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Expression profile of *MYB60* and *GUSP1* genes during early growth of cotton genotypes submitted to water stress

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Genet. Mol. Res. 18 (4): gmr18319
Received April 05, 2019
Accepted August 17, 2019
Published October 30, 2019
DOI http://dx.doi.org/10.4238/gmr18319

ABSTRACT. Cotton has high adaptability to adverse conditions; however, one of the main factors causing production loss is water deficits. To adapt to these conditions, plants go through a series of changes, many of them driven by genes that are expressed to increase drought tolerance. We examined the expression profile of the *MYB60* and *GUSP1* genes, which are involved in the abiotic stress pathway, focusing on drought tolerance. Four Upland and Mocó genotypes were submitted to water stress during early growth and further evaluated at 50% (phase 1) and 80% (phase 2) of stomata enclosure. Plants were previously phenotyped, based on vegetative, physiological and biochemical traits. Expression of *GUSP1* and *MYB60* transcripts was estimated by qRT-PCR. Plants were grown in 288 mL pots in a greenhouse and further submitted to water stress during 25 days. Although Mocó cotton is considered tolerant to drought and upland cotton is known to be drought-sensitive; we

found a different behavior in these genotypes. Mocó 1 was very sensitive to the imposed water deficiency stress, with severe reductions in leaf number, stem diameter and weight of roots and canopy, while Delta Opal (Upland) presented the smallest reductions in growth. Expression of *GUSP1* transcripts was higher in all stressed genotypes, in both phases, during the water stress period, with the genotype Mocó 2, presenting the highest level of expression, while *MYB60* transcripts were high expressed only in phase 1, decreasing in phase 2. Considering that differences in the expression of MYB60 can be detected earlier, because the peak of expression occurred at phase 1 of water stress, it is worth investigating the genetic diversity in cotton germplasm to select genotypes with drought tolerance and to estimate the relation with the expression of MYB60, since Mocó genotypes were considered tolerant, but in this work a Mocó 1 genotype presented drought-sensitive characteristics.

Key words: Gene expression; Gas exchange; Antioxidative enzymes; *MYB60*; *GUSP1*

INTRODUCTION

Drought is one of the primary stresses that influences the yield of various important crops. The response of plants to water stress includes broad physiological and metabolic adaptations. A lack of water leads to osmotic stress, producing redundant reactive oxygen species (ROS). The disturbances caused in cells due to ROS excess may lead to lipid peroxidation, protein oxidation, enzyme inhibition, DNA damage, and even cell death (Cooke et al., 2003; Rodrigues et al., 2016). The damage to DNA may result in either arrest or induction of transcription, induction of signal transduction pathways, replication errors, cell membrane destruction and genomic instability (Cooke et al., 2003).

Several plant species employ antioxidative defense systems in order to protect themselves against ROS. Antioxidative enzymes include superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase, monodehydro ascorbate reductase, dehydroascorbate reductase and glutathione reductase. Overexpression of these enzymes is associated with tolerance to various abiotic stresses (Wang et al., 2004; Yang et al., 2009; Rodrigues et al., 2016).

Many genes that are induced under drought conditions have been identified and used as candidates in molecular assays in order to assist the selection procedures of crop breeding. Some of them have been found to be involved in stress perception, signal transduction and transcriptional regulatory pathways (Aktas et al., 2009; Uribe et al., 2014). In *Arabidopsis thaliana*, Wohlbach et al. (2008) found evidence that ATHK1-His kinase, plays a role in regulation of water stress response during early growth, soon after perception by the plants. According to these authors, ATHK1-mediated response to osmotic stress is abscisic acid (ABA) dependent and functions by positively regulating genes involved in ABA biosynthesis to increase ABA hormone levels.

ABA is a broad-spectrum phytohormone involved in plant growth and development; it is also involved in various signal transduction pathways in response to

abiotic stress. The pathways of ABA from stress perception to gene expression involve various transcription factors, such as DREB, MYC/MYB, ABFs, NAC and CBF/DREB1. Overexpression of genes linked to stress tolerance in plants submitted to drought has been reported in various species, including *A. thaliana* (Abe et al., 2003; Jung et al., 2008), *Triticum aestivum* (Rahaie et al., 2010), and *Oryza sativa* (Wang et al., 2008).

