 DAP) and last (108 DAP) measurements (Figure 1b). At 80 DAP, the water-stressed 0-BR treatment exhibited the lowest water potential (-1.2875 ± 0.1087 MPa), while BR-treated plants showed higher values, with the 1.5 mg dose reaching -0.9275 ± 0.0131 MPa. At 108 DAP, the water-stressed 0-BR treatment had a water potential of -1.4550 ± 0.1202 MPa, whereas BR-treated plants showed less negative values, with the 0.5 mg dose reaching -0.7925 ± 0.0527 MPa. These results indicate that BR application helped restore water status after stress relief.

3. Discussion

3.1. Implications of Brassinosteroids on Biomass Partitioning and Yield Components Under Water Stress

Brassinosteroids (BRs) are plant hormones that actively regulate growth and the partitioning of photoassimilates under stress conditions [11]. The exogenous application of BRs (especially at 1 and 1.5 mg) slightly mitigated the negative effect of water deficit on biomass accumulation. Aerial dry mass, pod mass, and HSM increased numerically when BRs were applied in water-stressed treatments compared with the water-stressed 0-BR treatment; however, these differences were not statistically significant (Table 1). Only the well-watered control (CK) was significantly higher than all water-stressed treatments. The drastic reduction in shoot dry mass ($\approx 88\%$ relative to CK) underscores the severity of the water deficit, which was imposed during early flowering—a period of active vegetative and reproductive growth—and maintained at low volumetric water content (5–9%) for 21 days, sharply limiting carbon assimilation. This cumulative impact on whole-plant carbon balance explains why SDM was more severely affected than pod or seed numbers, which were partially sustained by remobilization of reserves from vegetative tissues. The observed numerical improvements with BRs, though not significant, suggest a potential modulation of carbon partitioning, consistent with the reported role of brassinosteroids in promoting photoassimilate translocation [12]. Additionally, the increase in HSM may be attributed to higher starch synthase activity in the endosperm, as reported in legumes under drought conditions treated with epibrassinolide (EBR) [13]. Furthermore, BRs reduce lipid peroxidation and maintain cell membrane integrity, facilitating assimilate flow from the leaves to the pods [14]. These results are consistent with those reported by Shahid et al. (2011) [15] in *Pisum sativum*, who found that 10 μM of 24-EBR to salt-stressed plants increased total dry mass by 21% compared to the well-watered control. Likewise, Xiong et al. [16] demonstrated that BRs improve biomass partitioning toward pods by negatively regulating genes in the abscisic acid pathway while increasing shoot biomass and seed production in grass pea plants subjected to 80% and 50% of full irrigation. The findings suggest that, under water stress conditions, the application of BRs mitigates the reduction in shoot dry mass and pod dry mass caused by stress in ‘San Isidro’ pea plants.

The number of pods is a yield component that is highly sensitive to water stress, especially during flowering and pod set [17]. Treatments with BR applications increased the number of pods per plant and per branch compared to the water-stressed 0-BR treatment. This observation is consistent with previous reports that BRs can reduce flower and pod abscission, potentially by regulating cell wall-related genes and modulating ABA signaling,

although we did not directly investigate these mechanisms [18]. Notably, variables related to the number of reproductive nodes per plant and per branch did not differ significantly, suggesting that BRs do not increase the number of potential sites for pod formation but instead improve retention and development of initially formed pods. This pattern has been observed in other legumes such as soybean, where brassinosteroids increase the number of pods per node without changing the number of nodes and decrease the number of pods with one or two seeds, implying a greater seed fill per seed [19]. In this regard, the results suggest that aspersions with BRs during early reproductive stages is convenient for maximizing yield under drought conditions.

The number of seeds is one of the parameters most closely related to the final yield in pea cultivation and in grain legumes in general [20]. In this study, brassinosteroid treatments increased the number of seeds per plant and per branch compared with the water-stressed 0-BR treatment; however, they reached only 53% of the well-watered control. Various mechanisms have been proposed in the literature to explain BR-mediated improvements in seed set under stress, including enhanced pollen viability, pollen tube growth, and reduced ovule sterility [10,21–23]. However, as we did not measure these processes directly, these explanations remain speculative and are included only to provide a physiological context for our observations. Recent studies in peas have shown that BR application increased seeds per pod by up to 50% compared with the water-stressed 0-BR treatment [24]. Furthermore, 1 mg of BRs was the most effective among the BR-treated treatments, suggesting that higher doses do not further increase pod number. Based on this, brassinosteroids are an effective tool to ensure seed filling in pea crops exposed to water deficit during flowering.

