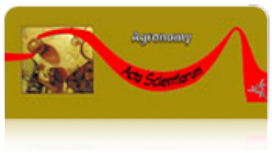

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Physiological analysis and gene expression analysis of *ZmDBP3*, *ZmALDH9*, *ZmAN13,* and *ZmDREB2A* in maize lines

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ABSTRACT. : The objective of this study was to evaluate gene expression related to water deficit tolerance in maize lines. For this, lines previously classified as tolerant (91-T and 32-T) and non-tolerant (24-NT and 57-NT) to water deficit were used. The seeds of the four lines were evaluated for emergence and emergence speed index, and the seedlings were evaluated for root and shoot length under two conditions of water availability (70 and 10% substrate water retention capacity). In transcript analysis, the expression of several genes associated with water deficit tolerance, *ZmDBP3*, *ZmALDH9, ZmAN13,* and *ZmDREB2A,* was evaluated by *qRT-PCR* for the 91-T and 57-NT lines. It can be concluded that soil water deficiency did not reduce root development. However, the shoot length was significantly lower under dry conditions. Through transcript analysis, the genes *ZmDBP3* and *ZmAN13* were identified as potential markers for the early selection of maize lines tolerant to water deficit.

Keywords: abiotic stress, Zea mays, qRT-PCR, water deficit.

Introduction

Low water availability is a climatic variable that affects maize grain production in Brazil. Water deficit directly causes changes in plants. It is considered the factor that most limits plant development, and water deficit is the main agent that causes grain production reduction (*Companhia Nacional de Abastecimento*[Conab], 2017).

In this context, one of the most relevant objectives in breeding programmes currently is to increase plant tolerance to environmental stresses. However, mechanisms that control tolerance, such as tolerance

to water deficit, are complex and involve the expression of several genes, making identification of the mechanisms difficult (Ashraf, 2010). Plants subjected to stress seek to adapt to the stress by expressing specific genes. Genes responsive to water deficit are largely regulated by abscisic acid (ABA). However, there are response genes that are not regulated by ABA, indicating the existence of several regulatory molecular mechanisms (Ding et al., 2012; Liu et al., 2013).

Many genes have been reported to potentially be important in increasing plant tolerance to water deficit, such as the genes of the *ZmDREB* family and the genes *ZmALDH9* and *ZmAN13* (Jin et al., 2007; Liu et al., 2009; Wang & Dong, 2009; Zhou et al., 2012). According to Wang and Dong (2009), the overexpression of the *ZmDBP3* gene, a member of the *CBF/DREB* family extracted from the leaves of maize seedlings, increased the tolerance to drought and cold in *Arabidopsis* transgenic plants.

Quantitative differences in gene expression provide different tolerance levels to water deficit among plants, making the discovery of superior genotypes regarding drought tolerance possible through gene expression analysis. Therefore, the objective of this study was to evaluate the gene expression associated with water deficit in seeds, ear tissues and seedlings of maize grown under normal cultivation conditions and under conditions of water deficit through gene expression analysis. Specifically, this study sought to define potential marker genes in maize seeds that could enable the early selection of genotypes tolerant to water deficit.

Material and methods

The research was conducted at the Center for Scientific and Technological Development in Agriculture (*Muquém* Farm) and at the Central Seed Laboratory of the Federal University of Lavras (UFLA) in Lavras city, State Minas Gerais, Brazil.

An experimental field for seed multiplication of four maize lines granted by Genesseds Ltda., Lavras, State Minas Gerais, Brazil, was installed. Among the lines used, two had been previously classified as tolerant to water deficit (91-T and 32-T), and two were not tolerant to water deficit (24-NT and 57-NT).

A spacing regime of 0.8 metres between rows and six plants per linear metre was used. Cover fertilization and other agricultural and phytosanitary treatments were carried out according to the needs of the crop. Self-fertilization was performed manually, and when the water content of the seeds reached 25%, harvesting was performed manually. Ear drying was performed in a maize ear dryer at 35°C until the water content reached 13%; then, the ears were threshed manually and treated with the fungicide Vitavax-Thiram 200 (300 mL per 100 kg of seeds), and several factors were analysed.

The seed germination and vigour of the four lines were evaluated with an accelerated ageing test, as described in the Rules for Seed Analysis - RAS (Brasil, 2009).

