

The level of environmental noise affects the physiological performance of *Glycine max* under water deficit

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Received: March 25, 2013; Accepted: April 16, 2013



ABSTRACT: Plants in natural environments are subjected to a multitude of environmental cues. However, studies addressing physiological analyzes are usually focused on the isolation of a stress factor, making it difficult to understand plants behavior in their extremely complex natural environments. Herein, we analyzed how environmental variability (noise) may influence physiological processes of *Glycine max* under water deficit conditions. The plants were kept in a greenhouse (semi-controlled environment – E_{sc}) and in a growth chamber (controlled environment – E_c) under two water regime conditions (100 and 30% of replacement of the water lost by evapotranspiration) for 30 days. The environmental variability was daily monitored with automatic sensors to record temperature, humidity, and irradiance. The physiological responses were analyzed by leaf gas exchanges, chlorophyll fluorescence, biomembrane integrity, and growth parameters. The results showed that water deficiency caused significant reductions in the physiological parameters evaluated. However, the environment with high variability (E_{sc}) caused more extensive damages to biomembranes, regardless the water regime likely compromising physiological efficiency. The lower variability of E_c promoted higher efficiency of total biomass production in both water regimes compared to the E_{sc} . Therefore, our results support the hypothesis that more variable environmental conditions can limit the growth of *Glycine max* in response to the fluctuation of resources, therefore amplifying the effect of water deficit.

KEYWORDS: controlled environment, environmental complexity, growth, photosynthesis, soybean.

INTRODUCTION

Plants in natural environments find a combination of stress factors arising from the variability of various environmental resources (Chapin 1991, Sultan et al. 1998). The impact of a particular type of disturbance on plant performance is the result of the interaction between the plant and the environmental complexity of resources availability, affecting different levels of plant organization (Vitolo et al. 2012). Particularly, water has been considered

the main factor, either alone or combined with other ones, limiting agricultural productivity around the world (Chaves and Oliveira 2004, Murchie et al. 2008, Flexas et al. 2009). Studies addressing water stress in combination with a second abiotic factor (Jiang and Huang 2001, Reynolds-Henne et al. 2010) and up to three additional ones (Wertin et al. 2010) have been documented. These kinds of analysis, which consider a more complex environment, have shown that certain combinations of factors are unique. For

example, the response of plants to a combination of drought and heat stresses is specific and cannot be directly extrapolated from plant reactions to the stress factors applied individually, especially considering that the mechanisms conferring acclimation of plants to the combination of multiple environmental factors are still poorly understood (Rizhsky et al. 2004, Mittler and Blumwald 2010, González-Cruz and Pastenes 2012).

Although many studies have identified the physiological responses of plants to water deficit (Lawlor and Tezara 2009, Pinheiro and Chaves 2011), it is not so evident how the environment variability and complexity can influence those processes. The responses of plants facing the environmental variability involve the way in which the different plant parts interact with one another, and the different time scales in relation to its development (Chaves et al. 2009). The strength, duration, and rate of progression of stress events have also an impact on the reactions of the plant, as well as its developmental stage and sensitivity to different environmental factors (Shannon 1997, Pinheiro and Chaves 2011).

According to Mittler (2006), most studies on abiotic stresses are performed under controlled conditions and do not reflect conditions that take place in the field. For instance, within a particular environment, large fluctuations in water availability, salinity, temperature, and even anaerobic conditions may occur. As a consequence, a high degree of heterogeneity among the levels of stress that cause impact on different plants in the same environment may be present. This heterogeneity, in turn, can affect plant performance and its yield (Mittler and Blumwald 2010). Therefore, there may be a considerable gap between the knowledge obtained from studies under controlled conditions and that necessary for the development of crops with increased tolerance to face the environmental complexity of field conditions. This gap may explain why some of the transgenic plants grown under such situations, which present an improved tolerance to a particular stress condition, fail to show the acquired tolerance when tested in the field (Mittler 2006). Thus, according to Mittler and Blumwald (2010), as the interactions among events from multiple abiotic stresses are critical for agricultural production they should be considered in developing new transgenic crops.

Soybean (*Glycine max* L. Merr.) is one of the most important economic crops in Brazil and worldwide. However, the occurrence of climatic adversities, like water deficit, remains a risk factor in soybean cultivation (Souza et al. 2013). In this context, the aim of this study was to characterize physiological responses (photosynthesis and growth) of soybean plants subjected to water deficit in

conditions with lower and higher environmental noise, that is, random variations (noncontrolled) of physical environmental factors other than drought. Furthermore, we tested the hypothesis that physiological responses of *G. max* to drought conditions would be affected by environmental noise, which synergistically could amplify the potential damage caused by water deficiency.

MATERIAL AND METHODS

Experimental design: Soybean plants (*Glycine max* (L.) Merr. cv. Codetec 202) were grown under greenhouse conditions in pots containing 12 kg of a 1:1 mixture (w/w) of red-yellow Ultisol soil-organic matter, being watered daily. The soil was fertilized according to the recommended chemical soil test procedures, maintaining the base saturation at 60% (Catuchi et al. 2012).