3.2. Photochemical and ATP Synthase Efficiency Mechanisms Induced by Brassinosteroids to Mitigate the Deficit

Chlorophyll fluorescence is a non-destructive parameter that allows assessment of the functioning of a plant's photosynthetic apparatus under stress conditions [25]. In the first measurement at 85 DAP (11 days after the start of water restriction), leaf Φ_{PSII} did not show significant differences among treatments (Table 2). However, leaf q_L showed significant differences, with only the 0.5 mg BR treatment exhibiting a significantly higher value (0.38) compared to the water-stressed 0-BR treatment (0.15), while the 1.0 and 1.5 mg doses did not differ from the water-stressed 0-BR treatment. In contrast, stipule Φ_{PSII} at 95 DAP showed significant differences, with the 0.5 and 1.0 mg per plant BR treatments showing higher values than the water-stressed 0-BR treatment (Table 3). Φ_{PSII} expresses the proportion of absorbed energy used in photochemistry, and its decrease is one of the earliest indicators of water stress [26]. The higher Φ_{PSII} values observed with BR application may reflect the known protective role of BRs against photoinhibition, possibly through enhanced antioxidant defense, as reported in other species [27]. However, as we did not measure antioxidant enzyme activities or ROS levels, this interpretation remains speculative. On the other hand, q_L indicates the number of PSII reaction centers that are in an oxidized (open) state and capable of accepting electrons; therefore, water deficit likely caused low q_L values, reflected in an excess of reducing power that saturates the transport chain as a consequence of water stress. In this regard, Zhou et al. [13] report that BR application maintains quinone A activity, which promotes electron flow toward Photosystem I and is reflected in increased q_L values. This aligns with findings reported by Pereira et al. [28] in soybeans, where applying 24-EBR at 100 nM increased Φ_{PSII} and q_L by 55% and 6.2%, respectively, compared with the water-stressed 0-BR treatment. The lack of statistical differences in measurements after 95 and 108 DAP suggests that, as the stress prolonged, all treatments reached a similar level due to the extension of water-deficit conditions.

Total electrochromic change (ECSt) estimates the proton gradient across the thylakoid membrane and the activity of ATP synthase [29]. At 108 days after planting (DAP) (after stress termination), we observed significant differences: the water-stressed 0-BR treatment showed the highest ECSt gradient values, suggesting greater proton accumulation because ATP synthase had a lower capacity to consume them. In contrast, plants subjected to water stress and treated with 1.5 mg of BRs showed lower ECSt and higher g_H^+ , indicating increased ATP synthase activity and more efficient use of the proton gradient for ATP synthesis [30]. These results suggest that, although water deficit caused proton accumulation in the water-stressed 0-BR treatment, brassinosteroids promoted a functional recovery of photophosphorylation, accelerating gradient consumption and improving energy efficiency. This effect is crucial because ATP produced by ATP synthase activates chloroplast ribosomes, which are needed for carbon fixation in the Calvin cycle and for repairing and biosynthesizing the D1 protein of photosystem II, which is damaged by photoinhibition during water stress [31]. The observed differences in ECSt and g_H^+ suggest that BRs may have facilitated a functional recovery of photophosphorylation after stress. Under water deficit, reduced ATP synthase activity (low g_H^+) appears to have led to proton gradient accumulation (high ECSt, slow ECS τ). In contrast, BR-treated plants showed higher g_H^+ and faster ECS τ , indicating more efficient proton flux and ATP synthesis. This interpretation is consistent with the known role of ATP in supporting Calvin cycle activity and protein repair, as described in the literature [31–33]. However, as we did not measure ROS production or D1 protein levels, these mechanistic links remain theoretical. Thus, by sustaining ATP supply, BRs alleviate photoinhibition and maintain photosynthetic efficiency under water stress. In this regard, Li et al. [31] report that brassinosteroids induce the expression of nuclear and chloroplastic genes encoding ATP synthase subunits under water stress. Maia et al. [34] found that EBR increases the relaxation rate of ECS in tomato and optimizes electron flow and proton gradient modulation, which correlates with higher ATP production and biomass. In leaves at 108 DAP, the 1.5 mg BR dose showed the highest g_H^+ values, indicating a positive effect on ATP synthase activity in that organ and at that time point. Therefore, electrochromic change parameters can serve as sensitive biomarkers for evaluating the effectiveness of BRs in mitigating water stress in peas [35].