The seeds of the four lines were evaluated under two conditions of water availability. In the first condition, the no water deficit condition, the substrate moisture was adjusted to 70% water retention capacity; in the second condition, the water-deficit condition, the water retention capacity was adjusted to 10%. In each condition, 50 seeds from each line were seeded in plastic trays containing the substrate sand and were allowed to germinate in a growth chamber at 25°C. After seven days, the seedlings were removed from the substrate and washed in running water.

The following characteristics were evaluated:

a) seedling emergence: the percentage of seedlings that had emerged by the seventh day after sowing; b) emergence speed index: the number of seedlings that emerged per day (Maguire, 1962); and c) root and shoot length: the average length of the main root and shoot of the seedlings on the seventh day after sowing, from the emergence analysis and expressed in centimetres (cm) per seedling.

For the germination and accelerated ageing test, the experiment used a completely randomized design with four replicates of 50 seeds. Regarding the emergence, emergence speed index, and root and shoot length analyses, the experimental design was completely randomized with a 4x2 factorial scheme; four lines were evaluated under two conditions of water availability with four replicates. Comparisons of averages were performed using the Tukey test at a probability of 5%, and the data were analysed with R software.

Gene expression related to water deficit tolerance was evaluated using *qRT-PCR* analysis, which was divided into four steps: extraction and purification of RNA, reverse transcription for cDNA synthesis, real-time PCR and analysis of the results.

Seeds from two lines with contrasting water deficit tolerance, 91-T and 57-NT, were sown in pots to grow and produce ears. In the period from 5 to 10 days after the issuance of styles/stigmas, only ear tips, approximately 3 to 5 cm in length, were collected.

Thus, the samples used for gene expression analysis were dried seeds, ear tips and seedlings collected seven days after sowing of two maize lines, 91-T and 57-NT. Seedlings were submitted to two contrasting conditions of water availability, no water deficit (70% water retention capacity) and water deficit (10% water retention capacity).

For *RNA* extraction, the samples were macerated in the presence of liquid nitrogen, and then the *PureLink Plant RNA* (*Invitrogen*) reagent was added following the manufacturer's specifications. The *RNA* integrity was verified on a 1% agarose gel (stained with *GelRed*, *Biotium*) and with a spectrophotometer using wavelengths of 260 and 280 nm.

After nucleic acid extraction, the samples were treated with *DNase*. For this purpose, a *TURBO DNA-Free Kit* (*Ambion*) was used according to the protocol recommended by the manufacturer. To verify the *DNase* treatment efficiency, a conventional *PCR* reaction was performed with all the samples, proving that there was no amplification of *DNA*.

After the extraction and purification process, the *mRNA* was used as a template for *cDNA* synthesis using a *High-Capacity cDNA Reverse*

Transcription Kit from *Applied Biosystems* following the protocol recommended by the manufacturer.

For gene expression analysis with *qRT-PCR*, the target genes were chosen based on a previous review of the literature considering their importance in maize water deficit tolerance. Sequences were found by searching the database of the maize genome, and based on these sequences, primers were designed using *Primer Express 3.0* software (*Applied Biosystems*) (Table 1).

For the expression analysis of the selected genes, an *ABI PRISM 7500 Real-Time PCR* system (*Applied Biosystems*) was used with *SYBR Green*. The cycling conditions were 2 min. at 50°C, 10 min. at 95°C, 40 cycles of 15 s at 95°C and 1 min. at 60°C, and 15 s at 95°C. At the end of the cycling, a denaturation curve from 60-95°C showed the *PCR* reaction specificity. The data were collected and stored in the *7500 Fast Software* program (Version 2.1). For each reaction, 1.0 µl of *cDNA* (diluted 1:5), 0.4 µL of *forward/reverse primer* (10 µM), 5 µL of *SYBR Green Master Mix* (*Applied Biosystems*) and 3.6 µL of ultrapure water were included for a final volume of 10.0 µL sample^{-1}. Negative controls and melting curves were included in all analyses.

Three biological replicates and three technical replicates were analysed for each gene under study, and the results were normalized using the *threshold* Cycle (Ct) of the expression of the reference genes ubiquitin (UBI) and beta-actin (β*ACT*).

The Ct is determined by the number of cycles at which the fluorescence generated within a reaction crosses the baseline (*threshold*). A validation experiment was carried out to prove that the amplification efficiencies of the target genes and the reference genes were similar and were close to 100%, enabling the selection of the optimal *cDNA* dilution for each reaction (1:5). The samples with the lowest expression for each gene were used as calibration samples, and the relative expression was analysed by the mathematical model proposed by Pfaffl (2001).

