Physiological Evaluation of Drought Tolerance in Brazilian Soybean Cultivars : Water Use Efficiency and Carbon Isotope Discrimination

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Abstract

A significant negative linear correlation between carbon isotope discrimination (CID) and water use efficiency (WUE) in soybean was demonstrated in preliminary pot experiments, suggesting that cumulative mean of WUE in field-grown soybean plant can be estimated by measuring of CID.

In two consecutive seasons (2003/2004 and 2004/2005), a total of ten cultivars of Brazilian soybean were field cultivated at Embrapa-Soja, Londrina, Parana, Brazil, under automated shelters for rain-out treatment (RS) for approximately one month after first flowering (R1 to R5 stage), as well as in no-shelter plots of irrigated (IR) treatment and rain-exposure/non-irrigated (NI) controls.

Leaves and pods were sampled on several occasions around the rain-out treatment till harvest for analysis of carbon isotope discrimination (CID). CID of leaves was around 21 and 22‰ in all cultivars during the flowering stage in the IR plot, whereas CID of pods was always 1 to 2‰ lower than that of leaves. Under NI plots, CID was almost the same as IR plots, especially in the 2003/2004 season when there was not a prominent drought problem. Drought-tolerant cultivars (BRS-183 and BRS-184) maintained a relatively high CID value compared to sensitive cultivars (BR-16 and BRS-232) under RS treatment in the field. These findings agreed with higher dry matter production in terms of photosynthetic rate. For both seasons, grain yield was positively correlated with CID in overall plots (3 treatments x 10 cultivars), although the correlation was significant only if CID for pods at the end of the rain-out treatment (most drought-stressed point) was considered.

From the current CID study, the mechanism of drought-tolerance of BRS-183 and BRS-184 cultivars is assumed to be based on maintenance of a superior internal water condition under stress. This hypothesis is supported by results for relative water content, always higher in drought-tolerant cultivars. Therefore, these cultivars are neither water-use efficient or water-saving, but have the ability for greater water uptake from drying soil, likely from higher root mass/activity or deeper root distribution.

Introduction

Terminal drought is one of the most significant limiting factors for many crops (reviewed by Subbarao *et al.* 2004), especially soybean yields (Doss *et al.* 1974, Sionit and Kramer 1977). Preventative measures include changing sowing dates and/or adoption of cultivars of varying maturities, effective methods for diminishing the influence of severe drought at the reproductive growth stage. However, as it is difficult to predict droughts, cultivars with high drought tolerance are strongly preferred.

Water use efficiency (WUE), the amount of dry matter produced by plants per unit of water, under water-limited conditions is closely associated with drought tolerance and type of tolerance mechanism. Although WUE comparison between soybean cultivars is important for elucidation of the drought tolerance mechanism, direct measurement of WUE in field-grown crops is not feasible.

When intracellular CO_2 concentration is high, RuBP carboxylase/oxygenase (RuBisCO), the key enzyme of photosynthetic carbon assimilation in C_3 plants, fixes more $^{12}CO_2$ than $^{13}CO_2$, a higher carbon isotopic discrimination (CID) rate. If the CO_2 concentration is decreased by stomatal closure or other causes, the enzyme fixes $^{12}CO_2$ and $^{13}CO_2$ at a ratio more close to that of air, i.e. CID is lowered. Farquhar and Richards (1984) found that WUE and CID are inversely correlated in wheat, with the same relationship reported in several C_3 plant species (reviewed by Farquhar *et al.* 1989). This is because higher WUE is theoretically attained by a lower transpiration, related to higher stomatal resistance (see Fig. 1 for a schematic explanation). To determine whether this relationship also exists for soybean plants, pot experiments, accommodating control of water supply and precise measurement of quantity of water transpired by plants, were performed.

Field experiments were conducted in Brazil using rain-out shelters, aiming to observe the traits associated with dry matter production and WUE, estimated by CID, in Brazilian soybean cultivars with differing degrees of drought tolerance.

