



## Flower and pod genes involved in soybean sensitivity to drought

Mayla Daiane Correa Molinari, Renata Fuganti-Pagliarini, Juliana Marcolino-Gomes, Daniel de Amorim Barbosa, Silvana Regina Rockenbach Marin, Liliane Marcia Mertz-Henning, Alexandre Lima Nepomuceno & Elíbio Leopoldo Rech Filho

To cite this article: Mayla Daiane Correa Molinari, Renata Fuganti-Pagliarini, Juliana Marcolino-Gomes, Daniel de Amorim Barbosa, Silvana Regina Rockenbach Marin, Liliane Marcia Mertz-Henning, Alexandre Lima Nepomuceno & Elíbio Leopoldo Rech Filho (2021) Flower and pod genes involved in soybean sensitivity to drought, Journal of Plant Interactions, 16:1, 187-200, DOI: [10.1080/17429145.2021.1921293](https://doi.org/10.1080/17429145.2021.1921293)

To link to this article: <https://doi.org/10.1080/17429145.2021.1921293>



© 2021 The Author(s). Published by Informa UK Limited, trading as Taylor & Francis Group



[View supplementary material](#)



Published online: 02 May 2021.



[Submit your article to this journal](#)



Article views: 460



[View related articles](#)



[View Crossmark data](#)

## Flower and pod genes involved in soybean sensitivity to drought

Mayla Daiane Correa Molinari<sup>a,b,\*</sup>, Renata Fuganti-Pagliarini<sup>a,\*</sup>, Juliana Marcolino-Gomes<sup>a</sup>, Daniel de Amorim Barbosa<sup>a,b</sup>, Silvana Regina Rockenbach Marin<sup>a</sup>, Liliane Marcia Mertz-Henning<sup>a</sup>, Alexandre Lima Nepomuceno<sup>a</sup> and Elíbio Leopoldo Rech Filho<sup>c</sup>

<sup>a</sup>Embrapa Soybean, Londrina, Brazil; <sup>b</sup>Department of General Biology, Londrina State University, Londrina, Brazil; <sup>c</sup>Embrapa Genetic Resources and Biotechnology, Brasília, Brazil

### ABSTRACT

In soybean, studies on drought-responses are conducted during vegetative season. Information related to genes triggered in response to water-deficit (WD) in flower and pod is lacking. We performed an RNASeq and an agro-physiological characterization at stages R2 and R4 of soybean cultivar BR16, under WD. Physiological results showed a decrease in gas exchange parameters. Agronomical results showed WD impaired yield. Global Gene Ontology analyses indicated that most of the Differential Expressed Genes (DEGs) were down-regulated in flowers but up-regulated in pods. qRT-PCR revealed that WD triggered hormone biosynthesis changes. Mechanisms such as a decrease in glyceollin levels and cell wall instability were observed. This data showed tissue-specific mechanisms correlated with phenotypic responses such as drought escape, early flowering hormone-induced, and loss in grain weight. We presented here a comprehensive expression profile of flowers and pods soybean genes, which could guide researchers in the development of plants more tolerant to drought.

### ARTICLE HISTORY

Received 9 December 2020  
Accepted 20 April 2021

### KEYWORDS

*Glycine max*; water deficit; drought escape; drought sensitivity; RNA-Seq; genes differentially expressed

## Introduction

Natural disasters, between 2005 and 2015, cost the agricultural sectors of developing country economies a staggering US\$96 billion in damage, crop loss, and livestock production worldwide according to Food and Agriculture Organization of the United Nations (FAO 2018). Drought, which has battered farmers in all corners of the globe, was one of the leading culprits. Eighty-three percent of all drought-caused economic losses documented by FAO's study were absorbed by agriculture, with a price tag of US\$29 billion (FAO 2018). In Brazil, where agriculture is a key factor in the national economy, the importance of real and potential prejudices due to drought can be speculated by analyzing some numbers. Especially, in soybean production, an important stanchion on agriculture and Brazilian economy, losses due to WD, added up US\$79 billion in financial losses during 1976/1977 and 2013/2014 crop seasons (Ferreira 2016). According to IBGE (Brazilian Institute of Geography and Statistics), in 2015, a year when Brazil went through a serious financial crisis, agriculture was the only economic sector that did not drop its contribution to GDP (Gross Domestic Product) mainly because of soybeans and corn production, 1.8% over the previous year (FAO 2016).

Besides this positive survival in the face of the financial crisis and increasing productivity numbers, upcoming climate predictions are not good for food producers. According to the last report from the Intergovernmental Panel on Climate Change (IPCC 2011), every year from 1961 to 2013, an additional 1 percent of the world's drylands slipped into drought, which will put production, food security and food access in danger. Thus, in the present context and future

picture of climatic changes, many alternatives can be used to mitigate and reduce production and financial losses due to drought in soybean. Several studies have been developed to understand soybean plant's response to drought aiming to develop strategies to reduce the harmful effects of WD, to protect vulnerable farming systems and the populations depending on farmers. During the vegetative stage, water shortage can delay seedling emergence and triggers the early transition to the reproductive period. When it occurs at the reproductive stage (flowering and pod formation) can reduce productivity, usually as a result of decreased numbers of flowers, pods, and seeds, induced by different processes. WD also affects key metabolism processes, such as photosynthesis reducing carbon fixation and the availability of photo-assimilates needed for seed formation, which also, impairs grain filling and therefore final productivity (Pinheiro and Chaves 2010).

Although flower, pod, and seed formation are among the main phases affected by WD, most of the available information about drought responses in soybean was assayed in the vegetative and reproductive developmental stage in roots and leaves (Manavalan et al. 2009; Savitri et al. 2013; Shanker et al. 2014; Kidokoro et al. 2015; Xu et al. 2018), leaving a gap about the genes and metabolic pathways involved in the mechanisms of response and acclimatization to WD in soybean reproductive organs, such as flower and pod. Thus, in this context, the present study aimed for the first time to reveal the gene expression profile in flowers (at R2 stage – full bloom) and pods (at R4 stage – pods completely developed) in soybean under WD. This data could help uncover metabolic pathways triggered in reproductive

**CONTACT** Renata Fuganti-Pagliarini  [renatafuganti@gmail.com](mailto:renatafuganti@gmail.com)

\*These authors contributed equally to this work.

 Supplemental data for this article can be accessed at <https://doi.org/10.1080/17429145.2021.1921293>.

structures and be a raw material for the development of strategies to increase drought tolerance in plants.

## Material and methods

### Biological material and greenhouse experiment

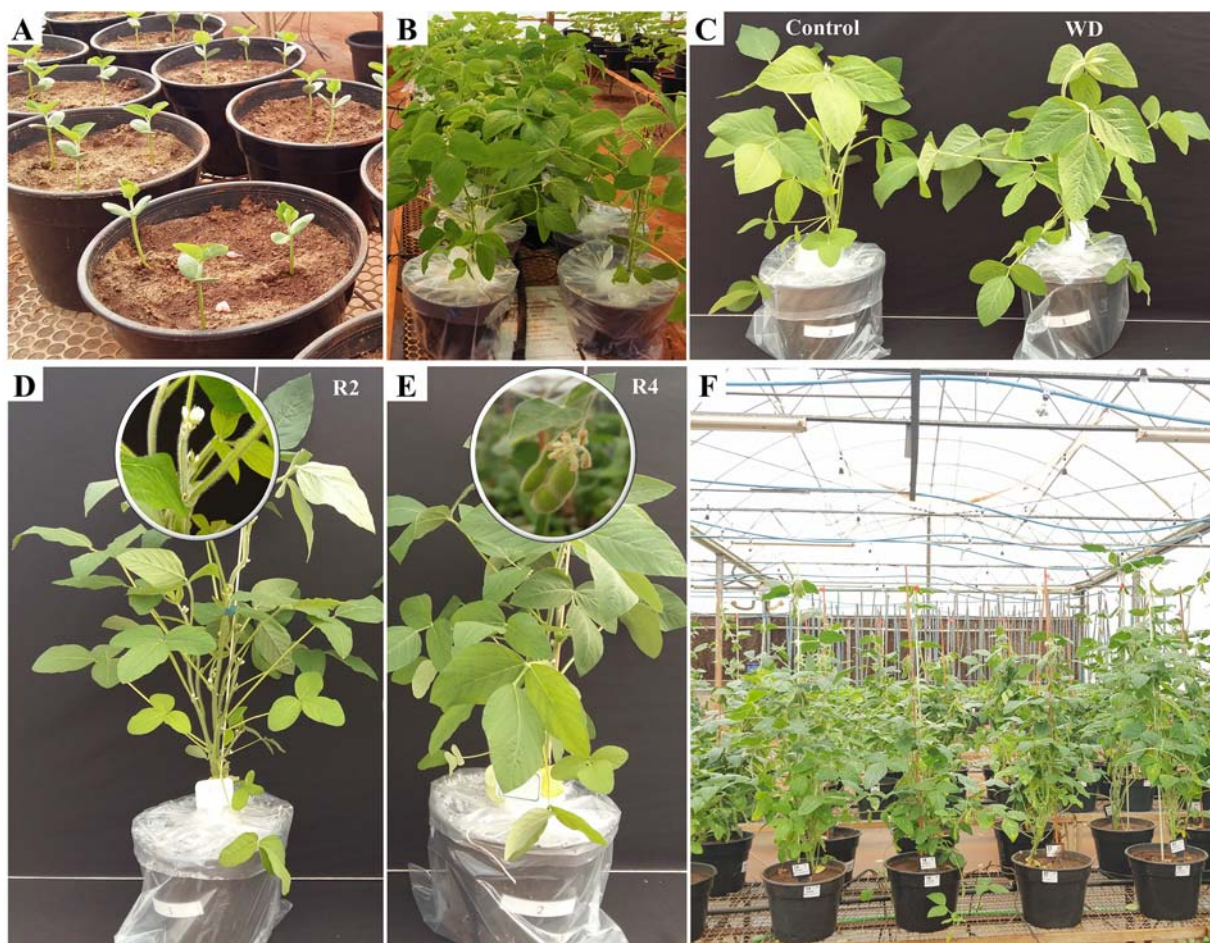
Soybean seeds from conventional cultivar BR16, considered drought-sensitive (Oya et al. 2004; Lima et al. 2019) were treated with fungicide Vitavax® Thiram 200 SC (200 g.L<sup>-1</sup>) (ADAPAR) for health quality purposes and then allowed to germinate on Germitest® paper in the dark in germinating chamber, for 4 days, at 28 ± 1°C and 100% relative humidity (RH). Seedlings were transferred to 5 kg pots filled with substrate mixture 1:1 (fertilized soil and washed sand), one plant per pot. The experiment was carried out in greenhouse conditions under a short-day condition (10 h light/14 h dark) at 28 ± 2°C.

Three experiments were carried out in parallel. The experimental design was in completely randomized blocks, in 2 × 3 factorial arrangement, i.e. two water conditions (water deficit – WD; control – C), three collecting points (R2 to collect flowers, R4 to collect pods and at end of the developmental cycle to assay agronomical parameters), with eight blocks. Pots were maintained at 100% field capacity (FC) through daily irrigation with a fixed water volume sufficient to saturate the substrate until plants reached the phenological stage V7. At this stage, one day before WD induction, all

pots were saturated with water at the end of the afternoon to allow excess water to be drained overnight. In the following morning, pots were wrapped in polyethylene bags, and the central region of each pot was covered with cotton around the stem base to prevent water loss by evaporation (Figure 1). Water deficit was imposed by completely withholding water for 7 days. After water deficit imposition, control plants were kept hydrated by watering them with 180 mL per day.

### Physiological and agronomical parameters analysis

Irrigation was kept in C plants, while was withheld in the WD group, which was monitored daily to the stomatal conductance ( $g_s$ ) until plants showed  $g_s$  values less than 200 mmol H<sub>2</sub>O m<sup>-2</sup>.s<sup>-1</sup> (condition considered as WD stressed) (Flexas et al. 2004; Salinet 2009). When plants were at the R2 developmental stage, i.e. in full flowering which means that there is an open flower at one of the two uppermost nodes, gas exchange parameters were assayed. Photosynthetic rate ( $A$ ), stomatal conductance ( $g_s$ ), sub-stomatal CO<sub>2</sub> concentration ( $C_i$ ), transpiration rate ( $E$ ), leaf temperature and vapor-pressure deficit were measured on the central leaflet of the third fully-expanded trifoliate leaf (apex-base direction) using a portable infrared gas analyzer (LCpro-SD, ADC BioScientific). These measurements were performed inside a greenhouse from 9.00 am (Brazilian daylight-saving time) at 1000 μmol



**Figure 1.** In (A), soybean plants growing in the early stages in greenhouse conditions, showing three plants per pot. Before drought treatment imposition, two plants were removed, keeping more homogenous plants to carry out the experiment, in an attempt to avoid bias due to growth. In (B), plants wrapped in polyethylene bags, and with the central region of each pot covered with cotton around the stem base to prevent water loss by evaporation. In (C), control and treated soybean plants, showing wilting. In (D and E), respectively soybean plants in R2 and R4 developmental stage, showing in detail, flower and pod. In F, general view of soybean plants growing in greenhouse. These plants were assayed for agronomical parameters.



