

TESTING DREB GENETICALLY MODIFIED SOYBEAN PLANTS FOR DROUGHT TOLERANCE IN BOTH GREENHOUSE AND FIELD CONDITIONS

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The development of drought tolerant plants is highly desirable as areas suffering with drought are due to increase in the future. One strategy being used for this purpose is the genetic engineering of plants with transcription factors that regulate the expression of several genes related to abiotic stress. The *DREB1A* and *DREB2A* genes driven by the stress inducible promoter, *rd29A* were introduced into a drought-sensitive soybean cultivar BR16 through biolistics. From the lines obtained we evaluated drought tolerance and yield performance of the genotypes: DREB1A-P58, DREB1A-P1142, DREB2A-P2193 and a crossing between the cultivar BR16 and DREB1A-P58, named 09D-0077. Drought was simulated in the greenhouse by exposing the plants to progressive soil drying in pot culture. Testing DREB plants in the field is particularly important, considering that few studies have reported results in realistic field conditions. Field performance was evaluated in plots under three different water regimes, irrigated, natural drought (non-irrigated) and stressed by sheltering the plots from rain at the vegetative or reproductive stages. Occurrence of natural drought was verified through meteorological data obtained at the site. We were able to show that DREB genetically modified (GM) plants had a higher survival rate than its non-GM isoline BR 16, after severe water stress in both greenhouse and field conditions, and that, under stress, DREB plants had a higher relative growth ratio (RGR). Although DREB plants did not outperform the cultivar BR16 in terms of yield, there was a clear tendency of superiority for some yield components such as number of seeds, number of pods with seeds and total number of pods when stress was applied at the vegetative stage. Further studies are needed to better characterize the conditions in which these plants may outperform the non-transformed parental. Plant drought responses are influenced by diverse plant-soil-atmosphere interactions and also by the time, intensity, duration and frequency of the stress. More insights into the mechanisms of stress tolerance of DREB soybean plants are been investigated in our on-going studies.

Testing DREB genetically modified soybean plants for drought tolerance in both greenhouse and field conditions



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Introduction

- Drought tolerant plants are highly sought as areas with drought and limitations in water supply are due to increase in the future
- One approach been used for this purpose is genetic engineering of plants using Transcription Factors (TFs). TFs regulate the expression of several downstream genes responsive to abiotic stresses
- One such class of transcription factors is the dehydration-responsive element-binding proteins (DREBs) that are transcriptionally up-regulated by water deficit or temperature
- DREB proteins are classified in two groups: *DREB1* (*DREB1A*, *DREB1B*, *DREB1C*, *DREB1D*, *DREB1E* e *DREB1F*) e *DREB2* (*DREB2A* and *DREB2B*)
- Overexpression of *DREB1A* in plants such as rice, *A. thaliana* and tobacco under the control of the CaMV 35S promoter resulted in increased tolerance to high salinity, freezing and drought, however it also caused growth retardation.
- An alternative to the use of the constitutive CaMV 35S promoter is the use of the stress inducible promoters such as the *rd29A* that apparently overcomes the difficulties encountered when using the 35S promoter.

Introduction (cont.)

- Overexpression of the DREB2A in plants such as *A. Thaliana*, sunflower, wheat and *Chrysanthemum* improved tolerance to drought and heat.
- Soybean plants over-expressing the DREB1A and DREB2A genes under the control of the rd29A promoter has been generated in our lab and have been tested for drought stress tolerance in greenhouse and field.
- In the greenhouse they showed the slow wilting phenotype.
- Our studies are focused in the DREB1A lines P58 and P1142, one DREB2A line (P2193) and a crossing DREB1A-P58 x BR 16, named 09D-077.
- We compare this lines with the cultivars BR 16 (used in the transformation and considered drought sensitive), and Embrapa 48 considered drought tolerant

Characteristics being analyzed



Growth: height, number of nodes, leaf area, leaf area index, fresh and dry weight of shoots and leaves

Plant physiology and biochemistry: RWC, chlorophyll, gas exchange, water and osmotic potential, transpiration, transpiration efficiency, RGR, sap flow, NDVI, proline, ureides (allantoic acid and allantoin), electrolyte leakage

Soil conditions: matric potential, gravimetric humidity

Environ. conditions: T°C/RH%

Yield components: seed number, number of pods with seeds, total number of pods, 100seed weight, AHI)

Main aims

•To verify if the acquirement of water stress tolerance can be achieved without any detrimental effects on plant growth, development and yield



•For this we have been testing the plant s performance under field conditions in drought and non-drought environments

•To give insights towards the tolerance mechanism of the transformed plants



Stress simulation

Stress simulation

- **Field:**

Water stress simulation, in the field, by sheltering the plots from rain at the V and R stages using rain-out shelters

*2009/2010: Reproductive
2010/2011 and 2011/2012:
Vegetative and Reproductive*

- **Greenhouse:**

Greenhouse plants were exposed to progressive soil drying in pots

Testing DREB plants in the field is particularly important, considering that few studies have reported results in realistic field environmental conditions (Saint Pierre et al 2012).



Greenhouse experiments



Material and Methods - Greenhouse experiment

- Soybean seeds of BR16, P58 and P1142 lines germinated on filter paper in growth chamber (25 ± 1 °C and RH 100%).
- Seedlings cultivated into 3.0 kg pots containing soil:manure:sand (2:2:1; 39% WHC) in well-watered conditions (70% WHC) until V_4 stage (Fehr et al. 1971).
- T and RH recorded every 5min (thermo-hygrograph Hobo U14-002, Onset®).
- $VPD = (100 - RH) / 100 * VP_{sat}$ (kPa). (VP_{sat} calculated using psychrometric chart at <http://physics.holsoft.nl/physics/ocmain.htm>)
- At 30 DAP (V_4), pots divided in three subsets:
 - 1) Six plants/genotype were harvested, divided in roots and shoots to estimate the initial dry plant biomass;
 - 2) Drought stressed treatments;
 - 3) Control.
- Randomized Complete Block Design, in factorial arrangement, with three Reps.
- Treatments:
 - a) Two water regimes (control-C and drought stressed-DS) and;
 - b) Three genotypes (cultivar BR 16 and DREB1A lines: P58 and P1142)

