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The role of inorganic phosphate on photosynthesis recovery of common bean after a mild water deficit

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Abstract

A foliar spray of inorganic phosphate (Pi) was applied at the pollination stage of two common bean genotypes A320 and Ouro Negro, growing in pots in a greenhouse. Two days after the foliar Pi supply, a mild water deficit was imposed for 7 days and photosynthesis was evaluated during water stress and recovery. The net CO_2 assimilation rate (A) and stomatal conductance (g_s) were not affected by Pi supply during the dehydration of either genotype. On the fourth day of water deficit, A320 presented a high value of A despite lower g_s, indicating a small effect of g_s on A for this genotype, while Ouro Negro showed a low value for both A and g_s. However, after rehydration A and g_s for A320 and A for Ouro Negro, both supplied with Pi, were higher than for non-Pi-supplied plants. In addition, the O_2 evolution (A_c) of rehydrated A320 with foliar Pi supply was also higher than for non-Pi-supplied plants, and the non-photochemical quenching (NPQ) was higher for rehydrated A320 without foliar Pi. The results revealed an up-regulation of the recovery of photosynthesis after water deficit induced by the foliar Pi supply, which was genotype-specific.

Keywords: Chlorophyll fluorescence; Drought; Gas exchange; Oxygen evolution; *Phaseolus vulgaris*

1. Introduction

Common bean (*Phaseolus vulgaris* L.) is an important food crop grown under rainfed conditions, but even a mild water deficit may cause a significant reduction in its net photosynthetic rate [1]. In addition to this environmental constraint, common bean is considered a poor soil phosphorus extractor due to its low cation exchange capacity and small root volume, with low yield in phosphorus deficient soils [2], like tropical soils. Therefore, it is reasonable to assume that a synergetic effect between water stress and low phosphorus availability could occur, aggravating the effects of drought on bean production. These effects can vary among bean genotypes due to their differential response to drought stress [3], as well as their different soil phosphorus uptake capacities [2].

Water deficit induces a reduction in the net CO_2 assimilation rate (A) [1], and also in the O_2 evolution (A_c) of common bean, especially when it is imposed at the pollination stage [4]. The effect on A can be partially explained by a low intercellular CO_2 concentration (C_i) due to stomatal closure [5], but an almost entire cessation of biochemical activity in photosynthesis during drought was demonstrated, despite a high C_i [6]. In addition, in a study with sunflower leaf discs with and without epidermis, a non-stomatal limitation of photosynthesis under water deficit was also observed [7]. Accordingly, limitation of CO_2 assimilation under drought could be partially due to the

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Abbreviations: A, net CO_2 assimilation rate; A_c, oxygen evolution; C_i, intercellular CO_2 concentration; ETR, apparent electron transport rate; g_s, stomatal conductance; NPQ, non-photochemical quenching; Pi, inorganic phosphate; PPFD, photosynthetic photon flux density; PT, phosphate translocators; Ψ_l, leaf water potential

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inhibition of ribulose bisphosphate synthesis, associated with low ATP content, probably due to the loss of ATP synthase activity [8].

However, decreased ATP synthesis in the chloroplast may also be caused by a low availability of free cytoplasmatic inorganic phosphate (Pi) exchanged by triose-P from the chloroplast through the phosphate translocators (PT), which use Pi as a substrate [9]. Under drought, there is an arrest of photoassimilate export from the leaves [10] and an accumulation of sugars-P, especially fructose-2,6-bisphosphate [11], which regulate antiport PT systems and sustain photophosphorylation and the Calvin cycle, by regulating incoming Pi and carbon exporting in chloroplasts [12].

Several studies have investigated the relationship between phosphorus status and photosynthetic metabolism [13], but few have evaluated the effects of water deficit in this relationship [14]. In leaves, the rate of end-product synthesis (e.g. sucrose, starch and amino acids) determines the rate at which Pi is recycled back to the reactions of photosynthesis [12], but photorespiration may increase the rate of the metabolic turnover of phosphates in bean leaves with a low availability of Pi [15]. Recently, it was demonstrated that phosphate-fed leaves of grapevine subjected to low temperature had an increase of 71–80% on photosynthesis, depending on environmental conditions [16]. Therefore, the objective of this study was to evaluate the response of the gas exchange, chlorophyll fluorescence and O_2 evolution at the pre-flowering stage of common bean genotypes with an adequate Pi sowing fertilization, and submitted to a mild water deficit applied 2 days after an extra foliar Pi supply.

