WATER DEFICIENCY AT DIFFERENT DEVELOPMENTAL STAGES OF *GLYCINE MAX* CAN IMPROVE DROUGHT TOLERANCE ([1])

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ABSTRACT

Developmental windows are specific periods of sensitivity during normal plant development in which a perturbation may be adaptively integrated. In these periods, sub-lethal environmental perturbations may improve the capacity to grow at lethal conditions. The aim of this study was to test the hypothesis that previous non-lethal water deficit applied in different developmental stages in soybean plants could enables them to improve the tolerance to environmental perturbations. In order to test this hypothesis we carried out an experiment with soybean plants submitted to water deficit in different stages of plant development, evaluating yield and physiological aspects. Our results indicated that water deficit experienced on V4 stage (vegetative) induces more suitable response, enabling plants to develop a process of tolerance improvement to a further water shortage period, probably through a reduction of growth, which maintains a conservative strategy of energy use. On the other hand, water deficit in R1 stage (reproductive), increased the plant susceptibility to posterior water withholding. This "strategy" was the opposite of the one employed by plants on V4 stage, i.e., to maintain growth rate probably at the expense of a higher energetic cost.

Key words: crop yield, photosynthesis, plant stress, soybean.

RESUMO

DEFICIÊNCIA HÍDRICA EM DIFERENTES ESTÁGIOS DE DESENVOLVIMENTO DE SOJA PODE AUMENTAR A TOLERÂNCIA À SECA

Janelas de desenvolvimento são períodos específicos durante o ciclo de vida das plantas em que uma perturbação ambiental pode ser incorporada através de um processo de adaptação. Nesses períodos, perturbações ambientais subletais podem capacitar as plantas a crescer em condições letais. O objetivo deste trabalho foi testar a hipótese de que plantas de soja (*Glycine max*) submetidas à deficiência hídrica não-letal em diferentes estágios de seu desenvolvimento poderiam otimizar sua tolerância a estresses ambientais posteriores. Para testar essa hipótese, foi conduzido um experimento com plantas de soja submetidas à deficiência hídrica em diferentes estágios de desenvolvimento, avaliando-se alguns aspectos fisiológicos e produtivos do cultivo. Nossos resultados indicaram que a ocorrência de deficiência hídrica no estádio V4 (vegetativo) induziu uma resposta mais adequada, permitindo que as plantas desenvolvessem um mecanismo de tolerância a um segundo período de falta de água, sendo a redução do crescimento provavelmente relacionada à manutenção de uma estratégia de conservação de energia. Entretanto, a ocorrência de déficit hídrico no estádio R1 (reprodutivo), aumentou a suscetibilidade da planta a novo período de deficiência hídrica. Essa "estratégia" foi contrária àquela evidenciada no estádio V4, i.e., manter a taxa de crescimento provavelmente à custa de maior demanda energética.

Palavras-chave: estresse vegetal, fotossíntese, produtividade, soja.

1. INTRODUCTION

Water deficiency is a common environmental factor that constrains plants to express their ecophysiological potential, causing short and long term effects in different hierarchical levels, from biochemical to morphological ones, and affecting crop yield (JONES and CORLETT, 1992; SOUZA and CARDOSO, 2003; PIMENTEL, 2004). Water deficiency leads invariably to a decrease in photosynthetic rate, although levels of tolerance may vary in different plant species (KAISER, 1987; CHAVES, 1991; LARCHER, 1995; CHAVES et al. 2002). Among the factors that contribute to this photosynthesis reduction stomatal closure can be considered as a direct response to leaf water potential reduction induced by drought (SANTOS et al., 2004, 2006). Stomatal conductance reduction limits CO_2 supplying, lowering intercellular CO_2 concentration (Ci) consequently constraining net CO_2 assimilation (JONES, 1998; CORNIC, 2000; CHAVES et al., 2002), which decreases plant growth and productivity.