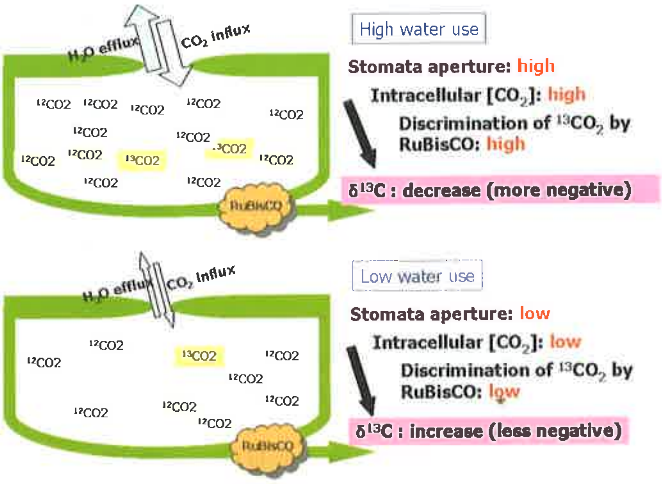

Fig. 1. Schematic explanation of relationship between water use and $\delta^{13}C$ under well-watered (top) and water-stressed (bottom) conditions.

Materials & Methods:

Pot experiments

Preliminary pot experiments were conducted in a glasshouse in Tsukuba, Japan. Four soybean cultivars were used: Conquista (drought tolerant and adapted to lower latitudes), BRS-183 (drought tolerant and adapted to higher latitudes), BRS-185 (drought-sensitive) and Aurora (a cultivar from Paraguay with a deep root system). From the beginning of flowering (44 days after sowing), a proportion of each cultivar was water-stressed by withdrawal from regular irrigation. WUE was calculated using the equation:

WUE= [Total above-ground matter] / [Total water transpired] ($g\ L^{-1}$)

Field experiments

Field experiments were conducted in two consecutive growing seasons (2003/2004 and 2004/2005) at the Soybean Research Center, Brazilian Agriculture Research Cooperation (Embrapa Soja) in Londrina, Parana, Brazil.

Ten determinate soybean cultivars bred by Embrapa Soja were used. All were adapted to and recommended for southern regions (lower latitudes) of Brazil, specifically Parana State. Previous studies by Embrapa Soja characterized BR-16 as markedly less drought tolerant and BRS-183 and BRS-184 be drought tolerant (Oya *et al.*, 2004). Standard cultural practices for soybean in Brazil were employed, including soil fertilization and Rhizobium inoculation.

Three water regimes (IR, NI and RS) were assigned to main plots with four replicates and ten cultivars arranged randomly in sub-plots in each main plot. Plants in the IR plots were irrigated manually when soil water potential decreased to -0.05 MPa at a depth of 30 cm. No water was applied to plants in the NI plots under rain-fed conditions. Plants were artificially drought-stressed by sheltering from rain in RS plots for approximately one month after first flowering.

Physiological measurements

Gas exchange rate (photosynthesis and respiration) was measured for the youngest expanded trifoliate using Li-Cor 6200 under natural light and CO_2 condition in field experiments, plus using Li-Cor 6400 under artificial light in glasshouse experiments.

An approximately 3.5 cm^2 leaf sample was punched out from field-grown soybean plants to estimate internal plant water status. Leaf samples were immediately measured for fresh weight (FW), then immersed in distilled water at 25℃ in the dark for 24 hours. Full turgid weight (TW) of the sample was measured, then samples were dried in a convection oven at 85℃ for 48 hours to determine dry weight (DW). Relative water content (RWC) of leaves was calculated as follows:

RWC (%) = (FW-DW)/(TW-DW) x 100

CID measurement

Leaf or pod samples were analyzed for carbon isotope discrimination (CID) at JIRCAS, Tsukuba, Japan, using an isotope ratio mass spectrometer (IRMS; Delta XP^{plus}, ThermoFinnigan, Hamburg, Germany) connected to an element analyzer (EA; Model 1112, Carlo Erba, Milan, Italy). Total carbon in leaf or pod samples was incinerated in the EA furnace, then separated out as pure CO_2 gas. An adequate quantity of gas was introduced to the IRMS to measure $^{13}CO_2/^{12}CO_2$ ratio based on their different molecular mass (45/44). Normally the ratio (R) of $^{13}C/^{12}C$, in plants and other living tissue is expressed as $\delta^{13}C$ (‰) using the equation below, where $R_{standard}$ is internationally accepted to be 1.1237×10^{-2} of a Belemnite from the PeeDee formation (PDB).

$$\delta^{13}C(‰) = (R_{sample} - R_{standard}) / R_{standard} \times 1000$$

CID (Δ) is calculated against $\delta^{13}C$ of air ($\delta^{13}Ca$) fixed at -8‰, and converted to a value of approximately +20‰ in C_3 plants using the equation.

$$\Delta (‰) = (\delta^{13}Ca - \delta^{13}C)/(1+ \delta^{13}C/1000)$$

Results and Discussion:

Yield

Yields in 2003/2004 and 2004/2005 seasons were compared (Fig. 2a & 2b). Yields of IR (irrigated) plots, representing the maximum potential yield for each cultivar, were similar in each season. Yield of RS (rain-out) plots, representing the harvest most likely to be retarded by severe drought, also had similar values between seasons.