2. Materials and methods

2.1. Plant material and growth conditions

The study was conducted with the *P. vulgaris* (L.) genotypes A320 and Ouro Negro, which have almost the same cycle. A320 is a line that maintains high leaf water potential under drought condition and Ouro Negro is a new black seeded cultivar commonly cultivated in Brazil [3]. They were sown in 10 L pots (one plant per pot), containing 8 kg of a growth medium consisting of a soil-less mixture (Plantimax; Eucatex Inc., Brazil). Each pot was fertilized as usual with an equivalent of 20 kg NH_4PO_4 ha^{-1}, 30 kg K_2O ha^{-1}, 90 kg P_2O_5 ha^{-1} and 1500 kg ha^{-1} of dolomitic lime, and supplied with 300 mL of a micronutrient solution proposed by McCree [17]. Additional fertilization, using soluble urea equivalent to 40 kg N ha^{-1}, was provided 25 days after seedling emergence (DAE), as proposed by Vieira [18]. The essay was conducted in a greenhouse, with a mean air temperature of 26 °C during the cycle of the plants. The pots were irrigated daily until the beginning of drought treatment imposed 34 DAE, when the plants were at the pollination (pre-flowering) stage. After 7 days of drought, when the leaf water potential (Ψ_l) was around −1.1 MPa (a mild water deficit for common bean), the plants were rehydrated until the end of their cycle.

2.2. Foliar phosphorus supply

For the extra Pi supply treatment, half of the plants were sprayed with 12.5 mL of 10 g Pi L^{-1} solution, as ammonium dihydrogen phosphate ($(NH_4)H_2PO_4$), while the other half were sprayed with 2.64 g N L^{-1}, as urea ($(NH_2)_2CO$), to compensate for N added in the Pi treatment. In both solutions some drops of the wetting agent Tween 80 were added. The Pi sprayed dose was the maximum possible without causing leaf burning. Plants were sprayed at night with the respective solutions at 32 DAE, 2 days before withholding irrigation.

2.3. Leaf water status

The pre-dawn water tension in the xylem was measured daily with a Scholander pressure chamber (Soilmoisture Equipment Corp., Santa Barbara, CA, USA) in the fourth trifoliolate leaf from the base of the plant, a mature but not senescent leaf. These measurements were assumed to measure the leaf water potential (Ψ_l).

2.4. Leaf gas exchange

Net CO_2 assimilation rate (A) and stomatal conductance (g_s) were evaluated using an open gas exchange system with a 6 cm^2 clamp-on leaf cuvette (LI-6400, LICOR Inc., Lincoln, USA). Leaf gas exchange was measured in the middle leaflet of the fifth trifoliolate leaf from the base of the plant, which was a mature but not senescent leaf. These measurements were performed on the day of foliar Pi spray (Day −2), at the onset of drought (well-hydrated plants, i.e. Day 0), on the fourth, fifth and seventh day of drought, as well as on the first, second and third day after rehydration, between 09:00 and 10:30 h to avoid high vapor pressure deficit (VPD) and photoinhibition at midday. During measurements, the air mean temperature was 30 °C and the photosynthetic photon flux density (PPFD) was maintained at 1100 μmol m^{-2} s^{-1}, corresponding to the mean PPFD in the greenhouse at the leaf level, using a red-blue led light source built into the leaf cuvette. The VPD in the cuvette was kept between 0.5 and 2.0 kPa, because during the water stress treatment, on the fourth day of drought, the air temperature increased to 32 °C and, consequently, the VPD was around 2.0 kPa, but on the days before and after the air temperature and VPD were lower. The air collected outside the greenhouse was passed through a buffering gallon and then pumped into the system, with a CO_2 concentration around 360 μmol mol^{-1}. The other microclimatic conditions prevailing in the leaf cuvette were the same as the outside atmosphere.

2.5. Oxygen evolution

The O_2 evolution (A_c) was measured using a gas-phase Clark-type oxygen electrode in a LD2/3 leaf chamber (Hansatech, Norfolk, UK) with an external light source (LS3, Hansatech). A leaf disc with an area of 10 cm^2 was placed into the leaf chamber at CO_2 saturation concentration, where the oxygen electrode was previously calibrated. The chamber was equipped with a water jacket, controlled by an external water bath (MA-127, Marconi, Piracicaba, Brazil) at the same temperature as the gas exchange measurements.