$m^{-2}.s^{-1}$  photosynthetically active radiation (PAR). The intrinsic water use efficiency was obtained through the ratio  $A/g_s$ , as the ratio of carbon assimilation to the correspondent water loss at the leaf level (Medrano et al. 2015). At this point, flowers were collected from WD and C plant groups, individually from each block and the experiment was discarded. After that, other WD parallel experiments were kept until plants reached the R4 developmental stage, i.e. in the full pod, which means pods are 3/4 inch (2 cm) at one of the four uppermost nodes, pods were collected. Physiological parameters were again evaluated at this collecting point and the experiment was discarded. A third experiment with WD and C plant groups was conducted until the end of the reproductive cycle, to evaluate agronomical parameters such as plant height, number of nodes (NN), number of pods with and without seeds, dry mass of pods with and without seeds, number of seeds (NS), dry mass of seeds (DMS), the total number of pods (TNP), dry mass of 100 seeds (DM100S), the mean distance between nodes and average number of seeds per pod. These agronomical results were subjected to statistical normality test (Shapiro and Wilk 1965), variance analysis (ANOVA) and means of 8 biological replicates comparison by Tukey test ( $p \leq 0.05$ ) using Sasm-Agri and R Studio v.3.5.1 software (Canteri et al. 2001; Racine 2012).

### mRNA-Seq libraries sequencing

Flowers and pods (without seeds) from soybean plants subjected to WD and C conditions were collected respectively, at the development stages R2 and R4, immediately frozen in liquid  $N_2$  and stored at  $-80^\circ C$ . Total RNA was extracted using Trizol® reagent (Life Technologies, Grand Island, NY, USA) according to the manufacturer's specifications.

RNA quantification was performed using the NanoDrop spectrophotometer, according to the following parameters of quality and purity: concentration  $>600$  ng  $\mu L$ , ratio 260/280 ranging between 1.8 and 2, and ratio 260/230  $\geq 2.0$ . RNA was treated with DNA-free turbo DNase kit (Life Technologies, Grand Island, NY, EUA). The RNA integrity was evaluated in agarose gel electrophoresis 1% (p/v) with ethidium bromide (1  $\mu g/mL$ ) (Sambrook et al. 1989). Quality samples were again evaluated at Agilent Bioanalyzer 2100 (Agilent Technologies, Inc.) and only samples with RNA Integrity Number (RIN)  $\geq 7.00$  were used to synthesize the mRNA-Seq libraries. High-quality RNA samples (1000–1800 ng) were sent to Georgia Genomics Facility (GGF), at the University of Georgia (USA), to prepare libraries and sequence the mRNA-Seq libraries. Sixteen libraries, which corresponded to 4 biological repetitions (RNA equimolar libraries) of each treatment and biological material (4 libraries using flowers under WD, 4 libraries using flowers under C, 4 libraries using pods under WD and finally 4 libraries using pods under C) were synthesized using KAPA Stranded mRNA-Seq Illumina® platform kit (Illumina). Libraries were sequenced in Illumina NextSeq 500 1.9 poli-A 75 bp paired-end (Illumina, San Diego, CA, EUA), with about 1X genome coverage.

### Bioinformatics analysis

From each library, forward and reverse reads were overlapped to generate longer consensus fragments using COPE v.1.2.5 software (Liu et al. 2012). The quality of

these consensus fragments was evaluated using FastQC v.0.11.5 software (Andrews 2010; Patel and Jain 2012). Removal of adaptors and low-quality sequences were carried out through Trimmomatic version 0.36 software (Bolger et al. 2014) standardizing cuts every 4 bases in the extremities of the sequences that presented quality score less than 20 (Phred Quality Score,  $Q \geq 20$ ). The quality of resulting fragments was checked again using FastQC v.0.11.5. Soybean reference genome (*Glycine max* Wm82.a2.v1) was downloaded from Phytozome ([https://phytozome.jgi.doe.gov/pz/portal.html#!info?alias=Org\\_Gmax](https://phytozome.jgi.doe.gov/pz/portal.html#!info?alias=Org_Gmax)). Genome indexing and alignment of reads were performed using HISAT2 software v.2.1.0., with the final recovery of reads with unique alignments (Kim et al. 2015). Unnatural duplications from the PCR step were removed using the Samtools v.1.5 software (Li et al. 2009). Mapping was carried out using Stringtie v.1.3.3 software (Pertea et al. 2015). The differential expression analysis was performed by EdgeR software v.3.22.3 (Robinson et al. 2010) in RStudio v.3.5.1 (Racine 2012). For each cultivar, DEG was obtained by comparing the C and WD conditions for each tissue (flower and pod). Genes considered differential expressed presented  $\log_2$  fold-change ( $\text{Log}_2\text{FC}$ )  $\leq -2$  and  $\geq +2$  values with false discovery rate (FDR)  $\leq 0.05$  excluding negative  $\log\text{CPM}$  (Molinari 2021). The annotation of the biological function of DEG was performed using the Phytomine tool available at Phytozome (Goodstein et al. 2011). A parametric analysis of gene enrichment (PAGE) was performed using differentially expressed genes sets exclusive from flower and pod and their respective  $\text{Log}_2\text{FC}$  (Kim and Volsky 2005).

### Candidate genes selection process

Nine differentially expressed genes in flower and pods with biological annotated function were considered to be tested. For flowers, selected genes were: Glyma.06G049900 [(1-Aminocyclopropane-1-Carboxylate Synthase 8-Related - ACS8, E.C.4.4.1.14), (primer F 5'ACATATCTCCTGGCTCTTC T3'/primer R 5'GGTAATTGAGTCCGCAAAG3')], Glyma.11G129300 [(beta-glucosidase - BG1, EC.3.2.1.21), (primer F 5'ATAGCCAACATGGTTATGGA3'/primer R 5'AGAAGTTGGTGATGTGAGAC3')], Glyma.10G295300 [(Glycinol 4- dimethylallyltransferase - G4DT, EC.2.5.1.36), (primer F 5'TTGTTGTGAAGGCAATCTCT3'/primer R 5'TGCCAATCATTTGTGTATGGA3')], Glyma.10G193800 [(Embryonic Flower 2 - EMF2), (primer F 5'GTTGATGGGAAGGGGAATAC3'/primer R 5'CAGAAGCAAAGACC AAGAACC3')] and Glyma.09G196500 [(MYBS2 putative), (primer F 5'GATCTGACAACTCTCCTCC3'/primer R 5'TGTCGGCCATTATTGGTAAA3')]. For pods, selected genes were: Glyma.08G044000 [(pectinesterase - PME, EC .3.1.1.11), (primer F 5'CTCAAACGCTCAATGAACTC 3'/primer R 5'AGTGAAACCATTTTGGCATG3')], Glyma.19G009000 [(formate dehydrogenase - FDH, EC .1.1.1.9), (primer F 5'ATTCCTGATGCCAATGTC AT3'/primer R 5'TCAACGTGATCAGAACCAAT3')], Glyma.12G146400 [(ABA 8'-hydroxylase - CYP707A4, EC .1.14.14.137), (primer F 5'TGAAGTTGGAATCCTCAC AG3'/primer R 5'TTGTCCACAATCCGGTAATT3')] and Glyma.03G042700 [(WRKY transcription factor 33), (primer F 5'GAGCCACTAAAGAAACAGGA3'/primer R 5'GGTTT GATTGAGGCTAATGC3')]. Sequences were obtained from Phytozome and specific primers were designed using

Primer3Plus software (<https://primer3plus.com/cgi-bin/dev/primer3plus.cgi>). Homo and heterodimers were checked using Multiple Primer Analyze software (<https://www.thermofisher.com/br/multiple-primer-analyzer.html>).

### Validation of RNA-Seq gene expression by qRT-PCR

Total RNA (RNA Integrity Number (RIN)  $\geq 7.00$ ) was extracted from soybean flower and pod samples using Trizol<sup>®</sup> reagent, treated with DNase I kit (Invitrogen, Carlsbad, CA) to remove possible DNA remained. After that, cDNA was synthesized using Super Script<sup>®</sup> III First-Strand Synthesis System (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. qRT-PCR reactions were composed of cDNAs, 0.2  $\mu\text{M}$  F and R primers, and 1x reaction buffer Platinum<sup>®</sup> SYBR Green<sup>®</sup> qPCR SuperMix UDG (Invitrogen, Carlsbad, CA). Gene expression calibration was performed using  $\beta$ -actin (Glyma.15G050200 – primer F 5'GAGCTATGAATTGCCTGATGG3'/ primer R 5'CGTTTCATGAATTCCAGTAGC3') and *Fyve* (Glyma.13G114700 – primer F 5'TTCTGTCTTCTGCAAGTGGTG3'/ primer R 5'GATCCCTCATCCATACATTTTCAG3') genes, as described by Marcolino-Gomes et al. (2015). Gene expression relative quantification was performed using three random biologicals and three technical replicates ( $n = 9$ ). Reactions were made through 7900HT thermocycler equipment (Applied Biosystems). Cycling conditions used were denaturation at 95°C for 20 s (s) followed by 40 cycles of 95°C for 3 s, 60°C for 26 s, and 1 cycle for Melt curve at 95°C for 15 s, 60°C for 1 min and 95°C for 15 s. PCR primers efficiencies were estimated using LinRegPCR software v.2012.0 (Ruijter et al. 2009), considering as efficient primers displaying values  $\geq 85\%$ . The expression level was determined using the formula  $2^{-\Delta\Delta C_t}$  adapted according to the primer's efficiencies (Livak and Schmittgen 2001). Statistical analysis was performed using the *t*-test ( $p \leq 0.05$ ) from Sasm-Agri software (Canteri et al. 2001). The Pearson correlation between qRT-PCR and RNA-Seq log<sub>2</sub>FC expression was performed in RStudio v.3.5.1 (Racine 2012).

## Results

### Physiological and agronomical data analysis

Physiological data obtained revealed that in flowers (R2) and pods (R4), WD treatment decreased gas exchange parameters when compared to C conditions (Figure 2). In the R2 developmental stage, photosynthetic rate (*A*) decreased from 19.90 to 11.71  $\mu\text{mol CO}_2 \text{ m}^{-2} \cdot \text{s}^{-1}$  in C and treated plants, respectively, differing statistically between treatments. WD also impaired stomatal conductance (*g<sub>s</sub>*), declining from 0.31 to 0.07  $\text{mol H}_2\text{O m}^{-2} \cdot \text{s}^{-1}$  in treated compared to C flowers, characterizing the WD condition as moderate (Flexas et al. 2004). Stomatal closure severely impacted sub-stomatal  $\text{CO}_2$  concentration (*C<sub>i</sub>*), which was reduced by more than 50% in the WD condition, from 260.94 in C to 122.93  $\mu\text{mol CO}_2 \text{ mol air}^{-1}$  in WD plants. There was also a negative impact on the transpiration rate, which dropped from 3.88  $\text{mmol H}_2\text{O m}^{-2} \cdot \text{s}^{-1}$  (C) to 1.33  $\text{mmol H}_2\text{O m}^{-2} \cdot \text{s}^{-1}$  (WD). WD condition increased vapor pressure (from 1.25 in C to 1.78 kPa in WD) and leaf temperature (from 28.38 to 29.99°C in C and treated plants, respectively), mainly due to altered cellular evapotranspiration. Intrinsic water-use efficiency was increased from 75.38

under C to 165.43 under WD treatment, being statistically different (Tukey test at 5%).

Similar results were obtained in the physiological analyzes performed during the R4 developmental stage. C and treated plants presented average stomatal conductance values of 0.45 and 0.14  $\text{mol H}_2\text{O m}^{-2} \cdot \text{s}^{-1}$ , respectively. Significant reduction in *C<sub>i</sub>* was also identified, decreasing from 274.45 in C to 147.21  $\mu\text{mol CO}_2 \text{ mol air}^{-1}$  in WD. Transpiration rate was also reduced, from 5.66 (C) to 2.81  $\text{mmol H}_2\text{O m}^{-2} \cdot \text{s}^{-1}$  in WD. Likewise was observed in the R2 developmental stage, in the R4 stage, an increase in vapor pressure (from 1.28 in C to 1.98 kPa in WD plants) and leaf temperature (from 27.21 to 29.88°C in C and treated plants, respectively) was also observed. The change in these parameters resulted in a decrease in the average photosynthetic rate, which dropped from 25.35 in C plants to 17.30  $\mu\text{mol CO}_2 \text{ m}^{-2} \cdot \text{s}^{-1}$  in plants under WD (Figure 2) and a rise in intrinsic water-use efficiency, which increased from 66.88 in C conditions to 141.23 under WD treatment, being statistically different (Tukey test at 5%).