The MYC/MYB proteins are widely found in both plants and animals and play important roles in many physiological processes under normal or adverse growth conditions, such as in upregulation of genes involved in response to abiotic stress. In transgenic plants of *Arabidopsis*, Abe et al. (2003) reported overexpression of *AtMYC2* and *AtMYB2*, which was induced through ABA-responsive genes. They concluded that AtMYC2 and AtMYB2 proteins function as transcriptional activators in ABA-inducible gene expression under drought stress in plants. In *T. aestivum*, Rahaie et al. (2010) analyzed the expression of 10 *MYB* genes in plants submitted to salt or drought stresses and found that several genes were up-regulated, including *TaMYBsdu1*, an important regulator in the adaptation of wheat to both salt and drought stresses. In addition to the transcription factors, several genes have been found to be involved in drought tolerance, such as *LEA*, *NCED*, *P5CS*, *USP* (Maqbool et al., 2009; Isokpehi et al., 2011; Jung et al., 2015). USP is a small protein that is upregulated when the cell is exposed to stress agents, enhancing cell survival during prolonged exposure to such conditions (Nystrom and Neidhardt, 1992, 1993; Jung et al., 2015). Isokpehi et al. (2011) found two *Arabidopsis* USP genes, *At3g62550* and *At3g53990*, that encode an ATP-binding motif up-regulated in a drought microarray dataset. In *Gossypium arboreum*, Maqbool et al. (2009) identified two closely related genes, *GUSP1* and *GUSP2* from water-stressed leaves. Expression assays revealed a high level of *GUSP* gene expression in leaves, roots, and stems exclusively in plants following water stress. However, the highest levels of drought-inducible expression were found in the leaves.

Cotton (*G. hirsutum*) is a fiber crop grown worldwide; it is often exposed to environmental stresses such as soil salinity, heat and drought. This species has two botanical types with contrasting differences in drought tolerance. Genotypes from *G. hirsutum* spp. Marie Galante (Mocó) are perennial and more adapted to dry environments, while upland genotypes are annuals and show varying levels of drought tolerance (Rodrigues et al., 2016). In environments prone to drought, the use of short cycle and drought tolerant genotypes is an adequate strategy in order to avoid losses in fiber production. The traits that affect the tolerance of crops to drought are quantitatively inherited and polygenic in nature. The identification of drought-responsive genes could provide important molecular markers for use in cotton breeding (Aktas et al., 2009; Uribe et al., 2014).

Aktas et al. (2009) used two contrasting cotton genotypes in order to identify compounds with ROS-scavenging ability and found that the drought-tolerant genotype had higher levels of polyphenols, proline, carotenoids and antiradical capacity. Uribe et al. (2014) used a tolerant genotype (Acala 1517-99) in order to identify drought-responsive genes under drought stress conditions; they found 110 drought-responsive genes, 79% of which were drought-repressed and 21% were drought-induced. They found that genes for CAT and NADPH were also suppressed by drought in cotton plants and suggested that the antioxidant and radical scavenging systems in Acala 1517-99 are not highly active in alleviating oxidative stress after prolonged water stress treatment.

Considering the availability of drought tolerance-genes reported in literature and also the necessity to validate such findings in order to assist the selection procedures of cotton breeding, in this work we analyzed the expression profile of *MYB* and *USP* transcripts in cotton genotypes submitted to water stress during early growth. Additionally, we evaluated plant growth under water-stress conditions.