Stipules generally showed significant differences in more physiological variables than leaves. However, because we did not perform a formal organ $\times$ treatment interaction analysis, we interpret this observation descriptively rather than as evidence of differential sensitivity. The apparent stronger response of stipules may reflect differences in organ-specific physiology or developmental timing, but confirming this would require dedicated statistical testing in future studies. Twenty days after the stress was initiated (95 DAP), most fluorescence parameters showed statistically significant differences in variables such as ECSt, ECS, g_H^+ , Φ_{PSII} , Φ_{NPQ} , Φ_{NO} , Fv'/Fm' , and NPQt. The higher ECSt values in the water-stressed 0-BR treatment suggest proton gradient accumulation that ATP synthase may not fully utilize for ATP synthesis, as reflected in higher ECS τ (slower relaxation) and lower g_H^+ and v_H^+ [36]. Similarly, the lower values of Φ_{PSII} , q_L , and the thickness of the stipule in the water-stressed 0-BR treatment are possibly due to reduced efficiency in electron transport, indicating that PSII is functioning poorly and the oxidation state of QA is less favorable due to damage or photoinhibition induced by the applied stress [37]. Additionally, the significant differences shown for LEF in the stipules indicate a higher rate of electron transport through photosystem II, suggesting increased photosynthetic activity. Overall, the results indicate that BR application specifically increased q_L in leaves at 85 DAP (a measure of open PSII reaction centers) and Φ_{PSII} in stipules at 95 DAP (effective quantum yield). Additionally, BRs enhanced ATP synthase activity (g_H^+) in both organs, particularly at 108 DAP in leaves and at 95 DAP in stipules. These responses suggest a

protective effect on specific components of the photosynthetic apparatus, rather than a general improvement in overall photochemical efficiency, which aligns with the role of BRs in mitigating abiotic stress [37].

In contrast, the significant differences in STD at 95 DAP suggest that BR application was more effective at reducing stipule temperature than the water-stressed 0-BR treatment. This may have occurred because BRs increase stomatal conductance and transpiration under water-deficit conditions, and this increased transpiration led to a decrease in stipule temperature [38]. Likewise, an increase in leaf temperature is the first symptom of water stress onset [26], so measuring it is important because it is non-destructive and allows timely corrections to mitigate more severe stress.

3.3. Role of Brassinosteroids in the Regulation of Gas Exchange and the Recovery of Water Status

Water deficit significantly decreased A_n and g_s in both the leaf and the stipule, while BR application (1.5 and 1.0 mg) mitigated the reduction in A_n and g_s compared to the water-stressed 0-BR treatment. The reduction in A_n and g_s under water deficit is consistent with the well-known stomatal closure response mediated by ABA, which limits CO_2 entry and reduces photosynthetic carbon fixation [39,40]. This response also leads to an over-reduction of the electron transport chain, increased photorespiration, and decreased Φ_{PSII} [41]. These physiological responses are typical of water-stressed plants and provide a framework for interpreting our gas exchange data. However, we did not measure ABA concentrations, ROS, or ion fluxes, so the specific signaling events underlying the observed changes remain speculative. BR application has been reported to mitigate water stress by preserving chloroplast function, improving Rubisco activity, and reducing photoinhibition [42]. Consistent with this, Kumar et al. [43] observed that foliar application of EBR increased net photosynthetic rate in maize under drought. However, because we did not measure chloroplast ultrastructure, Rubisco activity, or antioxidant enzyme activities, these interpretations rely on previous reports and should be treated as a theoretical framework rather than direct evidence from our data. Therefore, plants treated with BRs show higher photosynthetic rates and more efficient physiological recovery after water stress conditions [42], which occurs because BRs modulate ABA sensitivity by increasing membrane ATPase activity, maintaining the proton gradient necessary for K^+ entry [44], and modulating nitric oxide (NO) production, a messenger that promotes stomatal opening [45]. Notably, the stipule showed a response similar to the leaf, suggesting that this organ also actively contributes to carbon fixation under stress conditions. Furthermore, the results also suggest that the stipule may show distinct stomatal responses compared to the leaf, possibly reflecting organ-specific physiology, but this interpretation remains speculative without direct measurements of receptor density or signaling components.

