



## AGRONOMIC EVALUATION OF GENETICALLY MODIFIED SOYBEAN GENOTYPES IN RESPONSE TO WATER DEFICIT

Luís Fernando Santos de Lima<sup>1,2†</sup>, Leonardo Cesar Ferreira<sup>3†</sup>, Renata Fuganti-Pagliarini<sup>1</sup>, Silvana Regina Rockenbach Marin<sup>4</sup>, Maria Cristina Neves de Oliveira<sup>4</sup>, Liliane Marcia Mertz-Henning<sup>4</sup>, José Renato Bouças Farias<sup>4</sup>, Norman Neumaier<sup>4</sup>, Kazuo Nakashima<sup>5</sup>, Liliane Moreira Nunes<sup>2</sup>, Alexandre Lima Nepomuceno<sup>4\*</sup>

**ABSTRACT:** Due to the importance of the soybean complex on the world economy, there is concern about the significant losses of the full production potential due to abiotic stresses, so that drought is the main challenge for production. Genetic engineering allows insertion of specific genes in order to develop more drought tolerant cultivars. This study aimed at evaluating field agronomic responses of conventional soybean cultivars (BR 16 and Embrapa 48) under water deficit conditions, in comparison to the soybean lines 1Ab58 and 1Bb2193, genetically modified (GM) for drought tolerance with *rd29A:AtDREB1A* and *rd29A:AtDREB2A* constructions. The conventional cultivar Embrapa 48 was the genotype most benefited by the abundance of water (IRR), but with no differences in yield compared to other plant materials under such conditions. Although the crossing 09D-0077 (BR 16 x 1Ab58) presented the highest leaf area index (LAI) values under IRR, such a genotype had a similar performance to GM lines (1Ab58 and 1Bb2193), including in yield, thus indicating that backcrossing and transforming elite lines showed similar responses to water deficit. Further studies are needed in order to confirm the potential of GM lines and the crossing 09D-0077 to cope with drought under more severe water deficit conditions.

**Key-words:** Drought tolerance induction, transcription factors, in-field plant growth.

## AVALIAÇÕES AGRONÔMICAS DE SOJA GENETICAMENTE MODIFICADA EM RESPOSTA AO DÉFICIT HÍDRICO

**RESUMO:** Devido à importância do complexo soja na economia mundial, existe a preocupação com as perdas significativas do máximo potencial produtivo em decorrência de estresses abióticos, sendo a seca o principal desafio para a produção. A engenharia genética permite a inserção de genes específicos a fim de desenvolver cultivares mais tolerantes à seca. O objetivo deste estudo foi analisar a resposta em campo de cultivares convencionais de soja (BR 16 e Embrapa 48) sob condições de déficit hídrico, em comparação às linhagens de soja 1Ab58 e 1Bb2193, geneticamente modificadas (GMs) para tolerância à seca com as construções *rd29A:AtDREB1A* e *rd29A:AtDREB2A*. A cultivar convencional Embrapa 48 foi o genótipo mais beneficiado pela abundância de água (IRR), mas sem diferenças no rendimento em comparação aos outros materiais vegetais sob tais condições. Embora o cruzamento 09D-0077 (BR 16 x 1Ab58) tenha apresentado o maior índice de área foliar (LAI) sob IRR, tal genótipo apresentou desempenho similar ao das linhagens GMs (1Ab58 and 1Bb2193), inclusive quanto ao rendimento, indicando que o retrocruzamento e a transformação para obtenção de eventos elite mostraram respostas semelhantes ao déficit hídrico. Estudos adicionais são necessários a fim de confirmar o potencial de linhagens GM e do cruzamento 09D-0077 para lidar com a seca sob condições mais severas de déficit hídrico.

**Palavras-chave:** Indução de tolerância à seca, fatores de transcrição, crescimento vegetal no campo.

<sup>1</sup>National Council for Scientific and Technological Development (CNPq) / Embrapa Soja, Londrina, Brazil.

<sup>2</sup>Philadelphia University Center, Londrina, Brazil.

<sup>3</sup>Coordination for the Improvement of Higher Education Personnel (CAPES) / Embrapa Soja, Londrina, Brazil.

<sup>4</sup>Embrapa Soja, Londrina, Brazil.

<sup>5</sup>Japan International Research Center for Agricultural Sciences (JIRCAS), Tsukuba, Ibaraki, Japan.

\*Corresponding author: alexandre.nepomuceno@embrapa.br

†These authors have contributed equally to this work.

Recebido em: 01/08/2017.      Aprovado em: 05/12/2017.

## INTRODUCTION

Soybean (*Glycine max* L. Merrill) crop has been grown worldwide and represents the most increasing Brazilian commodity in the last few decades (CONAB, 2017). Technological advances in efficient crop managements have led to increases in its productivity (PRIOLLI et al., 2004). It has been widely used mainly due to its composition, which consisted of high oil, protein, mineral, and fiber levels, thus representing the feeding basis for humans and animals (ESTEVES and MONTEIRO, 2001). Recently, soybean has become an alternative feedstock for biodiesel production. In this sense, among several raw materials that can be used for biodiesel production, soybean represents around 90% Brazilian production (SCHAMBECK et al., 2015).

However, the full potential of soybean production has been severely affected by biotic and abiotic factors. Abiotic stresses, including drought and high temperatures, lead to more significant losses than biotic ones mainly due to the complexity in controlling climate variables (SALEKDEH et al., 2009). Several food production regions subjected to drought have suffered mainly due to climate changes (COLLINS et al., 2008; REYNOLDS and ORTIZ, 2010). In soybean production, drought-related problems have impaired crop yield worldwide. Brazil is the second largest world soybean producer, and has suffered productivity losses due to water deficit in recent years (FUGANTI-PAGLIARINI et al.,

2017). Thus, strategies to minimize drought-induced losses are needed. Suitable soil management practices, fertility, and the choice of better-adapted cultivars may increase water use efficiency (BONATO, 2000), which can be achieved by genetic engineering techniques to advance plant breeding (COMINELLI et al., 2013). In this sense, genetic engineering represents a tool capable of helping gene transfer and thus originating new plants more suited to stand weather adversities (FALEIRO and ANDRADE, 2011).

