Genetics/ Original Article

Drought tolerance of elite soybean cultivars with the introgression of transgene AtAREB1

Abstract – The objective of this work was to verify if the introgression of the *AtAREB1* gene in the 'LS93-0375' and 'BMX Desafio RR' elite soybean germplasms increases the tolerance of these plants to water deficit. The F4 progenies of these two elite cultivars and of the AtAREB1 transgenic line (BR16-*AtAREB1*) and its background ('BR16') were subjected to water deficit assays. The water deficit bioassays were performed in a greenhouse using the following six soybean lines: the genetically modified BR16-*AtAREB1* and its background 'BR16'; 'LS93' and its F4 progeny, LS93-*AtAREB1*; and 'BMX Desafio RR' and its F4 progeny, Desafio-*AtAREB1*. A randomized complete block experimental design was carried out in a 6x2 factorial arrangement, with the six soybean genotypes and two water conditions – control (C) and water deficit (WD) treatments – with nine replicates. Soybean genotypes containing the *AtAREB1* gene showed better physiological performances under drought stress and altered expressions of drought-responsive genes. The intogression of *AtAREB1* in soybean increases the plant drought tolerance, regardless of the genetic background in which the gene was introduced.

Index terms: *Glycine max*, bZIP hybridization, transcription factor, water deficit.

André Luís Hartmann Caranhato[1] (iD),
Jéssika Angelotti-Mendonça[2] (iD),
Liliane Marcia Mertz-Henning[2] ✉ (iD),
Silvana Regina Rockenbach Marin[2] (iD),
Carlos Lasaro Pereira de Melo[2] (iD),
José Salvador Simoneti Foloni[2] (iD),
Norman Neumaier[2] (iD),
José Renato Bouças Farias[2] (iD) and
Alexandre Lima Nepomuceno[2] (iD)

[1] Universidade Estadual de Londrina, Centro de Ciências Biológicas, Campus Universitário, Rodovia Celso Garcia Cid, PR-445, Km 380, CEP 86057-970 Londrina, PR, Brazil.
E-mail: andrelhcaranhato@gmail.com

[2] Embrapa Soja, Rodovia Carlos João Strass, Caixa Postal 231, CEP 86001-970 Londrina, PR, Brazil.
E-mail: jangelottimendonca@gmail.com, liliane.henning@embrapa.br, silvana.marin@embrapa.br, carlos.lasaro@embrapa.br, salvador.foloni@embrapa.br, norman.neumaier@embrapa.br, joserenato.farias@embrapa.br, alexandre.nepomuceno@embrapa.br

✉ Corresponding author

Tolerância à seca em cultivares-elite de soja com a introgressão do transgene AtAREB1

Resumo – O objetivo deste trabalho foi verificar se a introgressão do gene *AtAREB1* em dois germoplasmas-elite de soja, 'LS93-0375' e 'BMX Desafio RR', aumenta a tolerância dessas plantas ao deficit hídrico. As progênies F4 das duas cultivares-elite e da linhagem transgênica AtAREB1 (BR16-*AtAREB1*) e de seu background ('BR16') foram submetidas a deficit hídrico. Os bioensaios de deficit hídrico foram realizados em casa de vegetação, tendo-se utilizado as seis seguintes linhagens de soja: a geneticamente modificada BR16-*AtAREB1* e seu background 'BR16'; 'LS93' e sua progênie F4, LS93-*AtAREB1*; e 'BMX Desafio RR' e sua progênie F4, Desafio-*AtAREB1*. Utilizou-se delineamento experimental de blocos completos com tratamentos casualizados, em arranjo fatorial 6x2, com os seis genótipos de soja e duas condições hídricas – controle (C) e tratamentos de deficit hídrico (WD) –, com nove repetições. Os genótipos de soja que contêm o gene *AtAREB1* exibiram melhor desempenho fisiológico sob estresse hídrico e expressão alterada de genes responsivos à seca. A introgressão de *AtAREB1* na soja aumenta a tolerância à seca, independentemente do background genético em que o gene foi introduzido.

Termos para indexação: *Glycine max*, bZIP, hibridização, fator de transcrição, deficit hídrico.

Introduction

The activation of drought tolerance mechanisms (Fang & Xiong, 2015; Jogawat et al., 2021) involves the expression of several genes, which characterizes the drought tolerance as a qualitative trait. It means that this characteristic has a low heritability, turning the traditional breeding a labor-intensive and time-consuming process (Hu & Xiong, 2014). Therefore, genetic engineering provides an alternative or complementary approach to develop desired genes more efficiently (Fang & Xiong, 2015).