Transpiration is directly related to stomatal opening and the vapor pressure differential between the leaf and the environment. Treatments with the highest doses of BRs (1.0 and 1.5 mg) maintained significantly higher transpiration rates than those observed in the water-stressed 0-BR treatment, indicating that plants treated with BRs preserved greater stomatal activity, which favored evaporative cooling and the transpiration flow responsible for nutrient transport, especially those mobilized through the xylem [45]. Previous studies suggest that BRs can regulate transpiration through aquaporin expression and ABA-mediated signaling [45], but these mechanisms were not investigated in our experiment. Although reducing transpiration is an adaptive strategy against drought, excessive stomatal closure limits CO_2 diffusion and reduces carbon fixation; therefore, BRs act as stabilizers, balancing water use and photosynthetic productivity by maintaining moderate transpiration without compromising the plant's water status.

The highest transpiration rate observed in the stipules compared to the leaves suggests that these organs constitute a fundamental part of the photosynthetically active surface [46], so their ability to maintain stomatal opening and transpiration flow is crucial for sustaining CO₂ assimilation and temperature regulation [42]. The greater transpiration recorded in stipules treated with BRs could indicate better stomatal function and healthier water status in these organs, which favors photosynthetic processes under stress. However, the anatomical and molecular mechanisms behind this differential response between stipules and leaf blades remain unclear. The intercellular CO₂ concentration (C_i) is an indicator of the balance between CO₂ entry through the stomata and its consumption during carbon fixation, and it showed a behavior similar to the responses observed in A_n and g_s . It is possible that in the water-stressed 0-BR treatment, stomatal closure induced by water deficit drastically reduced CO₂ entry into the mesophyll, which decreased C_i and limited A_n due to stomatal restrictions [47]. In contrast, plants treated with BRs showed higher g_s , allowing greater CO₂ entry for the Calvin cycle, resulting in higher C_i and A_n values, which aligns with the positive effect of BRs on gas exchange and photosynthetic recovery described by Zeng et al. [42]. Regarding the behavior of the stipule, it showed C_i values slightly lower than those of the leaf and greater statistical sensitivity (Table 4), suggesting that the stipule may show distinct stomatal responses compared to the leaf, possibly reflecting organ-specific physiology. However, this interpretation remains speculative without direct measurements of receptor density or signaling components and requires further investigation. Overall, these results indicate that brassinosteroids mitigate stomatal limitations induced by water deficit in pea plants by improving photosynthetic efficiency, as they maintain higher C_i , which favors greater CO₂ availability for carbon fixation.

Water stress applied to pea plants resulted in the lowest Ψ_h values at 80 and 108 days after planting (DAP), while BR application of 1.5 and 0.5 mg of brassinosteroids (BRs) at the two evaluation times, respectively, increased Ψ_h values. The improved Ψ_h in BR-treated plants is consistent with the reported roles of BRs in regulating aquaporin expression and osmotic adjustment [45]. However, as we did not measure aquaporin activity, osmolyte concentrations, or antioxidant capacity, these interpretations are based on previous literature. The gradual recovery of Ψ_h observed in Figure 1b may indicate that BRs not only protect the plant during stress but also facilitate restoration of water status during the post-stress period, accelerating the plant's physiological recovery mechanisms. In this regard, Kumar et al. [43] found that 24-EBR to maize increased the relative water content, strengthened the antioxidant system, and promoted plant recovery after water deficit. Overall, BRs help maintain water status and increase drought tolerance in peas.