The water treatments were performed when the plants reached the phenological stage V4 (fourth fully expanded trifoliolate). Plants were submitted to two irrigation conditions, a 100% replacement of total water evapotranspired and another with 30% replacement, in a 30-day period of cultivation. Water replacement was done by the gravimetric method with daily weights of pots. Each day it was fully replenished according to the treatments, considering the daily weight variation of the pot in function of evapotranspiration (Catuchi et al. 2012).

Plants in both irrigation treatments (100 and 30% water replacement) were maintained simultaneously in two conditions with different levels of environmental variability (noise), being the highest one (E_{sc} , semi-controlled conditions) established under greenhouse conditions (22°07'3"S, 51°26'0"W). On the other hand, the environment with less variability (E_c , controlled conditions) was established in a growth chamber with controlled light (600 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$), day/night temperature (30/23 °C), air humidity (60%), and long-day photoperiod (16 hours). The mean values of light, temperature, humidity, and photoperiod of E_c were determined according to the monitoring of these parameters within the greenhouse (E_{sc}). Both environments (E_c and E_{sc}) were monitored daily, every ten minutes, by a HOBO system (model H08-004-02, Onset, USA) with automatic recording of temperature, light, and humidity throughout the experiment.

The treatments were arranged in a fully randomized factorial design two x two (two irrigation regimes and two levels of environmental variability), resulting in four ones. Seven replicates were carried out for each treatment, they were composed by one plant per pot.

The measurements of all the physiological parameters were performed after 30 days of cultivation in every growing environment and irrigation regimes. The recovery of the photosynthetic capacity was evaluated 15 hours (overnight) after rehydration.

Measurement of the physiological parameters: The relative water content (RWC) was determined according to Čatský (1960) by weighing the leaves using an analytical balance (± 0.1 mg precision). At the end of the treatments, the saturated leaf mass was obtained after the samples had been immersed in distilled water for 24 hours in darkness; the dry mass was established by drying in an oven at 60°C until a constant one was attained.

Photosynthetic response curves to CO_2 (A/C_i ones, where A corresponds to net assimilation of CO_2 and C_i to the intercellular CO_2 concentration) and others to light (A/PPFD , where PPFD is the photosynthetic photon flux density) were performed according to the standard procedures described in Prado and Moraes (1997) and Long and Bernacchi (2003). The A/C_i curves were carried out varying the CO_2 concentration within the sample chamber of an infrared gas analyzer (Li-6400XTR, Li-Cor, USA) from 0 to $1,500 \mu\text{mol mol}^{-1}$ (Long and Bernacchi 2003). The measurements were done under saturating irradiance of $1,200 \mu\text{mol m}^{-2} \text{s}^{-1}$ and vapor pressure deficit around 1.5 kPa, maintained with a dew point generator (model Li-610, Li-Cor, USA) coupled to Li-6400XTR infrared gas analyser (IRGA); and the leaf temperature was adjusted to 30°C . The curves were put according to the model proposed by Monteiro and Prado (2009). The variables obtained from A/C_i were the photosynthetic potential (A_{maxCO_2}), compensation point of photosynthesis to CO_2 (P_{comCO_2}), and saturation point of photosynthesis to CO_2 (P_{satCO_2}). The relative stomatal limitation of photosynthesis (L_s) was calculated as per Farquhar and Sharkey (1982). Furthermore, maximum ratio of Rubisco carboxylation (V_{cmax}) was calculated based on von Caemmerer (2000).

The A/PPFD curves were estimated at PPFD from 2,000 to $0 \mu\text{mol m}^{-2} \text{s}^{-1}$ provided by LED light source emitting in the blue-red spectrum for three to six minutes, depending on the speed for stabilizing the readings. During these measurements, the concentration of CO_2 in the sample chamber of the Li-6400XTR was maintained at $380 \mu\text{mol mol}^{-1}$, the temperature was set at 30°C , and the vapor pressure deficit maintained in 1.5 kPa. The curves were adjusted according to Prado and Moraes (1997) model. The variables obtained from the A/PPFD curves were the photosynthetic capacity (A_{maxL}), compensation point of photosynthesis to PPFD (P_{comL}), saturation point of photosynthesis to PPFD (P_{satL}), apparent quantum efficiency (AQE), and dark respiration (R_d). Other parameters were achieved on the saturation point of photosynthesis to PPFD, such as stomatal conductance (g_s), intercellular CO_2 concentration (C_i), transpiration (E), and intrinsic efficiency

of water use (I_{ewu}). Photorespiration (Pr) of the C3 plant metabolism was calculated using the method published by Sharkey (1988), which utilizes the gas exchange (A_{maxL} and R_d) values at the light saturation point. All A/C_i and A/PPFD curves were estimated using measurements taken from healthy fully expanded leaves of eight plants per treatment from 9:00 am to 1:00 pm.