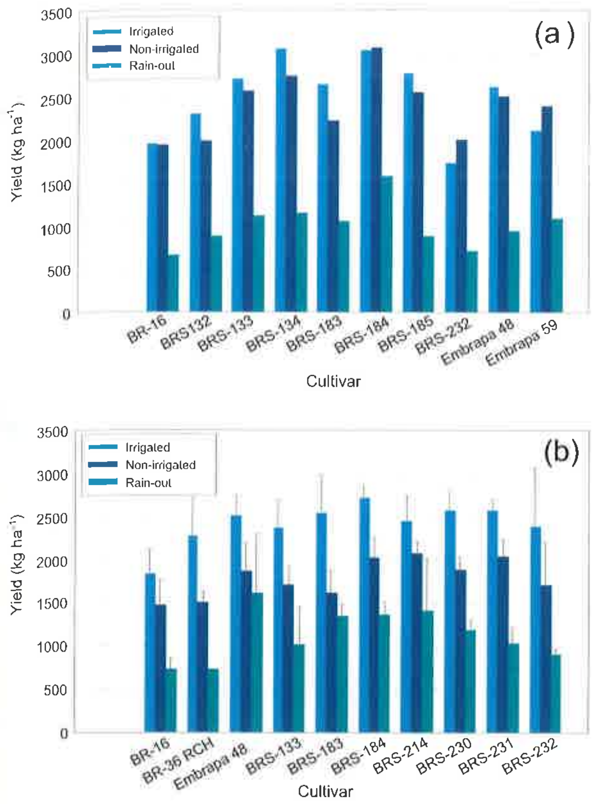

Fig. 2. Yield at harvest of Brazilian soybean cultivars under different water regimes in 2003/2004 (a) and 2004/2005 (b) crop seasons.

However, yield tendency in the three water regimes differed, with IR = NI (rain-fed) > RS (rain-out) in the non-drought 2003/2004 season (Fig. 2a) and IR > NI > RS in the drought-prominent 2004/2005 season (Fig. 2b). Therefore, rain-out treatment at the flowering stage is able to reproduce drought effects well.

Photosynthetic rate response to drought stress

Photosynthetic rate during rain-out treatment was examined for all 10 cultivars. Figure 3 shows the time course variation for rate of net photosynthesis of four soybean cultivars under three water regimes in the 2003/2004 season. Drought-sensitive BR-16 (3a) and BRS-132 (3b) cultivars, plus drought-tolerant BRS-183 (3c) and BRS-184 (3d) cultivars were evaluated. There was no difference in the rate between irrigated (IR) and non-irrigated (NI) treatments for all cultivars, indicating that rainfall was sufficient for soybean growth and there was no water stress in the 2003/2004 season at least around Londrina. With use of the rain-out shelters, soybean plants were subjected to water stress from 49 days after seeding (DAS). Though photosynthetic rate was decreased after rain-out treatment for all cultivars, drought-tolerant BRS-183 and BRS-184 cultivars maintained a relatively high rate (greater than 15 μg CO_2 m^{-2} s^{-1}), as compared to drought-sensitive BR-16 and BRS-132 cultivars at 18 days of the treatment. When the rain-out treatment was prolonged (27 days), photosynthetic rate was further