MATERIAL AND METHODS

Germplasm and experimental procedures

Seeds of four cotton genotypes were used (two Upland and two Mocó, Table 1) and grown in green house (Campina Grande, PB, 07º13'50", 35º52'52", 551 m), in tubes (0.3 L), containing commercial substrate (Basaplant, Base). After emergence, one plant was kept per tube. Plants were watered daily up to V2 phase (about 15 days after emergence) from which a total water suppression was established. A completely randomized experimental design was adopted, with two treatments (control and stress) and four repetitions. To growth analyses, plants were maintained under water suppression during 25 days, when the follows traits were collected: plant height, stem diameter, total number of leaves, weight of dry root and weight of dry shoots. Data were submitted to variance analyses, using F test ($P < 0.05$). Means were compared by Tukey test ($P < 0.05$). The software SISVAR 5.3 was adopted to statistical analyses (Ferreira, 2014). The air temperature and RH in greenhouse ranged from 28°C to 35°C and 43% to 64%, during assay.

Table 1. Origin, genealogy, and botanic type of the four cotton accessions (*Gossypium hirsutum*) of the active germplasm bank of Embrapa Cotton that were investigated.

Genotypes	Origin	Genealogy	Botanic type
Delta Opal	Cultivar/BASF, EUA	DP 5816 x Sicala 33	latifolium
CNPA Precoce 1	Cultivar/Embrapa, Brazil	GH 11-9-75	latifolium
Mocó 1	Accession/Embrapa, Brazil	Wild	Marie Galant
Mocó 2	Accession/Embrapa, Brazil	Wild	Marie Galant

Biochemical and molecular assays

The biochemical analyses started when plants reached 50% (Phase 1) and 80% (Phase 2) of stomata closure, measured in young leaves, during 8:00h -10:00, through IRGA (LCpro^{+}, ADC Bioscientific). The activity of the antioxidative enzymes (SOD and CAT) was estimated from crude extract of leave proteins, in both phases. Briefly, fresh leaves (200 mg) crushed in 2 mL potassium phosphate buffer (100 mM, pH 7.0) + ascorbic acid (0.1 mM), EDTA (0.1 mM) and PVP (10%). Concentration of samples was estimated by Bradford (1976). The methodology of free proline content was followed according Bates et al. (1973) method, and estimated through spectrophotometry (Biomate 3, USA) at 520 nm. SOD and CAT procedures were performed according Giannopolitis and Ries (1977) and Sudhakar et al. (2001), and estimated at 560 nm and 240 nm, respectively. Biological triplicates were adopted in all assays.

RNA extraction and RT-PCR assays

RNAs were extracted from the young leaves using Invisorb Spin Plant Mini kit (Invitek). cDNAs were synthetized with ImProm-II™ Reverse Transcription System, Promega. To RT-qPCR assays, a set of endogenous and specific primers were used (Table 2). The reactions were carried out using 1 µL cDNA, 0.2 µL of each primer and qPCR-SYBR-Green mix/ROX kit (Ludwig), according to manufacturer's recommendation. Reactions were performed as follow: 95°C for 10 min, followed by 40 cycles of 95°C for 15 s, 60°C for 1 min, and 72°C for 15 s. Biological triplicates were adopted.

The graphs, Cqs, and melt curve were automatically generated by software of Eco Real-Time PCR System thermocycler (Illumina, Inc., San Diego, CA, USA), based on normalization method with three reference genes (Livak and Schmittgen, 2001). The expression pattern was estimated by relative quantification. Normatized reference genes were used (Ártico et al., 2014).

Table 2. Sequences of specific and endogenous oligonucleotides used in the RT-qPCR assays of cotton genes.

Gene	Genbank	Sequence 5'→3'
GUSP1	EU107766.1	F: GCAAATCGGTGTAGCAATGG R: CTTCCTTCTCTCTGAACTCC
MYB60	NM_100755.2	F: ACCATGGACTCCTGAAGAAG R: CCAATAAGGCTTGCAAGTGA
ACTINA	AY305726	F: TTGCAGACCGTATGAGCAAG R: ACTCTCCGATCCAGACACTG
UBIQUITINA	DW505546	F: CAACGCTCCATCTTGTCCTT R: TGATCGTCTTTCCCGTAAGC
PP2A	DT545658	F: GATCCTTGTGGAGGAGTGGA R: GCGAAACAGTTCGACGAGAT

F- forward; R- reverse.