Usually, genes selected for studies aimed at obtaining plants more tolerant to adverse environmental conditions are those encoding enzymes involved in the biosynthesis of several osmotic protectors or genes encoding transcription factors (TFs). TFs are proteins involved in the initial steps of gene expression and regulation and in signal transduction in response to stresses, thereby, regulating the expression of several groups of genes. Thus, the use of TFs in genetic engineering studies aiming to increase plant tolerance to drought, salinity and freezing is a relevant strategy (SHINOZAKI and YAMAGUCHI-SHINOZAKI, 2007). Shinozaki and Yamaguchi-Shinozaki (2000; 2007) presented the DREB (dehydration-responsive element-binding) TFs, which is capable of activating a cascade of genes responsible for protection of cell structures during environmental stress conditions. In *Arabidopsis*, the expression of *DREB1/CBF* (C-repeat binding factors) genes is mainly

induced by cold stress, while *DREB2* genes are induced under drought, high-salinity, and heat stresses.

The use of gene constructions containing stress-induced promoters, such as *rd29A* combined with TFs, has shown increased ability of plants to tolerate periods of water deficit (SHINOZAKI and YAMAGUCHI-SHINOZAKI, 2007). Polizel et al. (2011) successfully introduced the construction *rd29A:AtDREB1A* via biolistics into embryos of BR 16, a drought-sensitive soybean cultivar (OYA et al., 2004), and observed that the promoter *rd29A* was activated under drought inducing the expression of the TF *AtDREB1A* and, then, activating drought-defense genes at higher levels than in wild-type (WT) plants. In greenhouse conditions, genetically modified (GM) plants had higher stomatal conductance and consequently higher photosynthetic and transpiration rates. In addition, they had higher chlorophyll content (POLIZEL et al., 2011).

The gene *AtDREB2A* has also been used to develop GM plants aimed at tolerating environmental stresses. It also has the conserved DNA-binding domain *ERF/AP2* and recognizes the sequence *DRE* of response genes. The genes *AtDREB2A* and *AtDREB2B* are specifically activated in response to drought and salinity, and are located in the chromosomes five and three of *Arabidopsis thaliana*, respectively (NAKASHIMA et al., 2009). Engels et al. (2013) successfully introduced the *rd29A:AtDREB2A* construction into BR 16 conventional soybean cultivar. Results from water deficit experiments in greenhouse showed that GM lines exhibited high expression of the transgene, with the roots of line 1Bb2193 showing the highest expression levels during water deficit.

Although these previous data obtained in greenhouses show the potential use of *rd29A:AtDREB1A* and *rd29A:AtDREB2A* constructions, such information was generated under monitored and controlled conditions of light, temperature, water, weeds, insects and diseases. According to

Passioura (2012), the results obtained under controlled conditions in greenhouses may not be representative of the way plants respond throughout season in field conditions. Thus, evaluations in field conditions are fundamental.

After characterized in the field, GM lines can be introduced into breeding programs, when the trait of interest from one GM elite variety (donor parent) will be incorporated through backcrosses into the favored genetic background of another (recurrent parent) (VOGEL, 2009). The segregating population however also needs to be challenged in field conditions as one of the stages of the breeding program. Backcrossing has higher efficiency than transforming elite lines, since most transformation protocols are optimized for specific laboratory lines, and several elite lines are not amenable for transformation (USDA, 2017).

Therefore, based on the current scenario in which new alternatives are needed for minimizing drought-induced damages, this study aimed at evaluating field agronomic responses of conventional soybean cultivars (BR 16 and Embrapa 48) under water deficit conditions, in comparison to the soybean lines 1Ab58 and 1Bb2193, genetically modified for drought tolerance with *rd29A:AtDREB1A* and *rd29A:AtDREB2A* constructions.

## MATERIAL AND METHODS

### Experimental site and growing conditions

The experiment was carried out in the field area located at the National Soybean Research Center - Embrapa Soja, Londrina, Paraná State (PR), Brazil (23°11' S, 51°11' W, 630 m altitude), a branch of the Brazilian Agricultural Research Corporation – Embrapa, during the 2012/13 crop season. Londrina has a Cfa climate according to the Köppen climate classification, i.e. humid subtropical climate, with rainfall in all seasons and the possibility of drought periods in the winter (PREFEITURA DE LONDRINA, 2017).

The treatments were defined by the factors: water condition and soybean materials. They were assigned to a completely randomized block design, according to a split-plot scheme, where the whole plots consisted of four water conditions and the subplots of five soybean materials. The water conditions were: irrigated (IRR, rainfall + watering when soil matric potential ranged from -0.03 to -0.05 MPa), non-irrigated (NIRR, rainfall only), and water deficits induced in the vegetative (WD-V) or reproductive (WD-R) development phases. The soybean materials were two cultivars reported as having contrasting responses to drought in Oya et al. (2004) - BR 16, more sensitive, and Embrapa 48, less sensitive; the transgenic lines obtained via biolistics and reported in Brasileiro (1998) - 1Ab58 (construction *rd29A:AtDREB1A*, T9 generation), 1Bb2193 (construction *rd29A:AtDREB2A*, T6 generation), and the crossing 09D-0077 (BR 16 x 1Ab58, F5 generation). The GM lines are isolines of the conventional cultivar BR 16.

Sowing was performed on November 05, 2012. Growing conditions followed the procedures for soybean production (EMBRAPA, 2011). WD-V (December 05-27, 2012) and WD-R (December 27, 2012 - harvest) were induced by rainout shelters programmed to cover plots during rainfall.