Soybean (*Glycine max* L.) is one of the most important crops in the world and Brazil is the main producer. Environmental perturbations such as water deficiency have caused major productivity losses world-wide (GOPEFERT et al., 1993). In soybean plants water availability is especially important in two developmental periods, between seed germination and seedling emergence and between flowering and pod filling. In these periods, both water deficit and flooding may be potentially harmful for crop yield homogeneity in the field, decreasing seed germination (DOSS and THURLOW, 1974; CÂMARA and HEIFFIG, 2000).

Soybean may be considered as a crop that tolerates water deficit since its flowering occurs during a relatively long period, enabling plants to avoid the effects of short term droughts. Late flowers could counterbalance the loss of some early flowers during short drought periods, maintaining a suitable pod-filling yield (MOTA, 1983). However, if the drought period is extended, water deficit can affect the crop often reducing plant height by shortening the inter-nodes and decreasing relative growth rate, leaf area index and photosynthetic rate. As consequence, a negative impact on grain yield is expected due to an impairment of whole plant metabolism (CONFALONE et al., 1998; DESCLAUX et al., 2000; NEUMAIER et al., 2000).

The aim of this study was to test the hypothesis that previous non-lethal water deficit applied in different developmental stages in soybean plants could enables them to improve the tolerance to environmental perturbations. This could have interesting implications, for instance, to reduce environmental impacts on crop yields. Thus, to test this hypothesis we carried out an experiment with soybean plants submitted to water deficit in different stages of plant development, evaluating yield and physiological aspects.

2. MATERIAL AND METHODS

Seeds of *Glycine max* cv. IAC 18 were germinated in plastic pots with 8 kg of a mixture (3:1) of organic substrate (Eucatex Agro, Brasil) and sand. Seedlings (one plant per pot) were cultivated under greenhouse conditions. Plants were irrigated each two days with 600 mL of a modified Hoagland´s solution (McCREE, 1986) in order to maintain field capacity. The water deficiency treatments were carried out by suspending irrigation until the first signal of leaf wilting, which occurred after six days approximately. At this time, leaf water potential was around -1 MPa. Plants were subjected to water deficit in different developmental stages: at the first trifoliate leaf fully expanded (V1); at the 4th trifoliate leaf fully expanded (V4); at the first open flower (R1); and at the beginning of pod filling (R4:R5). Part of the plants subjected to water deficit at V1, V4 and R1 stages were submitted to a second period of water withholding at R4:R5 developmental stage, in order to test the hypothesis that pre-treatments in certain developmental stages could improve plants tolerance to water deficiency. A control group was maintained well-irrigated.

Plant leaf water potential was monitored with a pressure chamber model PMS-1000 (PMS Instruments, USA). Leaf gas exchange measurements were performed in the plants subjected to water deficit at V1, V4 and R1 developmental stages, as well as in the control plants. Plants at R4:R5 stage showed advanced leaf senescence, which is an inadequate condition to measure photosynthesis. Measurements of net CO_2 assimilation (A), transpiration rate (E), stomatal conductance (gs), and intercellular CO_2 concentration (Ci) were carried out with a portable gas exchange system, model CIRAS-2 (PPSystems, UK), operating in open mode. The measurements were performed from 10:00 to 12:00h in four healthy and fully expanded leaves per treatment under saturating photosynthetic photon flux density (PPFD) of 900 mmol m^{-2} s^{-1}, air temperature ranging from 28 to 31°C, and air vapor pressure deficit (VPD) of 2 kPa.

At the end of the experiment, the crop yield was evaluated in terms of dry matter production of shoots (DMS, mg $plant^{-1}$), roots (DMR, mg $plant^{-1}$), and pods (DMP, g $plant^{-1}$), shoot:root relation (S:R). Plants of all treatments were harvested separately, dried for five days at 60 ^{o}C, and then weighed.

The experiment was arranged in random design, with four replicates per treatment. Plants were randomly moved twice a week to different positions in greenhouse in order to minimize experimental errors. Data were submitted to ANOVA and mean values were compared by Tukey's test ($p < 0.05$). Moreover, ordination multivariate analysis by principal components (PCA) was used in order to verify the global influence of all parameters evaluated on the treatment differences. PCA is a linear dimensionality reduction technique, which identifies orthogonal directions of maximum variance in the original data, and projects the data into a lower-dimensionality space generated by a sub-set of the highest-variance components (MANLY, 1994). In this study a space of two dimensions (two principal components), which was demarcated by two axes labeled pc1 and pc2, was suitable to our analysis. The software PC-ORD version 3.12 (MJM Software Design, USA) was used.