It is important to acknowledge that our experimental design included only one well-watered control (CK) and four water-stressed BR treatments, rather than a full factorial combination (water stress $\times$ BR dose). A well-watered + BR control was not included, as our primary objective was to evaluate the potential of BRs to mitigate water deficit, rather than to assess the interaction between water status and BR dose under non-stress conditions. Consequently, we cannot formally test for interactions between water stress and BR application, nor can we separate the effects of BRs under well-watered conditions. Future studies should adopt a complete factorial design (2 water levels $\times$ 4 BR doses) to fully disentangle the main effects and interactions.

Greenhouse temperatures frequently exceeded 35 °C and occasionally reached ~45 °C (Figure 2), which may have imposed additional heat stress on the plants and potentially amplified the effects of water deficit on stomatal conductance and photosynthesis. The IRGA measurements were conducted at a constant 25 °C to isolate the effect of water deficit from temperature variability, but the combined stress experienced by the plants in the

greenhouse could have influenced the observed responses. Future studies should include a factorial design to separate the effects of water deficit and high temperature.

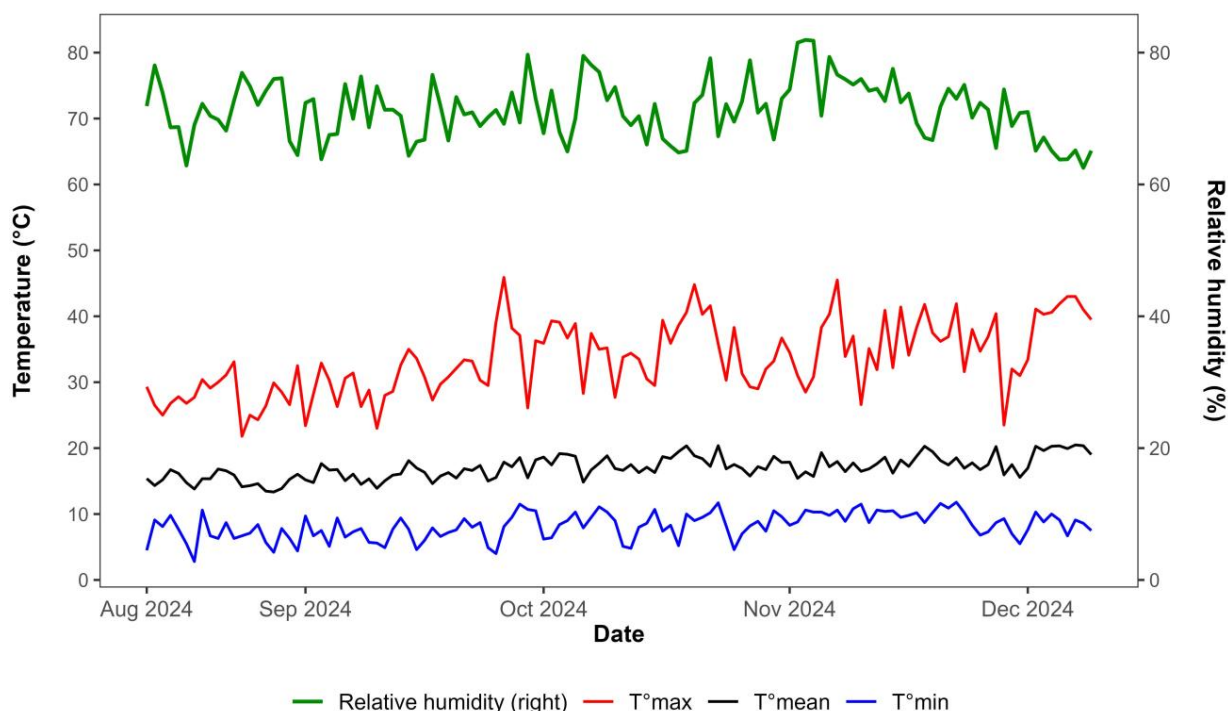


Figure 2. Behavior of environmental variables during the pea cultivation cycle in a greenhouse. The data span from seed planting (12 August 2024) to pod harvest (10 December 2024).