Measurements - Greenhouse experiment

- **Transpiration** - Prior to the stress initiation at 30 DAP, the pots of both C and DS treatments were saturated with water and left to drain the excess water overnight. In the following morning, the pots were enclosed in polythene bags to prevent evaporation from the soil surface. Thereafter, the pots were weighed every morning between 09:00 and 10:00 o'clock. In the control plants water loss by transpiration in a previous day was compensated in the following day by adding water to 70% of the field capacity. In the DS plants, field capacity of the pot was progressively adjusted to 40% and thereafter 20%. The transpiration of each plant was calculated as the difference in pot weights on successive days plus water added on the previous day.
- **Transpiration Efficiency** - At the end of the experiment the plants were harvested and divided in roots and shoots to estimate final dry plant biomass. The total transpiration was calculated as the sum of daily transpiration from the initial day when plants were bagged to the day when plants were harvested. The TE could then be calculated as the plant biomass accumulated between the first and final plant samplings, divided by the total transpiration during that period.
- **Growth Analysis** – RGR ($g\ g^{-1}\ day^{-1}$) as mean values across one harvest interval (t_2-t_1) by using the equation: $(\ln W_2 - \ln W_1) / (t_2 - t_1)$, where W_2 and W_1 are the dry matter of the plants at the 30 DAP and at after final harvesting, respectively.

Reduction in weight (%) was calculated as the rate between the whole plant dry weight (g) in the stressed condition by the whole plant dry weight (g) in the control condition multiplied by 100.

Phenotype was assessed by number of leaves, leaf area (length*width), height, and length of the internode.



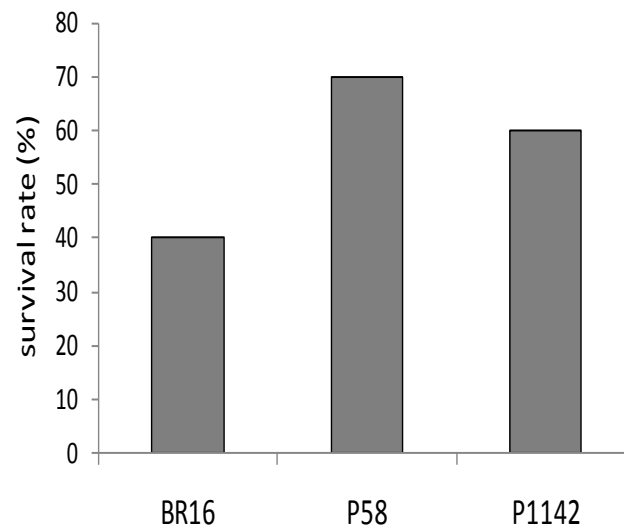
Material and Methods - Field experiments

- Randomized Complete Block Design – Treatments in split-plots , 4 Reps.
- Main Plots: Three water regimes
 - 1) Irrigated (I, soil-water potential was -0.03 to -0.05 Mpa);
 - 2) Non-Irrigated (NI, natural rainfall);
 - 3) Stressed in vegetative (EV) or reproductive (ER) stages (rain-out shelters)
- Sub-plots: BR 16 (drought sensitive); Embrapa 48 (tolerant); P58 (DREB1A); P2193 (DREB2A), and 09D-0077 (= P58-DREB1A x BR 16);
- Measurements: plant height (H); number of nodes (NN), leaf area (LA), leaf area index (LAI) and, shoot dry weight (SDW)
- At R8 stage: apparent harvest index (AHI), yield, number of seeds (NS), number of pods (NP), 100-seed weight (100-SW)

RESULTS

Greenhouse experiments

Quick survival rate test* at greenhouse-DREB1A



DREB plants had higher survival rate at greenhouse

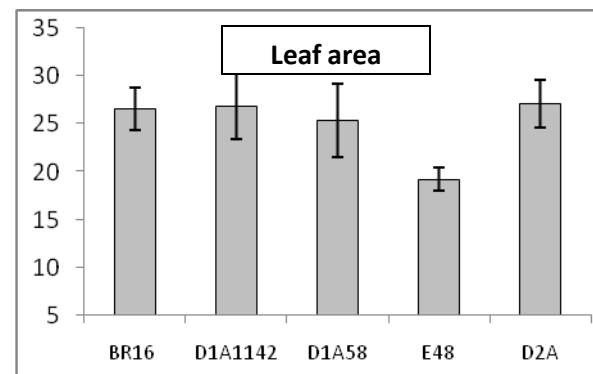
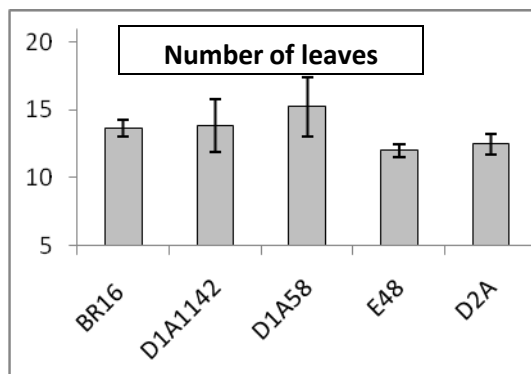
*10 seedlings cultivated into 1.0 kg pots (soil:sand:manure;1:3:1; 26% WHC) in well-watered conditions (70% of WHC) At V₃, prior to the stress initiation, the pots were saturated and thereafter irrigation was stopped, for six days Plants were re-irrigated and 3 days after the number of surviving plants was counted