3. RESULTS

Soybean plants exposed to water deficiency showed leaf water potential ($\emptyset_w$) around -1.0 MPa, corresponding to the beginning of leaf wilting, while control plants maintained $\emptyset_w$ values around -0.4 MPa (data not shown).

Water deficit induced significant ($p < 0.05$) decreases in leaf gas exchange in all plants at the different developmental stage (Figure 1). Stomatal conductance (gs) was significantly reduced in all developmental stages, mostly R1 which showed gs values lower than in V1 and V4 even in control plants (Figure 1A), indicating a developmental effect on stomatal aperture probably caused by plant growth regulators balance (INCOLL and JEWER, 1987; DAVIES and MANSFIELD, 1987). Similarly, transpiration (E) showed the same response pattern of gs, i.e., water deficit induced significant E reductions in the three developmental stages evaluated. Moreover, plants in R1 stage showed lower E values than plants in V1 or V4 developmental stages regardless water supplying (Figure 1B).

Water deficit affected net CO_2 assimilation (A) differently among developmental stages. While A values in V1 stage was not significantly affected by water deficit ($p > 0.05$), in stages V4 and R1 A values were strongly reduced, mainly in V4 stage, showing negative values. Under control conditions, A values were not different ($p > 0.05$) between V1 and V4 stages, being significantly lowered in R1 stage (Figure 1C). These results also indicate that photosynthetic activity is affected by developmental stages. Since A in V1 stage was not reduced by water shortage, the water use efficiency (WUE), calculated as A/E, of drought stressed plants was twofold higher (WUE

= 2.38) than in control plants (WUE = 1.13). Water deficit did not affect the intercellular CO_2 concentration (Ci), except in R1 which showed higher Ci values than control plants (Figure 1D). Under control conditions, Ci values in V4 stage were higher than in V1 and R1. However, Ci values were higher than 250 ppm in both well-watered and drought-stressed plants, suggesting that CO_2 assimilation was not impaired by limitation of substrate under water shortage.
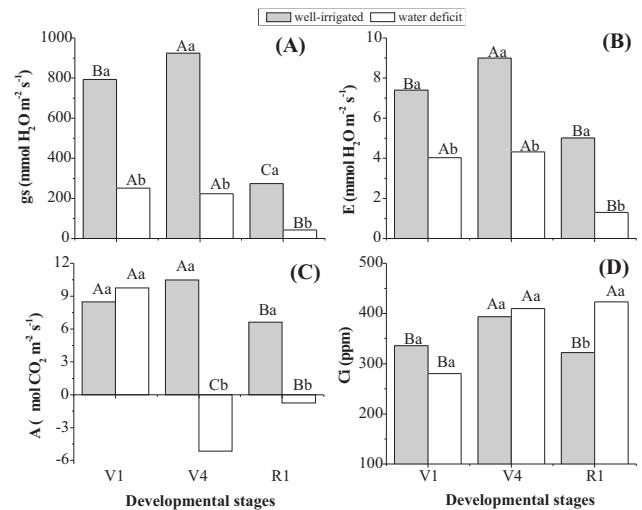
Figure 1. Leaf gas exchange of soybean plants (*G. max*) under well-irrigated (control) or water deficit conditions, imposed in different developmental stages (V1, V4 and R1). In A, stomatal conductance (gs); in B, transpiration rate (E); in C, net CO_2 assimilation (A); and in D, intercellular CO_2 concentration (Ci). Capital letters on the top of columns indicate significant differences ($p < 0.05$) among developmental stages at same water condition, while small letters indicate significant differences ($p < 0.05$) between well-irrigated and water-stressed plants in each developmental stage.