RESULTS AND DISCUSSION

Growth profile of plants

After seven days of water suppression, all genotypes showed wilting symptoms that increased till final of assay, however, the mortality rate of plants, based on permanent wilting stage (death), was 7.5% to Delta Opal, 15% to CNPA Precoce 1 and 2.6% to Mocó 1. To Mocó 2, no dead plant was found.

Growth measures were taken at end of assay, at 25 days of water suppression. Statistical differences were found to all growth traits (Table 3). Mocó 1 and CNPA Precoce 1 were more affected in most traits. This result is quite surprising because both genotypes belong to different botanical types and, as Mocó 1 was collected in the same region of Mocó 2 (Northeast semi-arid); we expected similar behavior between these two accessions. This results mean that, despite Mocó 1 and Mocó 2 are non-domesticated accessions, it is possible that Mocó 1 had inherited genes derived from herbaceous accesses, considering the proximity of collection site with cotton fields in that region.

Table 3. Relative losses (%) of growth traits in cotton genotypes submitted to 25 days of water suppression.

Genotype	Growth traits				
	PH	**TNL**	**SD**	**WDR**	**WDS**
Delta Opal	5.8 B	14.1 B	27.4 B	36.9 B	52.66 B
CNPA Precoce 1	13.9 A	42.7 A	34.0 B	50.7 A	65.66 A
Mocó 1	13.5 A	44.1 A	41.3 A	53.3 A	60.65 A
Mocó 2	12.7 A	29.5 B	27.2 B	29.8 C	55.02 B

Means with same letter do not differs statistically ($P < 0.05$). PH - plant height, NL- total number of leaves, SD - stem diameter; WDR - weight of dry root; WDS - weight of dry shoots.

Antioxidative enzymes

The profile of SOD and CAT in cotton plants, collected in leaves at 50% (phase 1) and 80% (phase 2) of stomata closure is seen in Figure 1. We found that SOD activity in scavenging of free radical was mild in both phases for Mocó 2, meaning that plants had broad ability to adjust the water stress, facilitating the action of CAT in neutralizing of ROS produced by cells due to water stress. This profile is an indication that this genotype has a greater ability to tolerate the imposed water stress, more than the others ones studied. In these genotypes, the enzymatic antioxidative system was activated to minimize the effects of free radicals (Rodrigues et al., 2016). After activation of a multigenic response to stress, several metabolites with important functions in the stabilization of enzyme and membrane complexes are synthesized in response to drought, ensuring the osmotic adjustment necessary to maintain turgor (Chaves et al., 2003).

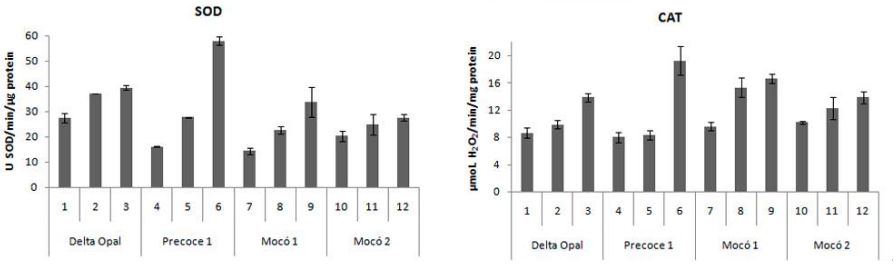

Figure 1. Activity of SOD and CAT in cotton plants submitted to water stress. 1, 4, 7 and 10 - Control; 2, 5, 8 and 11 - phase 1 (50% stomata closure); 3, 6, 9 and 12 - phase 2 (80% stomata closure).

Molecular Analyses

MYB60 and *USP* genes are involved in water stress tolerance in several crops, as *A. thaliana*, *Oryza sativa*, *G. arboreum*, *Salvia miltiorrhiza*, (Sauter et al., 2002; Maqbool et al., 2009; Cominelli et al., 2010; Wang et al., 2017). According to literature, *USP* appears improves the rate of cell survival during prolonged exposure to stress agents, and may endow plants with wide-ranging stress tolerance (Zahur et al., 2009). In *Arabidopsis*, *USP*-At3g53990 exhibits a chaperone function and is largely induced by heat, H_2O_2, and drought treatments (Jung et al., 2015). In this study we analyzed the profile of *MYB* and *USP* transcripts in latifolium and Marie Galant genotypes in young leaves at phase 1 and phase 2.