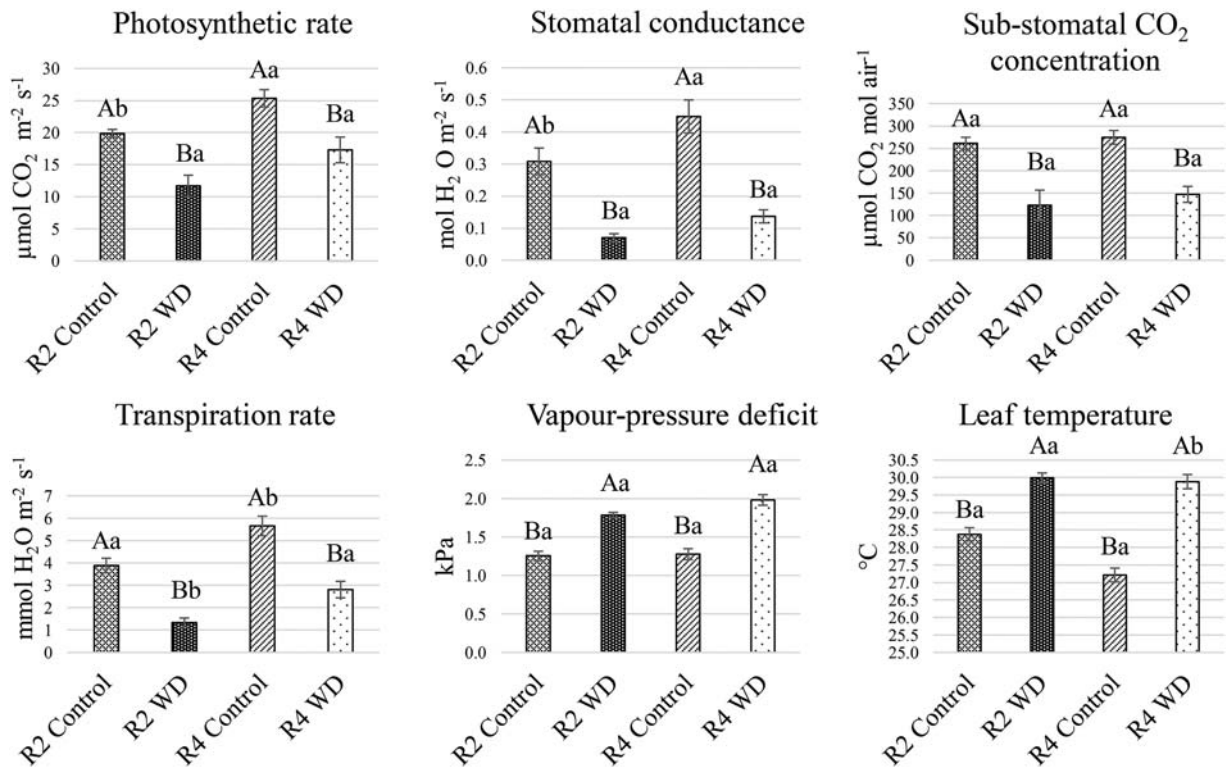
Drought responses in soybean BR16 sensitive cultivar were more pronounced in the R2 stage if compared to the R4 stage. Gas exchange reduction was higher in R2 compared to R4 being 9.44% for photosynthesis, 7.67% for stomatal conductance, 6.53% for sub-stomatal  $\text{CO}_2$  concentration, and 15.31% for transpiration, suggesting that flowers were more sensitive to WD than pods. Vapor pressure (12.86%) and leaf temperature (4.11%) reductions were also more prominent in R2 compared to R4.

WD treatment also impaired agronomical parameters (Table 1). R2 and R4 developmental stages were reached respectively, at 40 and 50 days after planting for all treatments (WD and C). WD condition reduced the average number of flowers per plant, from 12 to 8.44 flowers although no statistically significant (Table 1). Likewise, the total number of pods also decreased from 84.78 in C plants to 60 in WD treated plants (Table 1), differing statistically and strongly suggesting that WD might have induced pods abortion.

No differences between soybean plants under C and WD conditions were identified for average height ( $\sim 32$  cm), number of nodes ( $\sim 9$  nodes), the mean distance between nodes ( $\sim 3$  cm), number of pods without seed ( $\sim 16$ ) and dry mass of pods without seeds ( $\sim 29$  g). Statistical differences were observed in the number of pods with seeds (from 66.56 to 47.11), the total number of pods (from 84.78 to 60), dry mass of pods with seeds (from 33.18 to 24.62 g), number of seeds per plant (from 132.89 to 91.67) and dry mass of 100 seeds (from 25.50 to 19.34 g) (Table 1). These results indicated that the application of WD, even in a moderate and controlled manner, resulted in a negative impact on several agronomic parameters in soybean plants.

### Expression profile of soybean flower and pod genes under WD

COPE software analyses showed an average of 46% of paired-end reads overlapping (Additional file 1). These overlapped reads represent longer consensus fragments with more accuracy in the nucleotide sequence, increasing quality transcript assembly. Before overlapping, reads showed about 75 bp and after it, reads displayed a length about 130 bp. GC content after cleaning (45–47%) showed that no significant contaminations were present in the samples, thus not impairing reads



**Figure 2.** Gas exchange parameters assayed in soybean leaves at R2 and R4 developmental stages under WD and C conditions. Measurements of photosynthetic rate (A) – C.V% = 22.02, stomatal conductance (*gs*) – C.V% = 38.33, sub-stomatal CO<sub>2</sub> (C) – C.V% = 33.27, transpiration rate (E) – C.V% = 26.30, vapor-pressure deficit – C.V% = 9.74 and leaf temperature (°C) – C.V% = 1.64. Capital letters compared C to WD conditions within the developmental stage (R2 and R4) separately. Minor letter compared developmental stages within conditions (C and WD) separately. Means followed by the same letter stand for no significant difference according to means of 8 biological replicates comparison by Tukey test at 5%. Bars mean pattern error. Legend: ANOVA C.V%: Coefficient of Variation.

alignment. About 96.88% of reads showed Phred score  $\geq 20$ , which indicates 1 error for each 100-base call or 99% of real sequence accuracy.

**Table 1.** Agronomical parameters evaluated at the end of the soybean developmental stage.

	Treatment	Values <sup>a</sup> (±standard error)	Tukey <sup>b</sup> 5% <sup>b</sup>	ANOVA C.V% <sup>c</sup>
Plant height (cm)	C	32.00 ± 1.67	A	13.02
	WD	32.25 ± 1.44	A	
Number of nodes	C	9.22 ± 0.27	A	6.60
	WD	9.67 ± 0.23	A	
Mean distance between nodes (cm)	C	3.48 ± 0.18	A	15.16
	WD	3.00 ± 0.13	A	
Number of pods with seeds	C	66.56 ± 6.29	A	29.34
	WD	47.11 ± 3.93	B	
Number of pods without seeds	C	18.22 ± 2.22	A	31.90
	WD	12.89 ± 1.64	A	
Total number of pods	C	84.78 ± 6.52	A	24.60
	WD	60.00 ± 5.16	B	
The dry mass of pods with seeds (g)	C	33.18 ± 1.96	A	21.81
	WD	24.62 ± 1.72	B	
The dry mass of pods without seeds (g)	C	0.20 ± 0.08	A	125.86
	WD	0.11 ± 0.02	A	
Number of seeds per plant	C	132.89 ± 12.11	A	28.57
	WD	91.67 ± 7.12	B	
The dry mass of 100 seeds (g)	C	25.50 ± 0.50	A	20.23
	WD	19.34 ± 0.86	B	
Number of flowers	C	12.00 ± 1.37	A	41.93
	WD	8.44 ± 1.45	A	

<sup>a</sup>Average values for 8 plants evaluated (N = 8).  
<sup>b</sup>Capital letters compared C to WD conditions within developmental stages (R2 and R4) separately. Minor letter compared developmental stages within conditions (C and WD) separately. Means followed by the same letter stand for no significant difference according to means of 8 biological replicates comparison by the Tukey test at 5%. Bars mean pattern error.  
<sup>c</sup>ANOVA C.V%: Coefficient of Variation.

For libraries alignment, mapping and gene annotation, the genome file in FASTA format (Gmax\_275\_v2.0.fa.gz), and the annotation file in GFF3 format (Gmax\_275\_W-m82.a2.v1.gene.gff3.gz) were used. Library alignment was performed, and only single-aligned reads were selected. Multi-aligned and non-aligned reads have been discarded. Obtained results showed that for flower libraries, the percentage of unique read alignment ranged from 85 and 95% (average 94.18%) while for pods libraries, this value oscillated between 77 and 88% (average 88.31%) (Additional file 1). Consequently, the percentage of reads that did not align or showed multiple alignments were lower for flower libraries (between 5 and 15%) and higher for pods libraries (between 12 and 23%). The removal of unnatural PCR duplicates discarded approximately 50% of the total reads, reducing noise, and minimizing false positives. After PCR artifact removal (about 46.90%) the reads alignment against the reference genome was about 94.09%.

For all DEG identified under WD, in flowers, 163 and 62 DEG were down and up-regulated, respectively, while in pods, 23 DEG were down-regulated and 117 were up-regulated (Additional file 1). Considering DEG with biological function annotated, analyses showed 174 genes exclusively in flowers and 104 DEG identified exclusively in pods. Both analyzed tissues showed a remarkable difference when expression profile was assayed: for flowers, 42 genes were up-regulated and 132 down-regulated; while for pods, the opposite pattern was observed, with 84 genes being up-regulated and 20 down-regulated (Additional file 1).

Considering different biological functions, 106 and 98 were found in flower and pod, respectively. Twenty-nine genes corresponding to 14 different biological functions



were found in common for both flower and pod of drought-sensitive cultivar BR16. Among these genes, 8 biological functions presented different profiles (up/down-regulation) in each tissue, and 6 exhibited the same expression profile, being up or down-regulated in both flower and pod. Sequences without annotation were also identified, 33 genes (10 up and 23 down-regulated) being exclusively for flowers, 16 genes (14 up and 2 down-regulated) for pods, and 4 genes identified in common for both tissues, all showing up-regulation (Additional file 2).

For flowers exclusively, among the DEG showing up-regulation, some genes involved in WD responses were identified such as *Late Embryogenesis Abundant proteins* (Glyma.03G144400, Glyma.05G112000, Glyma.10G064400, Glyma.12G001600, and Glyma.13G363300), *Small Heat-Shock Protein Hsp20 Family* (Glyma.19G011400) and *9-cis-epoxycarotenoid dioxygenase (NCED) enzyme* (Glyma.08G176300 and Glyma.15G250100). Down-regulated genes that trigger drought-responses were also identified such as related to hormone (auxin transporter protein – Glyma.03G063900, ABA receptor – Glyma.11G233300 and ethylene – Glyma.18G148000), photosynthesis/stomata closure (protein phosphatase 2c – Glyma.07G164400), sugar biosynthesis and transporter (Glyma.12G032600, Glyma.13G213300 and Glyma.14G159900) (Additional file 2). The statistics of all genes in each Gene ontology category showed in Additional file 2 genes are represented in Figure 3(A).

In pods, a contrasting pattern was identified for some genes involved in abiotic-stress responses when compared to the flower expression profile. Sugar-related genes were up-regulated in pods (Glyma.03G137900 and Glyma.08G009900), as well as *ethylene hormone transcription factor* (Glyma.08G009900) and *ABA hydrolase* (Glyma.12G146400) and photosynthesis/stomata closure (*protein phosphatase 2c* – Glyma.06G290200 and Glyma.18G208100). Other exclusive genes related to WD responses were also up-regulated in pods, such as aquaporin (Glyma.20G170400), *Dehydrin* (Glyma.07G090400), *WRKY transcription factors* (Glyma.03G042700 and Glyma.18G208800) and osmotic-adjustment related (*solute carrier family 35* – Glyma.04G221100) (Additional file 2).

Some genes were shared between both tissues. Among common genes were *salicylate O-methyltransferase*, Protein of unknown function (DUF1298), *EMEA*, *WRKY*, *Zinc Finger Fyve domain protein*, *Sulfate-transporting ATPase*, *benzoate O-methyltransferase*, and *extensin related*, which showed contrasting expression profile between tissues and could be represented by different Glyma IDs. On the other hand, *LEA*, *AWPM-19*, *ATHB-12*, *inositol 3-alpha-galactosyltransferase*, and *EMB* genes were up-regulated in both tissues (Additional file 2).