In order to evaluate the photochemical activity, fluorescence analysis of chlorophyll a was performed simultaneously with the A/PPFD curves by applying a modulated light fluorometer (LI-6400-40) connected to the Li-6400XTR. The parameters were obtained on the saturation point of photosynthesis to PPFD, such as: potential (F_v/F_m) and effective ($\Delta F/F_m'$) quantum efficiencies of photosystem II (PSII), quantum antenna efficiency (F_v'/F_m'), photochemical (qP) and non-photochemical (NPQ) quenching of fluorescence and the electron transport rate – ETR (van Kooten and Snel 1990, Chaves et al. 2009, Baker and Rosenqvist 2004).

The alternative electron sink (AES) was calculated as the ratio between the effective quantum efficiency of PSII at the light saturation point ($\Delta F/F_m'$) and that of CO_2 fixation (φCO_2), as proposed by Ribeiro, Machado and Oliveira (2003). φCO_2 was calculated based on Edwards and Baker (1993).

Total chlorophyll content index (CCI) was determined from a fully expanded leaf from each plant in the study using a portable chlorophyll meter (Model CCM 200, OptSciences, United Kingdom).

With the aim of estimating the integrity of biomembranes, the cellular electrolyte leakage per unit dry mass – EL_{dm} (adapted from Matos et al. 2010) was measured. Ten leaf discs were collected from fully expanded leaves from each repetition and placed in glass vials with 10 mL of deionized water, which were then capped and kept at 25°C for 24 hours. After this period, the solution conductivity was determined (X_i) with a portable conductivity meter (CD-4301, LTLutron, Taiwan). The dry mass of the leaf disks was obtained by drying in at 60°C until reaching constant mass.

Plant growth analysis: Total leaf area (LA) was measured with a portable LA integrator (model LI-3000A, Li-Cor, USA), and the specific leaf mass (SLM) was estimated using the relation between dry mass and area of the leaves. To quantify the leaf dry mass (g), leaves were collected in paper bags and kept in a vented dryer (70°C) until reaching constant weight. At the end of the treatments, total plant dry mass was measured by drying the whole plants and then separating root from above ground tissue masses.

Data analysis: Data were analyzed using a two-way analysis of variance (ANOVA), and the means were compared using Tukey's test ($p < 0.05$).

RESULTS

Variability analysis of the environments: Although the averages of total daily irradiance (integrated values of PPFD_{day} throughout the day) and temperature (T) were roughly the same in both environments (Table 1), the coefficients of variation (CV) of the measured environmental factors were consistently lower in the controlled environment (E_C) when compared to semi-controlled one (E_{SC}), indicating that the environmental variability was comparatively smaller in E_C. The mean relative humidity (RH) was relatively higher in the E_{SC} when compared to E_C (Table 1). In the E_C, PPFD_{day} showed no variation as the light source provides constant illumination inside the growth chamber. Meanwhile, the CV of the irradiance during the days was 50% in E_{SC} (Table 1).

Plant water status and biomembranes integrity: In both environments, RWC was reduced under water deficit (Table 2); however, this effect was higher in E_{SC} with 32% decrease, whereas in E_C the RWC decline was 10%. The electrolyte leakage per unit of dry mass (EL_{dm}) showed no significant differences between soybean plants subjected to water deficit during 30 days, and plants grown under normal irrigation. Nevertheless, the EL_{dm} values were significantly lower when the plants were kept in E_C regardless of irrigation regime.

Photosynthetic responses: Data analysis of chlorophyll a fluorescence (Table 3) in E_{SC} indicates that the effective quantum efficiency of PSII ($\Delta F/F_m'$) in soybean plants under water deficit increased significantly, while the NPQ was reduced compared to well-watered plants. On the other hand, the values of these parameters in plants under water deficit in E_C presented no significant changes in comparison with the well-watered ones. Moreover, while the ETR remained stable in plants of both irrigation regimes, the AES increased in those grown under drought condition in both environments, especially in E_{SC} where the values increased two-fold. There was an increase in CCI in plants subjected to water deficit (Table 3) compared to well-watered ones in both water supply conditions, although the CCI values in E_C grown plants were smaller than those observed in E_{SC} for both irrigation treatments.

Table 1. Mean values of environmental variability and their coefficients of variation (CV) obtained during the experimental period in growth chamber (E_C) and greenhouse (E_{SC}). Photosynthetic Photon Flux Density (PPFD_{day}), total daily irradiance (mol photons m⁻² day⁻¹); T, daily temperature (°C); and Relative Humidity (RH), daily relative humidity (%)

| | E _C | | | E _{SC} | | |
|-------|-------------------------|--------|---------|-------------------------|---------|---------|
| | PPFD _{day} /CV | T/CV | RH/CV | PPFD _{day} /CV | T/CV | RH/CV |
| Day | 30.2 / 0 | 33 / 5 | 40 / 14 | 32.1 / 50 | 33 / 18 | 50 / 42 |
| Night | - | 23 / 3 | 70 / 10 | - | 23 / 13 | 88 / 13 |

Data obtained from the A/Ci curve (Table 4) revealed that the photosynthetic potential (A_{maxCO₂}) and the saturation point of photosynthesis to CO₂ (P_{satCO₂}) declined when soybean plants were subjected to water deficit in both growth environments. However, while the reduction of A_{maxCO₂} in E_C was 39%, under E_{SC} conditions this decrease was greater than 60%. Furthermore, the values of A_{maxCO₂} and P_{satCO₂} were significantly higher when the plants were maintained in E_C. The plants held the values of maximum ratio of Rubisco carboxylation (V_{cm_{max}}) when they were subjected to water deficit in E_C, while in E_{SC} they exhibited a significant decrease (27%). On the other hand, the compensation point of photosynthesis to CO₂ (P_{comCO₂}) and relative stomatal limitation of photosynthesis (L_s) did not show significant changes regarding the irrigation regimes and growing environments, although the L_s tended to be higher under drought condition in plants grown in E_C (30%), as seen in Table 4.