Soybean [*Glycine max* (L.) Merrill.] yield is severely affected by water deficit stress. To diminish yield losses, tremendous efforts for soybean breeding have been done to improve drought tolerance (Mertz-Henning et al., 2018). Several genes related to drought tolerance have been studied to get the drought-tolerant plant through genetic engineering (Zhu, 2002; Shinozaki &Yamaguchi-Shinozaki, 2007; Hu & Xiong, 2014). One of the promising genes used to generate drought-tolerant soybean plants is the transcription factor (TFs) AREB/ABF (abscisic acid-responsive element-binding protein/ ABRE binding factor) (Gao et al., 2011). The AREB TFs can recognize, in promoters region, a widely conserved sequence called ABA-responsive elements (ABRE - PyACGTGG /TC) of different stress-responsive genes regulating their expression under drought stress (Singh & Laxmi, 2015; Banerjee & Roychoudhury, 2017). In soybean plants, the overexpression of the *Arabidopsis AREB* gene (*AtAREB1*) resulted in better physiological performance both in greenhouse and field conditions, suggesting that it confers drought tolerance to soybean (Marinho et al., 2016; Fuganti-Pagliarini et al., 2017). Therefore, the introgression of this characteristic to elite soybean cultivars, via hybridization, may be an alternative to make them drought tolerant.

However, the introgression of a gene that confers drought tolerance to soybean plants is more complicated than the introgression of a qualitative gene to get resistance to herbicide or insects, for instance. This complexity refers to how plants may activate different strategies under drought stress. Plants under drought stress have their plant metabolism, growth, and development affected, since drought affects seed germination, seedling establishment, vegetative growth, flowering, and grain filling (Sreeman et al., 2018). For instance, the insertion of the transcription factor AREB1 from *Arabidopsis* in a soybean drought-sensitive genotype improves the stress tolerance in soybean plants. The transgenic plants can preserve water content in cells and, consequently, exhibit a better performance under water stress than genotypes without the *AtAREB1* gene (Marinho et al., 2016; Fuganti-Pagliarini et al., 2017; Fuhrmann-Aoyagi et al., 2021).

The objective of this work was to verify if the introgression of the *AtAREB1* gene in the 'LS93-0375' and 'BMX Desafio RR' elite soybean germplasms increases the tolerance of this plants to water deficit.

Materials and Methods

The genetically modified (GM) line BR16-*AtAREB1*, containing the drought tolerance gene *AtAREB1* (which was isolated from *Arabidopsis thaliana*, under the control of the CaMV35S promoter) was generated by the Laboratory of Biotechnology of Embrapa Soja, in partnership with the Japan International Research Center for Agricultural Sciences (JIRCAS). This GM line was generated via *Agrobacterium tumefaciens* transformation. BR16-*AtAREB1* genotype was also characterized under drought conditions by comparing it with its background 'BR16' that is considered susceptible to drought stress (Marinho et al., 2016). For the introgression, the GM line BR16-*AtAREB1* was used as a male genitor, in the simple hybridization process, with the elite genotypes 'LS93-0375' ('LS93') and 'BMX Desafio RR' ('Desafio'). To restore the genetic profile from the respective elite genotypes, three backcrosses were performed. The presence of the *AtAREB1* gene was confirmed by PCR test, in each F4 progeny used in the water deficit assay.

The water deficit bioassays were performed in a greenhouse, using the six soybean lines: BR16-*AtAREB1* and its background 'BR16'; the 'LS93' and its F4 progeny, named LS93-*AtAREB1*; and the 'BMX Desafio RR' and its F4 progeny, named Desafio-*AtAREB1*. The experimental design was carried out in randomized complete blocks, in a 6x2 factorial arrangement (six soybean genotypes and two water conditions – a control (C), and water deficit (WD) – with nine replicates. Briefly, to carry out the experiment, sterilized seed were germinated for 72 hours in Germitest paper, moistened with distilled water, and incubated at 25°C and 100% relative

humidity. After, the most vigorous and healthy seedlings were transferred to individual pots (15 cm external diameter x 10 cm base x 11 cm height) filled with 1 kg of a substrate composed of soil: sand: organic mixture (3:2:2). After transplanting, soybean plants were maintained in greenhouse at 28±2°C.