Results and discussion

The seeds of the four lines studied showed high germination values, among which there were no significant differences, and the values obtained were essential for the other tests carried out in this study. The initial vigour of the seeds was determined with the accelerated ageing test. There were significant differences in vigour; the seeds of the 91-T line, which is considered to be tolerant to water deficit, presented greater vigour with approximately 98% normal seedlings, while the seeds of the 57-NT line, which is considered intolerant to the water deficit, showed less vigour with 70% normal seedlings.

According to Oliveira et al. (2013), the genotype of a seed influences its physiological qualities, thus making selection for physiological qualities important in breeding programmes. According to Trachsel et al. (2016), vigorous seedlings quickly establish crops; they have a well-developed and

deep root system, which improves the absorption of water and nutrients under conditions of low water availability.

Regarding the emergence (% E) of the seedlings (Table 2), there were differences among the lines subjected to the no water deficit condition (70% water retention capacity); a higher emergence value was observed in 91-T line seeds than in 24-NT line seeds. It was verified that under the water deficit condition (10% water retention capacity), the four lines evaluated did not present significant differences in seedling emergence. In general, it was observed that the initial seed vigour of the lines could be considered high, resulting in high emergence values even under conditions of low water availability.

Table 1

Primers used in *qRT-PCR* analysis.

Gene	Function	Sequence (5'-3')	Reference
ZmDBP3	Binding element protein that responds to dehydration	F: CATGAGCTGGGATCTATACTAC R: CAAGGTATCAACGTCCTCA	Wang and Dong (2009)
ZmAN13	Regulatory function in response to abiotic stress	F: AGCTGTTGCCCAAGTCGAGTT R: GCTGGGTCCGGCAACAT	Jin et al. (2007)
ZmALDH9	Detoxifies aldehydes by oxidation of their carboxylic acids	F: CATCTACGTGCAGGAAGGGAT R: TTGGCTGACACTCGGGTTG	Zhou et al. (2012)
ZmDREB2A	Binding element protein that responds to dehydration	F: GCAGCCCGGAAGGAAGAA R: GATGACAGCTGCCACTGACGTA	Qin et al. (2007)
UBI	Reference gene	F: AAGGCCAAGATCCAGGACAA R: TTGCTTTCCAGCGAAGATGA	Manoli, Sturaro, Trevisan, Quaggiotti, and Nonis (2012)
Bact	Reference gene	F: TGTCCATCACTTGTGAAGCCTCCT R:ACGACCTTAGCCAATATCGCACCA	Hu et al. (2011)

(F) sequence of forward primer; (R) sequence of reverse primer .

Table 2

Average values for the emergence (%E), emergence speed index (ESI), root length (RL, in cm) and shoot length (SL, in cm) of four maize lines under two water availability conditions, no water deficit (NWD - 70% water retention capacity of substrate) and water deficit (WD - 10% water retention capacity of substrate). Federal University of Lavras, 2018.

Line	%E[1]		ESI		RL		SL	
	NWD	WD	NWD	WD	NWD	WD	NWD	WD
91	100 A a	99 A a	12.5 A a	12.2 A a	20.1 A b	22.7 A a	16.6 A a	13.1 A b
32	97 AB a	96 A a	11.1 B a	10.6 B a	20.1 A a	18.6 B a	12.8 B a	9.7 C b
24	95 B a	97 A a	10.7 B a	11.3 B a	16.2 B b	18.7 B a	12.5 B a	11.1 B b
57	98 AB a	98 A a	13.1 A a	13.0 A a	13.8 B a	14.7 C a	16.4 A a	12.9 A b
CV (%)	5.2		3.6		8.3		5.0	

Averages followed by the same lowercase letter in the line and the same capital letter in the column do not differ from each other based on a Tukey test at 5% probability. 1: Data transformed b a r c s i n X / 100

Regarding the emergence speed index (ESI) values presented in Table 2, there were significant differences among the lines. The highest values were observed for the 91-T and 57-NT lines in both water availability conditions (no water deficit and water deficit). The condition of water availability to which the seeds were submitted did not significantly influence the emergence values and emergence speed indexes for any of the lines evaluated. This result indicates that seedling performance was similar in the two water availability conditions, probably due to the high initial vigour of the seeds.