2.6. Chlorophyll fluorescence

Chlorophyll fluorescence was measured simultaneously with A_c using a modulated fluorometer (FMS 1, Hansatech, Norfolk, UK) attached to the LD2/3 leaf chamber. After 30 min of dark adaptation, the maximal quantum efficiency of the photosystem II (PSII) (maximum Φ_{PSII}, F_v/F_m) was obtained. The maximum fluorescence yield (F_m) was attained during a 2.5 s saturation pulse (18 000 μmol m^{-2} s^{-1}), following the measurement of F_o under weak, continuous illumination, with an actinic light of 5 μmol m^{-2} s^{-1}. The variable fluorescence yield was calculated for both dark ($F_v = F_m - F_o$) and light-adapted ($\Delta F = F'_m - F_s$) conditions. The steady-state (F_s) and maximum (F'_m) fluorescence yields were determined in light-adapted leaves under steady-state photosynthesis at 1100 μmol m^{-2} s^{-1}. Minimum fluorescence yield (F'_o), after exciting the photosystem I (PSI) with far-red light, was also measured. Based on the above fluorescence parameters, the potential (F_v/F_m) and effective ($\Delta F/F'_m$) quantum efficiency of PSII, the apparent electron transport rate (ETR = $\Delta F/F'_m \times$ PPFD $\times$ 0.5 $\times$ 0.84) and the photochemical [$q_p = (F'_m - F_s)/(F'_m - F'_o)$] and non-photochemical quenching [NPQ = $(F_m - F'_m)/F'_m$] were calculated, as proposed by Schreiber et al. [19].

2.7. Yield components

At the end of the plant cycle, the effects of both water deficit and extra Pi supply were evaluated on plant yield components: pod number and seed dry weight per plant.

2.8. Statistical analysis

The pots were arranged in a random block design with three replications. Data were subjected to analysis of variance (ANOVA), and means were compared by the Student–Newman–Keuls test when significance ($p < 0.05$) was detected.

3. Results

3.1. Leaf water status

The leaf water potential (Ψ_l) decreased during water withholding for both genotypes, but there were no differences in Ψ_l among genotypes. The Ψ_l was around −1.1 MPa after 7 days of water shortage (data not shown), when the plants were rehydrated, which characterizes a mild water deficit for bean [1].

3.2. Gas exchange and O_2 evolution

Under the mild water deficit, A was reduced in both genotypes. However, A of A320 was less sensitive to drought when compared to Ouro Negro (Fig. 1), presenting higher values until the fourth day. The g_s values also decreased during water stress, but there was no difference between genotypes (Fig. 2). After recovery, Pi-supplied plants of water-stressed A320 showed higher A ($p < 0.05$) values than non-supplied plants (Fig. 1a), and its g_s was also significantly higher than for all the other treatments (Fig. 2a). Also, Ouro Negro Pi-supplied plants on the third day of rehydration showed significantly higher A (Fig. 1b) than non-supplied plants, but no difference for g_s values on Pi-supplied plants was detected (Fig. 2b).