To guarantee the presence of the *AtAREB1* gene in BR16-*AtAREB1*, LS93-*AtAREB1* and Desafio-*AtAREB1*, leaf samples were collected at the V1 stage, according to Fehr & Caviness (1977) phenological scale, and used to isolate the total DNA, according to Doyle & Dickson (1987) protocol, followed by a PCR. For that, 1 μg of the genomic DNA of each sample was used as the template for PCR amplification ,using a primer to identify 35S: AtAREB1 cassette (Table 1). The PCR products were subjected to 1% agarose gel electrophoresis, where an amplicon of 607 bp was identified (Figure 1) (Marinho et al., 2016). Once confirmed the presence of the *AtAREB1* gene, the water deficit experiment was performed, as below described.

During the experiment, all pots were irrigated daily, close to field capacity until plantlets reached the V4 stage (Fehr & Caviness, 1977), except for 'LS93' plants, that belong to the 4.2 maturation group (Alliprandini et al., 2009), which were already instage R1 in the WD treatment application when the water stress was applied. The water deficit treatment (WD) was characterized by the total suspension of irrigation, while in the control treatment (C), the soil moisture was maintained close to the field capacity, following the methodology proposed by Marinho et al. (2016). Briefly, one day before the irrigation suppression, all pots were saturated with water, drained overnight, and, in the morning after, wrapped in polyethylene bags to prevent the loss of water by evaporation. The stomatal conductance (gs) values were daily monitored only in plants in the WD treatment, until they exhibited gs values below 0.2 mol H_2O m^{-2} s^{-1}, which typifies the water deficit stress (Flexas & Medrano, 2002), which was observed 5 days after the irrigation suppression, at the same moment as the leaves shriveled.

The measurements of photosynthetic rate (A), intercellular CO_2 concentration (*Ci*), transpiration rate (E), and stomatal conductance (*gs*) were carried out in the central leaflet of the third fully expanded trifoliate leaf (apex-base direction). All physiological parameters were measured using a portable infrared gas analyzer (LI-6400XT Portable Photosynthesis System, LI-COR, Lincoln, NE, USA) with a 90% red+10% blue light source, and a 2 cm^2 chamber. The relative water content (RWC %) was analyzed using three foliar discs of each plant, according to the method of Barrs & Weatherley (1962). These analyses were performed 5 days after the irrigation suppression.

Table 1. Primers used to confirm the insertion of the *AtAREB1* gene and primers used for RT-qPCR analysis.

Gene name	Description	Gene code	Primer sequence (5'-3')	Amplicon size (bp)	Amplification efficiency (%)
Primer to confirm the insertion of *AtAREB* gene					
35S:AtAREB1	Abscisic acid-responsive element-binding factor	*AT1G45249*	F: AGAAGAAAATCTTCGTCAACAT R: TAACAACTCATCCATGTTCATT	607	NA
Primer RT-qPCR Analyses					
Areb1	Abscisic acid-responsive element-binding factor	*AT1G45249*	F: GGAGGTGGAGGGTTGACTAGA R: CACTGCTCTGAAACTCATCAAACG	155	99
RAB18	Dehydrin	*Glyma.09G185500*	F: CAACTGGTGGCACTGGTTATGG R: TGGTCATGCTGACGATGTTCCT	103	97
HSP70	Heat-shock protein 70kD	*Glyma.17G072400*	F: TTTCGGGTTTGAATGTGTTG R: AGGTCAAAGATAAGCACGTT	115	90
NUDIX	NUDIX hydrolase 11	*Glyma.13G171900*	F: TGAGTGTTAGAAGGGCTACTGG R: AACTTTGCCAACGGCATC	108	88
FYVE	FYVE zinc finger	*Glyma.13G114700*	F: TTCTGTCTTCTGCAAGTGGTG R: GATCCCTCATCCATACATTTCAG	92	98

NA, not available.

Five days after the irrigation stop, and immediately after taking out polyethylene bags of each pot, a homogenous soil sample was collected, and the gravimetric soil moisture (GSM %) was determined following the protocol of Blake & Hartge (1986). Each physiological parameter was subjected to the analysis of variance ($\alpha = 0.05$) and to the Tukey's test ($\alpha = 0.05$), using the SAS 9.2 software.

After performing the physiological measurements, the third fully expanded trifoliate leaves of each soybean genotype of each water condition (C and WD) were collected, immediately frozen in liquid nitrogen, and stored at -80°C until total RNA isolation.