As seen in Figure 2, the expression of *GUSP1* transcripts was higher in both phases for Mocó 2, which was also more tolerant based on biochemical assays and growth analyses.

As to *MYB60*, transcript levels were found to be higher when the plants were in phase 1, decreasing slightly in the Marie Galant and abruptly in the latifolium, at phase 2 (Figure 2). This behavior corroborates with findings reported in the literature, in which *MYB60* is involved in the regulation of stomatal movement during stress. Their level of expression decreases in guard cells with increasing ABA levels, promoting stomatal closure (Oh et al., 2011). The high levels of expression found in both Delta Opal and Precoce 1 mean that genotypes suffered more during stress. According to Oh et al. (2011), the overexpression of the MYB60 gene confers sensitivity to the plant.

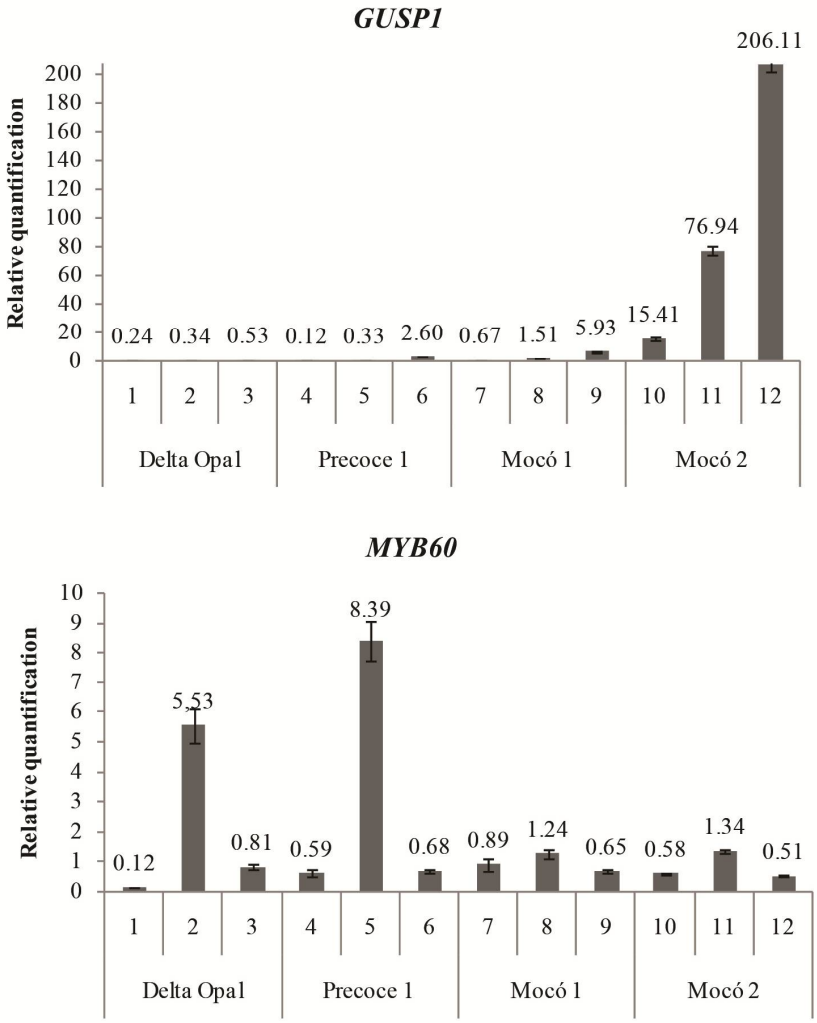

Figure 2. Relative expression quantification of *GUSP1* and *MYB60* genes involved in water stress in cotton. 1, 4, 7 and 10 - No stress; 2 and 5 (4th Day Water Stress - DWS), 8 and 11 (3rd DWS) - phase 1; 3 and 6 (7th DWS), 9 and 12 (6th DWS) - phase 2.