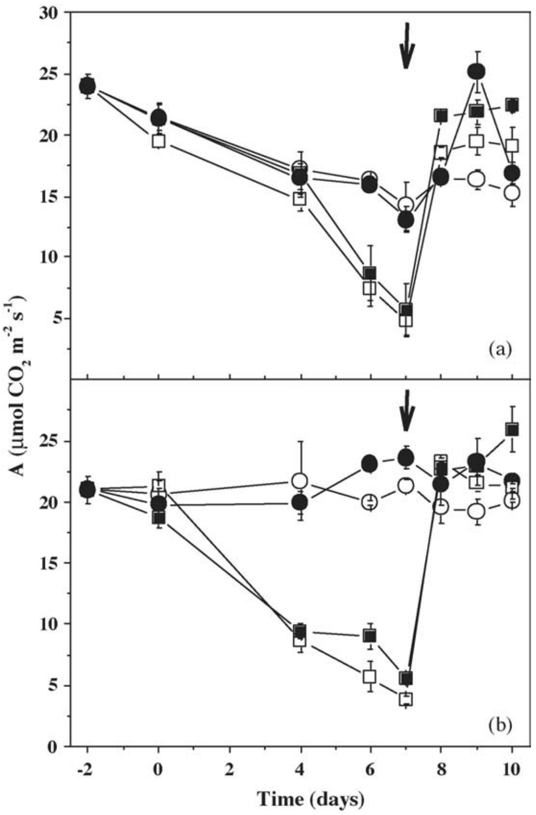

Fig. 1. Changes in net CO_2 assimilation rate (A) of bean genotypes: (a) A320 and (b) Ouro Negro, in well-watered plants (○, ●) and under drought (□, ■). Leaves were supplied with 10 g P L^{-1} (closed symbols) or 2.64 g N L^{-1} (open symbols) 2 days before water deficit (Day −2), and plants were re-watered after 7 days of drought stress. Points represent the mean value (±S.E.) of three replications.

The water deficit did not severely affect A_c of either genotype (Table 1). At the maximum stress, after 7 days of water withholding, there were no effects of the extra Pi supply on A_c of A320, but Ouro Negro with Pi supply presented higher A_c values than A320 with or without Pi foliar spray ($p < 0.05$) (Table 1). However, after recovery A320 with foliar Pi spray showed a significantly higher A_c value than for non-Pi-supplied plants, but Ouro Negro with extra Pi supply only showed a significantly higher A_c value when compared to A320 without Pi ($p < 0.05$).

3.3. Chlorophyll fluorescence parameters

The photochemical apparatus of both genotypes was relatively tolerant to water stress, and potential (F_v/F_m) and

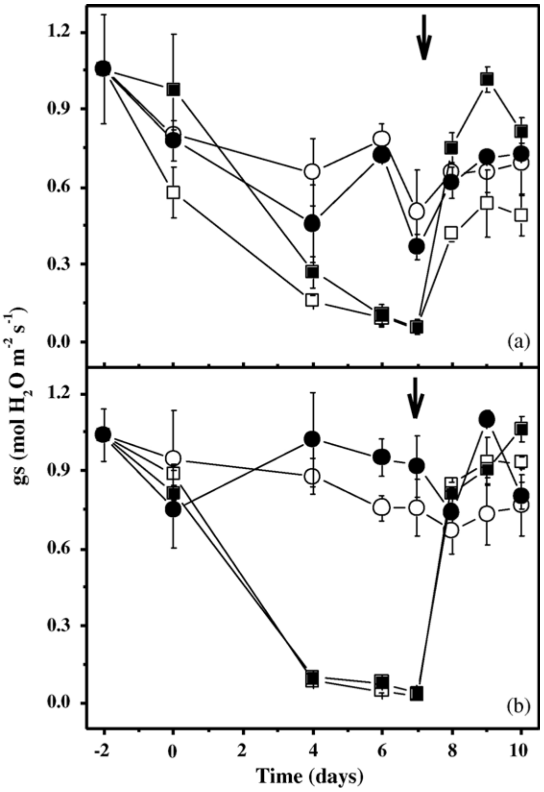

Fig. 2. Changes in stomatal conductance (g_s) of bean genotypes: (a) A320 and (b) Ouro Negro, in well-watered plants (○, ●) and under drought (□, ■). Leaves were supplied with 10 g P L^{-1} (closed symbols) or 2.64 g N L^{-1} (open symbols) 2 days before water deficit (Day −2), and plants were re-watered after 7 days of drought stress. Points represent the mean value (±S.E.) of three replications.

effective ($\Delta F/F'_m$) quantum efficiency of PSII were not affected by drought or Pi-supplying ($p > 0.05$) (data not shown). In agreement with the A_c response under water stress (Table 1), the apparent electron transport rate (ETR) was only significantly higher for Ouro Negro with Pi supply, when compared to A320 with Pi supply (Table 2). However, water deficit significantly reduced the non-photochemical quenching (NPQ) in Ouro Negro with Pi supply, when compared to A320. After rehydration, the NPQ of A320 without Pi spray was higher ($p < 0.05$) than for the plants with Pi supply and Ouro Negro with and without Pi (Table 2).