Table 2. Mean values of the electrolyte leakage per unit of dry mass (EL_{DM}) and relative water content (RWC) in plants under 100 and 30% water replacement and controlled (E_C) and semi-controlled (E_{SC}) environments

| Variables | 100% | | 30% | |
|--|--------------------|--------------------|--------------------|--------------------|
| | E _C | E _{SC} | E _C | E _{SC} |
| EL _{DM} (mS g ⁻¹) | 0.55 ^{Ab} | 5.19 ^{Aa} | 1.23 ^{Ab} | 5.69 ^{Aa} |
| RWC (%) | 87.3 ^{Ab} | 92.7 ^{Aa} | 79.3 ^{Ba} | 63.5 ^{Bb} |

Different capital letters indicate statistical difference (p<0.05) between the water treatments and the lowercase ones (p<0.05) between environments. Seven replicates were carried out for each treatment.

Table 3. Mean values of potential and effective quantum efficiencies of PSII (F_v/F_m , $\Delta F/F_m'$, respectively), photochemical and non-photochemical quenching (qP and NPQ, respectively), electron transport rate (ETR), alternative electron sink (AES), and chlorophyll content index (CCI) in plants under 100 and 30% water replacement and controlled (E_C) and semi-controlled (E_{SC}) environments

| Variables | 100% | | 30% | |
|---|---------------------|---------------------|---------------------|---------------------|
| | E _C | E _{SC} | E _C | E _{SC} |
| F_v/F_m | 0.731 ^{Aa} | 0.788 ^{Aa} | 0.745 ^{Ab} | 0.817 ^{Aa} |
| $\Delta F/F_m'$ | 0.450 ^{Aa} | 0.457 ^{Ba} | 0.487 ^{Aa} | 0.531 ^{Aa} |
| $\Delta F/F_m'$ | 0.302 ^{Aa} | 0.273 ^{Ba} | 0.388 ^{Aa} | 0.404 ^{Aa} |
| qP | 0.657 ^{Aa} | 0.596 ^{Aa} | 0.830 ^{Aa} | 0.760 ^{Aa} |
| NPQ | 1.65 ^{Ab} | 2.07 ^{Aa} | 1.63 ^{Aa} | 1.70 ^{Ba} |
| ETR (μmol m ⁻² s ⁻¹) | 63.05 ^{Ab} | 89.88 ^{Aa} | 70.7 ^{Ab} | 86.78 ^{Aa} |
| AES | 14.5 ^{Ba} | 10.7 ^{Ba} | 18.5 ^{Ab} | 21.8 ^{Aa} |
| CCI | 10.9 ^{Bb} | 16.9 ^{Ba} | 18.8 ^{Ab} | 37.7 ^{Aa} |

Different capital letters indicate statistical difference (p<0.05) among water treatments, and the lowercase ones indicate (p<0.05) between environments. Seven replicates were carried out for each treatment.

Table 4. Mean values of the photosynthetic potential ($A_{\max\text{CO}_2}$), compensation point of photosynthesis to CO_2 (P_{comCO_2}), saturation point of photosynthesis to CO_2 (P_{satCO_2}), relative stomatal limitation of photosynthesis (L_s), and maximum ratio of Rubisco carboxylation (V_{cmax}) in plants under 100 and 30% water replacement and controlled (E_c) and semi-controlled (E_{sc}) environments

| Variables | 100% | | 30% | |
|--|---------------------|---------------------|---------------------|--------------------|
| | E_c | E_{sc} | E_c | E_{sc} |
| $A_{\max\text{CO}_2}$ ($\mu\text{mol m}^{-2} \text{s}^{-1}$) | 22.80 ^{Aa} | 18.68 ^{Ab} | 13.82 ^{Ba} | 6.80 ^{Bb} |
| P_{comCO_2} ($\mu\text{mol mol}^{-1}$) | 8.1 ^{Aa} | 7.0 ^{Aa} | 7.8 ^{Aa} | 8.8 ^{Aa} |
| P_{satCO_2} ($\mu\text{mol mol}^{-1}$) | 69.7 ^{Aa} | 51.2 ^{Ab} | 46.2 ^{Ba} | 13.3 ^{Bb} |
| L_s (%) | 23.3 ^{Aa} | 27.9 ^{Aa} | 30.4 ^{Aa} | 28.7 ^{Aa} |
| V_{cmax} ($\mu\text{mol m}^{-2} \text{s}^{-1}$) | 78 ^{Aa} | 84 ^{Aa} | 63 ^{Aa} | 61 ^{Ba} |

Different capital letters indicate statistical difference ($p < 0.05$) among water treatments and different lowercase ones indicate ($p < 0.05$) between environments. Seven replicates were carried out for each treatment.