The total RNA was isolated using Trizol (Invitrogen Co., Carlsbad, CA, USA), according to the manufacturer's instructions. The integrity and the purity of the RNA were assessed in agarose gel, and the amount of RNA was quantified using a NanoDrop DNase Turbo spectrophotometer (Turbo DNA-free Kit, Thermo Fisher Scientific, Reinach, Switzerland) to eliminate residual genomic DNA. The cDNA synthesis was performed with 5 µg of total RNA from each sample using Superscript III First-Strand Synthesis System for RT-PCR (Invitrogen Life Technologies, Sigma-Aldrich, Barueri, SP, Brazil), according to the manufacturer's instruction; the cDNA was stored at
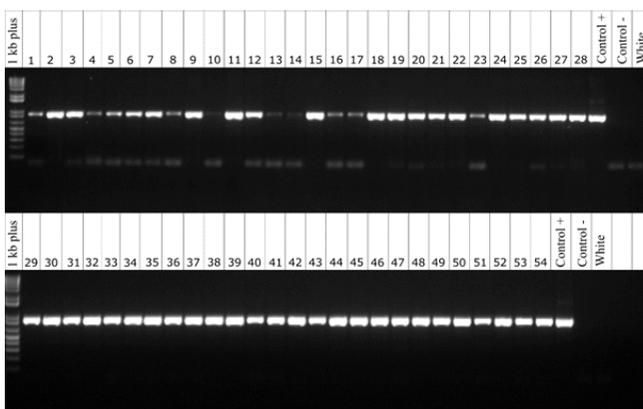

Figure 1. Validation of the *AtAREB1* gene presence via PCR products on electrophoresis agarose gel. The amplicon of 607 bp represents the *AtAREB1* presence. Samples 1 to 27 show the DNA from LS93-*AtAREB1* plants; samples 28 to 54 show the DNA from Desafio-*AtAREB1* plants. Control +, parent BR16-*AtAREB1*. Control -: wild type soybean. White: water.

-20°C until use. For the analysis of gene expression, the samples from nine replicates were grouped into three, to form three biological replicates.

Besides the *AtAREB1* expression, the stress-inducible gene *GmRAB18* and the *heat-shock protein 70 (GmHSP70)* relative expressions were also analyzed according to the method by Pfaffl (2001). The *FYVE* and *NUDIX* were used as reference genes (Fuhrmann-Aoyagi et al., 2021; Marcolino-Gomes et al., 2015); the primers used in the experiment are listed (Table 1). The reactions were carried out according to Marcolino-Gomes et al. (2015), in a 7300 RT-qPCR Thermocycler (Applied Biosystems/Life Technologies, Grand Island, NY, USA), using the following cycling parameters: 50°C for 2 min, 95°C for 10 min, 45 cycles at 95°C for 2 min, 62°C for 30 s, and 72°C for 30 s; the data were collected in the last (extension) phase. Standard curves were generated from fivefold serial dilutions of a cDNA pool to estimate the efficiency of the PCR amplification with each pair of primers.

The relative expression of the target genes was estimated by RT-qPCR and calculated according to Pfaffl (2001). The relative expression of the target genes (*AtAREB1, GmRAB18,* and *GmHSP70*) was shown as their proportion in control, used as a calibration sample, using two reference genes (*FYVE* and *NUDIX*). The experiment was performed with three biological and three technical replicates. The relative expression data were transformed using Log_{10} and subjected to both the analysis of variance and the Tukey's test at 5% probability, using SAS 9.2 software.

Results and Discussion

The physiological measurements (*Ci*, *gs*, E, and RWC) were similar for all genotypes in irrigated treatments (Figure 2), except for the photosynthetic rate (A) (Figure 2 A). In irrigated plants, the genotypes 'LS93', Desafio-*AtAREB1*, and BR16-*AtAREB1* exhibited the highest A values, while 'BR16', 'Desafio', and LS93-*AtAREB1* showed similar values. In contrast, under drought stress, BR16-*AtAREB1*, and Desafio-*AtAREB1* showed the highest values for photosynthetic rate – 15.07 and 14.22 µmol CO_2 m^{-2} s^{-1} –, respectively. Although LS93-*AtAREB1* plants have shown photosynthetic rates around 32% below BR16-*AtAREB1*, their A value was also almost 10-fold higher than the A value found in their parent 'LS93'. The