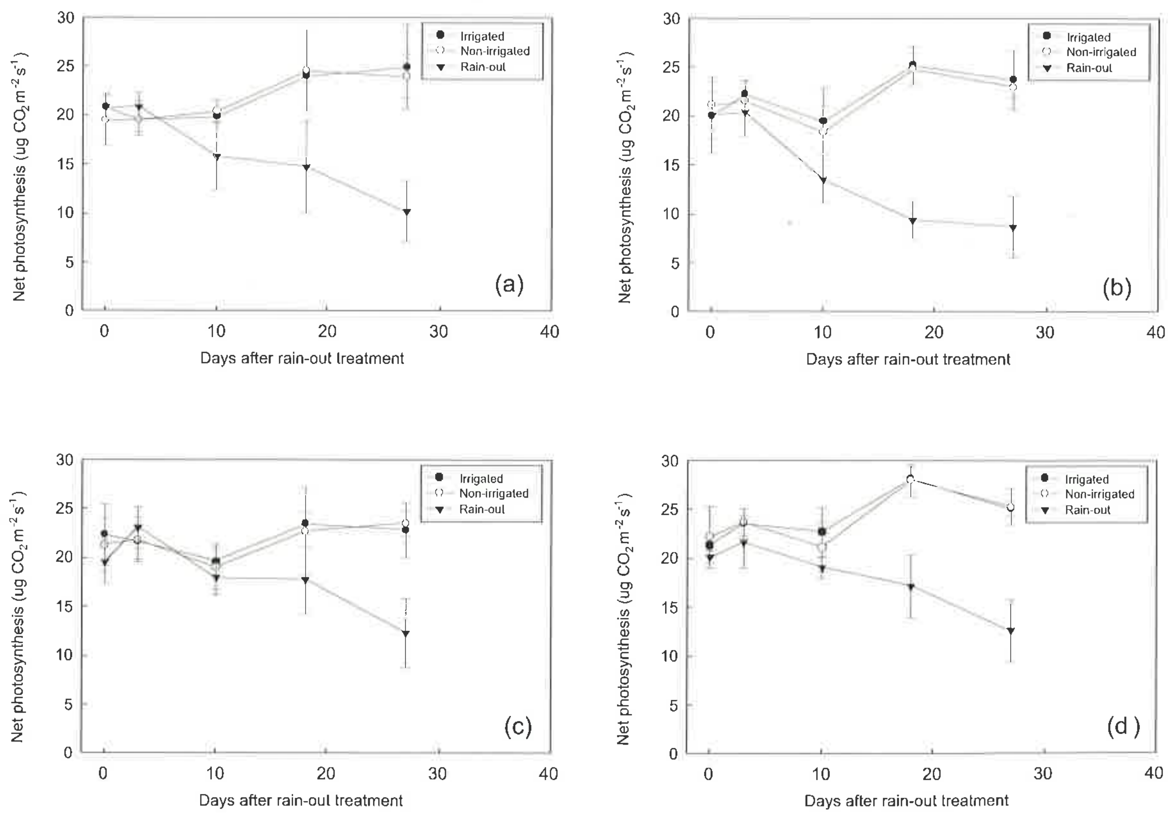

Fig. 3. Net photosynthetic rate during rain-out treatment in the 2003/2004 crop season for drought susceptible BR-16 (a) and BRS-132 (b); plus drought tolerant BRS-183 (c) and BRS-184 (d) soybean cultivars.

Table 1. Net photosynthetic rates (μg CO_2 m^{-2} s^{-1}) at 64 and 71 DAS (17 and 24 days of rain-out treatment, respectively) in leaves of drought-sensitive (BR-16 and BRS-232) and drought-tolerant (BRS-183 and BRS-184) soybean cultivars in the 2004/2005 season.

Water regime	Cultivar	At 64 DAS (17 days of treatment)	At 71 DAS (24 days of treatment)
NI	BR-16	14.4 ± 9.1	14.8 ± 6.0
	BRS-232	18.1 ± 6.9	15.6 ± 5.0
	BRS-183	23.4 ± 7.0	18.0 ± 1.3
	BRS-184	22.1 ± 3.2	19.1 ± 4.4
RS	BR-16	14.4 ± 4.1	10.2 ± 1.7
	BRS-232	20.2 ± 7.8	6.2 ± 2.5
	BRS-183	16.2 ± 6.7	12.7 ± 5.5
	BRS-184	24.2 ± 1.8	12.0 ± 5.2

reduced in all cultivars. BRS-183 and BRS-184 maintained higher photosynthetic rates of approximately 12 μg CO_2 m^{-2} s^{-1}, whereas those of other cultivars were 5 to 10 μg CO_2 m^{-2} s^{-1}.

The difference in drought tolerance between cultivars was reproduced in the 2004/2005 season (Table 1), as evaluated by photosynthetic dry matter production, although BRS-132 was replaced by BRS-232 as a drought-sensitive cultivar. Drought-tolerant BRS-183 and BRS-184 maintained a much higher photosynthetic rate (more than 12 μg CO_2 m^{-2} s^{-1}) than other cultivars, even after 24 days of rain-out treatment. These cultivars thus demonstrated an ability to maintain higher rates of dry matter production under drought stress.