Summarizing data interpretation by principal component analysis (Figure 2), we can verify that leaf gas exchange in V1 stage was less affected by water deficit than in V4 and R1. Considering the relative distances on pc1 axis in the ordination graphic (accounting for 63.6% of original data variation), plants in V1 stage under water deficit were closer to their control plants when compared to the plants subjected to water deficit in V4 and R1 developmental stages. In pc1 axis, A, E and gs (in this order) were the variables that showed the highest contributions to differentiate the treatments, according to their respective eigenvector values given by the PCA results. On the other hand, pc2 axis accounts for only 30% of original data variation, and Ci was the most significant parameter contributing to this variation. Thus, these results indicated that later developmental stages were more sensitive to water deficit considering leaf gas exchange measurements.

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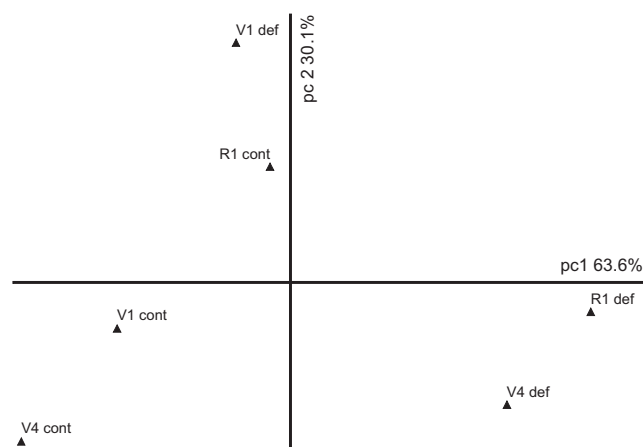
Figure 2. Graphic of ordination by principal component analysis (PCA) taking into account leaf gas exchange parameters of soybean plants (*G. max*) under well-irrigated (cont) or water deficit (def) condition imposed in different developmental stages (V1, V4 and R1). The first principal component (pc 1) accounted for 63.6% of the total variance contained in original data. Thus, data interpretation considered only the relative distances among the treatments along pc 1 axis.

A half of the plants subjected to water deficit in the V1, V4 and R1 developmental stages were submitted to a second period of irrigation withholding in R4:R5 stage in order to verify the possibility of changes in the degree of tolerance, comparing with plants which were subjected to a single period of water deficit in R4:R5 stage. This evaluation took into account impacts on crop yield. The results showed that water deficit did not affect root dry matter (DMR) production in any of the developmental stages (Table 1). On the other hand, shoot dry matter production (DMS) showed significant reduction when water shortage was imposed in V4 stage. However, when the plants in such developmental stage were subjected to a second period of water deficit in R4:R5 stage (V4:R4R5), DMS values did not show significant differences (p>0.05) in comparison to control plants. The dry matter production of plants in V1 stage was not affected by water deficit treatments, suggesting that this developmental stage is more stable than the others. DMS values in R4:R5 stage was not affected by water deficit (Table 1). In addition, the same pattern of response observed in DMS was verified in pod yield (DMP) (Table 1). The shoot:root relation was significantly decreased (p<0.05) by water deficit only in plants in V4 stage (Table 1).

The results of crop yield summarized by PCA (Figure 3) indicated that soybean plants in V1 stage, even after the second period of water deficit, were closer to control plants than the other treatments. On the other hand, plants in V4 stage was significantly affected by water deficit when compared to control plants, considering the relative distances to pc1 (which accounted for 68.8% of original data variation). However, a remarkable approximation to control conditions was verified when the second water deficit period was applied in R4:R5 stage (V4:R4R5).