Total rainfall during the experimental period was assessed through the water balance (WB) of the 2012/13 crop season. The WB (THORNTHWAITE and MATHER, 1955) was calculated at 10-day periods by spreadsheets as proposed by Rolim et al. (1998).

### Transgene presence identification

The GM soybean lines obtained via biolistics were evaluated to confirm the presence of transgenes, in the Plant Biotechnology Laboratory of Embrapa Soja, Londrina, PR, Brazil. Leaf genomic DNA was extracted through the method of Doyle and Doyle (1987). A conventional PCR analysis was performed to confirm the presence of positive events using a set of specific primers for the coding region of transgenes (Table 1).

**Table 1.** Sequence of the primers used to confirm positive events.

Identification	5' – 3' sequence	Amplicon
DREB2A-F	ATGGCAGTTTATGATCAGAGTG	920bp
DREB2A-R	TTAGTTCTCCAGATCCAAGTAAC	
DREB1A-F	ATGAACTCATTTTCTGCTTTTTCTG	649bp
DREB1A-R	TTAATAACTCCATAACGATACGTCG	

PCR reaction (25µL final volume) was composed of reaction buffer 1X, 5µM of each primer, 0.4mM dNTPs, 2mM magnesium chloride, 1U Taq DNA polymerase, around 60 ng DNA, and 13.8µL sterile water to fit the final volume. Amplifications were performed in thermal cycler (model Veriti Applied Biosystems), and cycling consisted of initial denaturation at 95°C for 5 min, followed by 35 cycles at 95°C for 30s, 55°C for 30 s, 72°C for 30s, and final extension at 72°C for 7 min. The reaction products were submitted to agarose

gel electrophoresis at 1.2% in SB buffer 1X, stained with ethidium bromide, and visualized under UV light. After amplified, the fragments were analyzed.

### Agronomic traits

Leaf area index (LAI), number of pods with seeds per plant (NPS), dry weight of pods with seeds per plant (DWPS), and yield (kg ha<sup>-1</sup>) using seed weight and moisture in the useful harvesting area (6 m<sup>2</sup>) were evaluated. NPS and DWPS were evaluated in the harvest period (March 05 -

April 03, 2013) as plants reached the R8 stage. Soybean stages of development were determined as proposed by Fehr and Caviness (1977). LAI was evaluated on January 28, 2013 (R2-R3 stage) and calculated as the ratio of leaf area by soil surface area occupied by plants.

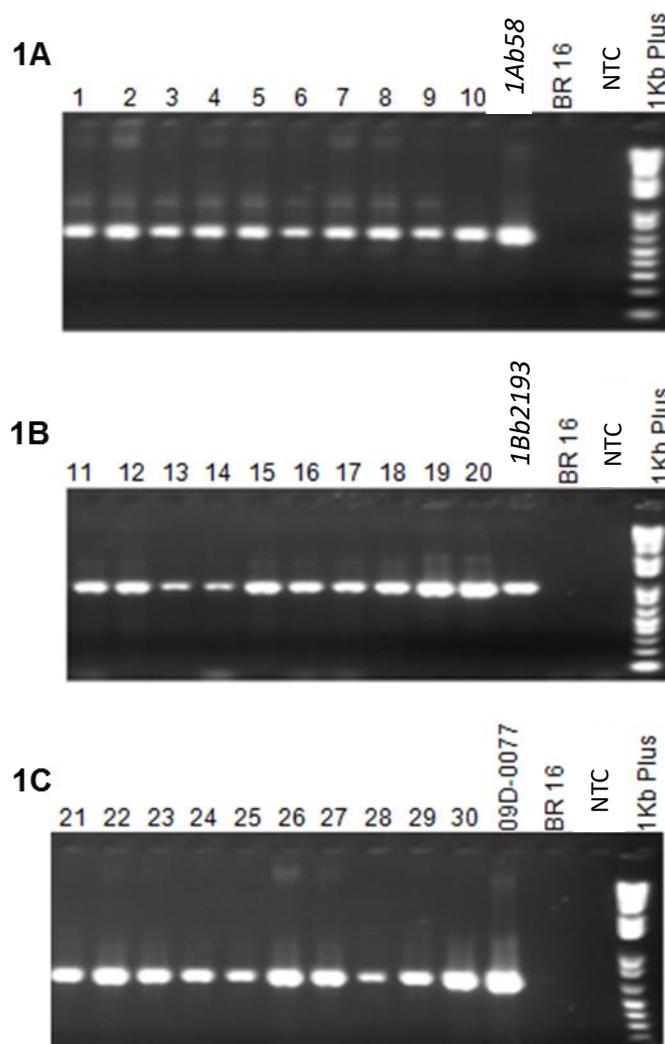
### Statistical analysis

The data were analyzed according to the analysis of variance (ANOVA) model,

after the verification of its adequacy to each variable. The means of the treatments were compared by the Duncan test ( $p \leq 0.05$ ), and the results are presented as mean  $\pm$  standard error.

### RESULTS AND DISCUSSION

The presence of transgenes in the GM lines was confirmed using conventional PCR with specific primers (Figure 1).