Relationship between water use efficiency and carbon isotope discrimination

Results from pot experiments are shown in Figure 4 (WUE) and Figure 5 (CID). WUE (g L^{-1}), dry matter assimilated per liter of water, was significantly higher in Conquista than Aurora and BRS-183 cultivars under well-watered conditions (Fig. 4). This likely indicates a difference in water requirements of soybean cultivars. In contrast, for water-stressed plants after 24 days of limited water supply, WUE increased in all cultivars, with no significant difference between cultivars. Further studies should concentrate on the effect of water stress on WUE and growth. Instantaneous observation at 24 days after water stress also indicated that WUEi was highest in Conquista (10.2 μmol CO_2/mol H_2O), whereas 8.8, 8.0 and 7.2 μmol CO_2/mol H_2O in BRS-185, BRS-183 and Aurora cultivars, respectively.

As shown in Figure 5, CID (Δ) of well-watered Conquista leaves was significantly lower (17.7‰) than other cultivars (approximately 19.0‰). Even under optimal water conditions, intracellular CO_2 concentration of Conquista cultivar would be lower presumably due to its unique stomatal response. Under water stress, Δ decreased to be between 17.2‰ and 17.9‰ for all cultivars, indicating an increase in stomatal resistance. This finding was confirmed by instantaneous measurement of

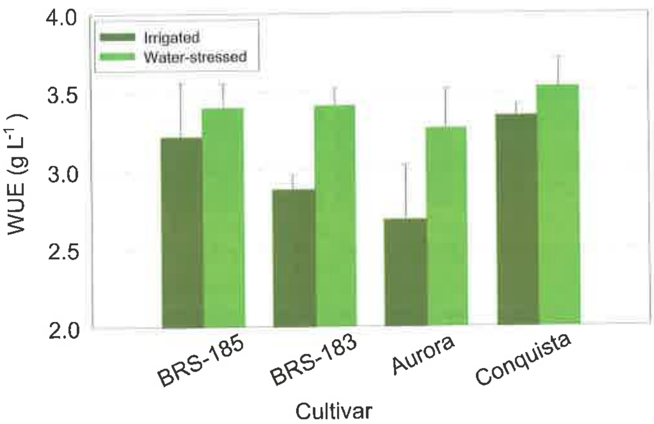

Fig. 4. Water use efficiency of pot-grown soybean cultivars with or without water stress after 24 days.

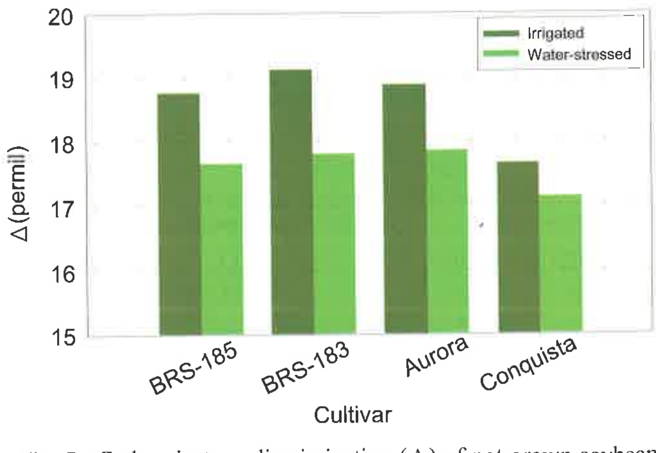

Fig. 5. Carbon isotope discrimination (Δ) of pot-grown soybean cultivars with or without water stress after 24 days.

transpiration rates during water stress treatment (data not shown).

Figure 6 shows the relationship between WUE and Δ in water-stressed (24 days) and non-stressed soybean cultivars at 68 days after sowing. A negative linear correlation was observed between these parameters with a high correlation

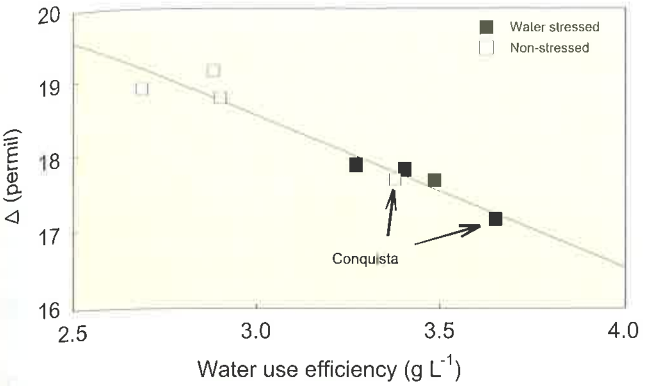

Fig. 6. Relationship between water use efficiency and carbon isotope discrimination (Δ) in water-stressed or non-stressed soybean plants grown in pots.

coefficient (r^2=0.929). Therefore, estimation of water use efficiency in field-grown soybean plants using the measurement of Δ appears practical.