parents and background without the *AtAREB1* gene – 'Desafio', 'LS93', and 'BR16' – exhibited the lowest photosynthetic rate, with a maximum of 3.88 μmol CO_2 m^{-2} s^{-1}. Plants of 'BR16' and 'LS93' under drought stress showed the highest intercellular CO_2 concentration (*Ci*) (Figure 2 B). BR16-*AtAREB1* had an intermediate value that was 27% lower than its background 'BR16'. Moreover, the progenies F4 with the *AREB1* gene – LS93-*AtAREB*, and Desafio-*AtAREB* – and the parent 'Desafio' had the lowest *Ci* values. Also, under drought stress, the stomatal conductance (*gs*) values were higher in BR16-*AtAREB1*, Desafio-*AtAREB1*, and LS93-*AtAREB1* (Figure 2 C), while the genotypes without the *AREB1* gene exhibited the lowest *gs* values, which did not reach 0.04 mol H_2O m^{-2} s^{-1}. However, even Desafio-*AREB1* and LS93-*AREB1* plants exhibited higher *gs* values, in comparison with

their respective parent without the *AtAREB1* gene. The *gs* values identified in Desafio-*AREB1* and LS93-*AREB1* were respectively 45 and 60% lower than that found in BR16-*AtAREB1*. These results indicate the superior performance of BR16-*AtAREB1* in comparison with the F4 progenies. The introgression of the *AtAREB1* gene in 'LS93' and 'Desafio' allowed soybean plants to maintain stomata partially open, and plants could maintain a longer photosynthetic activity. BR16-*AtAREB1* had the highest transpiration rate (E), followed by Desafio-*AtAREB1* and LS93-*AtAREB1*, both with E values close to 0.002 mmol H_2O $m^{-2}s^{-1}$, which represents a decrease of about 45%, in comparison with BR16-*AtAREB1* (Figure 2 D). The soybean genotypes without the *AREB1* gene – 'BR16', 'LS93', and 'Desafio' – showed the lowest transpiration rates, which were 89, 77, and 62% lower
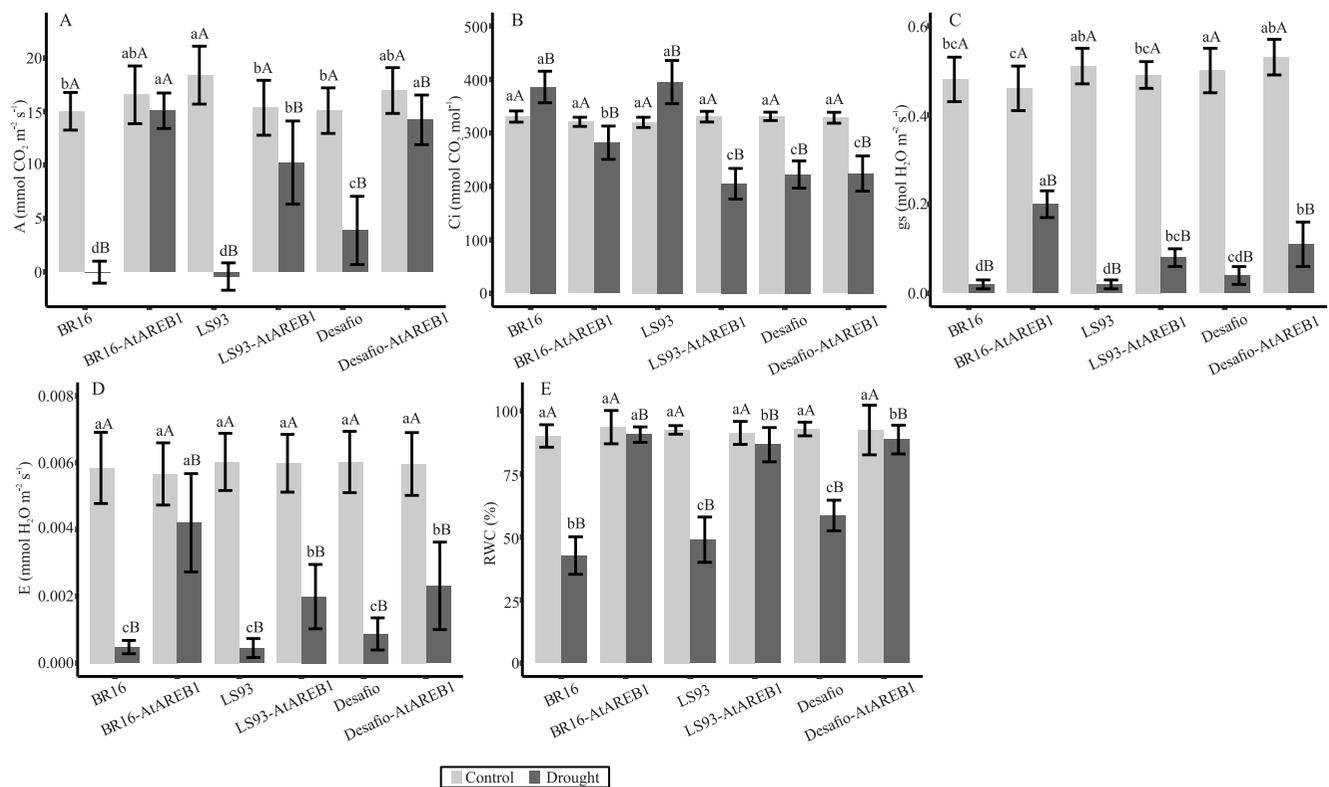
Figure 2. Physiological behavior of soybean plants with (BR16-*AtAREB1*, LS93-*AtAREB1*, and Desafio-*AtAREB1*) or without ('BR16', 'LS93', and 'Desafio') the *AtAREB1* gene: a) photosynthetic rate (A); b) CO_2 intercellular content (Ci); c) stomatal conductance (gs); d) transpiration rate (E); e) relative water content (RWC%). Uppercase letters compare the water treatments (control and drought) within the same genotype, and lowercase letters compare the genotypes within the same water treatment. Means followed by equal letters do not differ by the Tukey's test, at 5% probability. Bars represent the average value, and error bars represent the standard deviation. Experimental n=108.