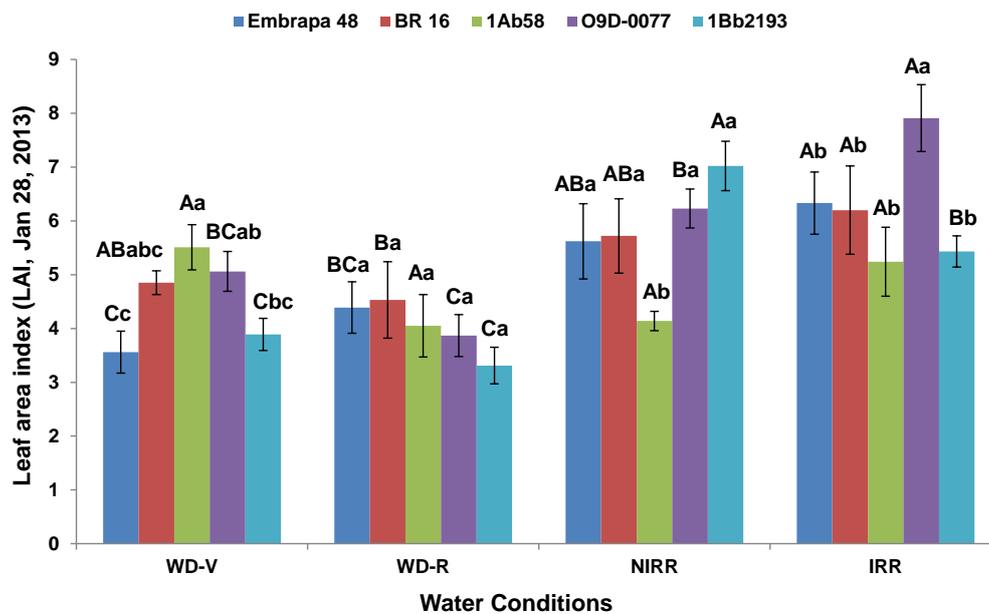
**Figure 1.** Conventional PCR with specific primers amplifying the coding region of the constructions *rd29A:AtDREB1A* (GM soybean line 1Ab58, rows 1-10, A) and *rd29A:AtDREB2A* (GM soybean line 1Bb2193, rows 11-20, B), and the crossing 09D-0077 (BR 16 x 1Ab58, rows 21-30, C). 1Ab58, 1Bb2193 and 09D-0077 – positive controls; BR 16 – negative control; NTC – no template control; 1 Kb Plus Marker.

The ANOVA of the four response conditions and the soybean materials, adding variables measured in this study showed evidences to the hypothesis that genetic significant interactions between the water factors contribute to the management of

abiotic stresses. Moreover, the effect of water stresses induced during the vegetative or reproductive phases were equally or more harmful than non-irrigated or irrigated conditions in the five genotypes, for the four response variables. However, while the stresses induced during the vegetative stages had a major effect on the LAI, NPS and DWPS response variables, it had a minor effect on the yield. On the other hand, stresses induced in the reproductive stages had its major effect on the yield and DWPS. The response of the soybean materials to both stress-induced treatments, as well as to the other treatments, varied according to the response variable.

The LAI is an important parameter to measure the effect of the treatment WD-V on

the soybean materials. As Figure 2 shows, the highest LAIs were obtained in the cultivar BR 16 and its GM isolines 1Ab58 and 09D-0077. Nevertheless, the GM line 1Ab58 was significantly less affected than 1Bb2193. This outcome is partly in agreement with the results reported in Fuganti-Pagliarini et al. (2017), who found no significant differences between mean LAIs of the transgenic lines 1Ab58 and 1Bb2193 and the cultivar BR 16, under the treatment NIRR in field conditions. In the present study, the highest LAI values were obtained for the crossing 09D-0077 under IRR. The conventional cultivar Embrapa 48 and the GM line 1Bb2193 produced the lowest LAI values under WD-V (Figure 2).



**Figure 2.** Leaf area index (LAI) evaluated on January 28, 2013 (R2-R3). WD-V: water deficit in the vegetative stage; WD-R: water deficit in the reproductive stage; NIRR: non-irrigated; IRR: irrigated.  $n = 4 \pm$  standard error. Means followed by the same uppercase letters among water conditions (in each genotype) and lowercase letters among genotypes (in each water condition) do not differ by the Duncan test ( $p \leq 0.05$ ).

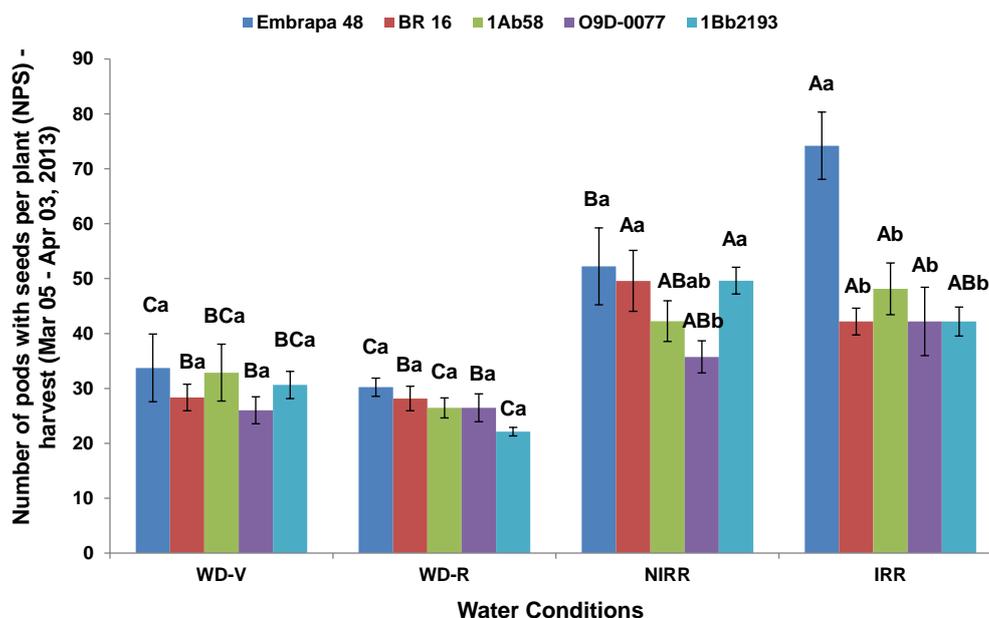
Likewise, Anyia and Herzog (2004) detected lower leaf area under drought evidenced by leaf growth reduction and abscission in cowpea genotypes. Kuss (2006) reported that severe water scarcity in field affects the balance between the production of assimilates and its demand for the development of reproductive organs due to a

reduction in photosynthetically active leaf area. In addition, according to the author, physiological changes such as stomatal closure and leaflet rolling due to tissue dehydration can also be observed, leading to early leaf abscission. As observed by Bianco et al. (2013), in the present study the conventional cultivar Embrapa 48 showed