The seedlings of the four lines from the emergence tray test grown in contrasting conditions of water availability were evaluated for root length (RL) and shoot length (SL) (Table 2). Under non-deficit conditions, greater root lengths were observed in the lines considered tolerant to low water availability, 91-T and 32-T, than in the intolerant lines, 24-NT and 57-NT. Under water deficit conditions, the 91-T line presented the long root length, while the 57-NT line presented the short root length. According to Bengough, McKenzie, Hallett, and Valentine (2011), it is essential to consider the root development of plants in response to drought in breeding programmes focused on water deficit tolerance because genotypes with greater root lengths may present higher tolerance to low-water conditions.

In the 91-T and 24-NT lines, the root length was significantly greater under water deficit conditions than under non-deficit conditions, thus suggesting that maize seedlings may show relatively elevated root growth when subjected to low water availability. Such root growth, according to Serraj and Sinclair (2002), occurs due to increased accumulation of osmolytes in the roots, allowing continuous or even increased development of the root system and thus providing the plants access to a larger water reservoir.

Regarding shoot length, the 91-T and 57-NT lines had longer shoots than the 32-T and 24-NT lines under both water availability conditions. It was observed that in all four lines analysed, shoot length was significantly higher in seedlings not subjected to the water deficit than in seedlings submitted to the water deficit, indicating that soil water deficiency reduces seedling development and cellular elongation (Marcos Filho, 2005).

Therefore, it was observed that low water availability reduced shoot development in all the lines analysed; however, this trend was not observed for root development, which increased under conditions of low water availability. This finding is consistent with the results observed by Sharp et al. (2004), who found that although roots became thinner depending on drought intensity and duration, root development was less affected than shoot development by drought. Plants, when exposed to water deficit, increase the concentration of abscisic acid (ABA) in the roots, increasing hydraulic conductivity and root growth. However, when ABA is transported through the xylem to the plant shoot, stomatal closure and a reduction in leaf expansion occur to prevent tissue dehydration (Barnabás, Jäger, & Fehér, 2008).

The present data reveal the effect of low water availability on seedling development and highlight the superior performance of the 91-T line. It is important to emphasize that root length analysis of maize seedlings can assist in the selection of superior genotypes in breeding programmes, since under conditions of low water availability, enhanced root length is generally observed in stress-tolerant seedlings.

For the gene expression analysis, two lines with contrasting water deficit tolerance were selected, the 91-T and 57-NT lines. The objective of the gene expression analysis was to analyse a multiple tissues, such as dried

seeds, ear tips and seedlings, from seedlings grown under water deficit and non-deficit conditions. Therefore, among the four lines analysed in the physiological tests, the two most different regarding their tolerance to low water availability, the 91-T line (with a high level of tolerance) and the 57-NT line (considered intolerant), were used. Considerable variation in gene expression was observed among the different evaluated treatments.

The relative expression levels of the genes are shown in Figure 1. Overexpression of the *ZmDBP3* gene, a member of subgroup A1 of the *CBF/DREB* family, has been found to trigger an increase in drought and cold tolerance in transgenic *Arabidopsis* plants (Wang & Dong, 2009). In the ear tips for the two analysed lines, *ZmDBP3* gene expression was essentially non-existent, and lower expression was observed in the intolerant line, 57-NT, than in the tolerant line in all treatments analysed. For the line classified as tolerant to water deficit, the 91-T line, greater gene expression was observed in dry seeds and in seedlings, and greater seedling expression was observed in non-deficit conditions than in water deficit conditions.

Therefore, the fact that the *ZmDBP3* gene was more highly expressed in the tolerant line suggests that this gene is a significant potential marker for the early selection of genotypes tolerant to water deficit. It is also worth mentioning that this gene is expressed in dry seeds, allowing early selection without the need to germinate the seeds and evaluate the resulting seedlings.

The superfamily of aldehyde dehydrogenase (*ALDH*) genes is characterized by its considerable role in the adaptation of plants to various abiotic stresses; one of the genes in this family is the *ZmALDH9* gene, which plays an important role in plant tolerance to water deficit (Zhou et al., 2012). Low expression of *ZmALDH9* was observed in the dried seeds of the two lines evaluated, while higher expression was observed in the ear tips, and greater expression in the ear tips occurred in the 57-NT line than in the 91-T line.