Table 5. Mean values of apparent quantum efficiency (AQE), dark respiration (R_d), photorespiration (Pr), stomatal conductance (g_s), intercellular CO_2 concentration (C_i), photosynthetic capacity ($A_{\max\text{L}}$), compensation and saturation point of photosynthesis to light (P_{comL} and P_{satL} , respectively), transpiration (E) and intrinsic efficiency of water use (I_{ewu}) in plants under 100 and 30% water replacement and controlled (E_c) and semi-controlled (E_{sc}) environments

| Variables | 100% | | 30% | |
|---|---------------------|---------------------|---------------------|---------------------|
| | E_c | E_{sc} | E_c | E_{sc} |
| AQE ($\mu\text{mol } \mu\text{mol}^{-1}$) | 0.049 ^{Ab} | 0.064 ^{Aa} | 0.035 ^{Bb} | 0.050 ^{Ba} |
| R_d ($\mu\text{mol m}^{-2} \text{s}^{-1}$) | 2.09 ^{Aa} | 2.28 ^{Aa} | 1.05 ^{Bb} | 2.07 ^{Aa} |
| Pr ($\mu\text{mol m}^{-2} \text{s}^{-1}$) | 3.25 ^{Ab} | 5.25 ^{Aa} | 2.04 ^{Bb} | 3.40 ^{Ba} |
| g_s ($\text{mol m}^{-2} \text{s}^{-1}$) | 0.218 ^{Aa} | 0.147 ^{Ab} | 0.054 ^{Ba} | 0.052 ^{Ba} |
| C_i (Pa) | 297 ^{Aa} | 183 ^{Ab} | 204 ^{Ba} | 138 ^{Bb} |
| $A_{\max\text{L}}$ ($\mu\text{mol m}^{-2} \text{s}^{-1}$) | 10.10 ^{Ab} | 17.30 ^{Aa} | 5.65 ^{Bb} | 10.98 ^{Ba} |
| P_{comL} ($\mu\text{mol m}^{-2} \text{s}^{-1}$) | 39.2 ^{Aa} | 32.4 ^{Aa} | 29.2 ^{Aa} | 39.6 ^{Aa} |
| P_{satL} ($\mu\text{mol m}^{-2} \text{s}^{-1}$) | 483.6 ^{Ab} | 633.4 ^{Aa} | 315.6 ^{Bb} | 496.9 ^{Ba} |
| E ($\text{mmol m}^{-2} \text{s}^{-1}$) | 3.47 ^{Aa} | 3.45 ^{Aa} | 0.95 ^{Ba} | 1.44 ^{Ba} |
| I_{ewu} ($\mu\text{mol mol}^{-1}$) | 46.9 ^{Bb} | 120.0 ^{Ba} | 93.4 ^{Ab} | 188.1 ^{Aa} |

Different capital letters indicate statistical difference ($p < 0.05$) among water treatments and the lowercase one indicate ($p < 0.05$) between environments. Seven replicates were carried out for each treatment.

According to information from $A/PPFD$ curves (Table 5), soybean plants subjected to water deficit in both environments showed an increase in the intrinsic efficiency of water use (I_{ewu}), while presented reduced AQE, stomatal conductance (g_s), intercellular CO_2 concentration (C_i), maximum assimilation rate at saturating light ($A_{\max\text{L}}$), saturation point of photosynthesis to light (P_{satL}), transpiration (E), photorespiration (Pr) and, particularly in E_c , dark respiration (R_d). In general, compared with E_{sc} , the plants cultivated in the environment with less variability (E_c) had smaller values of AQE, Pr , I_{ewu} , $A_{\max\text{L}}$ and P_{satL} . However, under E_c conditions, the plants had higher values of C_i and, only in well-irrigated plants, g_s was higher than those grown in E_{sc} .

Table 6. Mean values of root dry mass (DM_R), leaf dry mass (DM_L), stem dry mass (DM_S), total dry mass (DM_T), shoot/root ratio (S/R), total leaf area (LA), and specific leaf mass (SLM) in plants under 100 and 30% water replacement and controlled (E_c) and semi-controlled (E_{sc}) environments

| Variables | 100% | | 30% | |
|---------------------------|--------------------|--------------------|--------------------|--------------------|
| | E_c | E_{sc} | E_c | E_{sc} |
| DM_R (g) | 59.3 ^{Aa} | 21.7 ^{Ab} | 11.9 ^{Ba} | 4.2 ^{Ba} |
| DM_L (g) | 59.2 ^{Aa} | 21.6 ^{Ab} | 16.0 ^{Ba} | 7.5 ^{Ba} |
| DM_S (g) | 62 ^{Aa} | 29 ^{Ab} | 13 ^{Ba} | 8 ^{Ba} |
| DM_T (g) | 180 ^{Aa} | 72 ^{Ab} | 41 ^{Ba} | 19 ^{Ba} |
| S/R | 1.95 ^{Aa} | 2.42 ^{Ba} | 2.47 ^{Ab} | 3.71 ^{Aa} |
| LA (m^2) | 0.21 ^{Ab} | 0.50 ^{Aa} | 0.08 ^{Bb} | 0.18 ^{Ba} |
| SLM (g m^{-2}) | 272 ^{Aa} | 43 ^{Ab} | 190 ^{Ba} | 41 ^{Ab} |

Different capital letters indicate statistical difference ($p < 0.05$) among water treatments and the lowercase ones indicate ($p < 0.05$) between environments. Seven replicates were carried out for each treatment.

