# Tolerance to water deficiency between two soybean cultivars: transgenic *versus* conventional

## Tolerância à deficiência hídrica entre dois cultivares de soja: transgênico *versus* convencional

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## ABSTRACT

The main goal of the study was to evaluate the effects on the development and physiological aspects of soybean plants grown under water deficiency at greenhouse conditions, comparing the levels of tolerance to water deficiency between one transgenic and one conventional cultivar, enabling a systematic way to carried out physiological comparisons between soybean cultivars under drought. The study was divided into completely randomized using a factorial 2×2 design, with five replicates. The experimental design included two replenishment levels of daily irrigation (100% and 40%), applied to the leaves during the V4 developmental stage (fourth trifoliate fully expanded) and two cultivars, 'CD 202' conventional and 'CD 226RR' transgenic. The results showed that both cultivars had similar effects, caused by water deficiency, on dry mass production, but the transgenic cultivar tended to maintain higher biomass allocation in pods, as well as, higher efficiency of leaves to support dry mass production than conventional cultivar in both water conditions. Moreover, the higher maximum $CO_2$ assimilation values and lower membranes damages in the transgenic cultivar under water deficiency showed that the expected higher drought tolerance of the conventional cultivar was not supported by a more accurate physiological investigation.

**Key words**: *drought, Glycine max, photosynthesis, water relations.*

## RESUMO

O principal objetivo do estudo foi avaliar os efeitos sobre o desenvolvimento e aspectos fisiológicos de plantas de soja cultivadas sob deficiência hídrica em condições de casa de vegetação, comparando os níveis de tolerância à deficiência de água entre um cultivar transgênico e um convencional, gerando uma forma sistemática de realizar comparações fisiológicas entre cultivares de soja sob déficit hídrico. O delineamento experimental foi inteiramente casualizado, em esquema fatorial 2×2, com cinco repetições. Os tratamentos hídricos foram dois níveis de reposição de irrigação diária (100% e 40%), aplicados a partir do estádio V4 (quarto trifólio completamente expandido), em dois cultivares ('CD 202' convencional e 'CD 226RR' transgênico). Os resultados evidenciaram que ambos os cultivares tiveram as mesmas respostas na produção de massa seca, mas o cultivar transgênico tendeu a manter maior alocação de biomassa em vagens, assim como maior eficiência da área foliar para a produção de massa seca em ambas as condições de irrigação. Além disso, os maiores valores de assimilação máxima de $CO_2$ e menores danos às membranas no cultivar transgênico, sob deficiência hídrica, mostraram que a maior tolerância à seca esperada do cultivar convencional não foi apoiada por uma investigação fisiológica mais acurada.

**Palavras-chave**: *falta de água, fotossíntese, Glycine max, relações hídricas.*

## INTRODUCTION

Soybean (***Glycine max*** (L.) Merrill) cultivation occupies a large amount of land in Brazilian agriculture due to its global economic importance (CONAB, 2010). However, environmental constrains has been causing a significant loss of productivity worldwide. Water deficiency is considered the main factor contributing to suboptimal crop productivity (CHAVES & OLIVEIRA, 2004).

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According to CHAVES et al. (2002), water deficit may severely inhibit photosynthesis, due to the higher diffusive resistance to $CO_2$ entry that results in a reduction in the intercellular $CO_2$ concentration and, consequently, a decrease in the net assimilation of photosynthates, reducing crop yield. Prolonged periods of drought can directly affect soybean yield by reducing the plants' height, relative growth rate, leaf area index and photosynthetic rate. Consequently, a negative impact on seed production is expected due to a decrease in the metabolism of the whole plant (CONFALONE et al., 1998; DESCLAUX et al., 2000).

Some studies have addressing soybean physiological responses to water deficit (KRON et al., 2008; FIRMANO et al., 2009), however, despite of the soybean relevance to worldwide agriculture, there is no scientific studies about physiological differences between transgenic and conventional cultivars in response to drought in a single experiment. On the other hand, some practical studies have attempting to fulfil such gap information based on field observation, but without a consistent scientific approach (PITOL & BROCH, 2010).