Table 1. Crop yield components of soybean plants (*G. max*) subjected to well-irrigated (control) or water deficit conditions, imposed in different developmental stages (V1, V4, R1 and R4R5). V1:R4R5, V4:R4R5 and R1:R4R5 indicate the treatments in which plants were subjected to the second period of water shortage at R4R5 developmental stage

| Treatments | | Crop yield* (g DM plant-1) | | | |
		DMP	DMS	DMR	S:R
Well-irrigated		14.75 A	11.00 A	4.81 NS	2.32 A
	V1	12.06 AB	10.81 A	5.50	1.98 AB
	V4	3.38 C	5.55 C	5.28	1.05 B
	R1	12.30 AB	8.36 AB	5.48	1.67 AB
Water deficit	R4:R5	12.07 AB	10.05 AB	5.64	1.79 AB
	V1:R4R5	12.13 AB	10.28 AB	4.59	2.29 A
	V4:R4R5	12.29 AB	8.83 AB	5.48	1.65 AB
	R1:R4R5	9.30 B	7.64 BC	4.37	1.87 AB

* DMP: dry matter of pods. DMS: dry matter of shoots. DMR: dry matter of roots. S:R: relation between DMS and DMR. Significant differences (p<0.05) were represented by different capital letters; [NS]: means non-significant differences (p>0.05). Mean values of 4 replications.
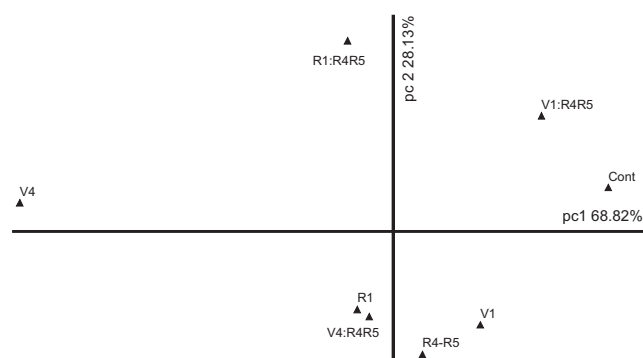
Figure 3. Graphic of ordination by principal component analysis (PCA) taking into account crop yield parameters of soybean plants (*G. max*) under well-irrigated (cont) or water deficit (def) conditions, imposed in different developmental stages (V1, V4, R1 and R4R5). V1:R4R5, V4:R4R5 and R1:R4R5 indicate the treatments in which plants were subjected to a second period of water shortage at R4R5 developmental stage. The first principal component (pc1) accounted for 68.8% of the total variance present in the original data. Thus, data interpretation considered only the relative distances among the treatments along pc1 axis.

4. DISCUSSION

Although water shortage have reduced stomatal conductance in the three developmental stages (Figure 1A), the results of leaf gas exchange measurements indicated that R1 stage under water deficit, and even under control condition, showed higher stomatal constrain to transpiration rate however, it was not sufficient to maintain the leaf water status. All plants under water deficit showed $Ø_w$ values around -1 MPa regardless of developmental stage. That effect of developmental stage on stomatal responses to water deficit was also observed in *Phaseolus vulgaris* (PIMENTEL et al., 1999). During normal plant development, the balance among plant hormones, such as auxins, cytokinins and abscisic acid (ABA), shows specific alterations according to each developmental stage (DAVIES, 1995). The relationship among plant hormones can affect stomatal movements and, as direct consequence, may modify gas exchange. While ABA induces stomatal closing, cytokinins stimulates stomatal opening as well as the presence of adequate concentration of endogenous auxins, mostly IAA (indol-acetic acid) (INCOLL and JEWER, 1987; DAVIES and MANSFIELD, 1987). It is known that R1 is a more advanced developmental stage in relation to V1 and V4, and plants in R1 stage have lower growth rates. Hence, it is likely that the relative levels of hormones, which stimulate plant growth, such as cytokinins and auxins, can be reduced and ABA levels can be relatively higher. Such hormonal balance could induce stomatal closing.

Data of net CO_2 assimilation (Figure 1C) and intercellular CO_2 concentration (Figure 1D) suggest that photosynthesis reduction was due to non-stomatal causes because Ci values in all treatments were at adequate levels for sustaining photosynthesis even under water deficit, as indicated by Ci values in well-watered plants. Therefore, it is reasonable to assume an occurrence of biochemical impairment on photosynthesis due to drought. Accordingly, SANTOS et al. (2006) observed a biochemical limitation of photosynthesis when bean plants reached a Y_w around -1.0 MPa as consequence of drought stress.