WUE in Conquista was much higher than other cultivars both under normal and water-stressed conditions (see arrows in Figure 6). Therefore, this cultivar must have some mechanism for saving water during dry matter production, to explain its drought tolerance.

Carbon isotope discrimination in field-grown plants

Plant samples were taken from field experiments with rain-out shelters both in the 2003/2004 and 2004/2005 seasons to estimate WUE under field conditions. Before, during, and after rain-out treatment plus at harvest, leaves and pods were analyzed for CID (Δ_L and Δ_P, respectively). It was generally observed an approximately 2 to 3‰ difference in CID values of leaf and pod tissues from the same plant sample. The above might be caused by differences in protein content and/or amino acid composition in distinct plant parts.

Results are described separately for 2003/2004 and 2004/2005 seasons, because of different climatic conditions, agronomic calendar and plant materials used between the seasons

2003/2004 season

Before treatment (only leaves available at 48 DAS), Δ_L ranged from 20.5 to 21.5‰ with no difference observed between cultivars or treatments (Fig. 7). After 25 days of drought treatment (74 DAS), Δ_L of RS plots was reduced to between 19.5 and 20.5‰, whereas Δ_L was unchanged in IR and NI plots. Δ_P of RS plots dramatically decreased (17.8 to 19.0‰), with the exception of BRS-183 (19.5‰), equivalent to Δ_P in IR and NI plots (Figure 8). After drought treatment, the same tendency was observed in RS plots. At 88 DAS (11 days after resuming from drought stress), Δ_P of BRS-183 was still markedly higher (19.2‰) than other cultivars (17 to 18‰) as shown in Figure 9. Δ_P of all cultivars in the RS plot recovered to 18.5 to 19.5‰ at

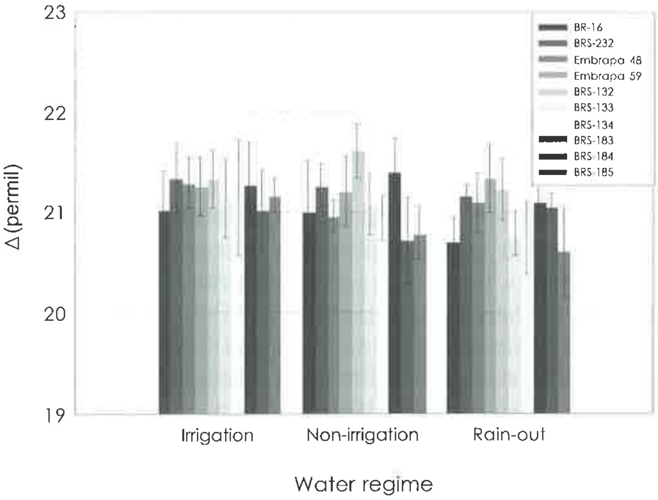

Fig. 7. Effect of water regime on carbon isotope discrimination (Δ) in soybean leaves at 48 DAS (before drought treatment).

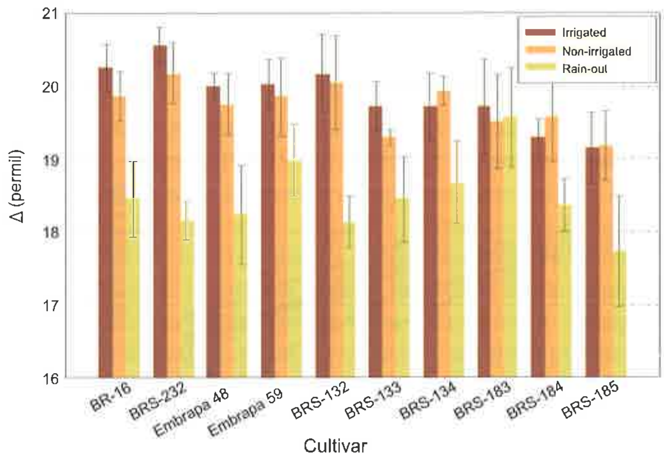

Fig. 8. Effect of water regime on carbon isotope discrimination (Δ) in soybean pods at 74 DAS (25 days after drought stress).