4. Materials and Methods

4.1. Location

The research was conducted in the experimental greenhouse of the ‘La María’ farm at the Pedagogical and Technological University of Colombia (UPTC), in the municipality of Tunja (Boyacá), located at coordinates 05°32′10″ north latitude and 73°21′37″ west longitude, at an altitude of 2696 m. The area has a cold and very dry climate, with an average annual precipitation of 645 mm, distributed across two dry seasons and two rainy seasons throughout the year. The zone’s average annual temperature was 12.9 °C, with lows around 8.5 °C and average highs of 17.8 °C (Figure 2).

4.2. Plant Material

The pea variety used was ‘San Isidro,’ which is the most widely planted variety in Colombia. It exhibits a vining growth habit, with white flowers and round, smooth, green seeds with a white hilum. The plants reach a height of 1.1 to 2 m. The harvest time is between 112 and 118 days. It adapts to altitudes of 2400 to 2800 m. It has a fresh yield of 3510 kg per hectare and a prolonged postharvest shelf life of 30 days, as it maintains its quality without germinating or undergoing oxidation processes, which gives it high commercial value. Additionally, this cultivar is appreciated for its pod load from the first internodes.

4.3. Experimental Design and Greenhouse Establishment

A completely randomized design was used with five treatments: a well-watered control (CK) and four water-deficit treatments receiving 0 (water-stressed 0-BR), 0.5, 1.0, or 1.5 mg of brassinosteroids (BRs) per plant. Each treatment had eight replicates, for a total of 40 experimental units. Each experimental unit consisted of eight nursery plastic pots

(15 cm wide $\times$ 20 cm high, volume 3.5 L), in which three plants were sown per pot on 12 August 2024, giving a total of 960 plants. The substrate was a 50:50 (*v/v*) mixture of black soil and rice husk. Pots were spaced 10 cm apart within rows and 1 m between rows. Seeds germinated fully on 13 September 2024.

Water deficit was imposed starting on 25 October 2024 (at early flowering) by withholding irrigation until the volumetric water content (VWC) reached 5–9% and was then maintained within this range by adding small volumes of water as needed for 21 days, until 15 November 2024. The well-watered control (CK) received daily irrigation of 1.5 L per pot to maintain VWC between 25% and 35%. VWC was monitored daily at 10 cm depth using a TDR 150-time domain reflectometry sensor (Spectrum Technologies, Inc., Aurora, IL, USA), with three random pots per treatment measured each day. After the 21-day stress period, all plants (including the stressed treatments) were rewatered daily with 1.5 L per pot to allow recovery until harvest (10 December 2024).

For brassinosteroid application, the spirostane analog DI-31 (BB16), chemically identified as [(25R)-3 β ,5 α -dihydroxy-spirostan-6-one], corresponding to the commercial product Biomex DI-31[®] (Minerales Exclusivos S.A., Bogotá, Colombia), was used. The intended doses were 0.5, 1.0, and 1.5 mg of active BR per plant. To deliver these amounts, stock solutions were prepared at concentrations of 5.0, 10.0, and 15.0 mg L^{−1}, respectively, in distilled water, with the addition of 3 mL L^{−1} of the adjuvant COSMO-IN[®] D (Cosmoagro S.A., Palmira, Colombia) to enhance foliar absorption. A single foliar spray was applied one day before irrigation was withheld, using a fixed volume of 100 mL per plant to deliver the corresponding dose per plant. The water-stressed 0-BR treatment received the same adjuvant-containing solution without the BR analog to ensure that any observed effects were exclusively attributable to the brassinosteroids. During application, the 0-BR plants were covered with plastic to avoid accidental contact with the product via wind drift.

4.4. Response Variables

The plants were collected on December 10, 2024, 121 days after planting (DAP), and the dry mass of the aerial part and the dry mass of seed pods with seeds were determined. For this, the samples were weighed on an Acculab VIC 612 scale with 0.01 g precision (Sartorius S.A., Madrid, Spain), after drying them at 70 °C for 48 h in a Memmert UNB500 oven (Mettler GmbH and Co. KG, Schwabach, Germany). Additionally, the HSM was determined for each experimental unit. The number of basal branches per plant, number of reproductive nodes per plant, number of reproductive nodes per branch, number of pods per plant, number of pods per branch, number of seeds per plant, and number of seeds per branch were also counted.

