



Genes related to high temperature tolerance during maize seed germination

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ABSTRACT. The identification of genes related to heat tolerance is fundamental for the development of high-quality seeds that are tolerant to heat stress condition. The objective of this study was to evaluate maize lineages and the gene expression involved in high temperature tolerance during germination using physiological tests, proteomics, and transcriptome analysis. Seeds from six maize lineages (30, 44, 54, 63, 64, and 91) with different levels of tolerance to high temperatures were used. Lineages 54 and 91 were observed to be more tolerant to high temperature conditions. The highest expression of α -amylase was observed in maize seeds from lineages 30 and 91 that were subjected to controlled deterioration. The highest expression of α -amylase was observed in maize seeds from lineages 30 and 91 that were subjected to controlled deterioration; with the controlled deterioration, the highest level of gene expression did not occur in the most tolerant materials; the association of lower expression of genes involved in heat-resistant protein systems was observed in seeds from lineage 44, which were more susceptible to high temperatures, and the highest gene expression

of LEA D-34, ZmAN13, and AOX-1 was observed in seeds from lineage 64 when submitted to controlled deterioration.

Key words: RT-qPCR; Proteomics; Abiotic stress

INTRODUCTION

The stress caused by high temperatures usually occurs over a period of time sufficient to cause irreversible damage to plant growth and development. Heat tolerance is generally defined as the ability of a plant to grow and produce at high temperatures (Peet and Willits, 1998).

Temperature is one of the most important factors in seed germination (Nelson, 2007), and can change the percentage, speed, and uniformity of germination (Marcos Filho, 2005). Cargnin et al. (2006) worked with seeds from eight progenitor and eight segregating populations (generation F3) of wheat and concluded that high temperature during seed germination leads to a reduction in the dry weight of seedlings and in the efficiency of endosperm use. Under high temperature conditions, the production of reactive oxygen species (ROS) is high, which can damage DNA, proteins, and lipids (Miller et al., 2008). Therefore, plant mitochondria have a proactive defense system of power dissipation, which significantly decreases the rate of ROS production in the mitochondria by the electron transport chain, thus from the maintenance of low level of reduction of ubiquinone (Keunen et al., 2013). In this system, the alternative oxidase (AOX) operates.

According to Allakhverdiev et al. (2008), heat tolerance in plant cells may be provided by the following factors: an increase in the expression of heat tolerance proteins, an increase in antioxidant activity, an increase in the unsaturation of the lipid membrane, expression and translation of genes related to tolerance, and thermal stability of proteins.

Immediately after exposure to high temperatures, changes occur at the molecular level, including altered expression of genes and changes in transcript accumulation, which increases the synthesis of stress-related proteins, such as tolerance strategy (Iba, 2002). High temperatures induce the accumulation of the LEA proteins *embryogenesis accumulated*. The mRNA of these proteins appears in embryonic tissues and decreases gradually after seed imbibition (Baker et al., 1988; Galau et al., 1991).

According to Boucher et al. (2010), an important feature of survival in the dry state is the ability to protect membranes during the transition phase in order to prevent loss of integrity. This role has been attributed to dehydrins, heatshock proteins, and LEA proteins. In maize, however, few studies have investigated the transcription, translation, and activation of these enzymes during germination under high temperatures. Other genes appear to be involved in achieving tolerance to high temperatures. Gene expression now can be studied due to advances in biotechnology. The study of these genes is only possible in protein level or cellular transcripts.

Maximizing the sensitivity of quantification methods has led to the development of increasingly complex techniques, including quantitative real-time PCR (qPCR), which has been used in the medical field for a long time, and has been used as a tool in studies investigating gene expression and quantification of specific sequences in plants (Gachon and Saindrenan, 2004). Thus, using the technique of real-time PCR, it is possible to identify genes that may be involved in the mechanism for achieving tolerance to high temperatures.

Xuan (2011) described the functional characterization of ZmAN13 from the ZnFAN1 family in maize, which is induced in response to abiotic stresses. Proteins of connecting element that

respond to dehydration (DREBs) play vital regulatory functions in the response to abiotic stress in plants. ZmDBP2 gene transcription was found to be highly induced by drought and moderately induced by salt and exogenous abscisic acid (Wang et al., 2011).