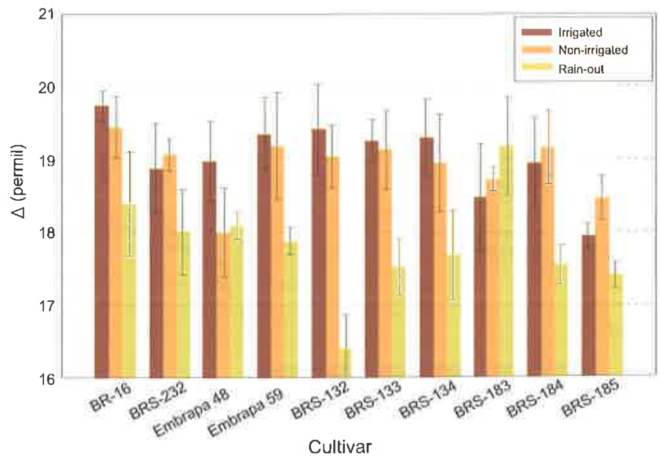

Fig. 9. Effect of water regime on carbon isotope discrimination (Δ) in soybean pods at 88 DAS (11 days after resuming from drought stress).

harvest (40 days after resumption), however BRS-183 was 19.8‰. From these results, BRS-183 is assumed to maintain a relatively high concentration of intracellular CO_2 even under drought stress. Therefore for BRS-183, Δ_P in the rain-out plot was equal to Δ_P for irrigated and rain-fed plots. Although BRS-183 had been demonstrate to be drought tolerant in several field trials (Oya *et al.* 2004), yields for this cultivar during this season were not great (Fig.1). This finding suggests that the final yield of soybean is likely to be determined not only by dry matter production but also other physiological, phenological and agronomical characteristics (e.g., harvest index), for which more investigations are necessary.

2004/2005 season

Similar to the previous season, effect of water-deficit was clearly apparent from CID values. Regardless of cultivar difference, Δ_L (CID in leaves) before rain-out treatment (42 DAS) averaged 21.9‰ in all treatments. Δ_L decreased gradually to 20.7‰ after 22 days of rain-out (RS) treatment and 21.1‰ in irrigated (IR) plots for overall cultivars on the same day. Time course of Δ_L in RS plots is presented in Figure 10. For CID in pods (Δ_P), effect of

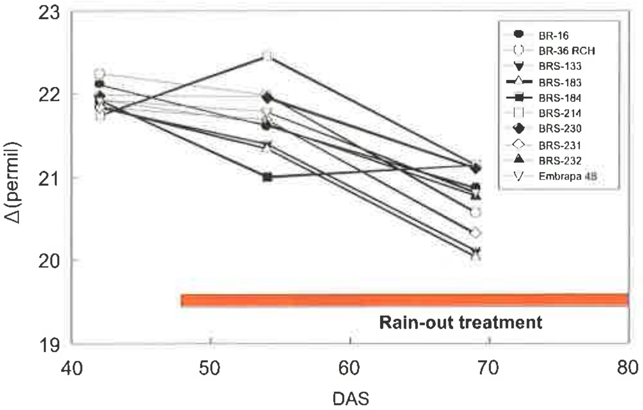

Fig. 10. Time course of carbon isotope discrimination (Δ) in leaves of soybean plants under rain-out treatment.

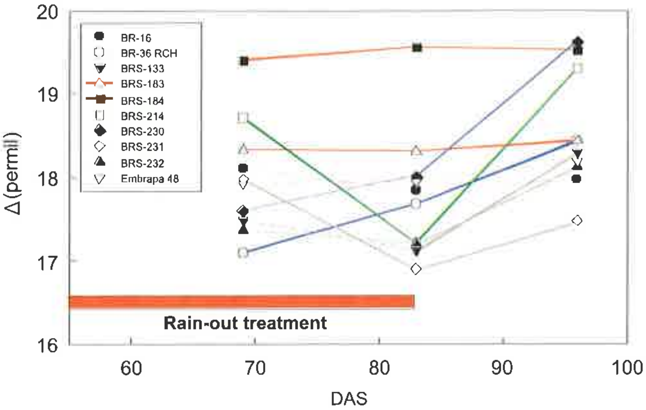

Fig. 11. Time course of carbon isotope discrimination (Δ) in pods of soybean plants under rain-out treatment.