Tolerance to abiotic stresses is one of the main targets of plant breeding in several annual or perennial crops. When face environmental stresses, plant cells activate several signaling pathways that trigger the expression of transcription factors. Water stress is considered widely critical because drought occurs in several environments, affecting the plant growth in critical phases during life cycle. Several descriptors have been adopted in order to aid in the identification of plants tolerant to water stress. As drought tolerance is a multigenic trait, involving a set of physiological and biochemical processes (Ullah et al., 2017), the adoption of several traits is generally necessary to attest the level of tolerance of the evaluated genotypes.

At the molecular level, plants respond differently depending on the level of tolerance, altering gene expression, up- or down-regulating, depending on the role of the gene involved in the processes of cell defense. The first step in activating a molecular response to an environmental signal (such as water deficit) is perception by specific receptors. After activation, these receptors initiate (or suppress) a cascade response to transmit the information through a signal transduction pathway (Chaves et al., 2003). The findings of genomics have contributed broadly to the validation of such results. From these studies, several genes involved in the path of plant defense have contributed to assist some selection procedures in several commercial crops, such as rice (*O. sativa*), cotton (*G. arboreum* and *G. barbadense*) and soybean (*Glycine max*) (Sauter et al., 2002; Maqbool et al., 2009; Chen et al., 2015).

The genes *GUSP1* and *MYB60*, initially identified in *A. thaliana*, have also homology in cotton. In plants, USP is upregulated when the cell is exposed to stress, increasing the survival rate (Nystrom and Neidhardt, 1992, 1993). In *G. arboreum*, Maqbool et al. (2009) suggest that *GUSP1* is widely expressed in leaves due to involvement with chloroplasts. In a study involving the expression of the GUSP1 protein in transgenic cotton seedlings submitted to water stress, Hassan et al. (2018) observed increased GUSP1 expression in leaves. The same was observed in our study when compared to the treatment without stress. In work on water stress in wild tomato (*Solanum pennellii*), Loukehaich et al. (2012) observed that overexpression of the *USP* gene increased plant tolerance to drought. Wang et al. (2017), in working with heterologous expression of *S. miltiorrhiza USP* genes in *E. coli*, observed that the increase in gene expression confers tolerance to salt stress and heat as well as the combination between them. In our work, using various genotypes of *G. hirsutum*, we found that Delta Opal, CNPA Precoce 1 and Mocó 1 had a slight increase in *GUSP1* expression, in both phases, unlike Mocó 2, which had a large increase, leading to the assumption that this genotype is the most tolerant to water stress, based on our data (Figure 2).

MYB expression has also been widely reported in response to abiotic stresses in several crops. According to Baldoni et al. (2015), the MYB family has a specific role in responses of plants to water stress, such as regulation of stomatal movement, control of suberin synthesis and cuticular wax production. Ours is the first report on the function of MYB60 in cotton. We found that *MYB60* transcripts were more expressed in leaves collected at phase 1, falling sharply with further water deficity stress, especially in sensitive plants (Figure 2). This result is coherent with what was found by Oh et al. (2011), who studied the involvement of two splice variants of the *MYB60* in *A. thaliana* under drought conditions. According to these authors, *MYB60* plays a crucial role in stomatal movement, based on over-expressing of each variant, resulting in enhanced sensitivity to water deficit

stress. Severe drought stress inhibited the expression of *MYB60*, resulting in stomatal closure and root growth inhibition.

Although our work focused on expression of *MYB60* only in leaves, there are several reports of *MYB60* expression in roots and flowers from other species, with negative or positive regulation, depending on the stress condition in which the plant was submitted (Cominelli et al., 2005; Oh et al., 2011; Zhao et al., 2014). Dspite the availability of information about these genes in several crops, reports in *Gossypium*, focusing on tolerance to abiotic stress are limited. More research should be conducted in this area, taking into account the importance of cotton to world agribusiness and the current environmental chances that face many crops. Validation of drought tolerance genes can contribute to selection in cotton-breeding programs focusing on semi-arid environments.