The chlorophyll *a* light-adapted fluorescence phase was evaluated in leaves and stipules at three different times in each one of the experimental units (at 85 days after planting, DAP, when 10 days had passed since the start of water stress; at 95 DAP when the imposed water stress ended; and at 108 DAP, when 13 days of plant recovery had occurred after the end of water stress) following the modified RIDES 2.0 protocol to use a constant photosynthetically active radiation (PAR) of 800 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ [48]. This was done using a modulated MultispecQ 2.0 fluorometer (Photosynq Inc., East Lansing, MI, USA), which measured chlorophyll fluorescence parameters of Photosystem II, including the operational quantum yield of Photosystem II (Φ_{PSII}), which is the proportion of absorbed light used for photochemistry (electron transport) and is a direct indicator of photosynthetic efficiency under light conditions; the linear electron flow (LEF), representing the rate of electron transport through Photosystem II and serving as a direct measure of overall photosynthetic activity; the quantum yield of regulated non-photochemical energy loss in Photosystem II (Φ_{NPQ}), which is the proportion of light energy dissipated as heat through

regulated protective mechanisms (regulated non-photochemical quenching), acting as an essential escape valve to protect the photosynthetic apparatus from excess light; and the quantum yield of non-regulated energy dissipation (Φ_{NO}), indicating the fraction of energy lost through non-regulated pathways such as fluorescence, where an increase in Φ_{NO} can suggest damage or inefficiency in photoprotection mechanisms. The sum of $\Phi_{\text{PSII}} + \Phi_{\text{NPQ}} + \Phi_{\text{NO}}$ always equals 1 (100% of absorbed energy); the maximum efficiency of open reaction centers of PSII in the light-adapted state (F_v'/F_m'), calculated as $(F_m' - F_o')/F_m'$, which, unlike the classic F_v/F_m (requiring dark adaptation), measures the potential efficiency of centers remaining open while the leaf is exposed to light, making it more representative of field conditions; the fraction of open PSII reaction centers (q_L), also known as the Lake coefficient, indicating the proportion of reaction centers ready to accept electrons, where a high q_L value means most centers are open, signaling good photosynthetic activity and low light saturation; and the non-photochemical quenching coefficient (NPQt), an adaptation of the NPQ parameter calculated without dark adaptation, reflecting energy dissipation as heat [49].

The fluorometer was also used to determine the parameters of electrochromic change that indicate the activity of adenosine triphosphate (ATP) synthase, including the measurement of the total electrochromic change magnitude (ECSt), which represents the estimation of the proton gradient (proton motive force (pmf)) accumulated across the thylakoid membrane during illumination, expressed in milli-absorbance units (mAU). A high gradient is necessary for ATP synthesis; the relaxation time constant of ECS (ECS τ) is the time it takes for the proton gradient to dissipate after turning off the light. A rapid decay (small τ) indicates high ATP synthase activity, as it quickly consumes the gradient to produce ATP; the proton conductance of ATP synthase (g_{H^+}), defined as the inverse of ECS τ ($g_{\text{H}^+} = 1/\tau$), represents the membrane's permeability to proton flow, which is a direct measure of the enzyme's efficiency; and the steady-state proton flux (v_{H^+}), calculated as ECSt/ECS τ , which is directly proportional to ATP production and serves as an excellent indicator of the leaf's capacity to generate chemical energy [49]. Chlorophyll fluorescence and electrochromic parameters were measured only in plants subjected to water stress (BR0, BR0.5, BR1.0, BR1.5); the well-watered control (CK) was not included in these measurements, as the study focused on the response to BR application under stress.

Morphological and environmental parameters were also measured, such as the leaf angle (LEFANG), which is the inclination of the leaf relative to the ground. A value of 0° means the leaf is parallel to the ground (horizontal), while 90° indicates it is perpendicular (vertical). This is a key parameter for understanding light interception and responses to water or thermal stress: leaf thickness (LEAFTHICK), expressed in millimeters (mm), which provides insight into the physical structure of the leaf, as greater thickness is often associated with higher density of photosynthetic tissue per unit area, potentially influencing CO_2 assimilation capacity and drought resistance; and leaf temperature differential (LTD), which is the difference between leaf temperature and air temperature ($T_{\text{leaf}} - T_{\text{air}}$) [50]. A negative value indicates the leaf is cooler than the air, a strong indicator of active transpiration, as water evaporation cools the leaf surface. Conversely, a value near zero or positive suggests stomatal closure due to water stress, and relative chlorophyll content is expressed in soil plant analysis development (SPAD) units, an indirect, non-destructive estimate of chlorophyll concentration in the leaf based on light absorbance at specific wavelengths. A high value correlates with good nitrogen nutritional status and high photosynthetic capacity [49].