Table 1
O_2 evolution (A_c) of bean genotypes Ouro Negro and A320 suffering maximum water stress (on the seventh day of water withholding) and after recovery (3 days after re-watering plants) in non-Pi-supplied or Pi-supplied plants

Genotype	Pi foliar spray	Water stressed	After recovery
A320	Non-supplied	17.00 c	19.33 c
	Pi supplied	18.55 c	26.97 ab
Ouro Negro	Non-supplied	21.45 bc	27.76 ab
	Pi supplied	26.08 ab	32.40 a

Pi-supplied leaves were sprayed with 10 g P L^{-1} and non-Pi-supplied leaves with 2.64 g N L^{-1}. Data represent the mean value of three replicates. In columns, mean values followed by different letters show statistical difference by the Student–Neuman–Keuls test ($p < 0.05$).

Table 2
Apparent electron transport rate (ETR) and non-photochemical quenching (NPQ) of bean genotypes A320 and Ouro Negro suffering maximum water stress (on the seventh day of water withholding) and after recovery (3 days after re-watering plants) in non-Pi-supplied or Pi-supplied plants

Parameter	Genotype	Pi foliar spray	Water stressed	After recovery
ETR	A320	Non-Pi supplied	293.48 ab	279.83 ab
		Pi supplied	291.75 b	295.83 ab
	Ouro Negro	Non-Pi supplied	309.02 ab	311.05 ab
		Pi supplied	320.79 a	318.74 ab
NPQ	A320	Non-Pi supplied	0.92 b	1.10 a
		Pi supplied	0.89 b	0.89 b
	Ouro Negro	Non-Pi supplied	0.69 bc	0.79 b
		Pi supplied	0.57 c	0.74 bc

Pi-supplied leaves were sprayed with 10 g P L^{-1} and non-Pi-supplied leaves with 2.64 g N L^{-1}. Data represent the mean value of three replicates. In columns, mean values followed by different letters show statistical difference by the Student–Neuman–Keuls test ($p < 0.05$).

Table 3
Yield components (pod plant^{-1} and seed dry weight plant^{-1}) and percentage of loss of bean genotypes A320 and Ouro Negro subjected to water deficit and supplied with 10 g Pi L^{-1}, or 2.64 g N L^{-1} by spraying leaves 2 days before the beginning of water withholding

Genotype	Pi foliar spray	Pod number		Seed dry weight (g)	
		Control	Water stressed	Control	Water stressed
A320	Non-Pi supplied	31 Aa	22 Bb	17 Aab	11 Ab
	Pi supplied	23 Bb	20 Bb	15 Aab	13 Ab
Ouro Negro	Non-Pi supplied	30 Aa	18 Bb	23 Aa	13 Bb
	Pi supplied	33 Aa	18 Bb	18 ABab	15 Bab

Data represent the mean value of three replicates. In columns, mean values followed by different capital letters are statistically different, whereas means values in rows followed by different minuscule letters show statistical difference for each yield component by the Student–Neuman–Keuls test ($p < 0.05$).

3.4. Yield components

At the end of the plants cycle, there was no difference in pod number and seed dry weight per plant for Pi supply in either water-stressed genotype, but the seed dry weight of both genotypes with Pi supply was very close to that of control plants irrigated continuously. In addition, water-stressed Ouro Negro without Pi supply presented a significant reduction in seed dry weight when compared to control plants irrigated continuously (Table 3).

4. Discussion

The results of the gas exchange measurements during water deficit (Figs. 1 and 2) showed a reduction in A and g_s for both genotypes, which were not affected by Pi supply, and a full recovery with rehydration. The lack of effect of Pi supply during the mild water deficit could be due to photorespiration, which may increase the rate of the metabolic turnover of phosphates in the chloroplast [15]. In another essay with the same Pi foliar spray applied 2 days before a water deficit, the Pi-supplied leaves of Ouro Negro revealed a Pi content of 0.67% (DW), while control plants, without foliar Pi supply, contained 0.47% (DW) of Pi, respectively, at day zero of the drought [14]. The Pi content of these leaves were both considered adequate levels of Pi in leaves of common bean [2]. Therefore, this study evaluated the effect of an extra Pi supply, by foliar spray at the pre-flowering stage of plants with an adequate Pi sowing fertilization before water deficit, revealing that this practice can improve the recovery of photosynthesis after drought.