than their *AtAREB1* genotypes – BR16-*AtAREB1*, LS93-*AtAREB1*, and Desafio-*AtAREB1*, respectively. Similarly, under drought stress, the greatest relative water content in leaves (RWC) was found in BR16-*AtAREB1* plants, followed by the progenies LS93-*AtAREB1* and Desafio-*AtAREB1*, whose values were close to 90% (Figure 2 E), while 'BR16', 'LS93', and 'Desafio' exhibited the lowest RWC values, which were up to 60%. LS93-*AtAREB1* and Desafio-*AtAREB1* showed greater physiological performance in comparison with their parents without this gene, which confirms the enhancement of soybean drought tolerance via the *AtAREB1* gene introgression. The introgression resulted in plants with higher values for stomatal conductance (*gs*), photosynthetic rate (A), relative water content (RWC), and transpiration rate (E), and lower intercellular content of CO_2 (*Ci*), under water deficit stress.

The first response of all plants under water deficit stress is the lowering of the transpiration rate due to stomatal closure (Sharma et al., 2020). The stomatal closure also leads to carbon starvation, due to the limitation of CO_2 absorption and, consequently, it limits the photosynthesis, which further affects other processes (Steppe et al., 2015). Moreover, soybean plants with the *AtAREB1* gene exhibited less water loss than those without the gene, expressed by their higher RWC, characterizing the water loss avoidance mechanism for drought tolerance (Fang & Xiong 2015; Jogawat et al., 2021). Additionally, BR16-*AtAREB1* plants exhibited a lower transpiration rate than their background 'BR16' (Marinho et al., 2016), confirming that the mechanism involved in drought tolerance, conferred by the *AtAREB1* introgression, is the avoidance. And, drought-sensitive genotypes showed a reduced photosynthesis process (Pinheiro & Chaves, 2011), whereas drought tolerant genotypes can maintain the photosynthetic activity at similar levels found in well-watered plants.

No interaction between genotypes and water treatments were found for gravimetric soil moisture (GSM). The plants with the introgression of the *AtAREB1* gene (Desafio-*AtAREB1* and LS93-*AtAREB1*) exhibited a slightly higher GSM (Table 2). The GSM represents the differential depletion of water from the soil of soybean genotypes (Fuhrmann-Aoyagi et al., 2021).

The BR16-*AtAREB1*, LS93-*AtAREB1*, and Desafio-*AtAREB1* genotypes had no difference for the relative expression of *AtAREB1* gene (Figure 3). Also, the transgenic plants exhibited lower *AtAREB1* gene expression in comparison with the control. The drought-responsive genes involved in the *AtAREB1* response pathway – *GmRAB18* and *GmHSP70* –, were upregulated in the parental genotypes 'BR16', 'LS93', and 'Desafio' under stress, in comparison with well-watered plants used as control (Figure 4 A and B). Similarly, in soybean plants with the *AtAREB1* gene, both genes were also upregulated in comparison with the control. Despite the upregulation of those genes, in BR16-*AtAREB1* and Desafio-*AREB1*, the *GmRAB18,* and *GmHSP70* expression levels were about three-fold lower than those of their respective backgrounds/parents without the *AtAREB1* gene ('BR16' and 'Desafio') (Figure 4 A and B). The overexpression of the *AtAREB1* gene from *Arabidopsis* in soybean that resulted in drought-tolerant plants was recorded by Marinho et al. (2016) and Fuganti-Pagliarini et al. (2017). Similarly, in the present research, the introgression of the gene *AtAREB1* through breeding resulted in drought-tolerant progenies. The expression of the *AtAREB1* gene in progenies strongly induced by drought was similar to that of the parent BR16-*AtAREB1*, enhancing drought tolerance of these two elite genotypes (Figure 3).