Thus, the main goal of our study was to evaluate the effects on growth and physiological aspects of soybean plants grown under water deficiency, comparing one transgenic and one conventional cultivar, indicating a protocol to carried out physiological comparisons between soybean cultivars under drought.

**MATERIAL AND METHODS**

The study was conducted in a greenhouse in the UNOESTE Campus II, located in Presidente Prudente, state of São Paulo, at latitude 22º07'S and longitude 51º26'W, and 433m above sea level.

The experiment was performed in pots containing 10.5kg of a 1:1 mix of Red-Yellow Argisol plus a vegetal substrate. The mixture had the following chemical characteristics: pH ($H_2O$)=6.2; base saturation (V)= 84%; P=128mg $dm^{-3}$; cation exchange capacity (CEC), exchangeable cations, K, Ca, Mg, H+AI=125; 106; 10.8; 55; 40; 20mmol$_c$ $dm^{-3}$, respectively; Mn=7.8mg $dm^{-3}$ and organic matter=85mg $dm^{-3}$.

The study was divided into completely randomized using a factorial 2×2 design, with five replicates. The experimental design included two replenishment levels of daily irrigation (100% and 40%) based on daily variation of pots weight, from V4 developmental stage (fourth trifoliate fully expanded) (FIRMANO et al., 2009), and two cultivars, 'CD 202' conventional (Conv.) and 'CD 226RR' transgenic (RR).

The first one is considered drought tolerant and the late one is drought sensitive (PITOL & BROCH, 2009). Seeds, inoculated at planting with bacteria of the genus Bradyrhizobium, were treated with the fungicide Vitavax Thiran 200 SC to keep the plants healthy during the initial stages of development. Plants were established from the initial germination of five seeds per pot, with posterior maintenance of only one plant per pot.

Physiological analysis (water relations and photosynthetic capacity) were performed two weeks after beginning the induction of water deficiency and, at the end of the plant growth cycle, we evaluated crop yield.

Leaf water potential measurements were performed using a pressure chamber (model 1000, PMS Instruments, USA) before dawn and at 12:00 pm. Total chlorophyll content was determined by evaluating one fully expanded trifoliate leaflet from each plant in all treatments, using a portable chlorophyll meter (Model CCM 200, OptSciences, UK).

Cellular electrolyte leakage was evaluated by measuring the electrical conductivity of an aqueous solution containing ten leaf discs collected from each replicate in all treatments using a portable electric conductivity meter (LTLutron, model CD-4301). This technique allowed us to evaluate the integrity of cellular membranes, since the lower the electric conductivity of a solution, the fewer electrolytes leak from the membrane, thus indicating their degree of integrity.

The photosynthetic capacity was measured using photosynthetic (A) responses to photosynthetic photon flux density (PPFD) curves (A-PPFD). Measurements were performed using a portable infrared gas analyser meter (IRGA, model LI-6400, LI-Cor, USA). The A-PPFD curves were performed between 10:00 am and 2:00 pm, in one leaflet from the youngest expanded trifoliate from each plant replicate. The A-PPFD curves were estimated using the following PPFD values: 0, 25, 50, 100, 200, 400, 600, 800, 1000, 1200, 1400, 1600, 1800 and 2000µ mol photons $m^{-2}$ $s^{-1}$ for each gas exchange measurement with an IRGA. The A-PPFD curves were adjusted according to PRADO & MORAES (1997). We calculated several parameters from the curve adjustment: Apparent Quantum Efficiency (AQE), Light saturation Point (Psat), Light compensation Point (Pcom), Dark respiration (Rd), and the Maximum CO2 Assimilation (Amax). Additionally, we calculated the water use intrinsic efficiency as A/gs (WUIE, µmol $mol^{-1}$), and apparent carbolixilation efficiency as A/Ci (ACE,µmol $L^{-1}$) at light saturation point, where A=net $CO_2$ assimilation, gs=stomatal conductance, and Ci=intercellular $CO_2$ concentration.