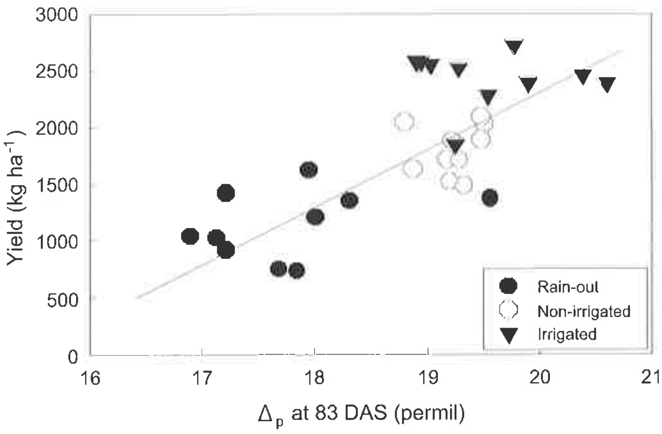

Fig. 12. Relationship between yield and carbon isotope discrimination (Δ) in pods of soybean plants at 83 DAS (end of rain-out treatment).

rain-out treatment was most obvious just after RS treatment (83 DAS), thereafter Δ_P tended to recover until harvest (Fig. 11). Δ_P in RS of drought-tolerant BRS-184 was outstanding and stable (19.5‰) at least during measurement, followed by another drought-tolerant cultivar, BRS-183, with no decrease in Δ_P at 83 DAS. Some cultivars showed a quick increase in Δ_P after rain-out treatment finished (BRS-214 and BRS-230). Yields correlated with Δ_P at each measurement with the best linear correlation (r^2=0.556, $p<0.001$) at 83 DAS and the final yield (Fig. 12). CID can be applied as a quantitative criterion for drought tolerance in soybean in the field. However, for each water regime, the relationship between yield and CID was less obvious, compared to overall results. Therefore, further characterization would be necessary to establish the CID technique for use of varietal screening.

Relative water content

RWC of soybean plants at the stage before flowering

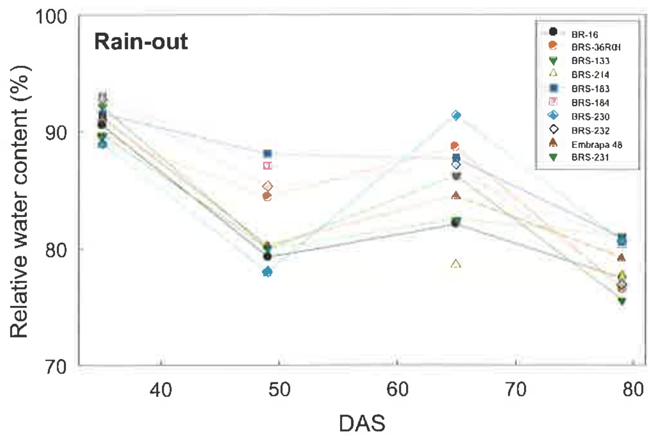

Fig. 13. Change in relative water content in leaves of soybean plants subjected to rain-out treatment.

did not differ between cultivars (approximately 92% for all water regimes). During the flowering stage, RWC of all cultivars in all water regimes decreased, presumably from ageing of leaves. In RS plots (Figure 13), drought-tolerant BRS-183 and BRS-184 cultivars had relatively higher RWC (89%) than drought-sensitive BR-16 (81%) cultivar at 49 and 65 DAS, the beginning and middle of rain-out treatment. This indicates superior water status in leaves of drought-tolerant cultivars, likely due to better water uptake ability.

Results from field experiments for two consecutive years demonstrate that estimation of water use efficiency (WUE) from analysis of carbon isotope discrimination (CID) or Δ value is practical. Drought tolerant cultivars, BRS-183 and BRS-184 were not superior in water use efficiency, however tolerance is likely due to enhanced water uptake ability from underground characteristics. Attempts should be made to differentiate water uptake ability between cultivars for more straightforward characterization of drought tolerance, using new techniques such as xylem exudation rate (Tobita *et al.* 2002).

Cultivar Conquista demonstrated an alternative, say, water-saving mechanism, indicated by markedly decreased Δ after water stress. This may lead to the way of pyramiding of different traits associated with drought tolerance, for breeding of elite cultivars better adapted to more diverse drought-prone environments. It is worthwhile to compare dry matter production and yield among drought-tolerant cultivars with different mechanisms. However, the cultivar Conquista could not be cultivated well in the field of Embrapa-Soja with rain-out shelters, because of its inadaptability to higher latitudes.

Δ is a useful option for screening of drought tolerance as: 1) it reflects water use efficiency over the entire crop season; 2) it is an objective and quantitative index; and 3) it is easy to measure if IRMS is available. Gene tagging associated with CID has already commenced in a research group in Australia (Farquhar, 2006). Yield is an integrated outcome of complicated physiological processes, with carbon isotope discrimination of the photosynthesizing enzyme only one measurement option, although a very useful one, especially in water-deficient environments.

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