The diminution of A during dehydration can be ascribed to the reduction of g_s, especially during a mild water deficit [1,5]. However, on the fourth day of water shortage, there was a significantly reduction in g_s of A320 (Fig. 2a), while A was still high (Fig. 1a). This result indicates the high intrinsic water use efficiency (IWUE) of A320, as demonstrated by Pimentel et al. [3], and a minimum effect of g_s on A on this day for this genotype. The other genotype, Ouro Negro, showed severely reduced A on the fourth day of water deficit (Fig. 1b), in agreement with Lauer and Boyer [6] and Tang et al. [7], who proposed a metabolic restriction of photosynthesis under drought. Therefore, there is a distinct genotypic-specific response of common bean submitted to water deficit, especially at the pollination stage [4,10]. In addition, A320 stomata were probably more sensitive to VPD variation, confirming its higher IWUE than Ouro Negro [3], because on the fourth day of water deficit when an increase in air temperature (to 32 °C) and VDP (to 2.0 kPa) occurred, even the irrigated control plants of A320 showed a reduction in g_s not shown by Ouro Negro (Fig. 2).

However, a beneficial effect of the extra Pi supply on A320 was shown by the significantly higher A and g_s values during rehydration, when compared to non-Pi-supplied plants, although Ouro Negro showed a significantly higher A value, though not for g_s, on Pi-supplied plants only on the third day of rehydration, which could be a genotypic-specific response. The higher A values ($p < 0.05$) shown in Pi-supplied water-stressed plants for both genotypes (Fig. 1) during recovery, may be due to a metabolic effect controlled by cytoplasmatic Pi content [16]. The occurrence of this beneficial effect of an extra Pi supply only during rehydration limits the interest of the foliar Pi spray, revealing that more studies are required to clarify this phenomenon.

The water deficit did not cause substantial changes in chlorophyll fluorescence parameters F_v/F_m and $\Delta F/F'_m$ for either genotype (data not shown). These results are in agreement with Cornic and Briantais [20], who demonstrated no significant effect on the photosynthetic photochemistry during a mild water deficit. However, the beneficial Pi effects on photosynthesis were also evident on A_c of A320 during recovery, when A320 with extra Pi supply presented a significantly higher A_c value than plants without Pi (Table 1). Ouro Negro revealed no difference in A_c values for Pi and non-Pi-supplied plants, reinforcing the genotypic-specific response observed for the gas exchange measurements (Figs. 1 and 2).

The flow of absorbed energy in electron transport chain (evaluated by ETR) and the non-photochemical chlorophyll fluorescence quenching (NPQ) were not reduced by the mild drought stress in either genotype (Table 2). In fact, high ETR and low NPQ values may be associated with high photosynthetic capacity, as shown by Ouro Negro plants with foliar Pi spray, but they were no different from plants without Pi. Yordanov et al. [11] reported that the ETR declined much less than A under drought, as in the present results (Fig. 1 and Table 2). However, on recovery, A320 with foliar Pi supply showed a lower NPQ than plants without Pi (Table 2), which indicates a reduction in processes that protect the leaf from light-induced damage or of the damage itself [11]. During recovery, there was no increase in ETR (Table 2) of Pi-supplied A320 plants associated with the higher A values (Fig. 1a) probably because ETR was saturated and it is a parameter calculated from the efficiency of the photosystem II photochemistry, i.e. the proportion of light absorbed by chlorophyll, which is more influenced by incident PPFD than by an increase in maximal A.

It should be noted that any plant physiological mechanism that avoids the deleterious effects of drought is important to crop yield, especially when it is active at a key stage of the crop cycle, like the pollination stage. The reduction of A caused by water deficit at the pollination stage (pre-flowering stage) will induce embryo abortion, and thus a lower yield [10]. The small increase in A and A_c of Pi-supplied plants shown during the recovery period (Fig. 1 and Table 1) decreased the reduction of seed dry weight of water-stressed A320 and Ouro Negro (Table 3). There was no effect of water stress on seed dry weight on either genotype, when they were supplied with foliar Pi, while water-stressed Ouro Negro without a foliar Pi supply showed a reduction in seed dry weight (Table 3). However, only three replicates of each treatment in this study were available to evaluate the yield components and growth in pots in greenhouse varies enormously, as can be seen by the lowest pod number value of A320 Pi-supplied control plants, which showed no difference from the values of water-stressed plants.

These results support the hypothesis that there is a positive effect of an extra foliar Pi supply before drought stress on the recovery of common bean photosynthesis, though it is genotype-dependent. However, more studies using a larger number of replicates are required to clearly understand the effects of an extra foliar Pi supply on the biochemical reactions of photosynthesis in leguminous plants suffering water deficit.

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