Phenotype in well watered conditions

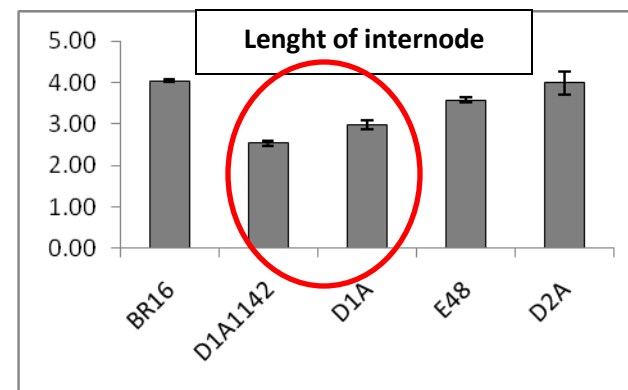
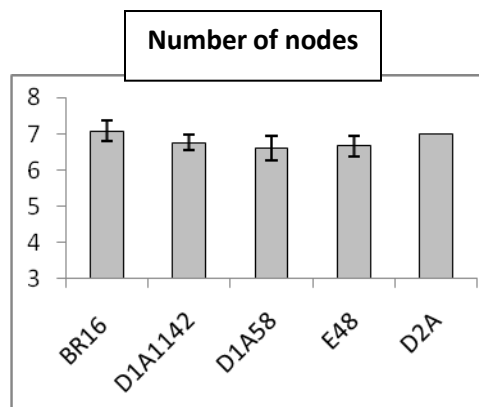
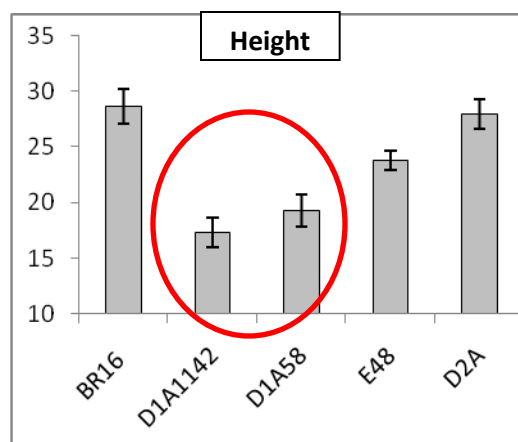


Are DREB1A plants smaller/shorter? Do they have growth retardation?

Growth



n=6
Bars=sem



DREB1A plants are shorter due to shortening of the internodes

Phenotype after suspension of irrigation

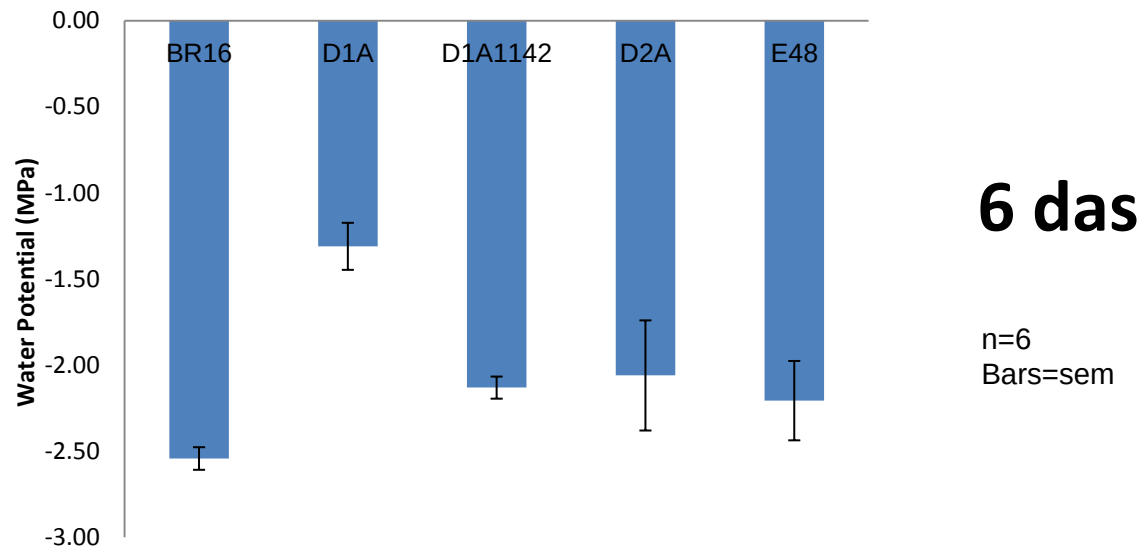


6 days after suspension of irrigation

BR 16 started to wilt, while DREB1A and Embrapa 48 don't

Water Potential

Leaf water potential was determined using a PsyPro (PST-55-SF, Wescor, Inc., Logan, UT) according to Nepomuceno et al. (1998).

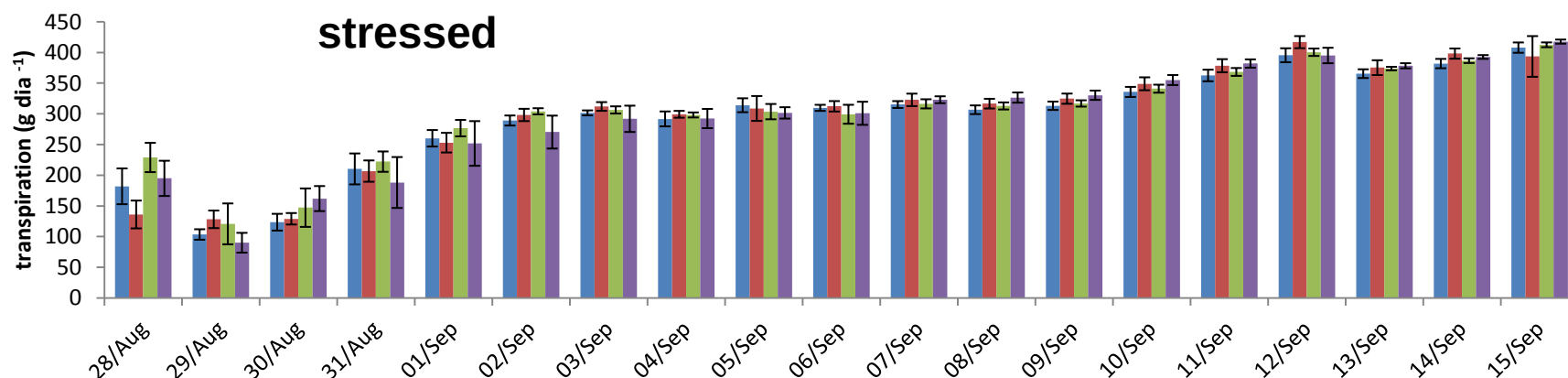
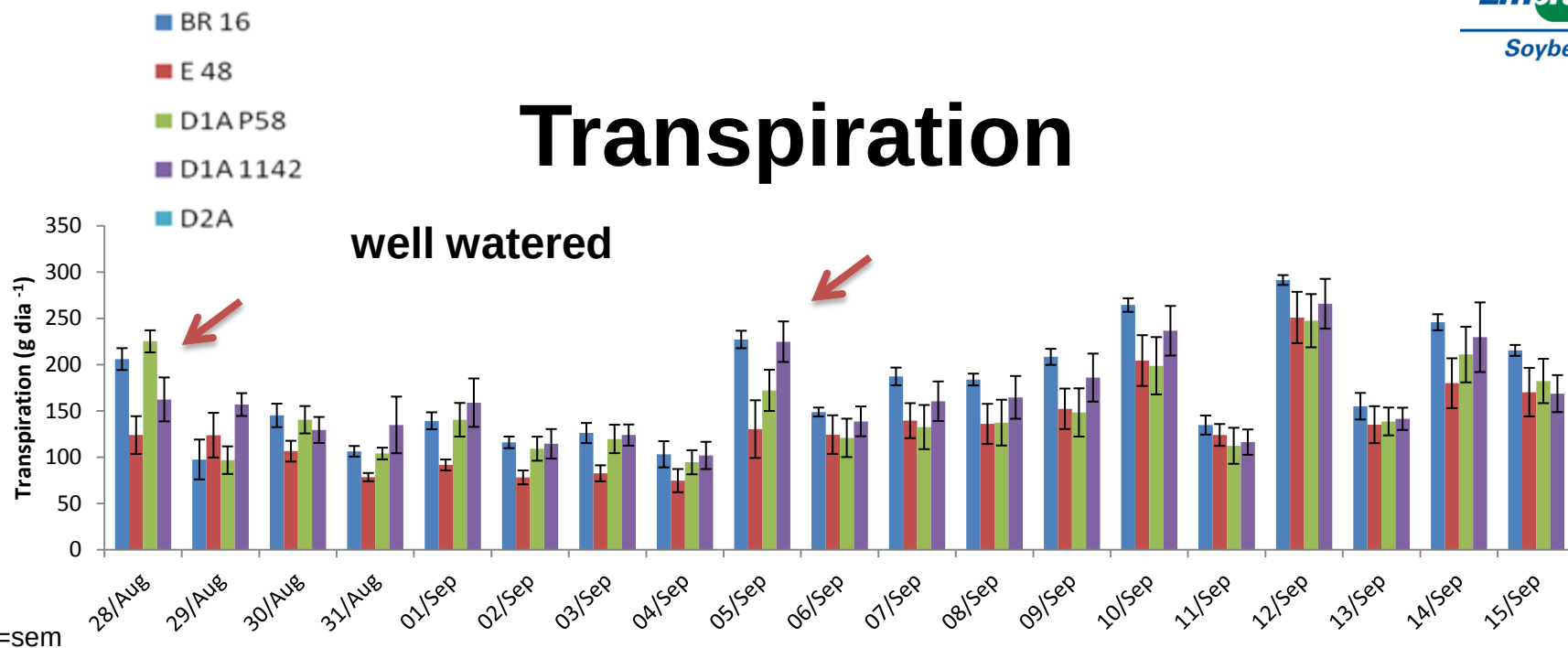