Therefore, the aim of this study was to evaluate maize lineages and gene expression involved in tolerance to high temperatures during germination using physiological tests, proteomics, and transcriptome analysis.

MATERIAL AND METHODS

This research was conducted in the experimental area and in the Central Seed Laboratory of the Agriculture Department at the Federal University of Lavras (UFLA), in Lavras, MG. Seeds from six different maize lineages (30, 44, 54, 63, 64, and 90) that have different levels of tolerance to high temperatures were used. For the physiological assessment of maize seeds in relation to different temperatures during germination, the following tests were performed:

Water content was determined by the oven method at 105°C for 24 h (Brazil, 2009). Results are expressed as mean percentage (wet basis). A germination test was performed at five different temperatures (25°, 30°, 35°, 40°, and 45°C), and for each temperature the test was conducted with four replications of 50 seeds. The seeds were sown between paper moistened with distilled water at a ratio of 2.5-times the weight of the dry paper. The rolls were stored in plastic bags and kept in B.O.D chambers each regulated at one of the five temperatures. Normal seedling evaluations were performed 4, 7, and 14 days after planting. Normal seedlings were considered to have a standard primary root length of 0.5 cm. Results are expressed as mean percentage.

After the germination test was performed at different temperatures, 35°C was able to distinguish between the six lineages in terms of tolerance to high temperature at the first germination count. Based on these results, a temperature 35°C was used for subsequent tests and analyzes.

Physiological assessment of maize seeds submitted to a controlled deterioration test was performed using the following tests: Controlled deterioration involved increasing the water content of seeds to 25%. The predetermined water content is reached by adding an amount of water to the seed as calculated by the formula:

$$W2 = 100 - A/100 - B \times W1$$

where A is the initial water content of seeds (based on wet weight); B is the amount of water required; W1 is the initial seed weight (g); W2 is the final seed weight (g); and W2 - W1 is the water to be added (Wang et al., 1991; Hampton et al., 1992). Next, the seeds were packed in aluminum, heat sealed, and maintained for 24 h at 10°C to standardize water content. The seeds were aged at 42°C for 48 h in a germination chamber (B.O.D.-Marconi). After this period, the seeds were maintained at room temperature until the water content reached close to 13%, and seeds were later submitted to a germination test at 35°C. Normal seedling evaluations were performed 4 and 7 days after planting. A primary root length of 0.5 cm was adopted as a standard of normal seedlings.

The length and dry mass of shoots and roots were determined in seeds that were and were not submitted to controlled deterioration. The test was conducted with four replicates of 15 seeds, and the seeds were sown between paper moistened with distilled water at a ratio of 2.5-times the weight of the dry paper. The rolls were stored in plastic bags and kept in a B.O.D chamber set at

35°C (+ or -1°C) for 7 days. Lengths of the shoot and the root systems were performed on a single assessment 7 days after sowing. The dry mass of the shoot and the root system was determined separately. The tissues were separated from the seeds, packed in paper bags, and placed in the oven at 60°C until weight stabilization.

For proteomic analyses, seeds that were and were not submitted to controlled deterioration were used. The following enzymes and proteins were extracted and their levels assessed:

Heat resistant protein was assessed using seed embryos. The extraction was performed according to the method described by Blackman et al. (1991) and the calculation was performed according to the method described by Alfenas (2006). Enzyme α -amylase: the seeds were imbibed for a period of 72 h at 25 °C. After this period, the embryonic axes and scutella were discarded and the remaining endosperms were used for extraction. The calculations were performed according to the method described by Alfenas (2006). Enzymes catalase (CAT), esterase (EST), and superoxide dismutase (SOD): whole seeds were used for extraction. Calculations were performed in accordance with the method described by Alfenas (2006). Transcriptomic analysis in relation to heat tolerance was performed by the qRT-PCR technique in four steps: RNA extraction and purification, reverse transcription for cDNA synthesis, RT-PCR, and analysis of results. For RNA extraction, seeds from six lineages that were and were not submitted to controlled deterioration were used.