For each GO term identified, genes under each category showed up and down-regulated pathways for pods and only down-regulated pathways for flowers, under WD (Figure 3(A)). Protein binding, membrane, and cellular processes were exclusively up-regulated in pods. Both tissues shared the following GO terms: transition metal ion binding, transferase activity, ion binding, hydrolase activity, and cell part; being up-regulated in pods and down in flowers. Transporter activity, ribonucleotide and purine nucleotide binding, primary metabolic process, nucleotide and nucleotide-binding, nitrogen compound metabolic process, metabolic

process, macromolecule metabolic process, gene expression, catalytic activity, carbohydrate metabolic process, binding, ATP binding, adenylyl ribonucleotide, and adenylyl nucleotide binding were exclusively down-regulated in flowers (Figure 3(A)). In short, GO terms reflected gene expression, as in flowers most GO terms were down-regulated as the DEG (75%) (Figure 3(A)), similarly in pods, up-regulated genes displayed up-regulated GO terms.

### Gene expression validation by qRT-PCR

qRT-PCR carried out for DEG in flower and pod showed the same expression profile (down or up-regulation), in different levels, identified in the RNA-Seq, validating the data obtained. These results also suggested that the pipeline analyses applied in this paper were robust enough to reach a strong positive Pearson correlation (0.75) between qRT-PCR and RNA-Seq techniques using log<sub>2</sub>FC gene expression. Genes from RNA-Seq was selected by higher up/down-regulation with FDR ≤ 0.05.

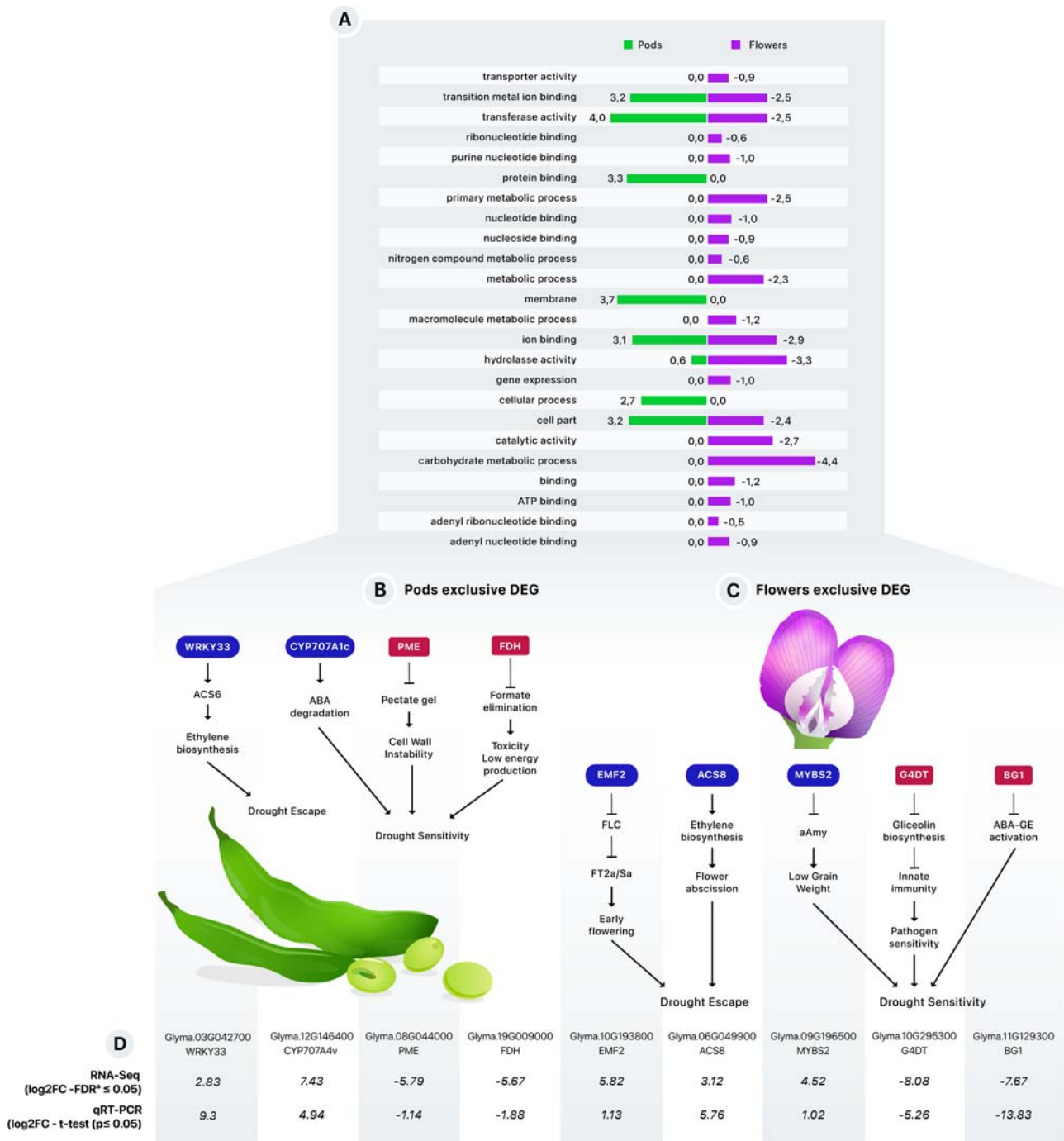
In flowers, Glyma.06G049900 (*1-Aminocyclopropane-1-Carboxylate synthase 8* – ACS8 – E.C.4.4.1.14) was up-regulated, showing, respectively 3.12x and 5.76 log<sub>2</sub>FC in RNA-Seq and qRT-PCR; as well as Glyma.10G193800 (*Embryonic Flower 2* – EMF2) which presented 5.82x and 1.13 log<sub>2</sub>FC, and Glyma.09G196500 (*MYBS2 putative*) which displayed 4.52x and 1.02 log<sub>2</sub>FC in RNA-Seq and qRT-PCR, respectively. Glyma.11G129300 (*beta-glucosidase* – BG1 – EC.3.2.1.21) and Glyma.10G295300 (*glycinol 4-dimethylallyl-transferase* – G4DT – EC.2.5.1.36) were down-regulated in both techniques, presenting respectively, -7.67x and -13.83x and -8.08 and -5.26 log<sub>2</sub>FC, respectively, at RNA-Seq and qRT-PCR techniques (Figure 3(D)).

For pods, Glyma.12G146400 (*ABA 8'-hydroxylase* – CYP707A4 – EC.1.14.14.137) was up regulated showing, respectively 7.43x and 4.94 log<sub>2</sub>FC in RNA-Seq and qRT-PCR, as well as Glyma.03G042700 (*WRKY33*) which presented 2.83x and 9.3 log<sub>2</sub>FC. Down-regulated genes in pods were: Glyma.08G044000 (*pectinesterase- PME* – EC.3.1.1.11) showing -5.79x and -1.14 log<sub>2</sub>FC in RNA-Seq and qRT-PCR, respectively and Glyma.19G009000 (*formate dehydrogenase* – FDH – EC.1.17.1.9) which presented respectively, -5.67x and -1.88 log<sub>2</sub>FC, respectively, at RNA-Seq and qRT-PCR techniques (Figure 3(D)).

### Discussion

Soybean as an important crop worldwide is affected by WD, and most of the studies currently available on gene expression in response to drought were carried out in leaves and/or roots. Especially in reproductive organs, such as flower and pod, information on genes and the metabolic pathways triggered in response to WD is lacking. This study reported a physiological and agronomical characterization and transcriptome analyses in flower and pod of a drought-sensitive soybean cultivar, subjected to WD treatment.

Physiological data showed that imposed treatment was sufficient to induce primarily responses to cope with WD such as a decrease in gas exchanges. It is known that stomatal conductance reduction, which aims to reduce water loss by transpiration, is one of the first physiological responses in plants to reduce WD damages. Lower stomatal conductance



**Figure 3.** (A). Genetic ontology analysis of DEG identified in flower (R2 developmental stage) and pods (R4 developmental stage) of drought-sensitive soybean cultivar BR 16 subjected to water-deficit conditions. Data include both expression profiles, up and down-regulated genes. Green bars represent genes associated with GO terms identified in pods and purple ones in flowers. Positive and negative numbers associated with the bars, stand for up and down-regulated expression profiles of genes associated with the respective GO term category. (B). Drought-responsive pathways affected by WD condition in soybean cv. BR16. Legend: Blue circles stand for up-regulation and red ones for down-regulation. (C). Comparison of expression analysis performed using RNA-Seq and qRT-PCR techniques for genes differentially expressed in flower and pod of drought-sensitive soybean cultivar BR 16 subjected to WD treatment compared to C condition.

values, however, result in lower CO<sub>2</sub> uptake, which contributes to the reduction of the photosynthetic assimilation rate (Flexas et al. 2004), which may also contribute to enhancing water use efficiency (WUE) in response to WD (Liu et al. 2005). These physiological responses were also reported by Paiva Rolla et al. (2014), which described that photosynthesis and stomatal conductance were reduced in soybean plants under WD conditions. Photosynthetic rate and stomatal conductance also decreased in soybean plants under moderate WD (45% ± 2 field water capacity) (Zhang et al. 2016). Besides, corroborating with this study, transpiration rate reduction and an increased in foliar temperature was also

described for soybean subjected to 4 different WD treatments (irrigation with 100%, 50%, 25% and 10% of water) (Inamullah 2005). In field conditions, in a previous study carried out in a dry season, cultivar BR16 showed higher instantaneous WUE and intrinsic WUE (Fuganti-Pagliarini et al. 2017). As observed in our study, plants facing low to moderate WD will frequently enhance WUE (Brock and Galen 2005; Liu et al. 2005; Yin et al. 2005; Medrano et al. 2015), probably due to a protective mechanism against stress, that allows plants to save water, improve water efficient use and to convert available CO<sub>2</sub> into photoassimilates into pods and grain production (Chaves et al. 2009).



Drought occurrence during water-scarcity-sensitive developmental phases, such as flowering and pod filling can also seriously compromise growth and final yield parameters. Here, a decrease in the number of pods with seeds, dry mass of pods with seeds, number of seeds, dry mass of seeds, the total number of pods, and dry mass of 100 seeds was observed in plants under WD compared to C conditions (Table 1). There is a positive correlation between pods, nodes and yield, and nodes and pods and seeds (Egli 2010; Kahlon et al. 2011); thus, a decrease in these parameters will reflect in yield losses. This association is also related to environmental conditions, photosynthesis, crop growth rate, and maturity. Growth parameters, however, appear to have been less affected by WD as plant height and number of nodes did not present reduction under treatment (Table 1). According to the literature, soybean water demand increases during plant development, reaching the maximum necessity at the flowering-pod filling phase, around 7–8 mm of water/day. Besides the developmental stage, water consumption also depends on atmosphere evaporative demand, climatic conditions of the site, sowing season, and rainfall distribution during crop season (Embrapa 2007). In short, results obtained in the greenhouse condition show that drought treatment was applied correctly and reflected in agro-physiological responses (Table 1).

### Expression profile and gene ontology analysis of DEG in flower and pod

This is the first study to report a large-scale gene expression profile in soybean flower and pod from a drought-sensitive cultivar. These reproductive organs showed a discrepant expression profile. In flowers, DEG and the average expression (log<sub>2</sub>FC) of GOs terms significantly expressed were down-regulated (Figure 3(A)), which may negatively affect many pathways. This data also suggested that in flowers, drought conditions tend to inhibit gene expression, being more sensitive to water lack. On the other hand, pods up-regulated genes and pathways indicating that the molecular machinery may have been activated to improve survival and pod formation, as a strategy to preserve and transmit genetic material to the next generation through grain formation, even if it means yield losses in the final production. According to Anjum et al. (2011), this response is disrupted by leaf gas exchanges, which not only impaired the size of the source and sink tissues, but also phloem loading, assimilate translocation and dry matter portioning, resulting in smaller and fewer grains, as described in the following paragraphs.