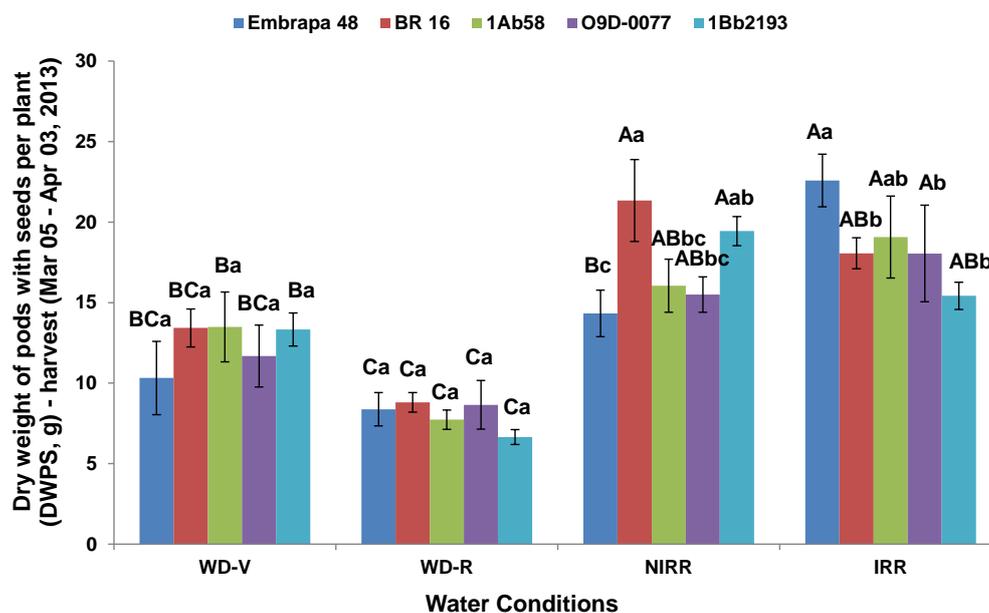
possible adaptive mechanisms to the control of leaf area expansion, thus preventing water loss by transpiration under water deficit. The GM line 1Bb2193 showed similar characteristics.

No differences among genotypes were detected for NPS under WD-V and WD-R treatments (Figure 3). The cultivar Embrapa

48 showed the best performance under IRR. BR 16 plants, GM lines and the crossing 09D-0077 showed no differences for DWPS under WD-V and WD-R (Figure 4). Considering the yield components presented in Figures 3 and 4 (NPS and DWPS), both of them were lower in all plant materials under WD-R.



**Figure 3.** Number of pods with seeds per plant (NPS) evaluated on March 05, 2013 (R8). WD-V: water deficit in the vegetative stage; WD-R: water deficit in the reproductive stage; NIRR: non-irrigated; IRR: irrigated.  $n = 4 \pm$  standard error. Means followed by the same uppercase letters among water conditions (on each genotype) and lowercase letters among genotypes (on each water condition) do not differ by the Duncan test ( $p \leq 0.05$ ).

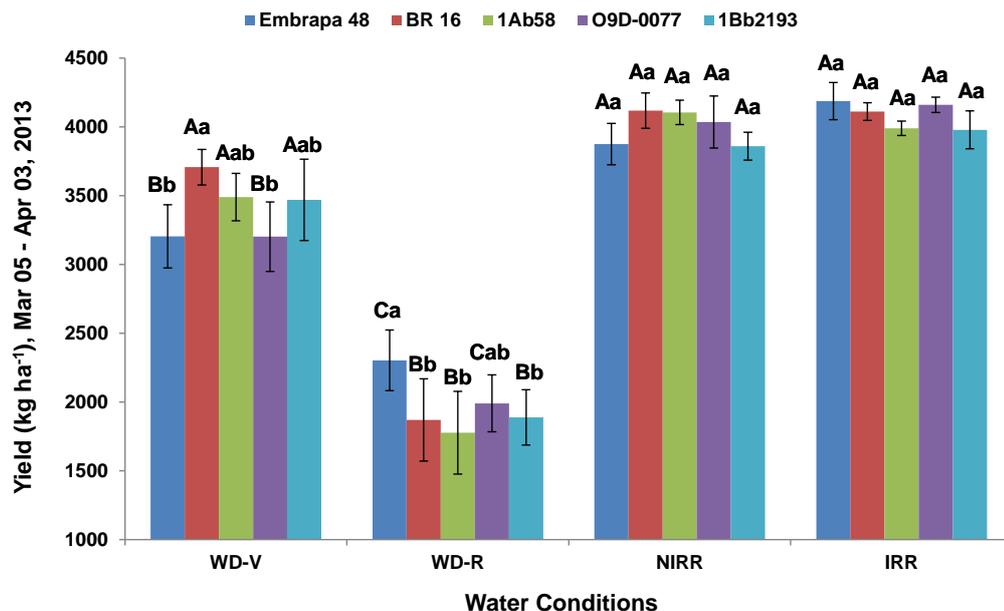


**Figure 4.** Dry weight of pods with seeds per plant (DWPS, g) evaluated on March 05, 2013 (R8). WD-V: water deficit in the vegetative stage; WD-R: water deficit in the reproductive stage; NIRR: non-irrigated; IRR: irrigated.  $n=4 \pm$  standard error. Means followed by the same uppercase letters among water conditions (on each genotype) and lowercase letters among genotypes (on each water condition) do not differ by the Duncan test ( $p \leq 0.05$ ).