Design of primers

For the analysis of gene expression by qRT-PCR, genes were selected based on literature review because of the importance in the study of plant tolerance to high temperatures. The primers were designed with help of the Primer Express 3.0 software (Applied Biosystems) following the search for gene sequences in <http://www.ncbi.nlm.nih.gov> site (Table 1). As controls endogenous genes UBI and DHA were used.

Table 1. Primer sequences used in qRT-PCR analysis.

Gene*	Identification	Sequence (5'-3')
<i>ZmAN13</i>	Regulatory role in response to abiotic stress	F: AGCTGTTGCCCAAGTCGAGTT R: GCTGGGTCCGGCAACAT
<i>AOX-1</i>	Alternative route of breathing/antioxidant	F: GCGAGGAGCAAACAGCAAA R: GGCCACTAGTGCGGTCAACT
<i>AOX-2</i>	Alternative route of breathing/antioxidant	F: CCAAGACGCTGATGGATAAGG R: AGCCATACCGCCTCTGGAA
<i>ZmDBP2</i>	Proteins of connecting element responsive to dehydration	F: CAATACCAGCGCACACCAGAT R: TCGTTATAAAAGGCTCGAGTGCTT
<i>LEAP (D-34)</i>	Heat-resistant proteins	F: CCGGCTCCACGCAGAAC R: TATAGGCGTAGCCCTGCATGA
<i>LEAP</i>	Heat-resistant proteins	F: TGCAGCTCCTAGTGCTTGATC R: TGAAGAGGACCTGGGATTG
UBI	Endogenous control	F: AAGGCCAAGATCCAGGACAA
<i>ADH-DA</i>	Endogenous control	R: TTGCTTTCCAGCGAAGATGA F: AGGACGCTGAGTTAAGACC

*LEAP (D-34) = late embryogenesis abundant protein D-34; LEAP = late embryogenesis abundant protein; UBI = ubiquitin; ADH = alcohol dehydrogenase; F = primer sequence forward; R = primer sequence reverse.

The tests performed to assess the physiological quality were conducted in a completely randomized factorial 6 x 5 design, using six genotypes and five temperatures, with four replications;

and a 6 x 2 design, using six genotypes and two conditions. The data were previously subjected to tests of residual normality and homoscedasticity of variance, and were submitted to variance analysis. Means were compared using the Scott and Knott test, at a 5% level of probability. Analyses were performed in the statistical program SISVAR® (Ferreira, 2000). Data from real-time PCR were analyzed using the 7500 SDS software program (Version 2.0.1) (Applied Biosystems).

RESULTS

Physiological analysis of maize seeds under different germination temperatures

Higher germination and vigor values were observed in seeds belonging to lineages 30 and 91 four days after the start of the test at 25°C (Table 2), and the lowest germination value was observed in seeds from lineage 44. At 30°C, there was no significant difference among the six lineages evaluated. At 35°C, a lower germination value was observed in seeds belonging to lineage 44, while higher germination percentages values were observed in seeds belonging to lineage 91, followed by those belonging to lineage 54.

Table 2. Percentage germination of seeds from six maize lineages subjected to different germination temperatures for 4, 7, and 14 days.