The cell wall elasticity module ($\varepsilon$), osmotic potential at saturation ($\Psi$s), turgor loss point (TLP) and maximum symplastic water fraction (Ws) were derived from pressure-volume (PV) curves according to SCHULTE & HINCKLEY (1985) and ANDERSEN et al. (1991).

Crop yield was evaluated taking into consideration the plant leaf area (LA) measured using a portable leaf area meter (Model LI-3000A, Li-Cor, USA), the total aerial part dry mass ($DM_{AP}$), analysis of the biomass partitioning, and the leaf area ratio (LAR) (BENINCASA, 2003).

Data were submitted to a variance analysis (ANOVA P=0.05) and the means were compared by Tukey test (P<0.05).

## RESULTS AND DISCUSSION

The leaf water potential ($\Psi$a) was only reduced in plants that were grown under the 40% irrigation regime when the $\Psi$a was evaluated at 12:00 pm, independent of the cultivar tested, indicating a moderate water deficit. At pre-dawn, $\Psi$a values ranged from -0.5 to -0.3MPa between 40 and 100% of water irrigation, while at mid-day $\Psi$a approaches -1.2MPa under water deficit.

The Apparent Quantum Efficiency (AQE), Light Saturation Point (Psat), Light Compensation Point (Pcom), Respiration (Rd) and Maximum $CO_2$ Assimilation (Amax) (Table 1) were affected in different ways by water deficit comparing both cultivars. Overall, the results showed typical plant responses to water deficiency, including a loss of photosynthetic capacity and a reduction in total biomass production (CHAVES & OLIVEIRA, 2004; FLEXAS et al., 2006). Insofar as the relative water content and water potential decrease, the photosynthetic rate of the leaves also decreases. This decrease is due to the stomata limiting the entrance of $CO_2$, as well as restricting the photochemical and biochemical activities of photosynthesis (KAISER, 1987; CHAVES et al., 2002). The conventional cultivar showed a more expressive drop in Amax values under water deficit from 26 to 4.7 $\mu$mol CO2 m$^{-2}$ s$^{-1}$ (81 %), while the Amax values in the transgenic cultivar show a 52% of decreasing (Table 1). The higher decreasing in photosynthetic capacity in the conventional cultivar was due to mainly non stomatal causes than stomatal ones, once AQE and ACE values showed significant reductions under water deficit and, on the other hand, WUIE was increased (P<0.05) (Table1). The increase in the WUIE in plants grown with only 40% water replenishment showed that the plants had the ability to regulate gas exchange by reducing stomatal conductance proportionally more than the assimilation of $CO_2$, generating water economy for each molecule of $CO_2$ assimilated. This efficiency occurs because the partial reduction in stomatal conductance limits transpiration proportionally more than it limits $CO_2$ entry into the leaf (CHAVES & OLIVEIRA, 2004). Moreover, Psat, Pcom, and Rd values in the transgenic cultivar remains stable under water deficit, while in the conventional cultivar those values were reduced (P<0.05) (Table 1).

Depending on the duration and intensity of water deficiency, metabolic limitations are frequently observed, correlated with the loss of ATP, which, in

Table 1- Mean values of Apparent Quantum Efficiency (AQE), Saturation Point (Psat, $\mu$mol photons m$^{-2}$ s$^{-1}$), Compensation Point (Pcom, $\mu$mol photons m$^{-2}$ s$^{-1}$), Respiration (Rd, $\mu$mol m$^{-2}$ s$^{-1}$), water use intrinsic efficiency (WUIE, $\mu$mol $CO_2$ mol$^{-1}$ $H_2O$), apparent carboxylation efficiency (ACE, $\mu$mol $\mu$L$^{-1}$), Maximum $CO_2$ Assimilation (Amax, $\mu$mol m$^{-2}$ s$^{-1}$), Chlorophyll content index (CCI), Cellular electrolyte leakage (CEL, $\mu$S), of conventional (CONV) and transgenic (RR) cultivar plants subjected to the two irrigation regimes (100% and 40%). The capital letters represent significant differences (p<0.05) for each cultivar between the levels of water replacement and lowercase letters represent significant differences (P<0.05) among cultivars at each level of water replacement.