ACKNOWLEDGMENTS

We thank Embrapa (Brazilian Agricultural Research Corporation) for financial support and the Coordination for Improvement of Higher Education Personnel (CAPES) for the scholarship granted.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

REFERENCES

Abe H, Urao T, Ito T, Seki M, et al. (2003). *Arabidopsis* AtMYC2 (bHLH) and AtMYB2 (MYB) function as transcriptional activators in abscisic acid signaling. *Plant Cell.* 15: 63-78.

Aktas YL, Dagnon S, Gurel A, Gesheva E, et al. (2009). Drought tolerance in cotton: involvement of nonenzymatic ROS-scavenging compounds. *J. Agron. Crop. Sci.* 195: 247-253.

Ártico S, Alves MR, Ribeiro-Alves M, Oliveira Neto OB, et al. (2014). Transcriptome analysis of *Gossypium hirsutum* flower buds infested by cotton boll weevil (*Anthonomus grandis*) larvae. *BMC Genomics.* 15: 1-24.

Baldoni E, Genga A and Cominelli E (2015). Plant MYB Transcription Factors: Their Role in Drought Response Mechanisms. *Int. J. Mol. Sci.* 16: 15811-15851.

Chaves MM, Maroco JP and Pereira JS (2003). Understanding plant responses to drought – From genes to the whole plant. *Funct. Plant Biol.* 30: 239-264.

Cominelli E, Galbiati M and Tonelli C (2010). Transcription factors controlling stomatal movements and drought tolerance. *Transcription.* 1: 1-5.

Cominelli E, Galbiati M, Vavasseur A and Tonelli C (2005). A guard-cell-specific MYB transcription factor regulates stomatal movements and plant drought tolerance. *Curr. Biol.* 15: 1196-1200.

Cooke MS, Evans MD, Dizdaroglu M and Lunec J (2003). Oxidative DNA damage: mechanisms, mutation, and disease. *FASEB J.* 17: 1195-214.

Chen T, Li W, Hu X, Guo J, et al. (2015). A Cotton MYB transcription factor, GbMYB5, is positively involved in plant adaptive response to drought stress. *Plant Cell Physiol.* 56: 917-929.

Ferreira DF (2014) Sisvar: a Guide for its Bootstrap procedures in multiple comparisons. *Cienc. Agrotec.* 38: 109-112.

Giannopolitis CN and Ries SK (1977) Superoxide dismutases: I. Occurrence in higher plants. *Plant Physiol.* 59: 309-314.

Hassan S, Samiullah TR, Ansari M and Rashid B (2018). Cloning, antibody production, expression and cellular localization of universal stress protein gene (USP1-GFP) in transgenic cotton. *Plant Biochem. Biotechnol.* 27: 175-185.

Isokpehi RD, Simmons SS, Cohly HH, Ekunwe SI, et al. (2011). Identification of drought-responsive universal stress proteins in Viridiplantae. *Bioinform. Biol. Insights.* 5: 41-58.

Jung C, Seo JS, Han SW, Koo YJ, et al. (2008). Overexpression of AtMYB44 enhances stomatal closure to confer abiotic stress tolerance in transgenic *Arabidopsis*. *Plant Physiol.* 146: 623-635.

Jung YJ, Melencion SMB, Lee ES, Park JH, et al. (2015). Universal stress protein exhibits a redox-dependent chaperone function in *Arabidopsis* and enhances plant tolerance to heat shock and oxidative stress. *Front Plant Sci.* 6: 1141.

Livak KJ and Schmittgen TD (2001). Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods.* 25: 402-408.

Loukehaich R, Wang T, Ouyang B, Ziaf K, et al. (2012). *SpUSP*, an annexin-interacting universal stress protein, enhances drought tolerance in tomato. *J. Exp. Bot.* 63: 5593-606.

Maqbool A, Zahur M, Husnain T and Riazuddin S (2009). GUSP1 and GUSP2, two drought-responsive genes in *Gossypium arboreum* have homology to universal stress proteins. *Plant Mol. Biol. Rep.* 27: 109-114.