According to Bonato (2000), water availability has great importance mainly in two periods of soybean development, i.e. germination-emergence and flowering-grain filling. Water requirement for soybean crop increases along plant development, thus reaching a maximum level during flowering-grain filling (NELSON, 2005), when plants have a daily demand of 7-8 mm of water; thus, water deficit in these periods implies greater losses (EMBRAPA, 2015). This fact might justify the lower values of NPS and DWPS in all plant materials under WD-R in the present study.

The WD-R treatment led to a great reduction in yield regardless of the genotype (Figure 5). Furthermore, BR 16 plants, GM lines and the crossing 09D-0077 showed no differences for yield under WD-R, a condition in which higher values were detected for the cultivar Embrapa 48. Furthermore, no significant difference was detected between genotypes under NIRR or IRR. Likewise, in other field study under the

same water regimes, Fuganti-Pagliarini et al. (2017) also did not find yield differences between GM (1Ab58 and 1Bb2193) and WT (BR 16 cultivar) plants under NIRR. Rolla et al. (2013) also evaluated the field performance of BR16, 1Ab58, and 09D-0077 plants, under similar water conditions of the present study and observed that, although GM plants did not outperform the cultivar BR 16 (WT plants) as to yield, the GM lines showed higher values for some yield components under WD-V, including the number of seeds, the number of pods with seeds, and the total number of pods. In comparison with the yield observed for soybean plants in Paraná State ( $3,336 \text{ kg ha}^{-1}$ ) during the 2012/13 crop season (CONAB, 2013), in general, similar values were observed in the present study for plants submitted to WD-V and higher values were detected under NIRR and IRR, so that a reduction in yield was noted only for plants submitted to WD-R (Figure 5).

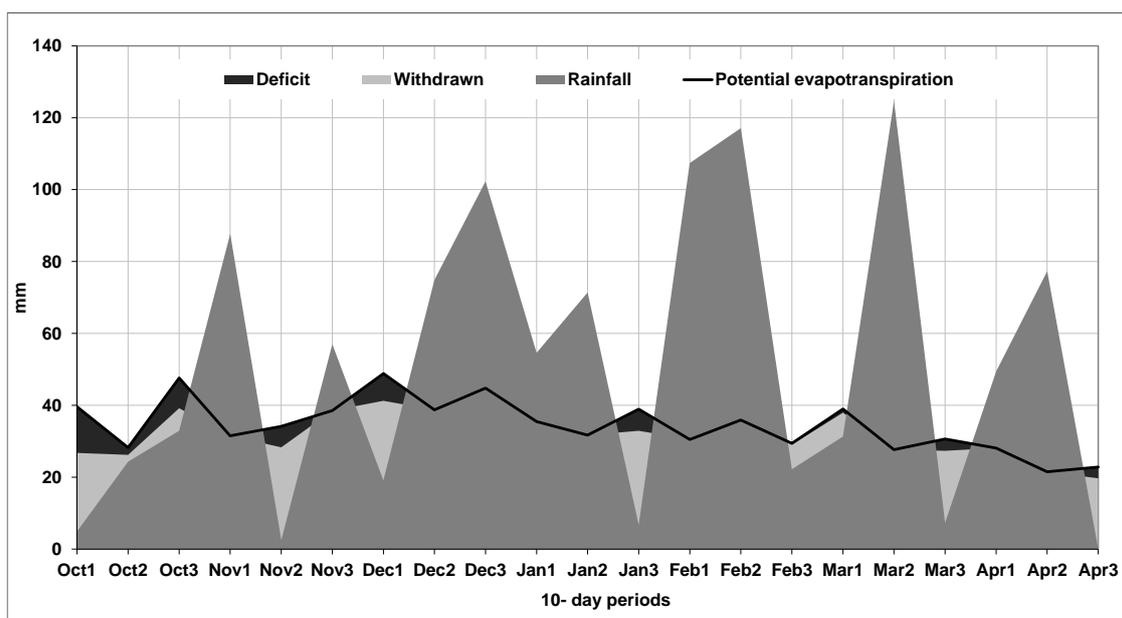


**Figure 5.** Yield ( $\text{kg ha}^{-1}$ ) evaluated on March 05, 2013 (R8). WD-V: water deficit in the vegetative stage; WD-R: water deficit in the reproductive stage; NIRR: non-irrigated; IRR:

irrigated.  $n = 4 \pm$  standard error. Means followed by the same uppercase letters among water conditions (on each genotype) and lowercase letters among genotypes (on each water condition) do not differ by the Duncan test ( $p \leq 0.05$ ).

According to Farias et al. (2007), soybean crop requires suitable water volume and regular rainfall distribution throughout the cycle to show a successful performance, meeting requirements mainly in the most critical stages - germination and grain filling. The authors reported an ideal water volume ranging from 120 to 300 mm to meet the requirements of soybean crop during the critical growth stage (R1-R6) with a proper distribution along such a period, which can range from 30 to 60 days from the beginning of the flowering according to the cultivar and weather conditions. In the present study, based on the 10-day period water balance (Figure 6) corresponding to the experimental

period, the crop season 2012/13 showed periods of intense rainfall, peaking around 87.7 and 57 mm on the first and third 10-day periods of November 2012, respectively; 102.3 mm on the third 10-day period of December 2012; 71.4 mm on the second 10-day period of January 2013; 117.1 mm on the second 10-day period of February 2013, and 124.6 mm on the second 10-day period of March 2013, corresponding to the harvesting period in R8 (March 05 - April 03, 2013). Then, in the present study, plants submitted to NIRR and IRR had a satisfactory rainfall distribution throughout their development (from planting to harvesting), thus corroborating their similar yield values.