DREB1A showed higher water potential. It seems that this line is using water conservation mechanisms and this could be the reason why it wilts later

- *A conservative use of water, when soil moisture is sufficient to fully supply plant water demand would maintain water in the soil profile for a longer period of time and might be advantageous under conditions of a long drought spell and/or terminal drought*

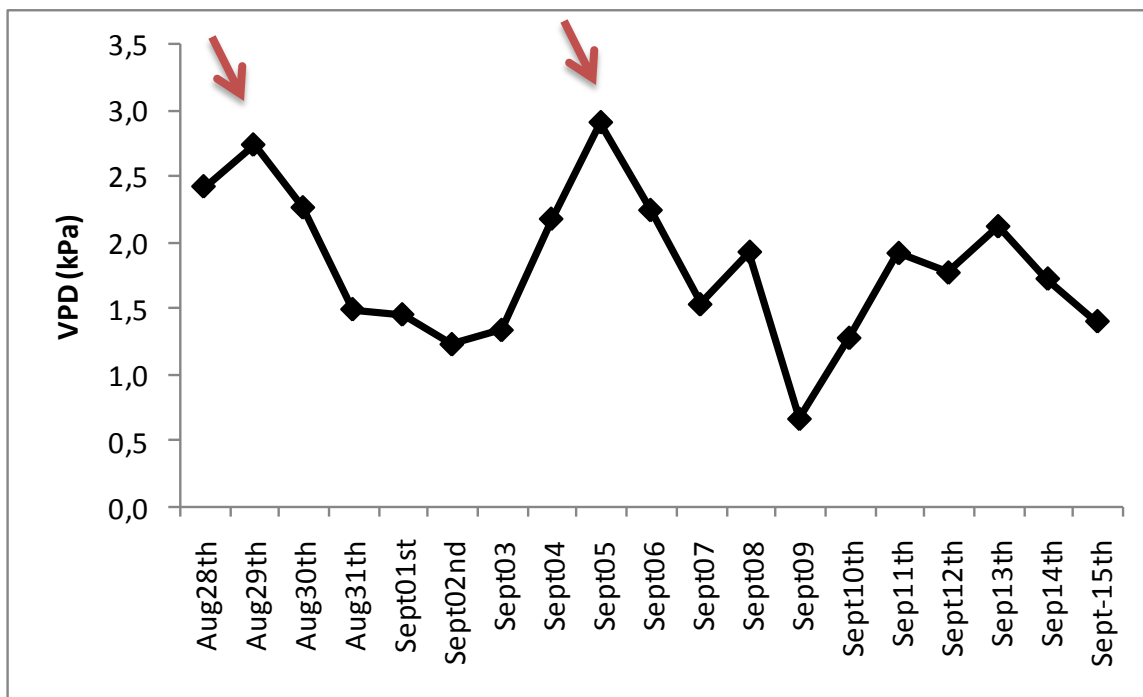
- A decline in the transpiration rates when the soil is relatively humid has been claimed as a mechanism that results in water conservation and this mechanism have been verified in maize (Ray; Sinclair, 1997), soybean (Vadez; Sinclair, 2001; Hufstetler et al., 2007) and peanuts (Bhatnagar-Mathur et al., 2007).
- Some drought tolerant soybean genotypes are able to limit an increase in transpiration when vapor pressure deficit is higher than 2 kPa (Fletcher et al. 2007; Sadok and Sinclair 2009)
- Therefore we analyzed transpiration, monitored temperature and humidity and calculated VPD

