Foliar phosphorus supply and CO_2 assimilation in common bean (*Phaseolus vulgaris* L.) under water deficit

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Received: 17 May 2006; Returned for revision: 03 August 2006; Accepted: 13 November 2006

Two common bean cultivars were grown in pots under greenhouse conditions. Plants were submitted to a foliar Pi spray two days before suspending irrigation, what enhanced net CO_2 assimilation rate of Ouro Negro cultivar but did not change significantly the photosynthesis of Carioca cultivar under both water deficit and rehydration periods. The results revealed that a foliar Pi spray induced an up-regulation of photosynthesis in common bean under mild water deficit, with this effect being genotype-dependent.

Key words: drought stress, ortophosphate, photosynthesis, water deficit

Suprimento foliar de fósforo e assimilação de CO_2 em feijoeiro (*Phaseolus vulgaris* L.) sob déficit hídrico. Duas cultivares de feijoeiro comum foram cultivadas em vasos, sob condições de casa de vegetação. As plantas foram submetidas à aplicação foliar com Pi dois dias antes da suspensão da irrigação, o que aumentou a assimilação líquida de CO_2 da cultivar Ouro Negro, mas não alterou significantemente a fotossíntese da cultivar Carioca durante os períodos de déficit hídrico e reidratação. Os resultados revelaram que a aplicação foliar de Pi induziu um efeito positivo na fotossíntese de feijoeiros sob déficit hídrico de intensidade moderada, sendo esse efeito dependente do genótipo.

Palavras-chave: déficit hídrico, fotossíntese, ortofosfato, seca

Water and phosphorus deficiencies are some of the most important constraints for plant growth in tropical regions (Fageria et al., 1997). More than 60% of common bean crops grown in developing countries of Latin America, Africa and Asia face drought in at least one stage of plant cycle (Singh, 1995). Therefore, a lower tissue Pi content of legumes cultivated in these regions can aggravate the damage caused by water deficit to photosynthesis, especially at the pollination stage, i.e. the pre-flowering stage for beans (Santos et al., 2004). After pollination, net CO_2 assimilation rate (A) is increased due embryo growth (Pimentel et al., 1999b), causing an increased leaf carbohydrate content (Westgate and Boyer, 1986). Therefore, the pre- and post-pollination phases of bean are considered very sensitive to water deficit (Pimentel et al., 1999a).

When plants are subjected to water deficit their stomata close to reduce water loss from leaves to atmosphere, but CO_2 diffusion from atmosphere to mesophyll cells is also expected to be reduced (Chaves et al., 2002; Lawlor and Cornic, 2002). As stomata close, internal CO_2 concentration (C_i) initially declines with increasing stress, but as drought becomes more severe, C_i might ultimately increase (Flexas and Medrano, 2002). Lauer and Boyer (1992) showed an increase in C_i under water deficit, indicating that stomatal conductance (g_s) did not inhibit A, which dropped to zero despite the high C_i. Probably, stomatal closure contributes to the limitation of *A* in the early phases of leaf dehydration, whereas both stomatal and non-stomatal (biochemical and/or photochemical) limitations take place in later phases of drought. In fact, the causes for reduction of photosynthetic metabolism under drought conditions are still a contentious issue (Lawlor and Cornic, 2002).

Rao and Terry (1994) showed a reduction both in *A* and ribulose-1,5-bisphosphate (RuBP) content under Pi deficiency, with a leaf accumulation of non-phosphorylated carbohydrates (starch and sucrose). Low leaf phosphate status seems to limit the Calvin cycle somewhere in the sequence of reactions between triose-P and RuBP formation (Rao and Terry, 1994). In addition, there is an accumulation of fructose-2,6-bisphosphate under drought (Yordanov et al., 2000), which controls antiport phosphate translocator systems and sustains photophosphorylation and the Calvin cycle by regulating incoming Pi and carbon exporting in chloroplasts (Flügge et al., 2003). Recently, Hendrickson et al. (2004), working with detached grapevine leaves under low temperature stress, showed a large stimulation of photosynthetic O_2 evolution (47 to 80%) by feeding Pi to the leaves. By contrast, Sivak and Walker (1986) showed that orthophosphate supply to the chloroplast can limit photosynthesis *in vivo* in some circumstances.

The objective of this study was to verify if an extra Pi supply by foliar spray just prior to water deficit imposition at the pollination stage of common bean (a drought sensitive and poor Pi extractor species; Fageria et al., 1997; Pimentel et al., 1999a) could stimulate photosynthesis under drought and alleviate the stress effects on yield.