**Figure 6.** Sequential 10-day period water balance between October 2012 and April 2013.

## CONCLUSIONS

Based on data regarding NPS and DWPS, the conventional cultivar Embrapa 48 was the genotype most benefited by the abundance of water (IRR), but with no differences in yield compared to other plant materials under such conditions. Although the crossing 09D-0077 (BR 16 x 1Ab58) presented the highest LAI values under IRR,

such a genotype had a similar performance to GM lines (1Ab58 and 1Bb2193), including in yield, thus indicating that backcrossing and transforming elite lines showed similar responses to water deficit. Further studies are needed in order to confirm the potential of GM lines and the crossing 09D-0077 to cope with drought under more severe water deficit conditions.

## ACKNOWLEDGMENTS

This study was supported by the Brazilian Agricultural Research Corporation - Embrapa (Embrapa Soja), the Japan International Research Center for Agricultural Sciences (JIRCAS), and the Science and Technology Research Partnership for Sustainable Development (SATREPS) of the Japan Science and Technology Agency/Japan International Cooperation Agency (JST/JICA). We thank Dr. Fernando Augusto Henning and Dr. Ivani de Oliveira Negrão Lopes for their contributions to the manuscript. We are also grateful to the National Council for Scientific and Technological Development (CNPq) for granting the scholarship to LFSL (undergraduate student, Process #165019/2015-8) and RFP (postdoctoral fellow, Process #158600/2014-2) and the Coordination for the Improvement of Higher Education Personnel (CAPES) for granting the scholarship to LCF (postdoctoral fellow, Process #23038004021201133). This paper was approved for publication by the Editorial Board of Embrapa Soja as manuscript number 184/2017.

## REFERENCES

- ANYIA, A.O.; HERZOG, H. Water-use efficiency, leaf area and leaf gas exchange of cowpeas under mid-season drought. *European Journal of Agronomy*, v.20, p.327-339, 2004.
- BIANCO, L.F.; TREVIZAN, F.H.; NICOLINO FILHO, C.J.; OLIVEIRA, T.B.M.; NEIVERTH, W.; CRUSIOL, L.G.T.; RIO, A.; SIBALDELLI, R.N.R.; CARVALHO, J.F.C.; FERREIRA, L.C.; NEPOMUCENO, A.L.; NEUMAIER, N.; FARIAS, J.R.B. Algumas características das cultivares de soja Embrapa 48 e BR 16 em diferentes regimes hídricos. In: **JORNADA ACADÊMICA DA EMBRAPA SOJA**, 8., Londrina. Resumos expandidos... Londrina: Embrapa Soja, 2013. p.137-141.
- BONATO, E.R. **Estresses da soja**. Passo Fundo: Embrapa Trigo, 2000.
- BRASILEIRO, A.C.M. **Manual de transformação genética de plantas**. Brasília: Embrapa-SPI/Embrapa-Cenargen, 1998.
- COLLINS, N.C.; TARDIEU, F.; TUBEROSA, R. Quantitative trait loci and crop performance under abiotic stress: Where do we stand? *Plant Physiology*, v.147, p.469-486, 2008.
- COMINELLI, E.; CONTI, L.; TONELLI, C.; GALBIATI, M. Challenges and perspectives to improve crop drought and salinity tolerance. *New Biotechnology*, v.30, p.355-361, 2013.
- CONAB. **Acompanhamento da safra brasileira: grãos, nono levantamento, junho 2013**. Brasília: Conab, 2013.
- CONAB. **Acompanhamento da safra brasileira: grãos, nono levantamento, junho 2017**. Brasília: Conab, 2017.
- DOYLE, J.J.; DOYLE, J.L. A rapid DNA isolation procedure for small amounts of fresh leaf tissue. *Phytochemical Bulletin*, v.19, p.11-15, 1987.
- EMBRAPA. **Tecnologias de produção de soja-região central do Brasil 2011**. Londrina: Embrapa Soja, 2011.
- EMBRAPA. **Boletim agrometeorológico da Embrapa Soja**. Londrina: Embrapa Soja, 2015.
- ENGELS, C.; FUGANTI-PAGILARINI, R.; MARIN, S.R.R.; MARCELINO-GUIMARAES, F.C.; Oliveira, M.C.N.; NAKASHIMA, K.; YAMAGUCHI-SHINOZAKI, K.; NEPOMUCENO, A.L. Introduction of the rd29A: AtDREB2A CA gene into soybean (*Glycine max L. Merrill*) and its molecular characterization in leaves and roots during dehydration. *Genetics and*