Transpiration



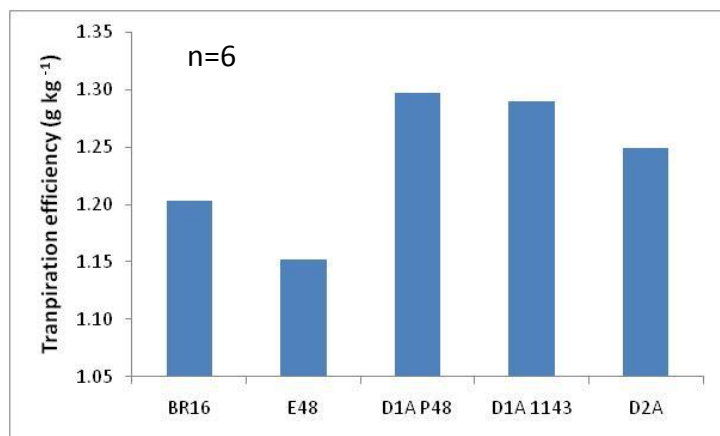
We found differences in transpiration among the material studied but only in well watered conditions and specially when VPD was high (days 28 Aug and 05 Sept)

Vapor pressure deficit (VPD)

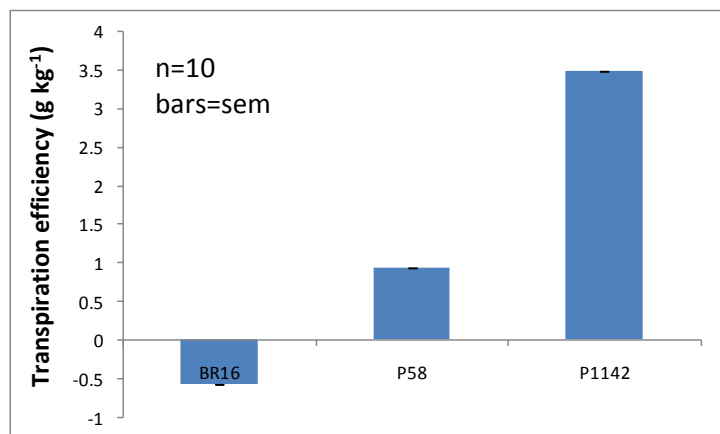


It seems that under high values of VPD the DREB plants have lower transpiration in well watered conditions

Transpiration efficiency (under water stress condition)



12 days after suspension of the irrigation-Greenhouse



3 days after suspension of the irrigation and high VPD-Phytotron

**TE= g of above ground dry weight/kg⁻¹
transpired water**

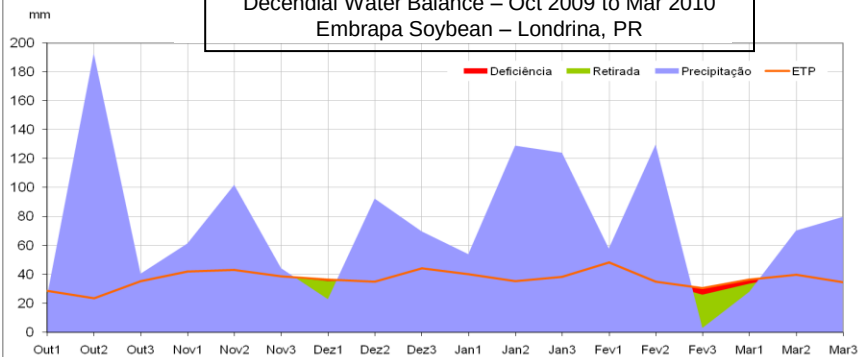
*BR16 showed negative TE
(CER was negative, or: respiration>photosynthesis)*

*According to Saint-Pierre et al., 2012: WUE is an
adequate trait measured at GH to select for a
stable yield under water stress in the field*

RESULTS

Field experiments

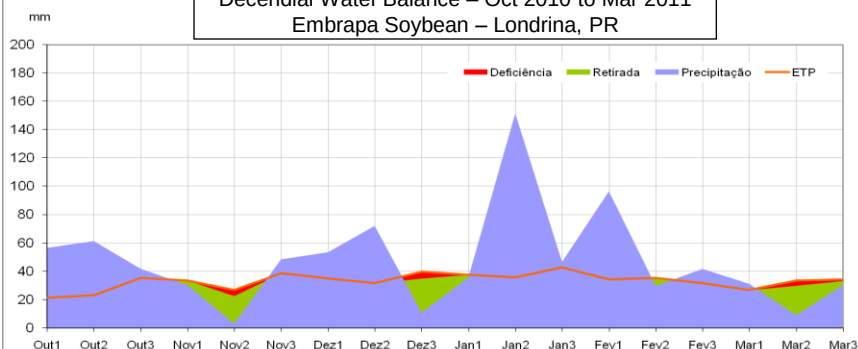
Decennial Water Balance – Oct 2009 to Mar 2010
Embrapa Soybean – Londrina, PR



2009/2010:

Excessive rainfall at the grain filling stage, known to be critical for final yield

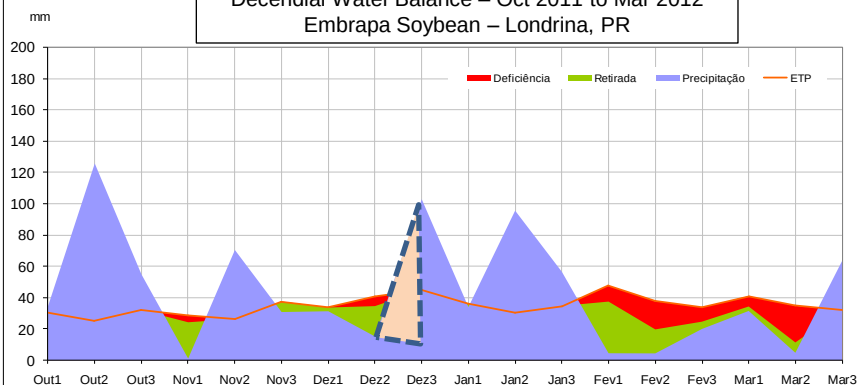
Decennial Water Balance – Oct 2010 to Mar 2011
Embrapa Soybean – Londrina, PR



2010/2011

Water deficit (5mm) at the pod filling stage.

