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Physiological, isozyme changes and image analysis of popcorn seeds submitted to low temperatures[1]

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ABSTRACT - This present study aimed to assess seed quality of popcorn seedlings subjected to low temperatures by examining their physiological changes, enzymes, and images. We used a completely randomized design with four replications in a factorial arrangement with four hybrids (P618, AP6002, AP8202, AP8203) and five temperatures (10, 13, 16, 19 and 25 °C). Their physiological quality was assessed through radicle protrusion, germination, dry mass of seedlings and emergence. We have assessed the images of seedlings through their hypocotyl and root lengths; and through rates of automatic force, uniformity and growth, provided by Groundeye. The isozyme expressions were determined for the catalase (CAT - IUBMB: EC 1.11.1.6), alcohol dehydrogenase (ADH - IUBMB: EC 1.1.1.1), malate dehydrogenase (MDH - IUBMB: EC: 1.1.1.37) and α-amylase (α-AMY - IUBMB: EC 3.2.1.1). Results show that low temperatures cause a negative effect on seed physiological quality in enzyme expression of CAT, ADH, MDH and α-AMY and on the performance of seedlings. These alterations compromise seed quality. Hybrids AP6002 and AP8203 should be recommended for sowing at up to 16 °C. The Groundeye software was efficient in the analysis of popcorn seedlings and in for the assessment of seed quality when submitted to low temperatures.

Index terms: *Zea mays*, seed quality, Groundeye, enzyme, cold.

Alterações fisiológicas, isoenzimáticas e análise de imagens de sementes de milho pipoca submetidas a baixas temperaturas

RESUMO - Objetivou-se neste trabalho determinar as alterações fisiológicas, isoenzimáticas e analisar imagens de plântulas de milho pipoca para avaliar o "seed quality" de sementes submetidas a baixas temperaturas de germinação. Utilizou-se delineamento experimental inteiramente casualizado com quatro repetições, em arranjo fatorial com quatro híbridos (P618, AP6002, AP8202, AP8203) e cinco temperaturas (10, 13, 16, 19 e 25 ºC). A qualidade fisiológica foi avaliada por meio de testes de protrusão radicular, germinação, massa seca de plântulas e emergência. Avaliou-se as imagens das plântulas pelos comprimentos do hipocótilo e raiz; e pelos índices de "seed quality" automático, uniformidade e crescimento, fornecidos pelo Groundeye. As expressões isoenzimáticas foram determinadas para catalase (CAT - IUBMB: EC 1.11.1.6), álcool desidrogenase (ADH - IUBMB: EC 1.1.1.1), malato desidrogenase (MDH - IUBMB: EC: 1.1.1.37) e α-amilase (α-AMY - IUBMB: EC 3.2.1.1). As baixas temperaturas influenciam: negativamente na qualidade fisiológica; na expressão isoenzimática da CAT, ADH, MDH e α-AMY; e no desempenho das plântulas. Estas alterações comprometem a qualidade das sementes. Os híbridos AP6002 e AP8203 podem ser recomendados para a semeadura até 16 ºC. O *software* Groundeye foi eficiente na análise de plântulas de milho pipoca e na avaliação do "seed quality" das sementes submetidas a baixas temperaturas.

Termos para indexação: *Zea mays*, *seed quality,* Groundeye, enzimas, frio.

Introduction

The sowing period of popcorn *(Zea mays* L. *everta)* is restricted by weather temperature and distribution of rainfall, which may vary in different regions of Brazil (Vaz-de-Melo et al., 2012). Production is increasing and an advantageous source of income, due to the added value of the product. Such growth is partly due to the selection and development of new

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cultivars adapted to the several weather conditions around the country. To increase the chances of crop success in regions of low temperatures, cultivars need to be tolerant to cold weather, especially in the stages of seed germination and emergence of seedlings. Thus, genotypes with such characteristic should be selected to be used for crops under such conditions, thus ensuring fair competition with other.