Lineages	Temperature (°C)				
	25°C	30°C	35°C	40°C	45°C
4 days					
30	97 ^{Aa}	99 ^{Aa}	76 ^{Cb}	0 ^{Ac}	0 ^{Ac}
44	83 ^{Db}	99 ^{Aa}	64 ^{Ec}	0 ^{Ad}	0 ^{Ad}
54	88 ^{Cb}	100 ^{Aa}	82 ^{Bc}	0 ^{Ad}	0 ^{Ad}
63	90 ^{Cb}	97 ^{Aa}	70 ^{Dc}	0 ^{Ad}	0 ^{Ad}
64	93 ^{Bb}	97 ^{Aa}	68 ^{Dc}	0 ^{Ad}	0 ^{Ad}
91	98 ^{Aa}	100 ^{Aa}	99 ^{Aa}	0 ^{Ab}	0 ^{Ab}
7 days					
30	99 ^{Aa}	99 ^{Aa}	99 ^{Aa}	90 ^{Ab}	0 ^{Ac}
44	100 ^{Aa}	100 ^{Aa}	100 ^{Aa}	81 ^{Bb}	0 ^{Ac}
54	100 ^{Aa}	100 ^{Aa}	99 ^{Aa}	94 ^{Ab}	0 ^{Ac}
63	99 ^{Aa}	98 ^{Aa}	98 ^{Aa}	92 ^{Ab}	0 ^{Ac}
64	99 ^{Aa}	98 ^{Aa}	98 ^{Aa}	92 ^{Ab}	0 ^{Ac}
91	100 ^{Aa}	100 ^{Aa}	100 ^{Aa}	96 ^{Aa}	0 ^{Ab}
14 days					
30	99 ^{Aa}	99 ^{Aa}	99 ^{Aa}	94 ^{Cb}	0 ^{Ac}
44	100 ^{Aa}	100 ^{Aa}	100 ^{Aa}	93 ^{Cb}	0 ^{Ac}
54	100 ^{Aa}	100 ^{Aa}	99 ^{Aa}	97 ^{Bb}	0 ^{Ac}
63	99 ^{Aa}	98 ^{Aa}	98 ^{Aa}	95 ^{Cb}	1 ^{Ac}
64	99 ^{Aa}	98 ^{Aa}	98 ^{Aa}	93 ^{Cb}	1 ^{Ac}
91	100 ^{Aa}	100 ^{Aa}	100 ^{Aa}	99 ^{Aa}	0 ^{Ab}

Results followed by the same lowercase letter on the line and uppercase letter on the column do not differ at 5% probability as determined by the Scott-Knott test.

At temperatures of 40° and 45°C, no germination was observed in seeds from the six lineages. The highest values of germination were observed at a temperature of 30°C. There were no significant differences between the tested lineages nor between the temperatures 25°, 30°, and 35°C when assessed on day 7 (Table 2). At 40°C, a lower germination value was observed in seeds from the lineage 44. The other lineages did not differ with each other. At 45°C, no seed germination was observed in the evaluated lineages. According to the results observed 14 days after sowing, there were no statistical differences in the germination of seeds from all lineages

germinated at 25°, 30°, 35°, and 45°C (Table 2). At 40°C, seeds belonging to lineage 91 had the greatest germination value, followed by those from lineage 54.

Physiological analysis of maize seeds submitted or not to the controlled deterioration test

On the fourth assessment day, lower germination percentages values were observed in seeds submitted to deterioration. Under this condition, higher germination values were observed in seeds belonging to lineages 44 and 63, and the lowest value was observed in those from lineage 64 (Table 3). In the seeds not submitted to aging, lower germination was observed in seeds from lineage 44, and no significant difference was observed for the results from seeds of other lineages (Table 3).

Table 3. Percentage germination of seeds from six maize lineages submitted (SDC) and not submitted (NSDC) for a controlled deterioration test at 4 and 7 days after installation of the test.

Lineages	Temperature (35°C)	
	NSDC	SDC
4 days		
30	98 ^{Aa}	29 ^{Db}
44	64 ^{Ba}	52 ^{Ab}
54	100 ^{Aa}	36 ^{Cb}
63	99 ^{Aa}	54 ^{Ab}
64	96 ^{Aa}	15 ^{Eb}
91	99 ^{Aa}	48 ^{Bb}
7 days		
30	99 ^{Aa}	83 ^{Bb}
44	100 ^{Aa}	86 ^{Bb}
54	99 ^{Aa}	83 ^{Bb}
63	98 ^{Aa}	72 ^{Cb}
64	98 ^{Aa}	23 ^{Db}
91	100 ^{Aa}	95 ^{Aa}

Results followed by the same lowercase letter on the line and uppercase letter on the column do not differ with each other at 5% probability by the Scott-Knott test.

On day 7, there was no significant difference in seeds from the tested lineages that were not submitted to controlled deterioration. However, in seeds submitted to controlled deterioration, lower germination values were observed in those from lineage 64 and the highest value was observed in those from lineage 91 (Table 3).

In relation to the dry matter of shoots, higher values were observed in seedlings generated from seeds that had not undergone controlled deterioration. Under this condition, a greater amount of dry matter was observed in seedlings from seeds belonging to lineage 91, followed by those from lineage 54. Higher dry matter values were observed in seedlings from seeds belonging to lineages 44 and 91 that had undergone deterioration (Table 4).