Table 2. Gravimetric soil moisture (GSM) in pots of genotypes with (BR16-*AtAREB1*, LS93-*AtAREB1*, and Desafio-*AtAREB1*) or without ('BR16', 'LS93', and 'Desafio') the *AtAREB1* gene, under control and drought[1].

Main Factor	GSM (%)
Genotype	
BR16	12.64c
BR16-*AtAREB1*	15.23ab
LS93	12.89c
LS93-*AtAREB1*	15.43ab
Desafio	13.76bc
Desafio-*AtAREB1*	15.63a
Water condition	
Control	23.22a
Drought	5.31b
CV (%)	23.10
Genotype x water condition	ns

[1]Means followed by equal letters do not differ by the Tukey's test, at 5% probability. [ns]Nonsignificant.

The *AtAREB1* gene belongs to the transcription factor AREB/ABF family, a set of regulatory genes that play a pivotal role in stress-inducible gene activation through the ABA-dependent pathway (Shinozaki & Yamaguchi-Shinozaki, 2007; Singh & Laxmi, 2015). The overexpression of the *AREB1* gene was also reported as a protection against oxidative stress, due to an increase of the antioxidant activity and activation of mechanisms of cell-water maintenance (Fuhrmann-Aoyagi et al., 2021). It occurs because the transcription factor AREB1 binds to the gene promoter region (ABRE) and it modulates the expression of a specific set of genes, converting stress-induced signals into cellular response (Singh & Laxmi, 2015).