Common bean (*Phaseolus vulgaris* L.) cvs. Carioca and Ouro Negro were grown under greenhouse conditions in 10 L pots (60 pots: two genotypes x two Pi levels x five sampling dates x three replications). The plants were watered regularly before the imposition of water deficit, when both genotypes were at the pre-flowering stage. The growth medium consisted of a soil-less mixture (Plantimax; Eucatex Inc., Brazil) fertilized with nutritive solution (McCree, 1986) before sowing, and at 25 d after emergence (DAE) of plants. Air temperature inside greenhouse varied from 14 to 41°C throughout the experiment. During gas exchange measurements, mean leaf-to-air vapor pressure deficit and mean air temperature were 4.3 ± 0.7 kPa and 27 ± 2°C, respectively.

For the extra Pi supply treatment, plants were sprayed at 34 DAE with solutions (12.5 mL) containing either 10 g Pi L^{-1} applied as $NH_4H_2PO_4$ or 2.64 g N L^{-1} applied as $(NH_2)_2CO$ (to compensate for the N added in the extra Pi treatment) (Santos et al., 2004). Leaf Pi content was measured in a similar assay with bean plants grown as outlined above (Santos et al., 2004). Both bean cultivars were submitted to water withholding 2 d after foliar Pi application. Plants were rehydrated when leaf water potential (Ψ_w) at predawn was around -1.0 MPa, which represents a mild water deficit for beans (Sharkey and Seemann, 1989). Predawn Ψ_w was measured daily with a Scholander pressure chamber (Soil Moisture Equipment Corp., Santa Barbara, CA, USA), according to Santos et al. (2004).

From 34 to 38 DAE, *A* and 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) in the middle leaflet of the fifth trifoliolate leaf. At the end of the plant cycle (90 DAE), the effects of both water deficit and extra Pi supply were evaluated on plant yield components: seed weight per plant, and number of pods and seeds per plant. The experiment was arranged in a randomized block design with three replications. Data were subjected to ANOVA, and means were compared and segregated by the Tukey test ($P < 0.05$) when significance was detected.

At the day of water deficit imposition (day zero, i.e. 2 d after foliar Pi supply), *A* and g_s were measured throughout the diurnal period in well-watered plants. Both cultivars showed similar patterns of *A* and g_s, independently of Pi treatments (Figure 1). These parameters increased from early morning until 1100 h, and then decreased afterwards. As there was no recovery of *A* and g_s during the afternoon (Figure 1), it may be proposed that bean plants behave as an anisohydric species (Tardieu and Simonneau, 1998).

Three days after withholding watering, Ψ_w reached about -1.0 MPa, regardless of Pi supply (Figure 2), when plants were then rehydrated. It should be emphasized that Ψ_w dropped rapidly, contrarily to what is expected under field conditions. In the potted plants, the development of the root system is much restricted and, therefore, not allowing plants to explore a great soil volume to gain a greater access of soil water. In addition, high atmospheric demand inside the greenhouse likely led to high rates of water use, thus hastening the development of internal water deficits. These conditions determined the time to attain a mild water deficit as found in this experiment. In any case, 2 d after rehydration, all plants recovered the

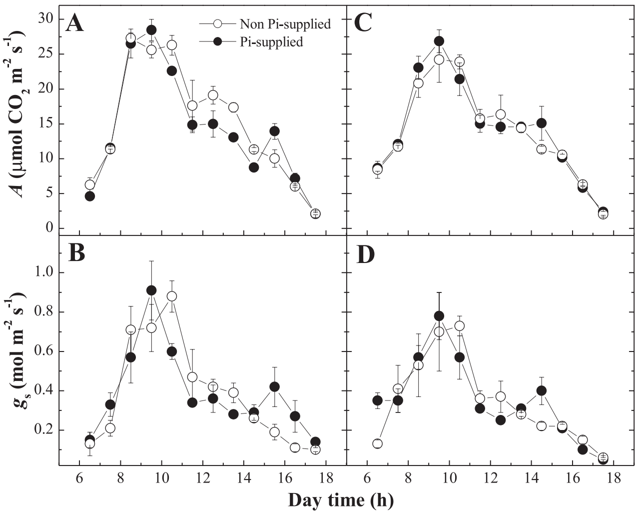

Figure 1. Diurnal course of net CO_2 assimilation (*A*) and stomatal conductance (g_s) of Carioca (**A, B**) and Ouro Negro (**C, D**) bean cultivars under well-watered conditions (day zero of water stress). Leaves were sprayed with 12.5 mL of solutions composed by 10 g Pi L^{-1} (Pi-supplied) or 2.64 g N L^{-1} (non-Pi-supplied). Each symbol represents the mean ± SE of three replicates.