| Water replenishment | --------------------100%-------------------- | | --------------------40%-------------------- | |
| --- | --- | --- | --- | --- |
| Cultivars | CONV | RR | CONV | RR |
| AQE | 0.054[Aa] | 0.039[Ab] | 0.038[Ba] | 0.038[Aa] |
| Psat | 1421.34[Aa] | 548.22[Ab] | 314.26[Ba] | 384.63[Aa] |
| Pcom | 46.39[Aa] | 22.85[Ab] | 22.79[Ba] | 22.91[Aa] |
| Rd | -2.38[Bb] | -0.96[Aa] | -1.06[Aa] | -0.98[Aa] |
| WUIE | 52.87[Ba] | 52.39[Ba] | 143.96[Aa] | 155.30[Aa] |
| ACE | 0.067[Aa] | 0.058[Aa] | 0.042[Bb] | 0.070[Aa] |
| Amax | 26.05[Aa] | 21.30[Aa] | 4.75[Bb] | 10.27[Ba] |
| CCI | 17.18[Bb] | 28.67[Ba] | 29.67[Aa] | 33.85[Aa] |
| CEL | 14.40[Ba] | 11.92[Aa] | 33.30[Aa] | 18.62[Ab] |

turn, decreases the regenerative ability of RuBP (PARRY et al., 2002). This reduction in ATP synthesis is due to decreased electron transport and photophosphorylation caused by the loss of membrane-associated reactions. The membranes suffer structural damage because of the reduction in free water (LAWLOR & CORNIC, 2002). The results from the electrolyte leakage analysis (Table 1) indicated that the cellular membranes of leaves from conventional cultivars had significant more damage caused by water deficiency than those from the transgenic cultivars grown under the same water conditions.

The results derived from the PV curves (Table 2) showed that, in general, the turgor loss point (TLP) was reduced in all treatments under water deficiency, without significant difference between both cultivars. On the other hand, Ws % and RWC at TLP values remains stable among treatments, except by an increase in RWC at TLP in the conventional cultivar under water deficit. The water deficiency tended to increase e (volumetric elasticity module), mainly in the conventional cultivar (Table 2). Changes in the cell wall elasticity play an important role in the plant's ability to respond to water deficit. Water loss in plant tissues controls the turgor pressure, affecting the elasticity of cell wall. However, water deficiency can result both in the increase and reduction of cell wall elasticity, and this can be a species-specific characteristic. The loss of elasticity (higher values of e) is related to a higher lignification of tissues, phenolic cross-linking and a reduction of extensin synthesis, while the loosening of the wall is associated with regions of growth, mediated by complex physiological and biochemical processes (MOORE et al., 2008). Our results indicate a loss of cell wall elasticity in response to the water deficiency in both cultivars, which, overall, led to a reduction in TLP values. Thus, plants undergoing water deficiency increased their tolerance to dehydration, losing cellular turgor at lower water potentials. According to DICHIO et al. (2003) high values of e

improve drought tolerance avoidance by stomatal control of transpiration because the TLP is reach faster, closing stomata quickly, avoiding excessive water loss.