Stomatal conductance (g_s) was measured at four time points during the experiment. At 85 DAP (11 days after the start of water stress), the net photosynthetic rate (A_n), stomatal conductance (g_s), transpiration rate (E), and intercellular CO_2 concentration (C_i) were deter-

mined between 7:00 and 11:00 am in each experimental unit using a portable gas exchange system CIRAS-4 (PP Systems International, LLC, Amesbury, MA, USA). Measurements were taken on both the fully expanded second leaf and the fully developed stipule corresponding to the same node to compare the physiological response of both organs to water deficit and brassinosteroid application. During measurements, the conditions inside the gas exchange chamber were kept constant: air temperature of 25 °C, photosynthetically active radiation (PAR) of 1500 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$, reference CO_2 concentration of 420 ppm (due to greenhouse conditions), relative humidity of 70%, and a stabilization time between 30 s and 1 min. The leaf area of the chamber used was 1.75 cm^2 . These IRGA-based gas exchange data (including g_s at 85 DAP) are presented in Table 4 and are also included in Figure 1a.

Additionally, stomatal conductance was measured at 80, 95, and 108 DAP using a portable leaf porometer SC-1 (Decagon Devices, Inc., Pullman, WA, USA), between 11:00 a.m. and 1:00 p.m., on the same leaves and stipules. These porometer-based g_s data are presented in Figure 1a alongside the IRGA-based measurement at 85 DAP. The well-watered control (CK) was included in the IRGA measurement at 85 DAP but was not measured with the porometer, as the porometer measurements focused on the temporal response of stressed plants. To determine the water status of the pea plants, water potential in leaves and stipules was evaluated at 80, 85, 95, and 108 DAP, between 11 am and 1 pm, using the Scholander pump Model 615 (PMS Instrument Company, Albany, OR, USA).

4.5. Statistical Analysis

The data obtained were subjected to a Shapiro-Wilk normality test to verify the assumptions of analysis of variance (ANOVA), which was used to determine whether there were statistically significant differences between treatments. Additionally, a repeated-measures ANOVA for g_s and Ψ_h was conducted to compare the measurements of each treatment across each sampling point. Subsequently, the Tukey means comparison test ($p < 0.05$) was applied to classify the treatments and time points. All analyses were carried out using the statistical software SAS OnDemand for Academics 9.4M8 (SAS Institute Inc., Cary, NC, USA), and the figures were created in the Visual Studio Code 1.98.2 environment with the open-source programming language Python 3.11, using the libraries Matplotlib 3.10.3, Scipy 1.13.1, and NumPy 2.2.0.

5. Conclusions

The application of brassinosteroids (BRs) mitigated the negative effects caused by water deficit on the yield of the ‘San Isidro’ pea variety, increasing dry mass, the number of pods and seeds, and the 100-seed mass. Additionally, these treatments improved the plant’s water status and facilitated recovery after stress. BRs enhanced q_L in leaves (at 85 DAP) and Φ_{PSII} in stipules (at 95 DAP), indicating a protective effect on specific components of the photosynthetic apparatus. They also increased photosynthetic rate, stomatal conductance, transpiration, and intercellular CO_2 concentration, contributing to greater gas-exchange efficiency. Furthermore, they reduced leaf temperature, reflected in improved stomatal regulation. Stipules showed significant responses in a larger set of physiological variables, suggesting they may be more responsive to BRs; nevertheless, the absence of a formal organ $\times$ treatment interaction precludes a definitive conclusion on differential sensitivity. Responses to BR doses varied among traits, and no single dose was consistently superior across all variables, indicating that further research is needed to define a specific recommendation for practical application. Our conclusions are limited to the evaluation of BR effects under water-stress conditions, as we did not implement a factorial design including well-watered + BR treatments.

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