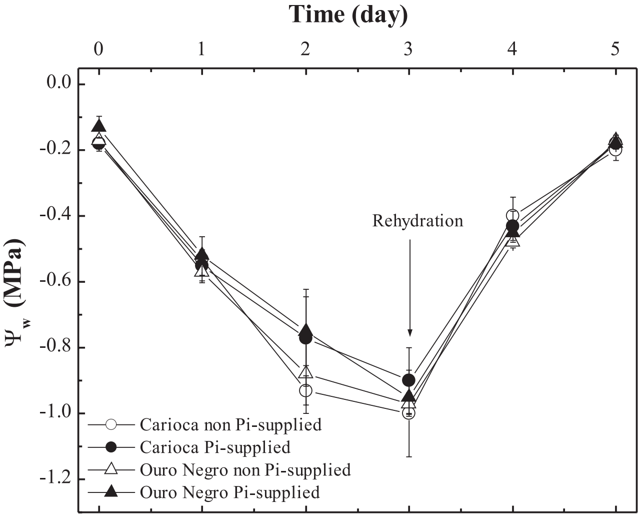

Figure 2. Changes in predawn leaf water potential (Ψ_w) of Carioca and Ouro Negro bean genotypes under water deficit. Leaves were sprayed with 12.5 mL of solutions composed by 10 g Pi L^{-1} (closed symbols, Pi-supplied) or 2.64 g N L^{-1} (open symbols, non-Pi-supplied). Arrow indicates plant rewatering. Each symbol represents the mean ± SE of three replicates.

same Ψ_w values as observed in day zero (Figure 2). The imposition of a mild water deficit induced a marked decrease in g_s in both genotypes already in the first day after applying the treatments, without a complete recovery even 2 d after rehydration (Figure 3A-F).

In general, the mild water deficit did not cause significant difference in yield components in both genotypes (data not shown). However, high air temperature inside greenhouse during the flowering period (> 35°C) could have hidden any effect of drought on yield. It is known that high air temperature causes flower abscission, a major determinant of yield in common bean (Osborne, 1989).

The foliar Pi spray induced distinct genotype responses when considering *A* upon suspending watering (Figure 3G-L). An extra Pi supply in Carioca cultivar caused higher *A* values only in the first day of water deficit at 0900 h, and in the third day at 1500 h (Figure 3G, I). Non-Pi-supplied plants showed higher *A* values in the first and second days after water shortage imposition at 1500 h, and in the second and third days at 0900 h. Thus, there was no consistent effect of Pi supply on *A* in drought-stressed Carioca plants. By contrast, in Ouro Negro, a consistent trend for higher *A* in Pi-treated plants was found under water-limited conditions (Figure 3J-L). In this cultivar, a trend for higher g_s (Figure 3D-F) in response to Pi spray was also found, what might in part explain its increased *A* (Figure 3J-L). Phosphate-induced changes in leaf gas exchange might be also considered as a metabolic response of photosynthesis to mild water stress, as stated by Lauer and Boyer (1992) and Tang et al. (2002). However, it is unclear for us why such response is genotype-specific, taking into account that the Pi supply brought about similar increases (~45%) in leaf Pi content for both Carioca and Ouro Negro genotypes (Santos et al., 2004). In any case, an increase in cellular Pi availability might enhance RuBP regeneration and/or Rubisco activity through higher chloroplastidic Pi concentration and so determining increases in CO_2 assimilation, as found in tomato under mild water deficit conditions by De Groot et al. (2002). Anyway, the higher *A* in Ouro Negro during water deficit and recovery did not increase its grain yield but increased the pod number per plant (data not shown), which is considered an important yield component for bean breeding programs. Nevertheless, further studies should be carried out, especially under field conditions,