Nystrom T and Neidhardt FC (1992) Cloning, mapping and nucleotide sequencing of a gene encoding a universal stress prot.ein in *Escherichia coli*. *Mol. Microbiol.* 6: 3187-98.

Nystrom T and Neidhardt FC (1993). Isolation and properties of a mutant of *Escherichia coli* with an insertional inactivation of the uspA gene, which encodes a universal stress protein. *J. Bacteriol.* 175: 3949-56.

Oh JE, Kwon Y, Kim JH, Noh H, et al. (2011). A dual role for MYB60 in stomatal regulation and root growth of *Arabidopsis thaliana* under drought stress. *Plant Mol. Biol.* 77: 91-103.

Rahaie M, Xue GP, Naghav MR, Alizadeh H, et al. (2010). A MYB gene from wheat (*Triticum aestivum* L.) is up-regulated during salt and drought stresses and differentially regulated between salt-tolerant and sensitive genotypes. *Plant Cell Rep.* 29: 835-844.

Rodrigues JD, Silva CRC, Pereira RF, Ramos JPC, et al. (2016). Characterization of water stress tolerant cotton cultivars based on plant growth and in activity of antioxidant enzymes. *Afr. J. Agric. Res.* 11: 3763-3770.

Sauter M, Rzewuski G, Marwedel T and Lorbiecke R (2002). The novel ethylene-regulated gene OsUsp1 from rice encodes a member of a plant protein family related to prokaryotic universal stress proteins. *J. Exp. Bot.* 53: 2325-2331.

Sudhakar C, Lakshmi A and Giridarakumar S (2001). Changes in the antioxidant enzyme efficacy in two high yielding genotypes of mulberry (*Morus alba* L.) under NaCl salinity. *Plant Sci.* 161: 613-619.

Ullah A, Sun H, Yang X and Zhang X (2017). Drought coping strategies in cotton: increased crop per drop. *Plant Biotechnol. J.* 15: 271-284.

Uribe LR, Abdelraheem A, Tiwari R, Gopalan CS, et al. (2014). Identification of drought-responsive genes in a drought tolerant cotton (*Gossypium hirsutum* L.) cultivar under reduced irrigation field conditions and development of candidate gene markers for drought tolerance. *Mol. Breed.* 34: 1777-1796.

Wang Q, Guan Y, Wu Y, Chen H, et al. (2008). Overexpression of a rice OsDREB1F gene increases salt, drought, and low temperature tolerance in both *Arabidopsis* and rice. *Plant Mol. Biol.* 67: 589-602.

Wang Y, Ying Y, Chen J and Wang X (2004). Transgenic *Arabidopsis* overexpressing Mn-SOD enhanced salt-tolerance. *Plant Sci.* 167: 671-677.

Wang XF, Su J, Yang N, Zhang H, et al. (2017). Functional Characterization of Selected Universal Stress Protein from Salvia miltiorrhiza (SmUSP) in Escherichia coli. *Genes (Basel.)* 8: 224.

Wohlbach DJ, Quirino BF and Sussman MR (2008). Analysis of the *Arabidopsis* histidine kinase ATHK1 reveals a connection between vegetative osmotic stress sensing and seed maturation. *Plant Cell.* 20: 1101-1117.

Yang Z, Wu Y, Li Y, Ling HQ, et al. (2009). OsMT1a, a type 1 metallothionein, plays the pivotal role in zinc homeostasis and drought tolerance in rice. *Plant Mol. Biol.* 70: 219-229.

Zahur M, Maqbool A, Ifran M, Barozai MY, et al. (2009). Isolation and functional analysis of cotton universal stress protein promoter in response to phytohormones and abiotic stresses. *Mol. Biol.* 43: 628-35.

Zhao Y, Xing L, Wang X, Hou YJ, et al. (2014). The ABA receptor PYL8 promotes lateral root growth by enhancing MYB77-dependent transcription of auxin-responsive genes. *Sci. Signal.* 7: ra53.