Among genes identified in our RNA-Seq experiment, *Late Embryogenesis Abundant (LEA)* proteins, *Small-HSPs-Heat Shock (HSPs)* Proteins, and *9-cis-epoxycarotenoid enzyme (NCED)* were up-regulated in flowers, and genes related to photosynthesis/stomata closure, sugar biosynthesis and transporter related genes were down-regulated in this tissue. Under a stressful environmental condition such as WD, *HSPs* act as molecular chaperones assisting protein folding, stabilizing membrane proteins, facilitating protein refolding, reestablishing normal protein conformation, and thus cellular homeostasis (Augustine 2016; Priya et al. 2019). Specifically, small *HSPs* play a distinctive function in the degradation of proteins, keeping membrane integrity under stress conditions (Nakamoto and Vigh 2007; Augustine

2016). Furthermore, *NCED* is an important key rate-limiting enzyme in the ABA biosynthesis pathway. Under WD conditions, an increase in *NCED* transcript levels leads to ABA biosynthesis and accumulation in plants, improving drought tolerance by triggering of WD-defense mechanism via hormone induction (Huang et al. 2018). *NCED* gene has been identified in many plant species and lines overexpressing it have shown improved drought tolerance in *Arabidopsis* (Tong et al. 2017), cowpea (Iuchi et al. 2001), tomato (Thompson et al. 2000), tobacco (Qin and Zeevaart 2002; Pedrosa et al. 2017), peanut (Wan and Li 2006), rice (Sultana et al. 2014), petunia (Estrada-Melo et al. 2015), and cotton (Souza et al. 2016). Specifically, in soybean plants GM to overexpress *NCED* gene, higher levels of ABA were identified in the WD-treated group (Molinari 2020). In addition, Lima, and co-workers (2019) reported in the conventional cultivar BR16, higher levels of ABA and *ACC* (ethylene precursor) in plants subjected to WD, with increased concentration following the severity of the treatment, corroborating the involvement of these genes under WD.

In flowers, the downregulation of photosynthesis/stomata closure-related genes reflected directly in gas exchange parameters decreases as WD can damage the basic organizational structure of photosynthetic apparatus, inhibiting carbon assimilation, and decreasing photosynthesis rate, usually by stomatal limitation (Wang et al. 2018a). In contrast, the up-regulation of these genes in pods suggested that in this developmental stage all resources were directed to pod filling. In this stage, leaf senescence also allows maximum recovery and remobilization of nutrients to pods formation, increasing in pod CO<sub>2</sub> metabolism, and reduce in energy and ATP use (Bennett et al. 2011). Yet, in soybean, the number of pods can also affect leaf photosynthesis. For instance, in WD condition, the removal of pods reduces CO<sub>2</sub> rates exchange within the plants, probably as a result of stomatal closure, a consequence of increased photoassimilate accumulation within the leaves brought by the availability of fewer sinks to export resources, indicating a dynamic system, in which pods signal their resource necessities to the leaves, initiating remobilization of photoassimilates relative to received signal strength (Bennett et al. 2011).

Aquaporin genes were up-regulated in pods. Under WD conditions, these proteins are responsible to maintain water movement across the plant body, stabilizing homeostasis of the cell membrane (Iwuala et al. 2019). Furthermore, under drought, the alteration in the levels of these proteins could enhance tolerance, as roots water uptake can be enhanced or reduced by the overexpression or loss of one or more PIP genes (Lu et al. 2018). Dehydrins were also up-regulated in pods under WD. These proteins are highly hydrophilic, thermostable and ABA-responsive, being induced under abiotic stress, and participating in membranes, enzymes, and nucleotides stabilization in cells (Hanin et al. 2011; Yu et al. 2018). The up-regulation of osmotic adjustment-related genes suggests that in pods, more than one mechanism was activated to cope with drought effects. Among these osmoprotectants, soluble sugars, which showed up-regulated profile in pods, under WD, regulate cell osmotic status, protecting membrane and contributing to the scavenging of free radical in plant cells (Zivcak et al. 2016). The molecular results, as physiological ones, suggested that pods were less impaired by WD treatment than flowers.

### Many genes are involved in the WD stress responses in flowering and pods stages

The qRT-PCR analyses of 9 genes differentially expressed (5 from flowers and 4 from pods) were carried out to validate RNA-Seq expression results. Figure 3(B, C) suggests a schematic chart that compiled all validated genes and the biological processes in response to drought in which these genes are involved.

Results suggested that part of BR16 cultivar sensitivity to drought may be due to the downregulation of important drought-responsive genes. In flowers, Glyma.10G295300 was expressed about 12x less under WD when compared to C conditions (average value between qRT-PCR and RNA-Seq log<sub>2</sub>FC expression). This gene encodes to G4DT enzyme (*glycinol 4-dimethylallyl transferase*, E.C.2.5.1.36) required to synthesize glyceollin 1 (Akashi et al. 2009; Lygin et al. 2010; Ahuja et al. 2012). In soybean, an important glyceollin is phytoalexin, a specific antimicrobial derivative from the flavonoid pathway, which disrupts or inhibits a wide range of pathogens colonization (Lygin et al. 2010; Sukumaran 2016; Yoneyama et al. 2016). The down-regulation of the phytoalexin pathway under WD may indicate a decrease in the innate immune system during stress (Zernova et al. 2014), suggesting crosstalk signaling between biotic and abiotic responses in plants. Lygin et al. (2010) stated that drought-tolerant plants showed a decrease in pathogen incidences, displaying a positive correlation between glyceollin levels and pathogen resistance. In *Arabidopsis thaliana*, plants infected with nematode showed more susceptibility to drought (Atkinson et al. 2013). Additionally, increased susceptibility to turnip mosaic virus (TuMV) by innate basal defenses, under combined heat and drought conditions was also reported (Prasch and Sonnewald 2013). In this way, BR16 susceptibility to WD may be, among other factors, due to a decrease in soybean innate immunity efficiency, since healthy plants have more probability to survive to hostile environmental conditions such as drought and pathogens infections (Prasch and Sonnewald 2013).

BG1 enzyme (Glyma.11G129300 – *beta-glucosidase*, E.C.3.2.1.21) was also repressed in flowers of BR16, being expressed about 22x less under WD compared to C conditions (average value between qRT-PCR and RNA-Seq log<sub>2</sub>FC expression). This enzyme hydrolyzes inactive abscisic acid which is conjugated with glucose (ABA-GE) to produce active ABA, in a reversible reaction (Lee et al. 2006; Seiler et al. 2011). Similar to other enzymes involved in ABA biosynthesis, functional deficiency of ABA-glucosidases also alters intracellular ABA level, affecting plant growth, development, and responses to adverse environmental conditions. Interestingly, WD dramatically decreases the enzymatic activity of *AtBG1* by inducing its polymerization, implying that high molecular weight form of *AtBG1* may play an important role in rapid ABA production under stress conditions (Lee et al. 2006). In *A. thaliana*, the loss function of *BG1* caused hypersensitivity to dehydration and defect in stomatal closure during WD (Lee et al. 2006; Xu et al. 2012). On the other hand, the overexpression of *BG1* resulted in higher ABA levels and drought tolerance (Lee et al. 2006; Wang et al. 2011; Han et al. 2012; Saradadevi et al. 2017). This data suggested that at this point, soybean plants may have balanced the down-production of ABA, by overexpressing the *NCED* gene in an attempt to keep ABA levels

enough to cope with WD effects. On the other hand, the downregulation of *BG1* gene in BR16 corroborates with its drought sensitivity and may be also related to early flowering identified for this cultivar, as an escape response strategy to drought, likewise observed in a previous study (Crusiol et al. 2017). Additionally, the downregulation of *BG1* probably led to the down-regulation of *SVP* flowering repressor as well, since they are strictly related genes, and consequently, flowering was induced (Wang et al. 2018b; Negin et al. 2019).

Considering genes down-regulated in pods, Glyma.08G044000, which encodes *PME* enzyme (*pectinesterase*, E.C.3.1.1.11) was expressed almost 7x less under WD when compared to C conditions (average value between qRT-PCR and RNA-Seq log<sub>2</sub>FC expression). This enzyme is involved in cell wall pectin remodeling (Kashyap et al. 2001), in the control of cell wall porosity and cell wall adhesion (Willats et al. 2001; Jarvis et al. 2003; Wu et al. 2010; Le Gall et al. 2015). Under abiotic stress, pectinesterases contribute to the stiffening of the cell wall by producing blocks of unesterified carboxyl groups that can interact with calcium ions forming a pectate gel, protecting and limiting the damage to cells (Bosch and Hepler 2005; Leucci et al. 2008; Moore et al. 2008; Wolf et al. 2012; Leng et al. 2017; Wu et al. 2018). Wheat, soybean, and tomato showed higher levels of pectin remodeling enzymes in drought-tolerant cultivars (Leucci et al. 2008; An et al. 2014; Iovieno et al. 2016; Landi et al. 2017). In an *Arabidopsis thaliana* mutant to pectinesterase gene, drought tolerance was impaired (Deslattes et al. 2018). Besides, a comparison of cell walls between two wheat cultivars differing in drought tolerance showed that the biosynthesis of pectic polymers under WD was less affected in the tolerant cultivar (Piro et al. 2003; Leucci et al. 2008). Similar results were found in wheat, with a drought-tolerant line displaying more pectin enzymes than the WD-sensitive one (Konno et al. 2008). This result may suggest that, among other factors, the low transcript levels of *PME* in BR16 could be contributing to its sensitivity to drought due to deficiencies in cell wall maintenance in pods.

Another repressed gene in pods, Glyma.19G009000 was expressed about 8x less under WD than C condition (average value between qRT-PCR and RNA-Seq log<sub>2</sub>FC expression). This gene encodes to FDH enzyme (*formate dehydrogenase*, EC.1.17.1.9), a mitochondrial and NAD-dependent enzyme that catalyzes the oxidation of formate to carbon dioxide in plants while reducing NAD<sup>+</sup> to NADH, serving to alleviate any detrimental effects accumulated formate has on the cell (Shiraishi et al. 2000; Alekseeva et al. 2011). Data show that, usually, the expression of FDH is drastically increased when plant tissue is subjected to stressful conditions, such as drought, and pathogen infection (des Francs-Small et al. 1993; Hourton-Cabassa et al. 1998; Suzuki et al. 1998; Li et al. 2001; Li et al. 2002; Choi et al. 2014). Besides that, FDH also acts in formate detoxification (Li et al. 2002), a toxic compound, that can inhibit cellular respiration and root growth, as observed in *Arabidopsis* (Li et al. 2002; David et al. 2010). Additionally, formate can also restrain water oxidation reaction on the donor side, as well as, in electron transfer on the acceptor side of photosystem II (Feyziev et al. 2000), decreasing photosynthesis, photoassimilates production and resulting in smaller pods and seeds dry mass under WD, in agreement to what was observed for BR16 (Figures 2 and 3(D)).

Glyma.06G049900 that encodes to ACS8 enzyme (*1-Aminocyclopropane-1-Carboxylate synthase 8* – E.C.4.4.1.14) was 6x more expressed in flower under WD than in C conditions (average value between qRT-PCR and RNA-Seq log<sub>2</sub>FC expression). This enzyme acts in ethylene biosynthesis, a hormone that enables senescence and abscission in flower and pod (Iqbal et al. 2017). Its biosynthesis is affected by several environmental factors including drought (Yoon and Kieber 2013; Song et al. 2016). Studies indicate that ACS synthase is transported from roots (via xylem) to aerial part in response to drought, increasing local ethylene biosynthesis (Tudela and Primo-Millo 1992). In *Arabidopsis*, transcriptional data showed that ACS8 (AT4G37770.1) is up-regulated in flowers under WD (Zhang et al. 2018), and appears to be circadian clock and photoperiod regulated (Thain et al. 2004). ACS8 up-regulation in BR16 cultivar may be inducing ethylene biosynthesis and as a consequence causing flower and pods abortion (Figure 3(D)).

Glyma.10G193800 that encodes to transcription factor EMF2 (Embryonic Flower 2) was also upregulated in flower being 7x more expressed under WD when compared to C conditions (average value between qRT-PCR and RNA-Seq log<sub>2</sub>FC expression). *Arabidopsis thaliana* AtEMF2 protein (AT5G51230.1) gene, a Polycomb SUZ2 protein (PcG) (Kim et al. 2010) forms complexes that maintain genes silenced throughout histone modifications (Chen et al. 2009; Costa and Dean 2019). Additionally, EMF2 interacts with genes related to ABA, such as *Abcisic Acid Insensitive 3* (ABL3), *Long Vegetative Phase 1* (LOV1), and Flowering Locus C (FLC), that control flowering and seed development. EMF repress FLC (flowering repressor) allowing flower activators, such as *Flowering Locus T* (FT) and Suppressor of overexpression of *constans 1* (SOC1) to induce flowering (Yoshida et al. 2001; Chanvivattana et al. 2004; Kim et al. 2010). Therefore, the up-regulation of EMF2 in BR16 cultivar may have induced early flowering, through FLC repression and FT and SOC1 induction. As already discussed, early flowering is a drought escape mechanism adopted by BR16 cultivar (Crusiol et al. 2017) to prioritize grain filling and avoid compromise final productivity.