It was observed that, after rehydration of the soybean plants subjected to water deficit, photosynthetic capacity ($A_{\max\text{L}}$) in plants grown in both environmental conditions exhibited full recovery, especially in E_c when $A_{\max\text{L}}$ was improved by 58% (Figure 1).

Growth analysis: Water stress reduced the dry mass of plants as a whole, as well as the total leaf area (LA) and specific leaf mass (SLM), while an increase was observed in the shoot-root ratio (Table 6). Soybean plants grown under E_c conditions had significantly more biomass accumulation compared to the E_{sc} ones, so that the values of total dry mass of the plants in both irrigation regimes in E_c were twice those observed in E_{sc} . On the other hand, the values of total LA of the plants in E_c were lower than in E_{sc} , even though they had a five-fold higher SLM (Table 6).

DISCUSSION

Environmental noise, that is, random changes in its factors, is a ubiquitous trait that all living organism is subjected throughout its life span. Even if it were possible to place organisms in a perfectly constant environment, they would still be subject to noise, because it also originates inside the organisms themselves (Wagner 2005). The main cause of internal noise is thermal motion caused by heat, increasing temperature inside organisms. This internal noise affects, for instance, the folding of macromolecules like RNA and proteins. Thus, under temperatures beyond physiological optimum the reactions of the organism metabolism can be seriously constrained (Wagner 2005). Therefore, the question raised in our study was how much the increased environmental noise could compromise the plant responses to water deficit.

Our results indicated that although the water deficit has reduced the amount of dry mass similarly in both growth

environments (about 75%), the one with less variability (E_c) was more favorable to the growth of soybean plants. The results of electrolyte leakage per unit of dry mass (EL_{dm}) (Table 2) showed that the environment with higher variability (E_{sc}) caused more damage to biomembranes, regardless of the irrigation regime, which probably resulted in increased energy cost of maintenance and repair, diverting a significant part of the assimilated carbon, thereby decreasing the production of biomass in this environment.

The severity level of water deficit applied to soybean plants in this work (30% of water replacement) was enough to reduce the values of RWC in both environments, mainly in E_{sc} . On the other hand, the EL_{dm} was not affected when plants were subjected to water deficit. Dehydration can induce conformational changes in the structure of the membranes at the biophysical level, which nevertheless are potentially reversible as soon as environmental conditions become more favorable (Leshem, 1992, Matos et al. 2010). Thus, damage to the structure of thylakoid membranes may induce changes in photochemical apparatus, reducing quantum efficiency and transport of electrons (Kaiser 1987, Sanda et al. 2011). In this study,

the maintenance of ETR in soybean plants under water deficit (Table 3) was observed, which could be explained by preserving cell membrane integrity. Therefore, although the water status (RWC) was reduced under water deficit, probably it may not have been severe to the point of disrupting biomembranes, in particular the thylakoids membranes.

The maintenance of the EL_{dm} values in soybean plants under water deficit in the two environments may also have been favored by the raise in alternative electron sinks, as indicated by the increase in the values of AES in plants under the same irrigation regime (Table 3). According to Lawlor and Cornic (2002), the main pathways that comprise the AES are photorespiration, water-water cycle, and metabolism of nitrate and sulfate. When the nonradiative deexcitation energy (NPQ) is not enough to cope with the excess of energy, these other sinks work as alternative routes from the electron transport chain of photochemical phase, protecting plants from overproduction of reactive oxygen species (ROS), which are compounds that have the potential of causing oxidative damage when reacting with biomolecules (Blokchina, Virolainen and Fagerstedt 2003, Smirnov 2005). Likely, this increase in AES (Table 3) was due to water-water cycle (Lawlor and Tezara 2009, Miyake et al. 2009, 2010), since Pr values were smaller under water deficit (Table 5).

The maintenance of ETR values, potential quantum efficiency of PSII (F_v/F_m), and photochemical quenching (qP) suggests that the period in which the soybean plants were subjected to water deficit in both growth environments was enough to start a process of acclimation. Flexas et al. (2006) reported that in many studies the results of photosynthetic responses to drought stress come from plants subjected to relatively short periods of experiment, yielding results related specially to the mechanisms of prompt reactions. However, under natural conditions, water stress develops gradually from weeks to months, when a process of acclimation may occur. Acclimated plants can improve their water relations and photosynthesis compared to nonacclimated ones, leading to smaller decreases in both carbon gain and growth (Flexas et al. 2006). The results observed in the recovery of photosynthetic capacity after rehydration (Figure 1) supported acclimation.