Decennial Water Balance – Oct 2011 to Mar 2012
Embrapa Soybean – Londrina, PR



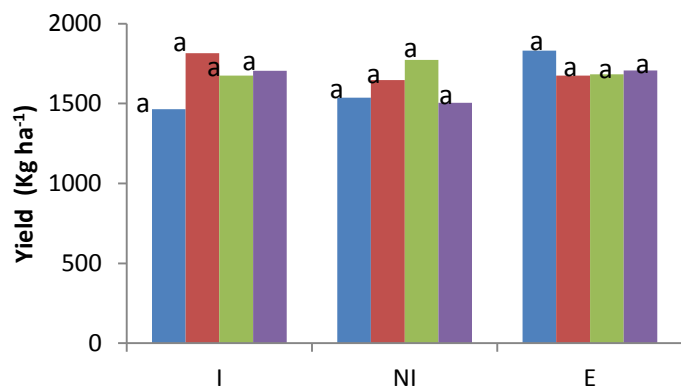
Dec 30th > 100 mm

2011/2012

*Water deficits at following:
Nov - 4.8 mm; Dec - 6.5 mm;
Feb - 38.3 mm; Mar - 30 mm*

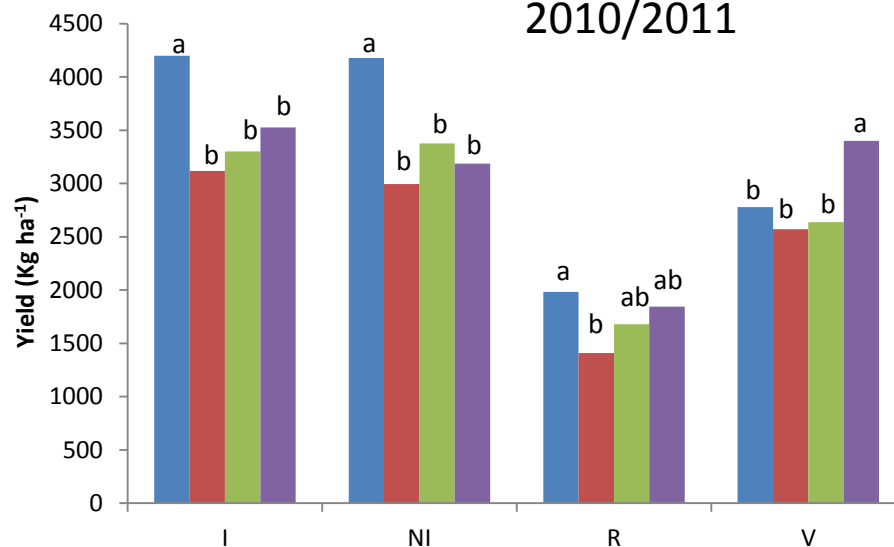
Seasons of 2009/2010 and 2010/2011 (no water deficits)

2009/2010



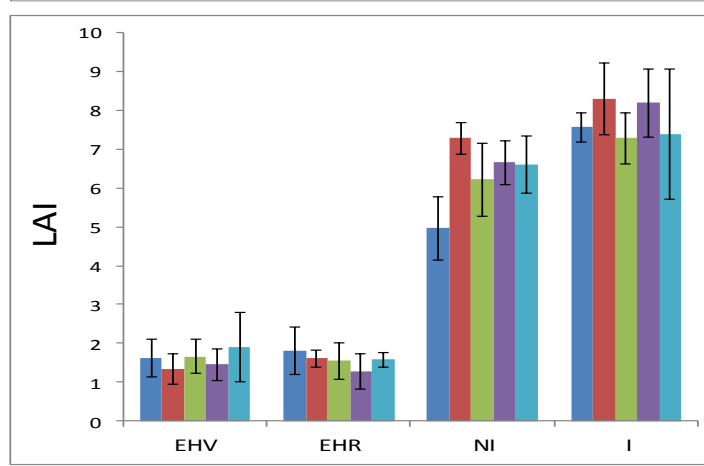
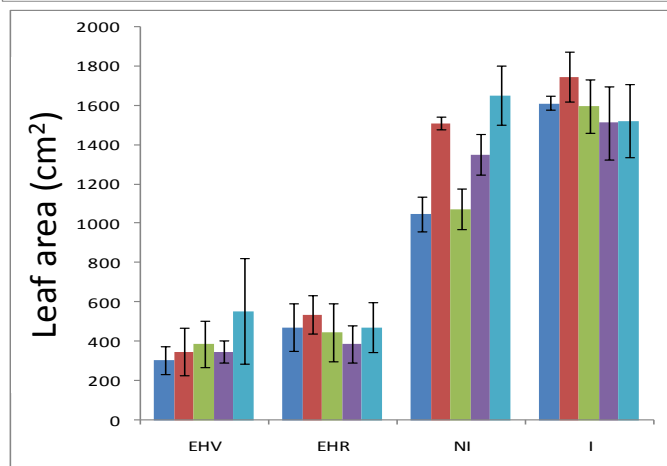
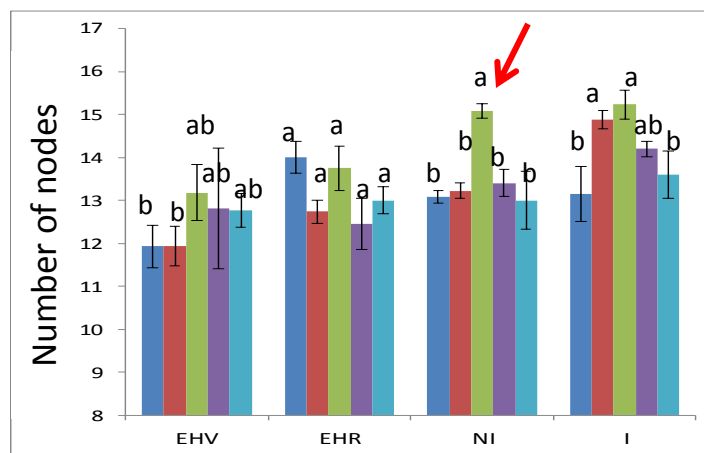
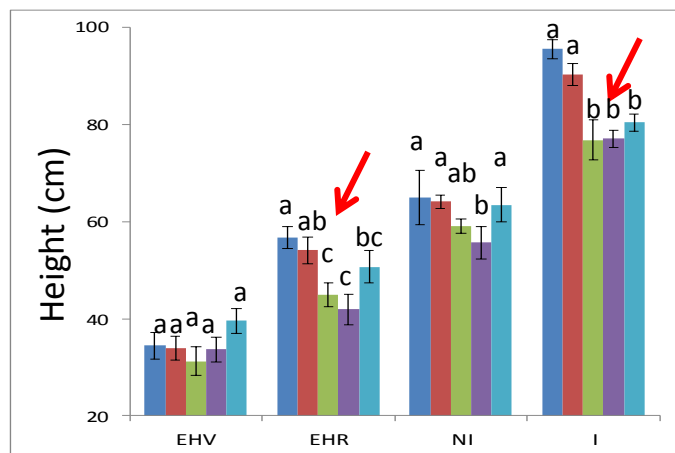
Embrapa 48
BR 16
P58 DREB1A
09D-0077
P2193 DREB2A