In flowers, Glyma.09G196500 showed an expression of 6x higher in WD when compared to C conditions (average value between qRT-PCR and RNA-Seq log<sub>2</sub>FC expression). This gene encodes to a putative MYBS2 transcription factor, ortholog to MYBS2 (AT5G08520) gene in *Arabidopsis thaliana*, which is responsible for sugar levels maintenance (Chen et al. 2017). In rice, MYBS2 gene represses amylase ( $\alpha$ Amy) production, an enzyme that hydrolyzes starch into sugars, decreasing as a consequence, grain weight in greenhouse and field conditions (Chen et al. 2019). The induction of MYBS2 in BR16 cultivar may have contributed to low dry-mass and a low number of seeds under WD compared to C conditions (Figure 3(D)), by repressing  $\alpha$ Amy activity and as a result, there was a reduction of available sugar to be mobilized into grains. In addition, Seo et al. (2012) and Gao et al. (2014) reported the involvement of MYB genes in drought response in plants, corroborating MYB roles in WD-defense mechanisms.

Glyma.12G146400, which encodes a cytochrome enzyme (CYP707A4), also known as ABA 8'-hydroxylase enzyme (EC.1.14.14.137) was expressed about 12x more in pods under WD when compared to C conditions (average value between qRT-PCR and RNA-Seq log<sub>2</sub>FC expression). In

*A. thaliana*, the loss of CYP707A4 function rescued drought hypersensitivity phenotype by increasing ABA levels (Umezawa et al. 2006). It is important to emphasize here that in both reproductive tissues, ABA metabolism was affected by WD in different ways. Differently from flowers where a balance between NCED overexpression to keep ABA levels and a decrease in ABA reactivation by BGI gene repression was identified; in pods, ABA catabolism was increased by up-regulation of CYP707A4. These mechanisms may have led to lower levels of ABA and as a consequence, drought sensitivity.

The last up-regulated gene validated in pods was Glyma.03G042700 which encodes the WRKY33 transcription factor and was 12x more expressed on average under WD compared to C conditions (average value between qRT-PCR and RNA-Seq log<sub>2</sub>FC expression). WRKY TFs play important roles in drought response and have been identified in several plants (Fan et al. 2015; He et al. 2016; Xu et al. 2016; Wei et al. 2017). In *Arabidopsis*, some authors have reported the involvement of WRKY factors in response to drought and osmotic treatment (Chen et al. 2009; Babitha et al. 2012; Zhao et al. 2020). In soybean, the characterization of these transcription factors showed high expression levels under WD and salinity conditions, emphasizing its participation in drought tolerance (Shi et al. 2018). Moreover, predictions from the STRING website, showed that WRKYs are involved in four signaling pathways: the jasmonic acid (JA), the salicylic acid (SA), the mitogen-activated protein kinase (MAPK), and the ethylene signaling pathways. According to these authors, WRKY33 binds directly to the ACS gene to promote ethylene synthesis (Fei et al. 2019). Both reproductive tissues of BR16 showed again a similar hormone response under drought conditions. Ethylene biosynthesis was increased in flower and pods by up-regulation of WRKY33 and ACS8 genes, respectively, which contributes to senescence and abortion of these tissues (Figure 3(D)). This increase in ethylene was also reported in BR16 conventional cultivar under drought by Lima and coworkers (2019). It is also important to highlight that early flowering (escape mechanism) is followed by senescence and abortion of less developed flowers, allowing more mature flowers to finish their development. According to the literature, this is a metabolic strategy to deliver water and nutrients for the development of fewer flowers/seeds rather than allocate nutrients to all young organs, under extreme environmental conditions (Richie et al. 1997; Neumaier et al. 2000; Su et al. 2013).

Data obtained here presented a broad expression profile of DEGs in flower and pod of drought-sensitive cultivar BR16, subjected to drought, showing some physiological and molecular mechanisms triggered in response to WD. These results will help researchers to understand how these tissues cope with water scarcity and can provide candidate genes for future projects aiming to develop soybean plants more tolerant to WD.

## Acknowledgements

We thank the National Council for Scientific and Technological Development (CNPq) for granting scholarships to Postdoctoral fellows R Fuganti-Pagliarini and J Marcolino-Gomes and the Coordination for the Improvement of Higher Education Personnel (CAPES) for granting a doctoral scholarship to MDC Molinari and DA Barbosa.



## Disclosure statement

No potential conflict of interest was reported by the author(s).

## Funding

This work was supported by CAPES and CNPq.

## References

- Ahuja I, Kissen R, Bones AM. 2012. Phytoalexins in defense against pathogens. *Trends Plant Sci.* 17:73–90.
- Akashi T, Sasaki K, Aoki T, Ayabe SI, Yazaki K. 2009. Molecular cloning and characterization of a cDNA for pterocarpan 4-dimethylallyl transferase catalyzing the key prenylation step in the biosynthesis of glyceollin, a soybean phytoalexin. *Plant Physiol.* 149:683–693.
- Alekseeva AA, Savin SS, Tishkov VI. 2011. NAD<sup>+</sup>-dependent formate dehydrogenase from plants. *Acta Nat.* 3:1–11.
- An P, Li X, Zheng Y, Matsuura A, Abe J, Eneji AE, et al. 2014. Effects of NaCl on root growth and cell wall composition of two soya bean cultivars with contrasting salt tolerance. *J Agron Crop Sci.* 200:212–218.
- Andrews S. 2010. FastQC: a quality control tool for high throughput sequence data. [accessed 2019 Oct 9]. <http://www.bioinformatics.babraham.ac.uk/projects/fastqc/>.
- Anjum SA, Xie XY, Wang LC, Saleem MF, Man C, Lei W. 2011. Morphological, physiological, and biochemical responses of plants to drought stress. *Afr J Agric Res.* 6:2026–2032.
- Atkinson NJ, Lilley CJ, Urwin PE. 2013. Identification of genes involved in the response of Arabidopsis to simultaneous biotic and abiotic stresses. *Plant Physiol.* 162:2028–2041.
- Augustine SM. 2016. Function of heat-shock proteins in drought tolerance regulation of plants. In: Hossain MA, Wani SH, Bhattacharjee S, Burritt DJ, editor. *Drought stress tolerance in plants*. Cham: Springer; p. 163–185.
- Babitha KC, Ramu SV, Pruthvi V, Mahesh P, Nataraja KN, Udayakumar M. 2012. Co-expression of AtbHLH17 and AtWRKY28 confers resistance to abiotic stress in Arabidopsis. *Trans Res.* doi:10.1007/s11248-012-9645-8.
- Bennett EJ, Roberts JA, Wagstaff C. 2011. The role of the pod in seed development: strategies for manipulating yield. *New Phytol.* 190:838–853.
- Bolger AM, Lohse M, Usadel B. 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics.* 30:2114–2120.
- Bosch M, Hepler PK. 2005. Pectin methylesterases and pectin dynamics in pollen tubes. *Plant Cell.* 17:3219–3226.
- Brock MT, Galen C. 2005. Drought tolerance in the alpine dandelion, *Taraxacum ceratophorum* (Asteraceae), its exotic congener *T. officinale*, and interspecific hybrids under natural and experimental conditions. *Am J Bot.* 92:1311–1321.
- Canteri MG, Althaus RA, Virgens filho JS, Giglioti EA, Godoy CV. 2001. SASM – Agri: Sistema para análise e separação de médias em experimentos agrícolas pelos métodos Scott-Knott, Tukey e Duncan. [accessed 2019 Oct 9]. [http://www.agrocomputacao.deinfo.uepg.br/dezembro\\_2001/Arquivos/RBAC\\_Artigo\\_03.pdf/](http://www.agrocomputacao.deinfo.uepg.br/dezembro_2001/Arquivos/RBAC_Artigo_03.pdf/).
- Chanvattana Y, Bishopp A, Schubert D, Stock C, Moon YH, Sung ZR, et al. 2004. Interaction of polycomb-group proteins controlling flowering in Arabidopsis. *Development.* 131:5263–5276.
- Chaves M. M, Flexas J., Pinheiro C. 2009. Photosynthesis under drought and salt stress: regulation mechanisms from whole plant to cell. *Annals of Botany.* 103(4):551–560. <https://doi.org/10.1093/aob/mcn125>.
- Chen YS, Chao YC, Tseng TW, Huang CK, Lo PC, Lu CA. 2017. Two MYB-related transcription factors play opposite roles in sugar signaling in Arabidopsis. *Plant Mol Biol.* 93:299–311.
- Chen LJ, Diao ZY, Specht C, Sung ZR. 2009. Molecular evolution of VEF-domain-containing PcG genes in plants. *Mol Plant.* 2:738–754.
- Chen YS, Ho THD, Liu L, Lee DH, Lee CH, Chen YR, et al. 2019. Sugar starvation regulated MYBS2 and 14-3-3 protein interactions enhance plant growth, stress tolerance, and grain weight in rice. *Proc Natl Acad Sci USA.* 116:21925–21935.
- Choi DS, Kim NH, Hwang BK. 2014. Pepper mitochondrial formate dehydrogenase1 regulates cell death and defense responses against bacterial pathogens. *Plant Physiol.* 166:1298–1311.
- Costa S, Dean C. 2019. Storing memories: the distinct phases of polycomb-mediated silencing of Arabidopsis FLC. *Biochem Soc Trans.* 47:1187–1196.
- Crusiol LGT, Carvalho JDFC, Sibaldelli RNR, Neiverth W, Rio A, Ferreira LC. 2017. NDVI variation according to the time of measurement, sampling size, positioning of sensor and water regime in different soybean cultivars. *Prec Agric.* 18:470–490.
- David P, des Francs-Small CC, Sévignac M, Thareau V, Macadré C, Langin T. 2010. Three highly similar formate dehydrogenase genes located in the vicinity of the B4 resistance gene cluster are differentially expressed under biotic and abiotic stresses in *Phaseolus vulgaris*. *Theor Appl Genet.* 121:87–103.
- des Francs-Small CC, Ambard-Bretteville F, Small ID, Rémy R. 1993. Identification of a major soluble protein in mitochondria from non-photosynthetic tissues as NAD-dependent formate dehydrogenase. *Plant Physiol.* 102:1171–1177.
- Deslattes A, Van Hulst MHA, Dixit SA, Martin DE, Munkvold JD, Dileo MV. 2018. U.S. Patent No. 9,909,138. Washington, DC: U.S. Patent and Trademark Office. [accessed 2019 Oct 9]. <https://patents.justia.com/patent/9909138>.
- Egli DB. 2010. Soybean reproductive sink size and short-term reductions in photosynthesis during flowering and pod set. *Crop Sci.* 50:1971–1977.
- EMBRAPA – Empresa Brasileira de Pesquisa Agropecuária. Sistemas de Produção. 2007. [accessed 2019 Oct 9]. [https://www.agencia.cnptia.embrapa.br/Repositorio/tpsoja\\_2007\\_000g0v67mto02wx5ok00gmbp4qhts2gj.pdf/](https://www.agencia.cnptia.embrapa.br/Repositorio/tpsoja_2007_000g0v67mto02wx5ok00gmbp4qhts2gj.pdf/).
- Estrada-Melo AC, Reid MS, Jiang CZ. 2015. Overexpression of an ABA biosynthesis gene using a stress-inducible promoter enhances drought resistance in petunia. *Hortic Res.* 2:15013.
- Fan X, Guo Q, Xu P, Gong Y, Shu H, Yang Y, Shen X. 2015. Transcriptome-wide identification of salt-responsive members of the WRKY gene family in *Gossypium aridum*. *PLoS One.* doi:10.1371/journal.pone.0126148.
- FAO – Food and Agriculture Organization of the United Nations. 2016. OECD-FAO Agricultural Outlook 2015–2024. [accessed 2019 Oct 9]. <http://www.fao.org/3/a-i4738e.pdf/>.
- FAO – Food and Agriculture Organization of the United Nations. 2018. The impact of disasters and crises 2017 on agriculture and food security. [accessed 2019 Oct 9]. <http://www.fao.org/3/I8656EN/i8656en.pdf/>.
- Ferreira RC. 2016. Quantificação das perdas por seca na cultura da soja no Brasil. <http://www.bibliotecadigital.uel.br/document/?view=vtls000211814/>.
- Feyziev YM, Yoneda D, Yoshii T, Katsuta N, Kawamori A, Watanabe Y. 2000. Formate-induced inhibition of the water-oxidizing complex of photosystem II studied by EPR. *Biochemistry.* 39:848–855.
- Flexas J, Bota J, Loreto F, Cornic G, Sharkey TD. 2004. Diffusive and metabolic limitations to photosynthesis under drought and salinity in C3 plants. *Plant Biology.* 6:269–279.
- Fuganti-Pagliarini R, Ferreira LC, Rodrigues FA, Molinari HB, Marin SR, Molinari MD, et al. 2017. Characterization of soybean genetically modified for drought tolerance in field conditions. *Front Plant Sci.* doi:10.3389/fpls.2017.00448.
- Gao Shuai, Zhang Yong Li, Yang Lu, Song Jian Bo, Yang Zhi Min. 2014. AtMYB20 is negatively involved in plant adaptive response to drought stress. *Plant and Soil.* 376(1-2):433–443. <https://doi.org/10.1007/s11104-013-1992-6>.
- Goodstein DM, Shu S, Howson R, Neupane R, Hayes RD, Fazo J, et al. 2011. Phytozome: a comparative platform for green plant genomics. *Nucleic Acids Res.* 40:1178–1186.
- Han YJ, Cho KC, Hwang OJ, Choi YS, Shin AY, Hwang I, et al. 2012. Overexpression of an Arabidopsis  $\beta$ -glucosidase gene enhances drought resistance with dwarf phenotype in creeping bentgrass. *Plant Cell Rep.* 31:1677–1686.
- Hanin M, Brini F, Ebel C, Toda Y, Takeda S, Masmoudi K. 2011. Plant dehydrins and stress tolerance: versatile proteins for complex mechanisms. *Plant Signal Behav.* 6:1503–1509.
- He GH, Xu JY, Wang YX, Liu JM, Li PS, Chen M, et al. 2016. Drought-responsive WRKY transcription factor genes TaWRKY1 and TaWRKY33 from wheat confer drought and/or heat resistance in Arabidopsis. *BMC Plant Biol.* doi:10.1186/s12870-016-0806-4.
- Hourton-Cabassa C, Ambard-Bretteville F, Moreau F, de Virville JD, Rémy R. 1998. Stress induction of mitochondrial formate dehydrogenase in potato leaves. *Plant Physiol.* 116:627–635.