In the present study, soybean overexpressing *AtAREB1* gene modulated the expression of the drought-inducible genes *GmRAB18* and *GmHSP70* that are involved in water deficit tolerance. The introgression of the *AtAREB1* gene in the elite genotypes 'LS93' and 'Desafio' resulted in the reduction of expression from the drought-responsive genes *GmRAB18* and *GmHSP70*, under stress conditions. These genes are stress-responsive and were activated in both GM and non-GM plants. However, the expression of those
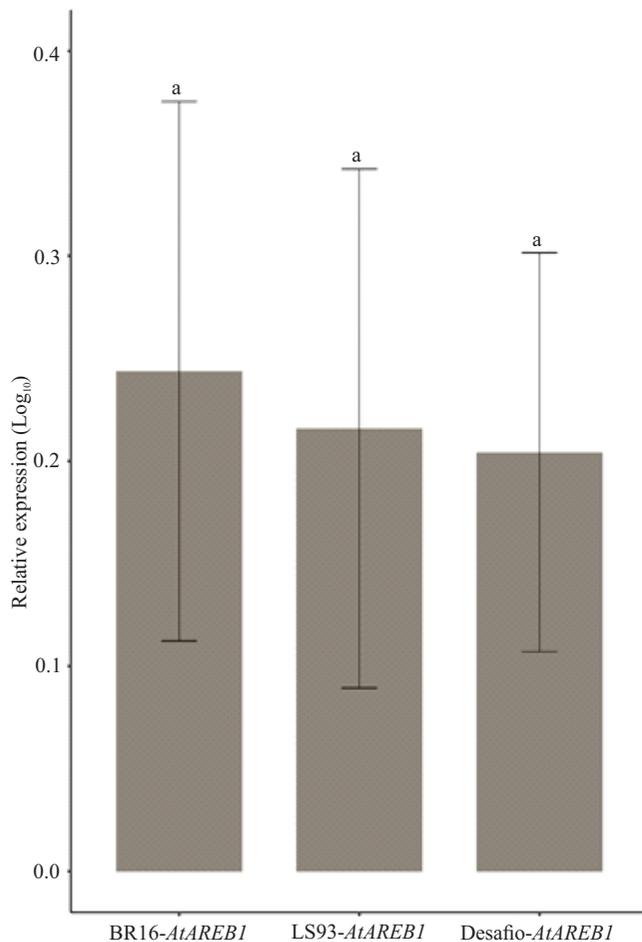
Figure 3. Relative expression of the *AtAREB1* gene from soybean plants BR16-*AtAREB1*, LS93-*AtAREB1*, and Desafio-*AtAREB1*. Means followed by equal letters do not differ by the Tukey's test, at 5% probability. Bars represent the average value, and error bars represent the standard deviation. Experimental n=45.
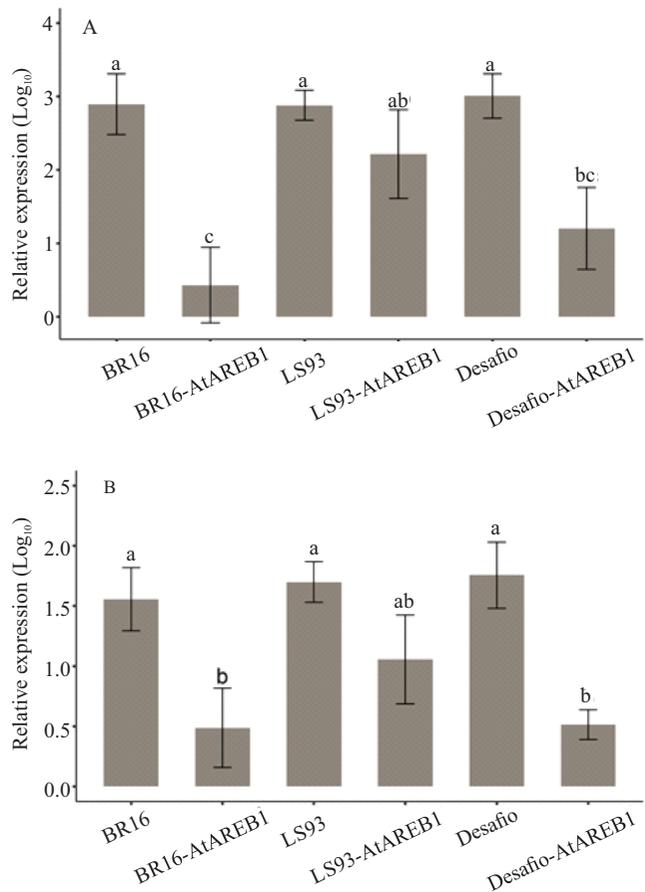
Figure 4. Relative expression level of two genes activated by the transcription factor AREB1 under drought conditions: A, *GmRAB18*; B, *GmHSP70*. Well-watered plants were used as control samples, and the relative expressions were transformed using Log_{10}. Means followed by equal letters do not differ, by the Tukey's test, at 5% probability. Bars represent the average values, and error bars represent the standard deviation. Experimental n=108.

stress-inducible genes in soybean BR16-*AtAREB1*, LS93-*AtAREB1*, and Desafio-*AtAREB1* was lower than that found in their respective background ('BR16') and parents ('LS93' and 'Desafio', respectively), indicating that the *AtAREB1* gene introgression allows of earlier stress response, making these elite soybean genotypes less sensitive to stress and, consequently, increasing their drought tolerance.

Both GmRAB18 and GmHSP70 proteins are described to act as molecular shields during stress, due to the many roles they play in stressed environmental resilience (Priya et al., 2019). These genes are also considered stress indicators due to their higher expression level under water deficit (Fuhrmann-Aoyagi et al., 2021). RAB18 protein belongs to a classical dehydrin of group 2 of LEA proteins, and it is one of the first proteins to be expressed under drought stress mediated by ABA induction (Graether & Boddington, 2014), whereas *GmHSP70*, a 70-kDa heat shock protein, belongs to a conserved class of chaperones, and it is crucial for folding newly synthesized polypeptides, refolding of misfolded or aggregated proteins, and assisting in the degradation of proteins (Sable & Agarwal, 2018). HSP70 proteins synthesis is also regulated by ABA, which induces the H_2O_2 production enhancing the HSP70 synthesis upregulating the activity of antioxidant enzymes (Hu et al., 2010).

Conclusions

1. The LS93-*AtAREB1* and Desafio-*AtAREB1* soybean (*Glycine max*) progenies exhibit better performance under drought stress than their respective parental genotypes without the *AtAREB1* gene.

2. The hybridization of the elite cultivars 'LS93' and 'Desafio' with BR16-*AtAREB1* results in drought-tolerant genotypes, due to the transgene *AtAREB1* introgression, through the drought avoidance mechanism.

3. The introgression of *AtAREB1* gene in soybean increases plant drought tolerance, regardless of the genetic background in which the gene is introduced.

Acknowledgments

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