Concerning the dry matter of root systems (Table 4) in seedlings generated from seeds that had not undergone controlled deterioration, a high value was observed in those from lineage 91. For seeds submitted to controlled deterioration, higher dry matter values were observed for seedlings from lineages 44, 54, and 91.

Shoot length (Table 4) was high in seedlings from lineage 91, both from seeds submitted to controlled deterioration and in those not submitted to controlled deterioration. Lower shoot length values were observed in seedlings from lineage 64 when submitted to controlled deterioration.

Table 4. Dry matter shoot (DMS), roots (DMR), length of shoot (LS), and length of root system (LRS) of seedlings from six maize lines derived from seeds submitted (SDC) and not submitted (NSDC) for a controlled deterioration test.

Lineages	DMS	NSDC
DMS		
30	0.89 ^{Ca}	0.04 ^{Bb}
44	1.07 ^{Ca}	0.29 ^{Ab}
54	1.76 ^{Ba}	0.08 ^{Bb}
63	1.22 ^{Ca}	0.04 ^{Bb}
64	1.11 ^{Ca}	0.01 ^{Bb}
91	2.67 ^{Aa}	0.36 ^{Ab}
DMR		
30	0.23 ^{Ca}	0.06 ^{Bb}
44	0.29 ^{Ca}	0.39 ^{Aa}
54	0.38 ^{Ca}	0.26 ^{Aa}
63	0.68 ^{Ba}	0.14 ^{Bb}
64	0.26 ^{Ca}	0.01 ^{Bb}
91	0.97 ^{Aa}	0.34 ^{Ab}
LS		
30	10.41 ^{Ca}	1.30 ^{Cb}
44	17.08 ^{Ba}	4.12 ^{Bb}
54	11.55 ^{Ca}	3.10 ^{Bb}
63	12.53 ^{Ca}	0.78 ^{Cb}
64	13.48 ^{Ca}	0.14 ^{Cb}
91	20.59 ^{Aa}	7.25 ^{Ab}
LRS		
30	7.50 ^{Ba}	0.83 ^{Cb}
44	5.30 ^{Ca}	4.50 ^{Aa}
54	8.94 ^{Ba}	1.50 ^{Bb}
63	8.55 ^{Ba}	0.61 ^{Cb}
64	9.35 ^{Ba}	0.45 ^{Cb}
91	20.59 ^{Aa}	1.89 ^{Bb}

Results followed by the same lowercase letter on the line and uppercase letter on the column do not differ with each other at 5% probability by the Scott-Knott test.

In seeds not submitted to controlled deterioration, the root system was a greater length in seedlings from lineage 91, and were shorter in seedlings from lineage 44 (Table 4). The root system was a greater length in seeds submitted to controlled deterioration in seedlings from lineage 44.

Proteomic analysis

There was higher expression of SOD in seeds from lineages 30 and 44 when submitted to controlled deterioration, which can be observed by the increased intensity of bands 1 and 2 (Figure 1A).

An increase in CAT expression was observed in seeds from lineages 30, 44, and 91 and reduced CAT expression was observed in seeds from lineages 54 and 63. In seeds from lineages 30 and 44, this increased expression may be explained because of the higher expression of SOD under the same conditions (Figure 1B). By the enzyme esterase zymogram (EST) is inferred the expression of two loci (top and bottom of the figure) (Figure 1C). Relative to the first locus, increased EST expression was only observed in seeds from lineage 63 submitted to controlled deterioration. For the second locus, some isoforms were not observed in deteriorated seeds. With the exception of lineage 54, lower expression of this enzyme at the second locus was also observed in seeds belonging to the other lineages.

Considering both loci in seeds not submitted to controlled deterioration, greater EST

expression was observed in seeds from lineage 64 and lower expression was observed in seeds from lineage 91. Expression of two additional isoforms (a and b) of α -amylase were observed in deteriorated seeds (Figure 1D) from lineage 91. Although these isoforms were both expressed, lower expression of α -amylase was observed in deteriorated seeds from lineage 91 and higher expression was observed in seeds from lineage 30.