In the 91-T line, there was greater expression in the seedlings, and the expression in the seedlings was higher in the seedlings grown under water deficit conditions. When plants are subjected to low water availability, accumulation of reactive oxygen species (ROS) increases, and according to Rodrigues et al. (2006), such an increases in the activity of the *ZmALDH9* gene are an efficient defence strategy to eliminate the toxic substances caused by ROS, promoting greater plant adaptation to low water availability.

In general, greater activity of the *ZmALDH9* gene was observed in the tolerant line (91-T) seedlings than in the intolerant line (57-NT) seedlings. This result is consistent with the assertion by Zhou et al. (2012) that greater gene expression is related to greater plant tolerance to water deficit stress.

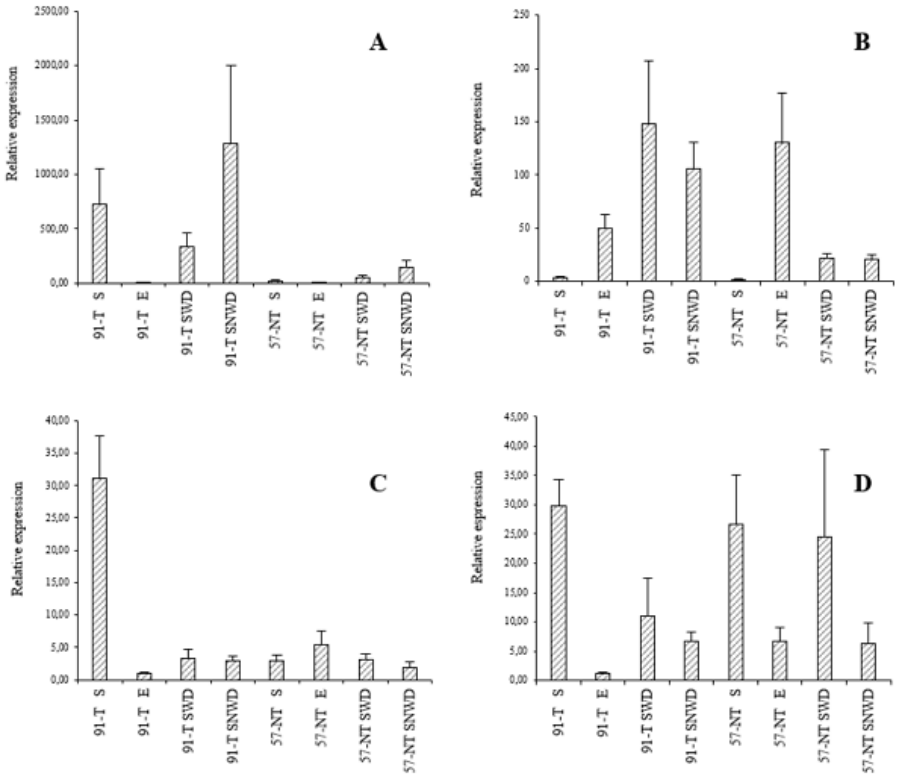

Figure 1

Expression of the genes *ZmDBP3* (A), *ZmALDH9* (B)*, ZmAN13* (C) and *ZmDREB2A* (D) in the dried seeds (S), ear tips (E) and seedlings of two maize lines (91-T and 57-NT) under two water availability conditions, water deficit (SWD) (10% water retention capacity of substrate) and no water deficit (SNWD) (70% water retention capacity of substrate).

The *ZmAN13* gene belongs to the *ZNF-AN1* family, and in general, in plants, most of the genes in this family are involved in responses to abiotic stresses (Jin et al., 2007). Quantitative analysis revealed low expression of this gene in the seedlings of both lines that were grown under the two water availability conditions (no water deficit and water deficit). Ear tip gene expression was relatively greater for the 57-NT line compared to the 91-T line.

Significant expression of the *ZmAN13* gene was observed in the dried seeds of the 91-T line, which is tolerant to water deficit, while in the 57-NT line, the expression in the dry seeds was lower. The fact that this gene was expressed at higher levels in the dry seeds of the tolerant line suggests that it has the potential to be a good marker for the early selection of genotypes tolerant to water deficit without the necessity of seedling evaluation, similar to the case for the *ZmDBP3* gene.