When water availability is low, leading to a reduction in stomatal conductance, the resulting decrease in photosynthesis may increase the nitrogen content of leaves (Chapin 1987). Additionally, Cheng and Fuchigami (2000) showed the relationship between the activation state of Rubisco and leaf nitrogen. Approximately 75% of foliar nitrogen in C3 plants was found in chloroplasts, and most of it is invested for Rubisco's synthesis (Fredeen et al. 1991, Dordas and Sioulas 2008). Our results concluded that the values of maximum ratio of Rubisco

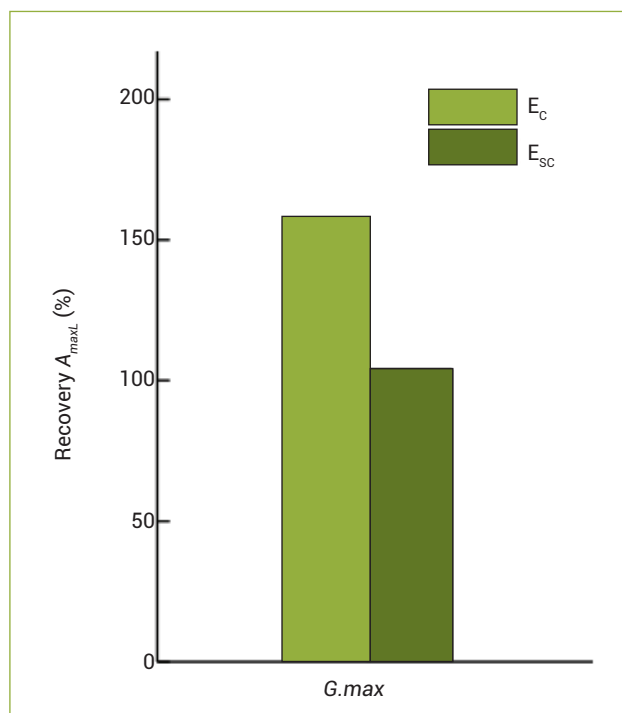


Figure 1. Recovery of photosynthetic capacity (A_{maxL}) of plants under water deficit in controlled (E_c) and semi-controlled (E_{sc}) environments. The measurements were performed after rehydration. Recovery was estimated from the A_{maxL} values of well-watered plants (100% hydration). The light green column represents recovery percentage in the E_c , and the dark green one indicates the recovery percentage in the E_{sc} . Seven replicates were carried out for each treatment.

carboxylation (V_{cmax}) decreased in soybean plants under water stress in E_{SC} (Table 4), meanwhile CCI values have increased (Table 3). Thus, we suggest that the nitrogen that was not used for carbon fixation has shifted to the synthesis of chlorophyll. According to Cheng and Fuchigami (2000), since Rubisco generates costs in terms of nitrogen (N) investment, N resources may be wasted if its content is greater than the required amount for carbon fixation.

The CCI results associated with the changes in the SLM (Table 6) show that while both environments (E_{SC} and E_{C}) had similar amounts of irradiance per day (PPFD_{day}) (Table 1), the large variability in this resource significantly affected the development of soybean plants, which showed clear signs of light acclimation divergent processes. In the controlled environment (E_{C}), where the irradiance was steady, plants exhibited significantly higher SLM values, while CCI ones were significantly lower than plants grown in the ambient with greater variability in the frequency of light distribution (E_{SC}). These results indicate that plants grown in E_{C} presented acclimated leaf traits to higher light availability, while those in E_{SC} had characteristics of leaves acclimated to a place with lower light availability (Souza et al. 2009). However, as E_{C} and E_{SC} showed basically the same total amount of light resource, we suggest that the large variability of PPFD_{day} in E_{SC} (CV = 50%) must have induced the soybean plants to optimize the process of prompt light harvest, since the light availability was irregular.

When a plant is subjected to low water availability, reduction in stomatal conductance is required to reduce loss of water by transpiration (E), while it also decreases the diffusion of carbon into the site of carboxylation in the chloroplast, causing significant diminishment in net photosynthesis (Yu et al. 2003). Thus, the reduction of stomatal conductance (g_s) in the soybean plants under water deficit (reductions of 75 and 60% E_{C} and E_{SC} , respectively) may have contributed to that photosynthetic capacity (A_{maxL}), since the C_i values were significantly lowered (Table 5). However, the outcomes obtained in our study indicate that the limitation of photosynthesis in soybean plants under water deficit occurred both by stomatal and non-stomatal factors, mainly related to biochemical limitation, since the photochemical activity was not affected by drought (Table 3). The values of photosynthetic potential (A_{maxCO_2}) declined proportionately to the decrease in V_{cmax} , indicating a biochemical limitation, especially in the controlled environment (Table 4). Moreover, when water stress reduces the activity of Rubisco independently of C_i , its reactions of carboxylation and oxygenation can be lowered in equal proportions (Ghannoum 2009), as indicated by the diminished values of Pr (Table 5), supporting the hypothesis of the biochemical limitation of photosynthesis

in soybean plants. These responses were consistent across both places. Also, under controlled environmental conditions the soybean plants showed values of A_{maxCO_2} higher than in the semi-controlled one (Table 4), regardless of irrigation treatments. This pattern was contrary to the A_{maxL} values (Table 5), showing that the differences in the environmental variability between E_{C} and E_{SC} were able to significantly affect photosynthetic processes in plants, besides the typical effects of water stress.