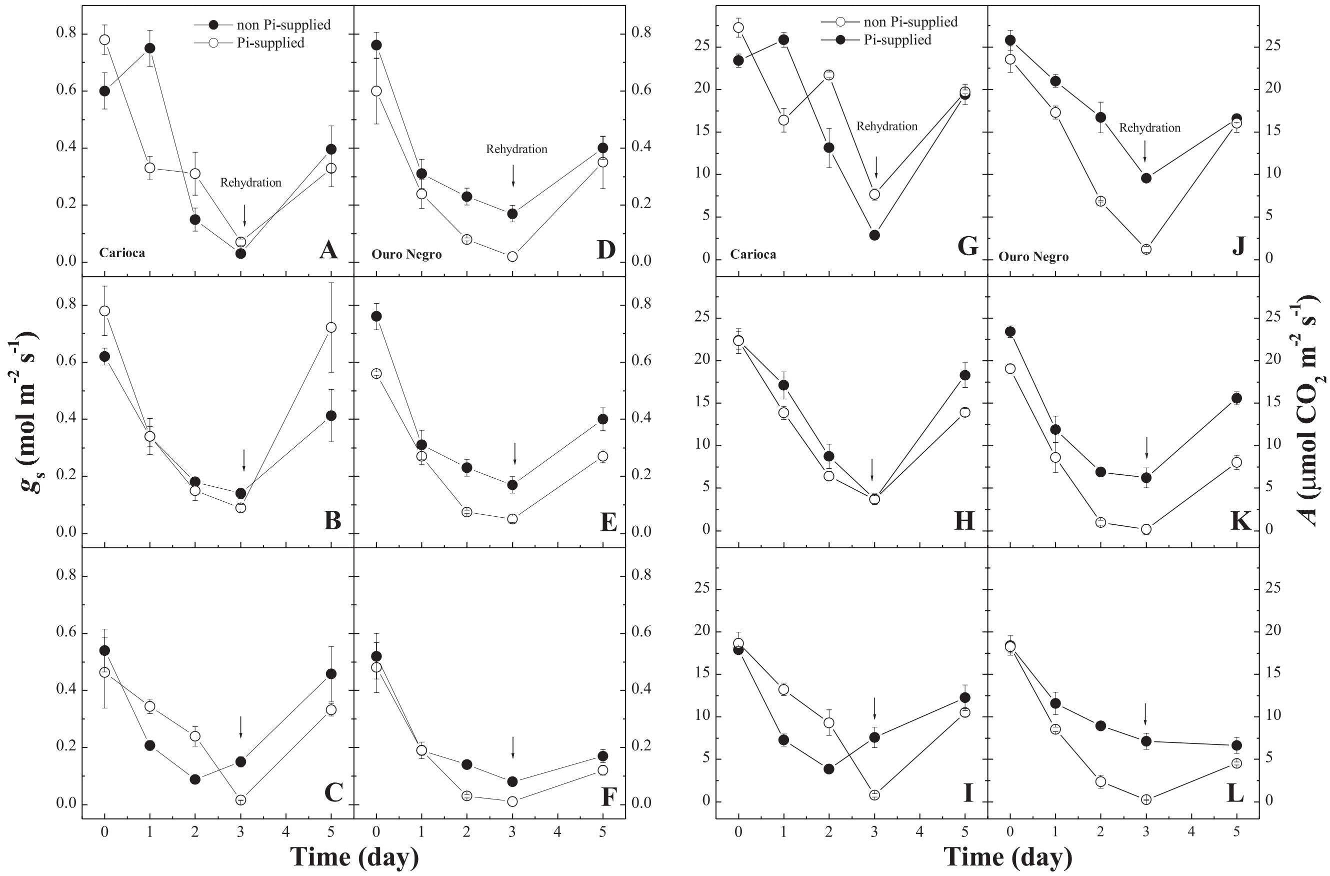

Figure 3. Stomatal conductance (g_s) and net CO_2 assimilation (A) in foliar Pi-supplied and non-Pi-supplied plants of Carioca (**A-C** and **G-I**) and Ouro Negro (**D-F** and **J-L**) bean genotypes under water deficit. Leaves were sprayed with 12.5 mL of solutions composed by 10 g Pi L^{-1} (Pi-supplied) or 2.64 g N L^{-1} (non-Pi-supplied). Arrow indicates plant rewatering (in the evening of the third day). Measurements were taken at 0900 h (**A, D** and **G, J**), 1200 h (**B, E** and **H, K**), and 1500 h (**C, F** and **I, L**). Each symbol represents the mean ± SE of three replicates.

since some reports have pointed out an absence of beneficial effect of extra foliar Pi supply on A (e.g., as in cultivar Carioca) or, alternatively, such effects are only seen after plant rehydration (Santos et al., 2004, 2006).

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