2010/2011



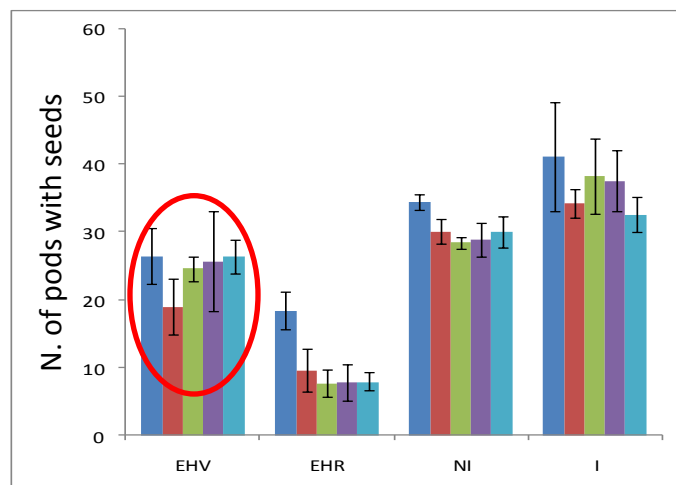
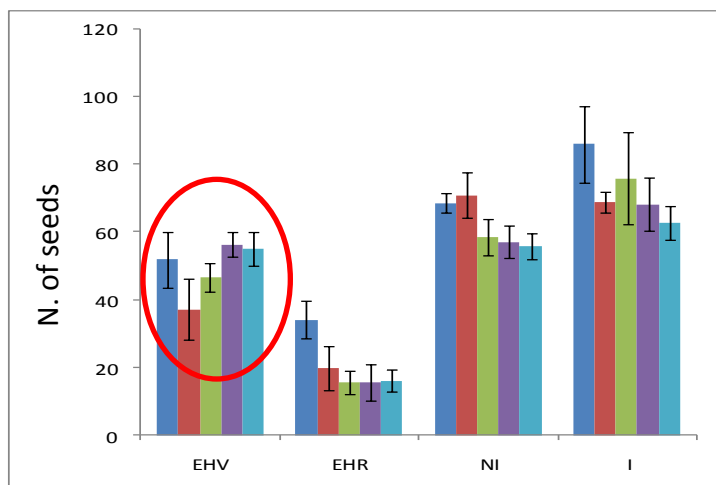
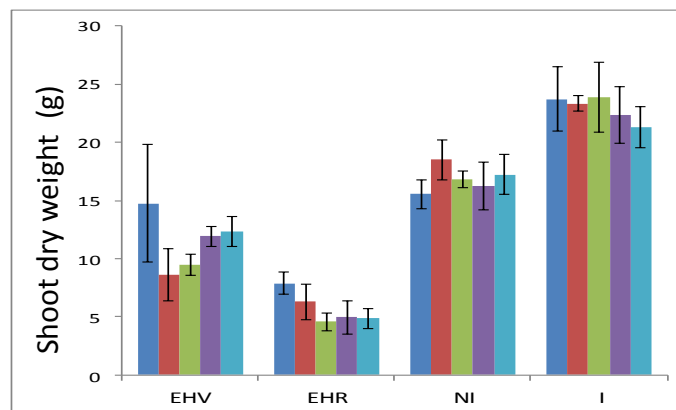
BR16 (better seed quality)
DREB1A
DREB2A
E48

Season of 2011/2012 (water deficit)



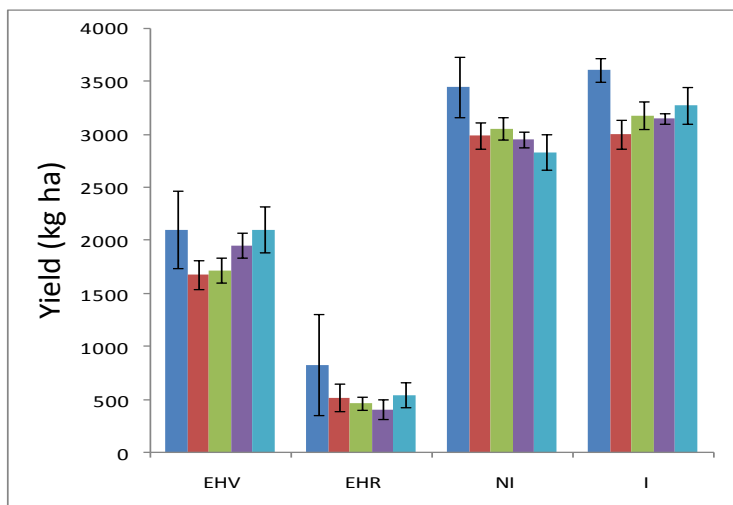
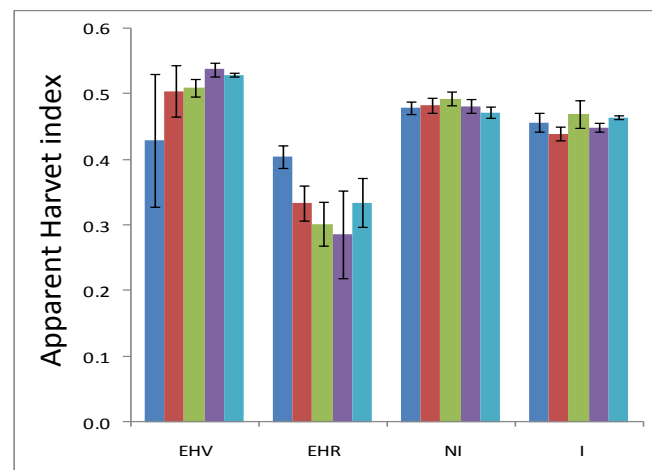
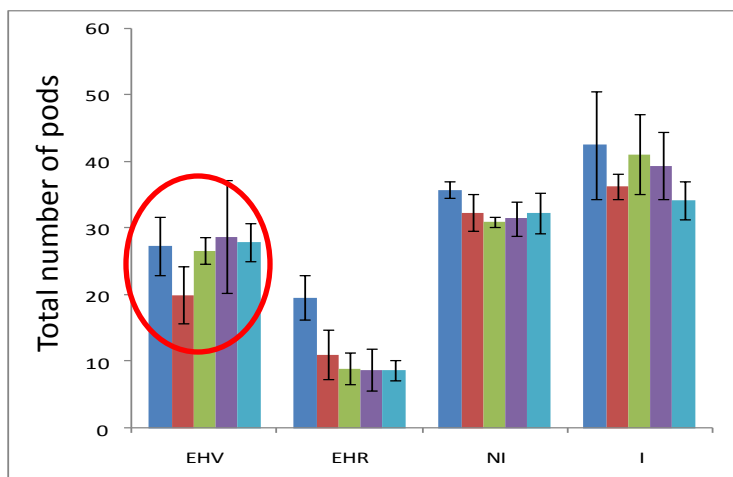
*DREB1A and 09D-0077 shorter than BR16
and DREB1 had higher number of nodes / NI*