- Huang Y, Guo Y, Liu Y, Zhang F, Wang Z, Wang H, et al. 2018. 9-cis-Epoxycarotenoid dioxygenase 3 regulates plant growth and enhances multi-abiotic stress tolerance in rice. *Front Plant Sci.* doi:10.3389/fpls.2018.00162.
- Inamullah IA. 2005. Adaptive responses of soybean and cotton to water stress: I. Transpiration changes in relation to stomatal area and stomatal conductance. *Plant Prod Sci.* 8:16–26.
- Iovieno P, Punzo P, Guida G, Mistretta C, Van Oosten MJ, Nurcato R, et al. 2016. Transcriptomic changes drive physiological responses to progressive drought stress and rehydration in tomato. *Front Plant Sci.* doi:10.3389/fpls.2016.00371.
- IPCC – Painel intergovernamental sobre mudanças climáticas. 2011. Mudanças Climáticas. [accessed 2019 Oct 9]. <http://www.brasil.gov.br/meio-ambiente/2011/11/painel-intergovernamental-sobre-mudancas-climaticas-ipcc/>.
- Iqbal N, Khan NA, Ferrante A, Trivellini A, Francini A, Khan MIR. 2017. Ethylene role in plant growth, development, and senescence: interaction with other phytohormones. *Front Plant Sci.* doi:10.3389/fpls.2017.00475.
- Iuchi S, Kobayashi M, Taji T, Naramoto M, Seki M, Kato T, et al. 2001. Regulation of drought tolerance by gene manipulation of 9-cis-epoxycarotenoid dioxygenase, a key enzyme in abscisic acid biosynthesis in *Arabidopsis*. *Plant J.* 27:325–333.
- Iwuala E, Odjegba V, Sharma V, Alam A. 2019. Drought stress modulates expression of aquaporin gene and photosynthetic efficiency in *Pennisetum glaucum* (L.) R. Br. genotypes. *Curr Plant Biol.* doi:10.1016/j.cpb.2019.100131.
- Jarvis MC, Briggs SPH, Knox JP. 2003. Intercellular adhesion and cell separation in plants. *Plant Cell Environ.* 26:977–989.
- Kahlon CS, Board JE, Kang MS. 2011. An analysis of yield component changes for new vs. old soybean cultivars. *Agron J.* 103:13–22.
- Kashyap DR, Vohra PK, Chopra S, Tewari R. 2001. Applications of pectinases in the commercial sector: a review. *Bioresour Technol.* 77:215–227.
- Kidokoro S, Watanabe K, Ohori T, Moriwaki T, Maruyama K, Mizoi J, et al. 2015. Soybean DREB1/CBF-type transcription factors function in heat and drought as well as cold stress-responsive gene expression. *Plant J.* 81:505–518.
- Kim D, Langmead B, Salzberg SL. 2015. HISAT: a fast-spliced aligner with low memory requirements. *Nat Methods.* 12:357–360.
- Kim SY, Volsky DJ. 2005. PAGE: parametric analysis of gene set enrichment. *BMC Bioinform.* doi:10.1186/1471-2105-6-144.
- Kim SY, Zhu T, Sung ZR. 2010. Epigenetic regulation of gene programs by EMF1 and EMF2 in *Arabidopsis*. *Plant Physiol.* 152:516–528.
- Konno H, Yamasaki Y, Sugimoto M, Takeda K. 2008. Differential changes in cell wall matrix polysaccharides and glycoside-hydrolyzing enzymes in developing wheat seedlings differing in drought tolerance. *J Plant Physiol.* 165:745–754.
- Landi S, Hausman JF, Guerriero G, Esposito S. 2017. Poaceae vs. abiotic stress: focus on drought and salt stress, recent insights, and perspectives. *Front Plant Sci.* doi:10.3389/fpls.2017.01214.
- Lee KH, Piao HL, Kim HY, Choi SM, Jiang F, Hartung W, Hwang I. 2006. Activation of glucosidase via stress-induced polymerization rapidly increases active pools of abscisic acid. *Cell.* 126:1109–1120.
- Le Gall H, Philippe F, Domon JM, Gillet F, Pelloux J, Rayon C. 2015. Cell wall metabolism in response to abiotic stress. *Plants.* 4:112–166.
- Leng Y, Yang Y, Ren D, Huang L, Dai L, Wang Y, Zhu L. 2017. A rice PECTATE LYASE-LIKE gene is required for plant growth and leaf senescence. *Plant Physiol.* 174:1151–1166.
- Leucci MR, Lenucci MS, Piro G, Dalessandro G. 2008. Water stress and cell wall polysaccharides in the apical root zone of wheat cultivars varying in drought tolerance. *J Plant Physiol.* 165:1168–1180.
- Li R, Bonham-Smith PC, King J. 2001. Molecular characterization, and regulation of formate dehydrogenase in *Arabidopsis thaliana*. *Can J Bot.* 79:796–804.
- Li H, Handsaker B, Wysoker A, Fennell T, Ruan J, Homer N, Durbin R. 2009. The sequence alignment/map format and SAMtools. *Bioinformatics.* 25:2078–2079.
- Li R, Moore M, Bonham-Smith PC, King J. 2002. Overexpression of formate dehydrogenase in *Arabidopsis thaliana* resulted in plants tolerant to high concentrations of formate. *J Plant Physiol.* 159:1069–1076.
- Lima LL, Balbi BP, Mesquita RO, da Silva JCF, Coutinho FS, Carmo FMS. 2019. Proteomic and metabolomic analysis of a drought tolerant soybean cultivar from Brazilian savanna. *Crop Breed Genet Genom.* doi:10.20900/cbagg20190022.
- Liu F, Andersen MN, Jacobsen SE, Jensen CR. 2005. Stomatal control, and water use efficiency of soybean (*Glycine max* L. Merr.) during progressive soil drying. *Environ Exp Bot.* 54:33–40.
- Liu B, Yuan J, Yiu SM, Li Z, Xie Y, Chen Y, Luo R. 2012. COPE: an accurate k-mer-based pair-end reads connection tool to facilitate genome assembly. *Bioinformatics.* 28:2870–2874.
- Livak KJ, Schmittgen TD. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the  $2^{-\Delta\Delta CT}$  method. *Methods.* 25:402–408.
- Lu L, Dong C, Liu R, Zhou B, Wang C, Shou H. 2018. Roles of soybean plasma membrane intrinsic protein GmPIP2; 9 in drought tolerance and seed development. *Front Plant Sci.* doi:10.3389/fpls.2018.00530.
- Lygin AV, Hill CB, Zernova OV, Crull L, Widholm JM, Hartman GL, Lozovaya VV. 2010. Response of soybean pathogens to glyceollin. *Phytopathology.* 100:897–903.
- Manavalan LP, Guttikonda SK, Phan TLS, Nguyen HT. 2009. Physiological and molecular approaches to improve drought resistance in soybean. *Plant Cell Physiol.* 50:1260–1276.
- Marcolino-Gomes J, Rodrigues FA, Fuganti-Pagliarini R, Nakayama TJ, Reis RR, Farias JRB, et al. 2015. Transcriptome-wide identification of reference genes for expression analysis of soybean responses to drought stress along the day. *PloS One.* doi:10.1371/journal.pone.0139051.
- Medrano H, Tomás M, Martorell S, Flexas J, Hernández E, Rosselló J, Bota J. 2015. From leaf to whole-plant water use efficiency (WUE) in complex canopies: limitations of leaf WUE as a selection target. *Crop J.* 3:220–228.
- Molinari MDC. 2021. Transcriptome analysis using RNA-Seq from experiments with and without biological replicates: A review. *Rev Ciênc Agrár Amaz J Agric Environ Sci.* 64:1–13.
- Molinari MDC, et al. 2020. Overexpression of AtNCED3 gene improved drought tolerance in soybean in greenhouse and field conditions. *Genet Mol Biol.* 43:1–12.
- Moore JP, Vitré-Gibouin M, Farrant JM, Driouich A. 2008. Adaptations of higher plant cell walls to water loss: drought vs desiccation. *Physiol Plant.* 134:237–245.
- Nakamoto H, Vigh L. 2007. The small heat shock proteins and their clients. *Cell Mol Life Sci.* 64:294–306.
- Negin B, Yaaran A, Kelly G, Zait Y, Moshelion M. 2019. Mesophyll abscisic acid restrains early growth and flowering but does not directly suppress photosynthesis. *Plant Physiol.* 180:910–925.
- Neumaier N, Nepomuceno AL, Farias JRB, Oya T. 2000. Estádios de desenvolvimento da cultura de soja. [accessed 2019 Oct 10]. [https://www.agencia.cnpia.embrapa.br/Repositorio/estudios.neumaier\\_000g4yai9ub02wx5ok0dkla0sd076il2.pdf/](https://www.agencia.cnpia.embrapa.br/Repositorio/estudios.neumaier_000g4yai9ub02wx5ok0dkla0sd076il2.pdf/).
- Oya T, Nepomuceno AL, Neumaier N, Farias JRB, Tobita S, Ito O. 2004. Drought tolerance characteristics of Brazilian soybean cultivars. *Plant Prod Sci.* 7:129–137.
- Paiva Rolla AA, Carvalho JDFC, Fuganti-Pagliarini R, Engels C, Do Rio A, Marin SRR, Neumaier N. 2014. Phenotyping soybean plants transformed with rd29A:AtDREB1A for drought tolerance in the greenhouse and field. *Trans Res.* 23:75–87.
- Patel RK, Jain M. 2012. NGS QC toolkit: a toolkit for quality control of next generation sequencing data. *PLoS One.* doi:10.1371/journal.pone.0030619.
- Pedrosa AM, Cidade LC, Martins CPS, Macedo AF, Neves DM, Gomes FP, Costa MG. 2017. Effect of overexpression of citrus 9-cis-epoxycarotenoid dioxygenase 3 (CsNCED3) on the physiological response to drought stress in transgenic tobacco. *Gen Mol Res.* doi:10.4238/gmr16019292.
- Pertea M, Pertea GM, Antonescu CM, Chang TC, Mendell JT, Salzberg SL. 2015. Stringtie enables improved reconstruction of a transcriptome from RNA-seq reads. *Nat Biotechnol.* doi:10.1038/nbt.3122.
- Pinheiro C, Chaves MM. 2010. Photosynthesis, and drought: can we make metabolic connections from available data? *J Exp Bot.* 62:869–882.
- Piro G, Leucci MR, Waldron K, Dalessandro G. 2003. Exposure to water stress causes changes in the biosynthesis of cell wall polysaccharides in roots of wheat cultivars varying in drought tolerance. *Plant Sci.* 165:559–569.
- Prasch CM, Sonnewald U. 2013. Simultaneous application of heat, drought, and virus to *Arabidopsis* plants reveals significant shifts in signaling networks. *Plant Physiol.* 162:1849–1866.
- Priya M, Dhanker OP, Siddique KH, Hanumantha RB, Nair RM, Pandey S, Nayyar H. 2019. Drought, and heat stress-related proteins:

- an update about their functional relevance in imparting stress tolerance in agricultural crops. *Theor Appl Genet*. doi:10.1007/s00122-019-03331-2.
- Qin X, Zeevaart JA. 2002. Overexpression of a 9-cis-epoxycarotenoid dioxygenase gene in *Nicotiana glauca* increases abscisic acid and phaseic acid levels and enhances drought tolerance. *Plant Physiol*. 128:544–551.
- Racine JS. 2012. RStudio: a platform-independent IDE for R and sweave. *J Appl Econ*. 27:167–172.
- Richie SW, Thompson HE, Benson GO. 1997. Como a planta de soja se desenvolve. [accessed 2019 Oct 10]. [http://brasil.ipni.net/ipniweb/region/brasil.nsf/0/1A183CA9FE55F39883257AA0003B5C23/\\$FILE/Como%20a%20Planta%20da%20Soja%20Desenvolve.pdf](http://brasil.ipni.net/ipniweb/region/brasil.nsf/0/1A183CA9FE55F39883257AA0003B5C23/$FILE/Como%20a%20Planta%20da%20Soja%20Desenvolve.pdf).
- Robinson MD, McCarthy DJ, Smyth GK. 2010. Edger: a bioconductor package for differential expression analysis of digital gene expression data. *Bioinformatics*. 26:139–140.
- Ruijter J, Van Der Velden S, Ilgun A. 2009. LinRegPCR: analysis of quantitative RT-PCR data. [accessed 2019 Oct 10]. [https://www.gene-quantification.de/LinRegPCR\\_help\\_manual\\_v11.0.pdf](https://www.gene-quantification.de/LinRegPCR_help_manual_v11.0.pdf).
- Salinet LH. 2009. Avaliação fisiológica e agrônômica de soja geneticamente modificada para maior tolerância à seca. [https://www.teses.usp.br/teses/disponiveis/11/11144/tde-10032009-142320/publico/Luana\\_Salinet.pdf](https://www.teses.usp.br/teses/disponiveis/11/11144/tde-10032009-142320/publico/Luana_Salinet.pdf).
- Sambrook J, Fritsch E F, Maniatis T. 1989. *Molecular cloning: a laboratory manual*. 2nd ed. Cold Spring Harbor Laboratory. Cold Spring Harbor: NY.
- Saradadevi R, Palta JA, Siddique KH. 2017. ABA-mediated stomatal response in regulating water use during the development of terminal drought in wheat. *Front Plant Sci*. doi:10.3389/fpls.2017.01251.
- Savitri ES, Basuki N, Aini N, Arumingtyas EL. 2013. Identification and characterization drought tolerance of gene LEA-D11 soybean (*Glycine max* L. Merr) based on PCR-sequencing. *Am J Mol Biol*. 3:32–37.
- Seiler C, Harshavardhan VT, Rajesh K, Reddy PS, Strickert M, Rolletschek H, Sreenivasulu N. 2011. ABA biosynthesis and degradation contributing to ABA homeostasis during barley seed development under control and terminal drought-stress conditions. *J Exp Bot*. 62:2615–2632.
- Seo Jun Sung, Sohn Hwang Bae, Noh Kaeyoung, Jung Choonyun, An Ju Hee, Donovan Christopher M, Somers David A., Kim Dae In, Jeong Soon-Chun, Kim Chang-Gi, et al. 2012. Expression of the Arabidopsis AtMYB44 gene confers drought/salt-stress tolerance in transgenic soybean. *Molecular Breeding*. 29(3):601–608. <https://doi.org/10.1007/s11032-011-9576-8>.
- Shanker AK, Maheswari M, Yadav SK, Desai S, Bhanu D, Attal NB, Venkateswarlu B. 2014. Drought stress responses in crops. *Funct Integr Genom*. 14:11–22.
- Shapiro SS, Wilk MB. 1965. An analysis of variance test for normality complete samples. *Biometrika*. 52:591–611.
- Shi WY, Du YT, Ma J, Min DH, Jin LG, Chen J, Zhang XH. 2018. The WRKY transcription factor GmWRKY12 confers drought and salt tolerance in soybean. *Int J Mol Sci*. doi:10.3390/ijms19124087.
- Shiraishi T, Fukusaki EI, Kobayashi A. 2000. Formate dehydrogenase in rice plant: growth stimulation effect of formate in rice plant. *J Biosci Bioeng*. 89:241–246.
- Song L, Prince S, Valliyodan B, Joshi T, dos Santos JVM, Wang J, Nguyen HT. 2016. Genome-wide transcriptome analysis of soybean primary root under varying water-deficit conditions. *BMC Genom*. 17:57.
- Souza A, Batista VG, Pinheiro MP, Suassuna JF, Lima LMD, Fernandes PD. 2016. Expression of NCED gene in colored cotton genotypes subjected to water stress. *Rev Bras Eng Agríc Amb*. 20:692–696.
- Su Z, Ma X, Guo H, Sukiran NL, Guo B, Assmann SM, Ma H. 2013. Flower development under drought stress: morphological and transcriptomic analyses reveal acute responses and long-term acclimation in Arabidopsis. *Plant Cell*. 25:3785–3807.
- Sukumaran A. 2016. Identification and characterization of the isoflavonoid-specific prenyltransferase gene family to prevent stem and root rot in soybean. <https://pdfs.semanticscholar.org/e90a/84f6561f7b3fcf06fa3c8009a0f137506a87.pdf?ga=2.71637395.18522414.0.1578782338-1146854597.1570919859/>.
- Sultana S, Turečková V, Ho CL, Napis S, Namasivayam P. 2014. Molecular cloning of a putative *Acanthus ebracteatus*-9-cis-epoxycarotenoid deoxygenase (AeNCED) and its overexpression in rice. *J Crop Sci Biotechnol*. 17:239–246.
- Suzuki K, Itai R, Suzuki K, Nakanishi H, Nishizawa NK, Yoshimura E, Mori S. 1998. Formate dehydrogenase, an enzyme of anaerobic metabolism, is induced by iron deficiency in barley roots. *Plant Physiol*. 116:725–732.
- Thain SC, Vandenbussche F, Laarhoven LJ, Dowson-Day MJ, Wang ZY, Tobin EM, Van Der Straeten D. 2004. Circadian rhythms of ethylene emission in Arabidopsis. *Plant Physiol*. 136:3751–3761.
- Thompson AJ, Jackson AC, Symonds RC, Mulholland BJ, Dadswell AR, Blake PS, Taylor I B. 2000. Ectopic expression of a tomato 9-cis-epoxycarotenoid dioxygenase gene causes over-production of abscisic acid. *Plant J*. 23:363–374.
- Tong SM, Xi HX, Ai KJ, Hou HS. 2017. Overexpression of wheat *TaNCED* gene in Arabidopsis enhances tolerance to drought stress and delays seed germination. *Biolog Plant*. 61:64–72.
- Tudela D, Primo-Millo E. 1992. 1-Aminocyclopropane-1-carboxylic acid transported from roots to shoots promotes leaf abscission in Cleopatra mandarin (*Citrus reshni* Hort. ex Tan.) seedlings rehydrated after water stress. *Plant Physiol*. 100:131–137.
- Umezawa T, Okamoto M, Kushiro T, Nambara E, Oono Y, Seki M, Shinozaki K. 2006. CYP707A3, a major ABA 8'-hydroxylase involved in dehydration and rehydration response in Arabidopsis thaliana. *Plant J*. 46:171–182.
- Wan XR, Li L. 2006. Regulation of ABA level and water-stress tolerance of Arabidopsis by ectopic expression of a peanut 9-cis-epoxycarotenoid dioxygenase gene. *Biochem Biophys Res Commun*. 347:1030–1038.
- Wang Z, Li G, Sun H, Ma L, Guo Y, Zhao Z, Mei L. 2018a. Effects of drought stress on photosynthesis and photosynthetic electron transport chain in young apple tree leaves. *Biol Open*. doi:0.1242/bio.035279.
- Wang P, Liu H, Hua H, Wang L, Song CP. 2011. A vacuole localized  $\beta$ -glucosidase contributes to drought tolerance in Arabidopsis. *Chin Sci Bull*. 56:3538–3546.
- Wang Z, Wang F, Hong Y, Yao J, Ren Z, Shi H, Zhu JK. 2018b. The flowering repressor SVP confers drought resistance in Arabidopsis by regulating abscisic acid catabolism. *Mol Plant*. 11:1184–1197.
- Wei S, Ma X, Pan L, Miao J, Fu J, Bai L, Chen M. 2017. Transcriptome analysis of *Taxillus chinensis* (DC.) danser seeds in response to water loss. *PLoS One*. doi:10.1371/journal.pone.0169177.
- Willats WG, McCartney L, Mackie W, Knox JP. 2001. Pectin: cell biology and prospects for functional analysis. *Plant Mol Biol*. 47:9–27.
- Wolf S, Hématy K, Höfte H. 2012. Growth control and cell wall signaling in plants. *Annu Rev Plant Biol*. 63:381–407.
- Wu HC, Bulgakov VP, Jinn TL. 2018. Pectin methylesterases: cell wall remodeling proteins are required for plant response to heat stress. *Front Plant Sci*. doi:10.3389/fpls.2018.01612.
- Wu HC, Hsu SF, Luo DL, Chen SJ, Huang WD, Lur HS, Jinn TL. 2010. Recovery of heat shock-triggered released apoplastic Ca<sup>2+</sup> accompanied by pectin methylesterase activity is required for thermotolerance in soybean seedlings. *J Exp Bot*. 61:2843–2852.
- Xu ZY, Lee KH, Dong T, Jeong JC, Jin JB, Kanno Y, Yun DJ. 2012. A vacuolar  $\beta$ -glucosidase homolog that possesses glucose-conjugated abscisic acid hydrolyzing activity plays an important role in osmotic stress responses in Arabidopsis. *Plant Cell*. 24:2184–2199.
- Xu H, Watanabe KA, Zhang L, Shen QJ. 2016. WRKY transcription factor genes in wild rice *Oryza nivara*. *DNA Res*. 23:311–323.
- Xu C, Xia C, Xia Z, Zhou X, Huang J, Huang Z, Zhang C. 2018. Physiological and transcriptomic responses of reproductive stage soybean to drought stress. *Plant Cell Rep*. 37:1611–1624.
- Yin C, Wang X, Duan B, Luo J, Li C. 2005. Early growth, dry matter allocation and water use efficiency of two sympatric *Populus* species as affected by water stress. *Environ Exp Bot*. 53:315–322.
- Yoneyama K, Akashi T, Aoki T. 2016. Molecular characterization of soybean pterocarpin 2-dimethylallyltransferase in glyceollin biosynthesis: local gene and whole-genome duplications of prenyltransferase genes led to the structural diversity of soybean prenylated isoflavonoids. *Plant Cell Physiol*. 57:2497–2509.



- Yoon GM, Kieber JJ. 2013. 14-3-3 regulates 1-aminocyclopropane-1-carboxylate synthase protein turnover in *Arabidopsis*. *Plant Cell*. 25:1016–1028.
- Yoshida N, Yanai Y, Chen L, Kato Y, Hiratsuka J, Miwa T, Takahashi S. 2001. EMBRYONIC FLOWER2, a novel polycomb group protein homolog, mediates shoot development and flowering in *Arabidopsis*. *Plant Cell*. 13:2471–2481.
- Yu Z, Wang X, Zhang L. 2018. Structural and functional dynamics of dehydrins: a plant protector protein under abiotic stress. *Int J Mol Sci*. doi:10.3390/ijms19113420.
- Zernova O, Lygin A, Pawlowski M, Hill C, Hartman G, Widholm J, Lozovaya V. 2014. Regulation of plant immunity through modulation of phytoalexin synthesis. *Molecules*. 19:7480–7496.
- Zhang B, Liu H, Ding X, Qiu J, Zhang M, Chu Z. 2018. *Arabidopsis thaliana* ACS8 plays a crucial role in the early biosynthesis of ethylene elicited by Cu<sup>2+</sup> ions. *J Cell Sci*. doi:10.1242/jcs.202424.
- Zhang J, Liu J, Yang C, Du S, Yang W. 2016. Photosynthetic performance of soybean plants to water deficit under high and low light intensity. *S Afr J Bot*. 105:279–287.
- Zhao KX, Chu SS, Zhang XD, Wang LP, Rono JK, Yang ZM. 2020. AtWRKY21 negatively regulates tolerance to osmotic stress in *Arabidopsis*. *Environ Exp Bot*. doi:10.1016/j.envexpbot.2019.103920.
- Zivcak M, Brestic M, Sytar O. 2016. Osmotic adjustment and plant adaptation to drought stress. In: Hossain MA, Wani S, Bhattacharjee S, Burritt DJ, editor. *Drought stress tolerance in plants*. Cham: Springer; p. 105–143.