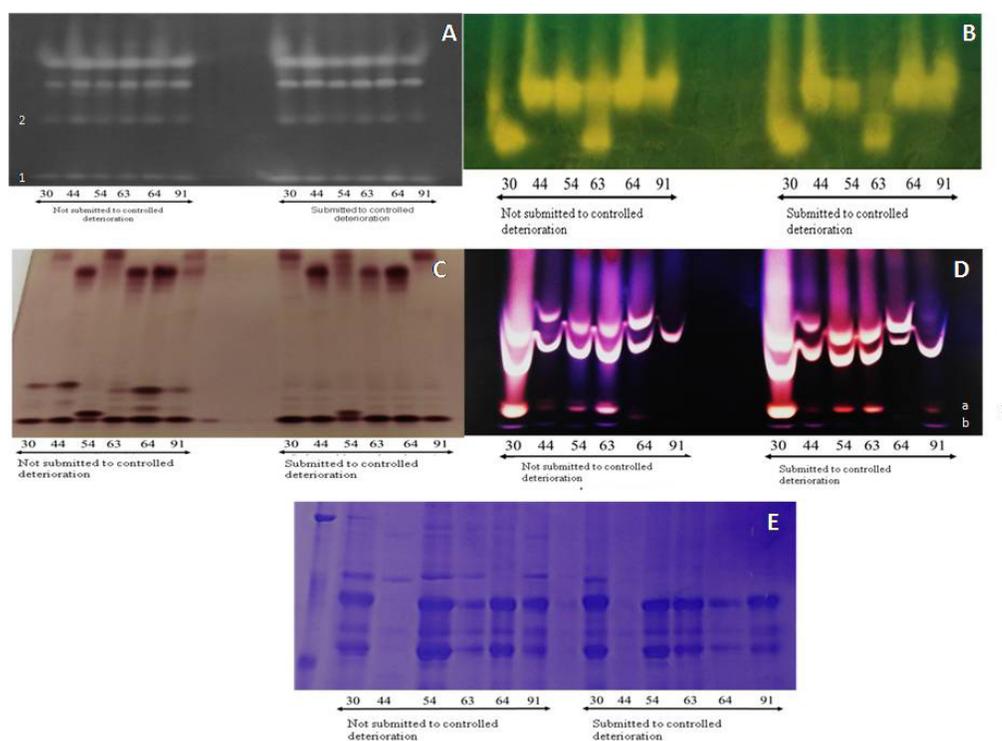


Figure 1. A. SOD enzyme activity in seeds from six maize lineages submitted or not to controlled deterioration. B. CAT enzyme activity in seeds from different maize lineages submitted or not to controlled deterioration. C. EST enzyme activity in seeds from different maize lineages submitted or not to controlled deterioration. D. α -amylase enzyme activity in seeds from different maize lineages submitted or not to controlled deterioration. E. heat-resistant protein activity in seeds from different maize lineages submitted or not to controlled deterioration.

Regardless of whether seeds were submitted to controlled deterioration, those from lineage 44 were found to have lower expression of heat resistant proteins (Figure 1E). In seeds that were not deteriorated, greater expression of these proteins was observed in seeds from lineages 30 and 54.

Transcriptomic analysis

Gene expression of LEA D-34 (Figure 2A) was higher in seeds submitted to controlled deterioration, and the highest expression was observed in seeds from lineage 64. Both in seeds not deteriorated as in deteriorated seeds the lowest gene expression LEA D-34 was observed in seeds of the lineage 44, classified as low tolerance to temperature of 40°C during the germination process.