Additionally, the results indicated that the reduction in the values of R_d in soybean plants under water stress in E_{C} were proportional to diminishments in A_{maxL} (Table 5), therefore both the ability to use carbon and the CO_2 assimilation capacity were affected by water shortage. As stated by Atkin and Macherel (2009), water deficiency induces changes in respiratory flow, which may reflect alterations in maximum activity of enzymes of respiration, in the supply of substrate (due to lower rates of carbon fixation and subsequent production of sugar), and/or demand for ATP (associated with growth, cellular maintenance, and ion transport). Still, in general, the most important factor for the carbon balance to become unfavorable in plants under drought stress is photosynthesis.

On the other hand, some studies have reported that low values of Pr and R_d in plants grown at low irradiance are able to reduce the carbon loss under adverse conditions (Chapin et al., 1987, Souza et al. 2008), and thus the photosynthetic carbon fixation is carried out more efficiently (Björkman 1981). The reduction of Pr and R_d in E_{C} compared to E_{SC} , mainly in plants under water deficit, suggests that the constancy of this environment favored a more positive carbon balance, since the DM_T of the soybean plants was higher in this environment. Meanwhile, although the mean values of PPFD_{day} were approximately the same in both environments, the increase in Pr and R_d in plants under E_{SC} may have contributed to making the carbon balance less favorable, since the scale of variation of the irradiance incident was higher in such ambient, which may have provided moments of light excess.

The maintenance of R_d in soybean plants under water deficit in E_{SC} indicates that the energetic cost may have been destined to maintenance and repair of damages caused by the higher variability of this environment. Another finding that supports this hypothesis was the significantly higher EL_{dm} values in E_{SC} (Table 2), showing additional damage in biomembranes in comparison to E_{C} . These numbers, in the environment with greater variability (E_{SC}), may indicate oxidative stress, suggesting that the AES was not enough to ensure the integrity of biomembranes in this environment. Thus, the higher variability in the irradiance availability, in synergy with greater temperature

fluctuations, may have generated excess energy in the photochemical apparatus, which should have increased ROS production, causing major damage to biomembranes in E_{SC} .

An important result of this study was the differences in the photosynthetic responses and in plant growth between both environments. It would be expected that plants with both higher carbon assimilation potential and efficiency of photochemical processes, as observed in E_{SC} , present higher values of DM_r . However, the accumulation of biomass was reduced in this environment. Thus, we suggest that a major amount of photoassimilates could have been allocated for maintenance and repair mechanisms in a place where the variation amplitudes of irradiance, temperature, and humidity were probably more stressful. The increased photosynthetic potential and efficiency of the photochemical apparatus may indicate an acclimation process in the soybean plants under E_{SC} conditions, since physiological responses tended to increase the biochemistry and photochemistry efficiency in order to optimize production resources required by these plants.

Chapin et al. (1987) reported some studies that showed the improvement of compensatory responses to multiple resource constraints. Accordingly, Peace and Grubb (1982) concluded that plants respond synergistically to adding multiple resources such as increased growth in response to extra nutrients and light. Therefore, a more constant environment could provide resources more adequately for growth, as can be observed in the soybean plants grown under E_C conditions. Despite the lower photosynthetic potential of plants in E_C , the

observed growth parameters suggest that the large amount of the photoassimilates should have been directed towards the accumulation of biomass, since the membrane damage and consequently the energy cost of maintenance and repair were reduced in plants cultivated in this environment.

Summarizing, water deficiency caused significant reductions in the amount of total plant biomass of *G. max*. However, the environment with lower variability (E_C) promoted a greater efficiency of total biomass accumulation in both irrigation regimes compared to the E_{SC} , despite the observed differences in photosynthetic capacity in each environment. Our results support the hypothesis that more variable environmental conditions may constrain soybean growth due to higher fluctuation of resources, thereby amplifying the effect of water deficit. Additionally, our outcomes showed that the analysis of water deficit impacts must consider not only the intensity of water restriction (moderate or severe), but equally the interplay between the effects caused by environmental complexity on the plant.

ACKNOWLEDGEMENTS

This study was supported by Fundação de Amparo à Pesquisa do Estado de São Paulo – Fapesp, Brazil (2008/57571-1). GM Souza and SC Bertolli were supported by fellowships granted by Conselho Nacional de Pesquisa e Desenvolvimento Tecnológico – CNPq, Brazil, and FAPESP (2009/11212-3), respectively. We thank doctor Luiz Gonzaga Vieira (Universidade do Oeste Paulista – Unoeste) for reviewing and commenting on the manuscript.

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