Season of 2011/2012 (water deficit)



Tendency for higher n° of seeds, n° of pods with seeds and total number of pods when stress was applied at the vegetative stage

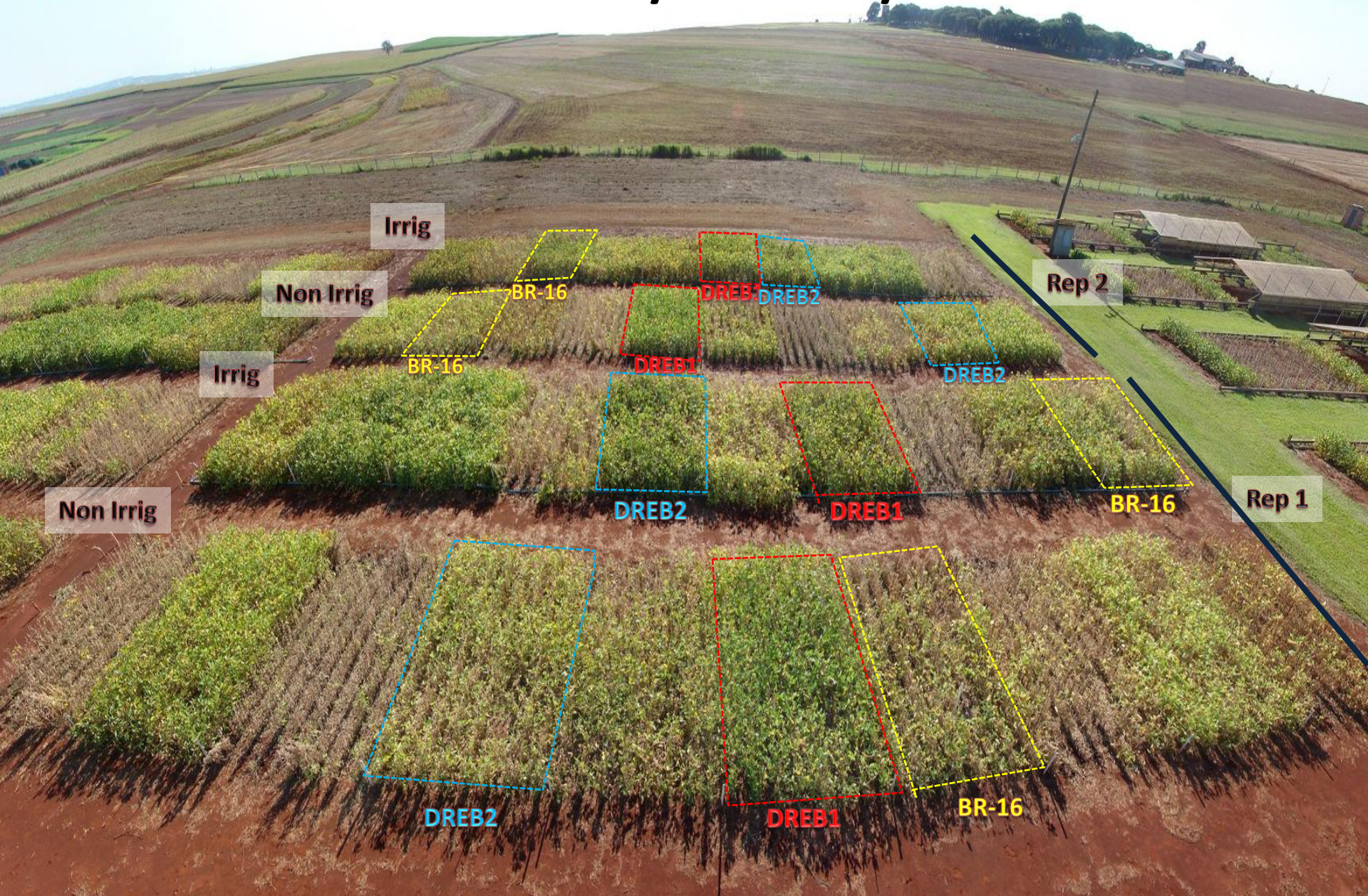
Season of 2011/2012 (water deficit)



- Embrapa 48
- BR 16
- P58 DREB1A
- 09D-0077
- P2193 DREB2A

*No significant differences
in yield*

Visual observation BR16/DREB1A/DREB2A



Comparing our data with the literature..



Wheat DREB1A transgenic lines did not generally outperform the controls in terms of grain yield under water deficit.

Events selected for WUE in the GH were identified as the lines that combined an acceptable yield—even higher yield under well irrigated conditions —and stable performance across the different environments generated by the experimental drought treatments.

Final remarks

Soybean DREB plants presented a slow wilting phenotype and did not show growth retardation or delayed flowering

Improvement of drought tolerance was further verified through several physiological characteristics at greenhouse

The tolerance mechanisms seems to involve water conservation at well watered conditions by the maintenance of lower transpiration rates at high VPD

Other mechanisms need to be further addressed

Since plant responses to drought are influenced by time, intensity, duration and frequency of the stress, plant stage, and by diverse plant-soil-atmosphere interactions, further studies are needed to better characterize the conditions (soil and atmosphere) in which DREB plants may outperform the non transformed parental lines

Insights into the mechanisms of stress tolerance both under greenhouse and field conditions is been sought in our on-going research work

Acknowledgments

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Thanks for your attention

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