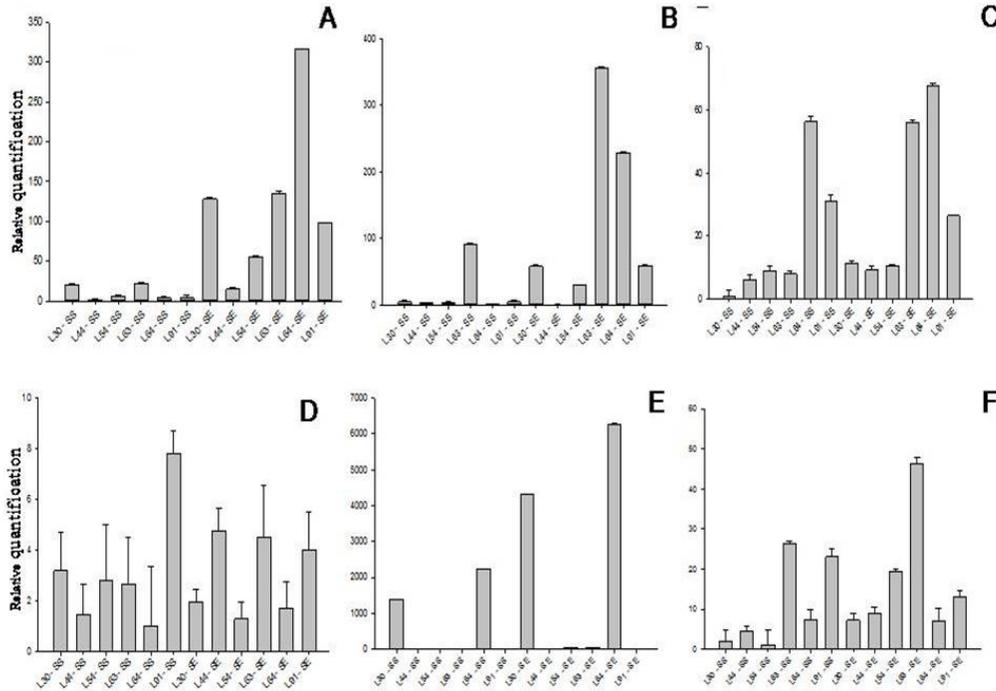


Figure 2. A. Gene expression. B. LEA D-34. C. LEA. D. ZmAN13. E. ZmDBP. F. AOX-1. G. AOX-2 in relation to seeds from six lineages that were submitted (SE) and not submitted (SS) to controlled deterioration.

Higher LEA expression was observed in seeds submitted to controlled deterioration, except in those from lineage 44 (Figure 2B). In all seeds regardless of the germination status, higher gene expression was observed in lineage 63. As shown in Figure 2C, higher ZmAN13 expression was observed in seeds from lineage 64 regardless of whether they were submitted to deterioration. The relative expression of ZmDBP2 is shown in Figure 2D. Gene expression varied depending on both the lineage and the deterioration status of the seeds. In seeds not submitted to controlled deterioration, higher expression of this gene was observed in lineage 91. Greater expression was observed in deteriorated seeds from lineage 44. AOX-1 expression was only observed in seeds from lineages 30 and 64 (Figure 2E), regardless of whether they had been submitted to deterioration. The highest expression of AOX-2 (Figure 2F) was observed in seeds from lineage 63, regardless of the state of deterioration.

The reliability of these results was confirmed through the use of an endogenous control gene, which was expressed at the same level regardless of the treatment used. In this study, the endogenous genes used were ubiquitin and alcohol dehydrogenase (ADH).

DISCUSSION

Physiological analysis of maize seeds under different germination temperatures

Higher values of germination were observed at 30°C, which is recommended for use

in maize seed germination tests since this temperature is expected to increase the expression of enzymes that favor germination. However, under field conditions in some regions where this crop is cultivated, the soil temperature can exceed 30°C during the germination process, which compromises the establishment of plants in the field.

Temperature is considered to be the most influential factor on the speed and percentage of seed germination and seedling emergence. A temperature range exists for all species in which the highest levels of germination and emergence occur, depending on the availability of an adequate moisture supply, and the presence of minimal adverse factors (Delouche, 2004). The ability of a seed to germinate under broad conditions is defined as the manifestation of its vigor, and is dependent, among other factors, on the environmental conditions found at the site where it was sown (De Simoni et al., 2011). Silva-Neta et al. (2014) performed a physiological assessment and ranked seeds from six maize lines (44, 54, 57, 63, 64, and 91) according to their tolerance to low temperature germination. Lines 44, 54, and 57 were ranked as more susceptible, and lines 63, 64, and 91 were ranked as more tolerant to low temperatures for germination. Sbrussi et al. (2014) observed that from 35°C upwards a sharp and significant decline in the germination percentage of the six lots of maize seeds was observed, whereas no germination occurred at the maximum temperature of 45°C.