Overall, the water deficiency significantly reduced the total aerial part dry mass and pod dry mass of the plants. However, the ratio of the pod dry mass to the aerial part dry mass ($DMp/DM_{AP}$) was significantly increased (P<0.05) for the conventional cultivar plants under 40% water replenishment, remaining stable in the transgenic one (Table 3). The results of the production analysis showed a tendency towards sustaining the allocation of resources to the pods in the transgenic cultivar, and an increase of this allocation in the conventional cultivar under water restriction, which is thought to be a drought-resistance mechanism for the maintenance of seed production. According to FRENCH & TURNER (1991), a moderate water deficiency can lead to a biomass reallocation to produce grain, which, in the case of *Lupinus angustifolius*, was expressed as an increase in the number of seeds per pod. The same pattern of reallocation has been observed in corn, mainly in strains more tolerant to water deficit (MACHADO et al., 1992). Total leaf area (LA) was higher in the conventional cultivar in both water conditions than transgenic one, but it was reduced (P<0.05) in both cultivars under water deficiency. The relationship between LA and $DM_{AP}$ (LAR) was also higher in the conventional cultivar, regardless water condition, indicating lower carbon assimilation efficiency of the available leaf area. However, in both cultivars under water deficiency, LAR values were reduced, indicating that the same leaf area can supports a higher dry mass production (Table 3).

## CONCLUSION

Concluding, our data showed that both cultivars had similar effects, caused by water deficiency, on dry mass production, but the transgenic cultivar tended to maintain higher biomass allocation in pods,

Table 2 - Turgor Loss Point (TLP), Maximum Symplastic Water Fraction (Ws %), Cellular Elasticity Module (ε) and Relative Water Content at the turgor loss point (RWC at TLP %), from conventional (CONV) and transgenic (RR) cultivar plants subjected to the two irrigation regimes (100% and 40%). The capital letters represent significant differences (P<0.05) for each cultivar between the levels of water replacement and lowercase letters represent significant differences (P<0.05) among cultivars at each level of water replacement.

| Water replenishment | ----------------100%---------------- | | ----------------40%---------------- | |
|---|---|---|---|---|
| Cultivars | CONV | RR | CONV | RR |
| TLP | -3.89[Aa] | -3.34[Aa] | -15,71[Ba] | -10.88[Ba] |
| Ws | 82.18[Aa] | 87.10[Aa] | 80.82[Aa] | 75.68[Aa] |
| ε | 2.10[Ba] | 1.53[Ba] | 14.11[Aa] | 9.575[Ab] |
| RWC at TLP | 60.26[Bb] | 79.20[Aa] | 73.33[Aa] | 73.89[Aa] |

Table 3 - Total aerial part dry mass ($DM_{AP}$ (g)), ratio of pod dry mass to aerial parts dry mass (DMV/DMAP), pod dry mass ($DM_V$ (g)), total leaf area (LA, cm²) and leaf area ratio (LAR, $cm^2 \, g^{-1}$) from conventional (CONV) and transgenic (RR) cultivar plants subjected to the two irrigation regimes (100% and 40%). The capital letters represent significant differences (P<0.05) for each cultivar between the levels of water replacement and lowercase letters represent significant differences (P<0.05) among cultivars at each level of water replacement.

| Water replenishment | ----------------100%---------------- | | ----------------40%---------------- | |
|---|---|---|---|---|
| Cultivars | CONV | RR | CONV | RR |
| $DM_{AP}$ | 55.57[Aa] | 50.14[Aa] | 27.85[Ba] | 20.62[Ba] |
| $DM_V$ | 20.31[Ab] | 27.59[Aa] | 14.92[Ba] | 12.54[Ba] |
| $DM_V/DM_{AP}$ | 0.358[Bb] | 0.552[Aa] | 0.547[Aa] | 0.611[Aa] |
| LA | 3383.60[Aa] | 2468.80[Ab] | 1495.80[Ba] | 937.40[Bb] |
| LAR | 62.68[Aa] | 50.58[Ab] | 54.19[Ba] | 44.29[Bb] |

as well as, higher efficiency of leaves to support dry mass production (LA/ $DM_{AP}$) than conventional cultivar in both water conditions. Moreover, the higher Amax values and lower membranes damages in the transgenic cultivar under water deficiency showed that the expected higher drought tolerance of the conventional cultivar (PITOL & BROCH, 2009) was not supported by a more accurate physiological investigation.

## ACKNOWLEDGMENTS

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