According to Liu et al. (2013) a family of transcription factors identified as *DREB*s (Dehydration Responsive Element Binding Proteins) is involved in gene activation related to water deficit tolerance. In the 91-T line, the expression of the *DREB* gene was higher in the dry seeds than in the ear tips. Regarding the seedlings, the expression of this gene was relatively higher in the seedlings grown under water deficit conditions than in the seedlings grown under non-deficit conditions.

In both the 57-NT line and the 91-T line, higher gene expression was observed in the dry seeds and seedlings subjected to low water availability than in those subjected to normal water availability. Expression in the ear tips was lower than that in the seeds.

The fact that the expression of this gene was higher in seedlings grown under conditions of low water availability than in seedlings grown under conditions of no water deficit for both of the lines analysed suggests that the activity of this gene was increased to combat the toxic effects of reactive oxygen species (ROS) that accumulated in seedlings grown under conditions of stress and to increase tolerance to dry conditions.

According to Qin et al. (2007), overexpression of the *ZmDREB2A* gene results in higher plant tolerance to water deficit. In general, the highest expression of this gene was observed for the dried seeds of the 91-T line, followed by the dried seeds of the 57-NT line. For the ear tips, higher expression occurred in the 57-NT line than in the 91-T line. In both analysed lines, the seedling expression was higher under stress conditions than under non-stress conditions, and the seedling expression under stress conditions was higher for the 57-NT line than for the 91-T line.

The results obtained in the physiological and gene expression analyses of this study underscores the complexity involved in the characteristic of water deficit tolerance. This complexity emphasizes the need for studies aimed at assisting plant breeding programmes in obtaining stress-tolerant genotypes.

Gene expression evaluation makes it possible to more quickly select materials with characteristics of interest, reducing the work, time and space required for such selection and making it an important tool. Expression analysis for genes such as the *ZmAN13* and *ZmDBP3* genes evaluated in this work enables the identification of plant lines that are tolerant to water deficit. *ZmAN13* and *ZmDBP3* can act as functional markers for the selection of genotypes tolerant to drought, mainly because the expression of these genes occurs in seeds, thus necessitating less time and space for selection.

Conclusion

Soil water deficiency did not reduce the root development of the lines analysed; however, the shoot length was significantly lower under dry conditions.

The genes *ZmAN13* and *ZmDBP3* were identified as potential molecular markers for the early selection of maize lines tolerant to low water availability.

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References

Ashraf, M. (2010). Inducing drought tolerance in plants: Recent advances. *Biotechnology Advances*, *28*(1), 169-183. DOI: 10.1016/j.biotechadv.2009.11.005

Barnabás, B., Jäger, K., & Fehér, A. (2008). The effect of drought and heat stress on reproductive processes in cereals. *Plant, Cell and Environment*, *31*(1), 11-38. DOI: 10.1111/j.1365-3040.2007.01727

Bengough, A. G., McKenzie, B. M., Hallett, P. D., & Valentine, T. A. (2011). Root elongation, water stress, and mechanical impedance: a review of limiting stresses and beneficial root tip traits. *Journal of Experimental Botany*, *62*(1), 59-68. DOI: 10.1093/jxb/erq350

Brasil. (2009). *Regras para a análise de sementes*. Brasília, DF: Mapa.

Companhia Nacional de Abastecimento [Conab]. (2017). *Acompanhamento de safra brasileira: grãos, décimo segundo levantamento, 2016/2017*. Brasília, DF: Conab.

Ding, Y., Cao, J., Ni, L., Zhu, Y., Zhang, A., Tan, M., & Jiang, M. (2012). ZmCPK11 is involved in abscisic acid-induced antioxidant defence and functions upstream of ZmMPK5 in abscisic acid signalling in maize. *Journal of Experimental Botany* , *64*(4), 871-884. DOI: 10.1093/jxb/ers366

Hu, Y., Zhang, L., Zhao, L., Li, J., He, S., Zhou, K., ... Li, L. (2011). Trichostatin A selectively suppresses the cold-induced transcription of the ZmDREB1 gene in maize. *PLoS One*, 6(7), 1-13. DOI: 10.1371/journal.pone.0022132

Jin, Y., Wang, M., Fu, J., Xuan, N., Zhu, Y., Lian, Y., ... Wang, G. (2007). Phylogenetic and expression analysis of ZnF-AN1 genes in plants. *Genomics*, *90*(2), 265-275. DOI: 10.1016/j.ygeno.2007.03.019