- Molecular Biology (Impresso), v. 36, p. 556-565, 2013. DOI: 10.1590/S1415-47572013000400015
- ESTEVEZ, E.A.; MONTEIRO, J.B.R. Efeitos benéficos das isoflavonas de soja em doenças crônicas. *Revista de Nutrição*, v.14, p.43-52, 2001.
- FALEIRO, F.G.; ANDRADE, S.R.M.; REIS JUNIOR, F.B. **Biotechnologia: estado da arte e aplicações na agropecuária**. Planaltina: Embrapa Cerrados, 2011.
- FARIAS, J.R.B.; NEPOMUCENO, A.L.; NEUMAIER, N. **Ecofisiologia da soja**. Londrina: Embrapa Soja, 2007.
- FEHR, W.R.; CAVINESS, C.E. **Stages of soybean development. Special Report 80**. Iowa: Iowa State University of Science and Technology, 1977.
- FUGANTI-PAGLIARINI, R.; FERREIRA, L.C.; RODRIGUES, F.A.; MOLINARI, H.B.C.; MARIN, S.R.R.; MOLINARI, M.D.C.; MARCOLINO-GOMES, J.; MERTZ-HENNING, L.M.; FARIAS, J.R.B.; OLIVEIRA, M.C.N.; NEUMAIER, N.; KANAMORI, N.; FUJITA, Y.; MIZOI, J.; NAKASHIMA, K.; YAMAGUCHI-SHINOZAKI, K.; NEPOMUCENO, A.L. Characterization of soybean genetically modified for drought tolerance in field conditions. *Frontiers in Plant Science*, v.8, p.1-15, 2017.
- KUSS, R.C.R. **Populações de plantas e estratégias de irrigação na cultura da soja**. 2006. 80 f. Dissertação (Mestrado em Engenharia Agrícola) - Universidade Federal de Santa Maria, Santa Maria, 2006.
- NAKASHIMA, K.; ITO, Y.; YAMAGUCHI-SHINOZAKI, K. Transcriptional regulatory networks in response to abiotic stresses in *Arabidopsis* and grasses. *Plant Physiology*, v.149, p.88-95, 2009.
- NELSON, K.A.; MOTAVALLI, P.P.; NATHAN, M. Response of no-till soybean [*Glycine max* (L.) Merr.] to timing of preplant and foliar potassium applications in a claypan soil. *Agronomy Journal*, v.97, p.832-838, 2005.
- OYA, T.; NEPOMUCENO, A.L.; NEUMAIER, N.; FARIAS, J.R.B.; TOBITA, S.; ITO, O. Drought tolerance characteristics of Brazilian soybean cultivars—evaluation and characterization of drought tolerance of various Brazilian soybean cultivars in the field. *Plant Production Science*, v.7, p.129-137, 2004.
- PASSIOURA, J.B. Phenotyping for drought tolerance in grain crops: when is it useful to breeders? *Functional Plant Biology*, v.39, p.851-859, 2012.
- POLIZEL, A.M.; MEDRI, M.E.; NAKASHIMA, K.; YAMANAKA, N.; FARIAS, J.R.B.; OLIVEIRA, M.C.N.; MARIN, S.R.R.; ABDELNOOR, R.V.; MARCELINO-GUIMARÃES, F.C.; FUGANTI, R.; RODRIGUES, F.A.; STOLF-MOREIRA, R.; BENEVENTI, M.A.; ROLLA, A.A.; NEUMAIER, N.; YAMAGUCHI-SHINOZAKI, K.; CARVALHO, J.F.C.; NEPOMUCENO, A.L. Molecular, anatomical and physiological properties of a genetically modified soybean line transformed with *rd29A:AtDREB1A* for the improvement of drought tolerance. *Genetics and Molecular Research*, v.10, p.3641-3656, 2011.
- PREFEITURA DE LONDRINA. Dados Geográficos. Available at: [http://www.londrina.pr.gov.br/index.php?option=com\\_content&view=article&id=40&Itemid=58](http://www.londrina.pr.gov.br/index.php?option=com_content&view=article&id=40&Itemid=58) . Accessed on December 05, 2017.
- PRIOLLI, R.H.G.; MENDES JUNIOR, C.T.; SOUSA, S.M.B.; SOUSA, N.E.A.; CONTEL, E.P.B. Diversidade genética da soja entre períodos e entre programas de melhoramento no Brasil. *Pesquisa*

- Agropecuária Brasileira, v.39, p.967-975. 2004.
- REYNOLDS, M.P.; ORTIZ, R. Adapting crops to climate change: a summary. In: REYNOLDS, M.P. **Climate Change and Crop Production**. Wallingford: CAB International, 2010, p.1-8.
- ROLLA, A.A.P.; CARVALHO, J.F.C.; FUGANTI-PAGLIARINI, R.; ENGELS, C.; RIO, A.; MARIN, S.R.R.; OLIVEIRA, M.C.N.; BENEVENTI, M.A.; MARCELINO-GUIMARÃES, F.C.; FARIAS, J.R.B.; NEUMAIER, N.; NAKASHIMA, K.; YAMAGUCHI-SHINOZAKI, K.; NEPOMUCENO, A.L. Phenotyping soybean plants transformed with *rd29A:AtDREB1A* for drought tolerance in the greenhouse and field. *Transgenic Research*, v. -, p. s11248-013-9723, 2013.
- ROLIM, G.S.; SENTELHAS, P.C.; BARBIERI, V. Planilhas no ambiente EXCEL para os cálculos de balanços hídricos: normal, sequencial, de cultura e de produtividade real e potencial. *Revista Brasileira de Agrometeorologia*, v.6, p.133-137, 1998.
- SALEKDEH, G.H.; REYNOLDS, M.; BENNETT, J.; BOYER, J. Conceptual framework for drought phenotyping during molecular breeding. *Trends in Plant Science*, v.14, p.488-496, 2009.
- SCHAMBECK, C.M.; RAMOS, D.T.; CHIARANDA, H.S.; MEZZARI, M.P.; FERNANDES, M.; CORSEUIL, H.X. Influência do biodiesel de soja na biodegradação anaeróbia do benzeno e toluene. *Engenharia Sanitaria e Ambiental*, v.20, p.315-321, 2015.
- SHINOZAKI, K.; YAMAGUCHI-SHINOZAKI, K. Gene networks involved in drought stress response and tolerance. *Journal of Experimental Botany*, v.58, p.221-227, 2007.
- SHINOZAKI, K.; YAMAGUCHI-SHINOZAKI, K. Molecular responses to dehydration and low temperature: differences and cross-talk between two stress signaling pathways. *Current Opinion in Plant Biology*, v.3, p.217-223, 2000.
- THORNTHWAITE, C.W.; MATHER, J.R. **The water balance**. Centerton: Drexel Institute of Technology, Publications in Climatology, 1955.
- USDA. Advanced Backcross Breeding - Introduction to Backcross Breeding. Available at: <http://passel.unl.edu/pages/informationmodule.php?idinformationmodule=959723462&topicorder=2&maxto=7> . Accessed on May 14, 2017.
- VOGEL, K.E. Backcross breeding. *Methods in Molecular Biology*, v.526, p.161-169, 2009.