High temperatures can also alter the composition and function of the lipid bilayer, which causes an increase in membrane fluidity, leading to disintegration of the lipid bilayer (Allakhverdiev et al., 2008). Furthermore, high temperatures may trigger the denaturation of enzymes and the expression of these enzymes may be reduced under these conditions.

Physiological analysis of maize seeds in response to deterioration state

Basavarajappa et al. (1991) found that accelerated aging of maize seeds led to a loss of membrane integrity. Free radicals, which are produced as a result of lipid peroxidation in aging, react with the lipids in cell membranes, leading to the destruction of cell membrane structure and of the seed reserves, resulting in reduced seed quality.

Proteomic analysis

SOD is the first enzyme involved in the antioxidant defense process, which acts to mitigate the action of SOD, catalyzing its conversion to hydrogen peroxide. When studying tolerance to low temperature during germination of seeds from six maize lines (44, 54, 57, 63, 64, and 91) Silva-Neta et al. (2014) found that there was a change in SOD expression in seeds from strain 63, which are tolerant to low temperatures, following seed imbibition for 24 h at 25°C, and that in most strains SOD expression was highest in seeds imbibed for 48 h at 25°C. Abreu et al. (2014) studied tolerance to drought in seeds from five maize lineages (44, 54, 63, 64, and 91) and observed high SOD activity in hydric potential control (OMPa). As this was reducing hydric potential, a reduction in band intensity was observed for lineages 63, 54, and 64, which did not occur in the lineages 91 and 44. Higher vigor of seeds from lineages 91 and 44 was observed under conditions of hydric restriction. In these seeds, there was higher expression of enzymes involved in the antioxidant system. In plants under hydric stress, increased activity of this enzyme was found to be related to increased tolerance to drought (Zhu, 2002). SOD leads to the formation of hydrogen peroxide and then a second enzyme, CAT, is expressed to protect the seeds, since it transforms hydrogen

peroxide into oxygen and water (Taiz and Zeiger, 2004; Buchanan et al., 2005). EST is involved in the hydrolysis of esters and is directly linked to lipid metabolism, including that of the phospholipid membrane.

Padilha et al. (2001) evaluated EST expression in hybrid maize seeds submitted to the controlled deterioration test for different imbibition times and seed water content. Changes in the expression of this enzyme were observed with the disappearance of some isoforms.

According to Franco et al. (2002), α -amylase promotes the hydrolysis of starch to provide the necessary carbohydrates for the developing embryo, thus allowing maize seed germination. Working with different maize seeds lineages, Andrade et al. (2013) found that the expression of heat resistant proteins, as detected by electrophoresis, occurred during the early stages of development (LL 3) in lineages tolerant to high drying temperatures, when compared to lineages that are not tolerant to high drying temperatures.

Transcriptomic analysis

The gene ZmAN13 belongs to the ZnFAN1 family. According to Jin et al. (2007) and Xuan (2011), genes in this family in maize are expressed in response to abiotic stresses, such as stress caused by temperature. Transcription of ZmDBP2 is activated by thermal tolerance to high temperatures and by salt stress. Overexpression of ZmDBP2 confers thermal tolerance to high temperatures in transgenic Arabidopsis plants (Wang et al., 2011). Conditions that cause oxidative stress, such as abiotic stresses, induce AOX activity suggesting that this enzyme may function to reduce the formation of ROS produced as a result of decreased or restricted respiratory activity (Moore et al., 2002).

In conclusion, these data show that seeds belonging to lineages 54 and 91 are more tolerant to high temperatures. The highest expression of α -amylase is observed in maize seeds from lineages 30 and 91 following submission to controlled deterioration. Following controlled deterioration, the highest levels of gene expression do not correlate positively with the most tolerant materials.

Low expression of genes involved in heat-resistant protein systems were observed in lineage 44, which is more susceptible to high temperatures. The highest gene expression of LEA D-34, ZmAN13, and AOX-1 was observed in seeds from lineage 64, following submission to controlled deterioration.

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