Liu, L., Hu, X., Song, J., Zong, X., Li, D., & Li, D. (2009). Over-expression of a *Zea mays* L. protein phosphatase 2C gene (ZmPP2C) in Arabidopsis thaliana decreases tolerance to salt and drought. *Journal of Plant Physiology*, *166*(5), 531-542. DOI: 10.1016/j.jplph.2008.07.008

Liu, S., Wang, X., Wang, H., Xin, H., Yang, X., Yan, J., ... Qin, F. (2013). Genome-wide analysis of ZmDREB genes and their association with natural variation in drought tolerance at seedling stage of *Zea mays* L. *PLoS Genetics*, 9(9), 1-17. DOI: 10.1371/journal.pgen.1003790

Maguire, J. D. (1962). Speed of germination - aid in selection and evaluation for seedling emergence and vigor. *Crop Science*, 2(2), 176-177. DOI: 10.2135/cropsci1962.0011183X000200020033x

Manoli, A., Sturaro, A., Trevisan, S., Quaggiotti, S., & Nonis, A. (2012). Evaluation of candidate reference genes for qPCR in maize. *Journal of Plant Physiology* , *169*(8), 807-815. DOI: 10.1016/j.jplph.2012.01.019

Marcos Filho, J. M. F. (2005). *Fisiologia de sementes de plantas cultivadas*. Piracicaba, SP: Fealq.

Oliveira, G. E., Von Pinho, R. G., Von Pinho, É. V. D. R., Andrade, T., Santos, C. D., & Veiga, A. D. (2013). Physiological quality and amylase enzyme expression in maize seeds. *Ciência e Agrotecnologia*, *37*(1), 40-48. DOI: 10.1590/S1413-70542013000100005

Pfaffl, M. W. (2001). A new mathematical model for relative quantification in real-time RT-PCR. *Nucleic Acids Research*, *29*(9), 2002-2007. DOI: 10.1093/nar/29.9.e45

Qin, F., Kakimoto, M., Sakuma, Y., Maruyama, K., Osakabe, Y., Tran, L. S. P., ... Yamaguchi-Shinozaki, K. (2007). Regulation and functional analysis of ZmDREB2A in response to drought and heat stresses in *Zea mays* L. *The Plant Journal*, *50*(1), 54-69. DOI: 10.1111/j.1365-313X.2007.03034.x

Rodrigues, S. M., Andrade, M. O., Gomes, A. P. S., DaMatta, F. M., Baracat-Pereira, M. C., & Fontes, E. P. (2006). Arabidopsis and tobacco plants ectopically expressing the soybean antiquitin-like ALDH7 gene display enhanced tolerance to drought, salinity, and oxidative stress. *Journal of Experimental Botany* , *57*(9), 1909-1918. DOI: 10.1093/jxb/erj132

Serraj, R., & Sinclair, T. R. (2002). Osmolyte accumulation: can it really help increase crop yield under drought conditions? *Plant, Cell & Environment*, *25*(2), 333-341. DOI: 10.1046/j.1365-3040.2002.00754.x

Sharp, R. E., Poroyko, V., Hejlek, L. G., Spollen, W. G., Springer, G. K., Bohnert, H. J., & Nguyen, H. T. (2004). Root growth maintenance during water deficits: physiology to functional genomics. *Journal of Experimental Botany* , *55*(407), 2343-2351. DOI: 10.1093/jxb/erh276

Trachsel, S., Sun, D., SanVicente, F. M., Zheng, H., Atlin, G. N., Suarez, E. A., ... Zhang, X. (2016). Identification of QTL for early vigor and stay-green conferring tolerance to drought in two connected advanced backcross populations in tropical maize (*Zea may*s L.). *PloS One*, *11*(3), 1-22. DOI: 10.1371/journal.pone.0149636

Wang, C. T., & Dong, Y. M. (2009). Overexpression of maize ZmDBP3 enhances tolerance to drought and cold stress in transgenic Arabidopsis plants. *Biologia*, *64*(6), 1108-1114. DOI: 10.2478/s11756-009-0198-0

Zhou, M. L., Zhang, Q., Zhou, M., Qi, L. P., Yang, X. B., Zhang, K. X., ... Wu, Y. M. (2012). Aldehyde dehydrogenase protein superfamily in maize. *Functional & Integrative Genomics* , *12*(4), 683-691. DOI: 10.1007%2Fs10142-012-0290-3

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