Water deficit may cause damages to the biochemical CO_2 fixation machinery, decreasing both activation and carboxylase activity of Rubisco, primarily due to the action of inhibitors (MEDRANO et al., 2002; PARRY et al., 2002). Reduction in the Rubisco efficiency may be caused by an increase in the mesophyllic resistance due to stomatal closure, constraining CO_2 uptake into chloroplasts and increasing the oxygenase activity of Rubisco with consequent increase in photorespiration. Moreover, the ribulose-1,5-bisphosphate regeneration may also be reduced by drought due to a decrease in ATP synthesis by ATPase. In fact, SANTOS et al. (2004; 2006) verified positive effects on photosynthetic activity and gas exchange of beans when extra Pi was supplied to increase its availability in leaves of plants under water stress. These integrated effects suggest a down-regulation mechanism of the whole photosynthetic apparatus due to water deficit (LAWLOR, 2002; MEDRANO et al., 2002). However, such hypothesis must be interpreted with caution since water deficit may lead to unreliable Ci measures due to patchy stomatal conductance or increased importance of cuticular transpiration (KAISER, 1987; MOTT and BUCKLEY, 1998; CORNIC, 2000).

The results of crop yield parameters showed that plants in different development stages were differently affected by water shortage. While plants in V1 stage showed stability after both water withholding periods, plants in R1 stage were not affected by the first water deficit, but when subjected to the second period of water shortage at R4:R5 stage dry matter production was significantly affected. On the other hand, dry matter production of plants in V4 stage was strongly affected by water deficit although when the plants were subjected to the second period of water shortage in R4-R5 stage dry matter production was not constrained. These results suggest two very distinct patterns of response to water deficit. Firstly, plants in R1 stage showed an apparent tolerance to water deficit maintaining dry mater production. Yet, the cost of this maintenance could have been very high, possibly increasing the susceptibility to further water shortage periods.

Secondly, plants in V4 stage showed a significant reduction in dry matter production which could lead to an improvement in plant tolerance to water deficit, likely due to physiological acclimation (AMZALLAG et al., 1993; AMZALLAG and SELIGMANN, 1998; LIZANA et al., 2006).

In crops such as soybean, the main selection criteria for drought resistance are parameters of plant growth and grain production (ACOSTA–GALLEGOS and ADAMS, 1991). Even though these parameters may reflect plant performance under stress, most are difficult and time-consuming to measure. Therefore, the evaluation of plants under drought conditions could be improved and accelerated if the physiological traits related to water deficit could be identified (LIZANA et al., 2006). The PCA analysis performed with leaf gas exchanges (Figure 2), which are instantaneous measurements, and crop yield parameters (Figure 3), which are long-term integrative measurements, showed basically the same result interpretation regardless of the different scales of observation.

However, according to SOUZA and CARDOSO (2003) a match between different scales of observation cannot be expected in all situations. Contrarily, different observation scales may lead to different interpretations of the same phenomenon. In our study and regardless of the scale of observation, plants in V1 stage were closer to control condition than V4 and R1 stages, mostly in relation to V4 stage after the first water deficit period (Figures 2 and 3).

Nevertheless, plants in V4 stage showed a sharp recovery after the second water shortage period (Figure 3), suggesting an improvement in water deficiency tolerance, a phenomenon related to the restitution phase after environmental disturbances named hardening (LICHTENTHALER, 1995). According to this author, stress responses can be differentiated between eu-stress and dis-stress. The former is an activating, stimulating stress and a positive element for plant development, whereas dis-stress is a severe and harmful element causing real damages in plants.

Thus our results indicated that water deficit experienced on V4 stage (vegetative) induces an eu-stress situation, enabling plants to develop a process of tolerance improvement to a further water shortage period, probably through a reduction of growth (Table 1), which maintains a conservative strategy of energy use. On the other hand, water deficit in R1 stage (reproductive), increased the plant susceptibility to posterior water withholding. This "strategy" was the opposite of the one employed by plants on V4 stage, i.e., to maintain growth rate probably at the expense of a higher energetic cost (NELSON, 1994).

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