Sowing under low temperature, below 10 °C, extends crop cycle, affecting the duration of plant growth stages. It negatively affects germination and seedling emergence, and may cause serious damage to production (Vaz-de-Melo et al., 2012; Silva-Neta et al., 2015). Low temperatures induce damage in the cell membrane and affect physiological functions delaying or hindering the germination process (Guan et al., 2009).

At low temperatures, imbibition may occur, but it may not be followed by embryo growth, or even, induce damage to the embryos or seedlings (Santos et al., 2015). Under these conditions seedling growth will be reduced, energy reserves will be directed to maintain the active metabolism in organs with preferential growth, and the respiratory-antioxidant system will suffer enzymatic activation. Furthermore, low temperatures may cause accumulation of ethanol resulting in the production of small quantities of ATP, followed by lipid peroxidation, advancing to the degradation of membranes and consequent cell death (Marini et al., 2013). Thus, exposure to low temperatures can cause alterations on the level of expression of some enzymes.

Low temperatures can reduce seedling growth rates (Magalhães et al., 2003). Seedlings with reduced length make it difficult for researchers to manually measure them, which may demand some extra time, with possible variations (Dornelas et al., 2005). The automation of this process is an option to reduce time spent on manual assessment and to eliminate the subjectivity of the analysis. Thus, the technique of image assessment of digitized seedlings allows differentiation of seed lots at different levels of seed quality (Hoffmaster et al., 2003). Thus, computerized image analysis may provide a safe assessment of seedlings originated from seeds germinated at low temperatures, and distinguish between lots, through an analysis procedure that is quick and accurate (Marcos-Filho et al., 2009). Therefore, image analysis enables the selection of normal seedlings at temperatures lower than those recommended for this crop. The genotypes with potential to develop to normal seedlings under these conditions may have advantage over others, because the flowering may occur before times more prone to drought and harvesting still be performed during off-season periods.

Knowing that temperature influences the feasibility and quality of seeds and seedlings, interfering with their metabolic and physiological processes has received special attention, due to the high relationship between this phenomenon and the quality of seeds (Mendes et al., 2009). Moreover, the aim of this present work was to verify physiological and isoenzyme changes, and analyze images of popcorn seedlings to assess seed quality when they are subjected to low temperature during germination.

Material and Methods

This research was conducted at the Central Seed Laboratory of the Department of Agriculture of University Federal of Lavras, in Lavras, MG, Brazil. We have included seeds of four hybrids of popcorn from AG ALUMNI SEED business (P618, AP6002, AP8202, AP8203) produced in the 2015 harvest in Indiana, USA.

Seed water content was determined by the oven method at 105 °C for 24 hours (Brasil, 2009), using two replicates of 50 seeds of each treatment. After this period the seeds were taken to desiccators until the samples were cooled and the dry weight of the samples was determined. Results were expressed as percentages.

The germination test was conducted with four replications of 50 seeds with seeds between germitest paper moistened with distilled water at a ratio of 2.5 $mL.g^{-1}$ of paper. Seeds were kept in a B.O.D type chamber in a 12 hours regime in the dark and 12 hours in the presence of light, adjusted to temperatures of 10, 13, 16, 19 and 25 ± 2 °C. Temperature of 25 ºC was used as a control, since it is considered the optimal temperature for the germination of this species (Brasil, 2009). Along with the germination test, we assessed the percentage of root protrusion on the fourth day after sowing. On the seventh day, we proceeded to the count of normal seedlings and the results were expressed in percentages (Brasil, 2009).

For the seedling emergence test, the seeds were seeded at a depth of 5 cm in plastic trays (25 x 45 x 10 cm) containing subsoil soil plus coarse sand in a 3:1 ratio (soil: sand). The trays were placed in B.O.D growth chambers with a photoperiod of 12 h and the temperature was adjusted according to each treatment: 10, 13, 16, 19, 25 ± 2 °C. The counts were performed at 20 days after test installation and the results were expressed as percentages of normal emerged seedlings.

Seedling dry weight was obtained from the normal seedlings in the germination test, placed in paper bags, and dried in air circulation oven at 62 °C for 48 hours. After this period, the samples were removed from the oven and placed to cool in a desiccator. Once cooled, the replications were weighed and the weight of the dry mass of the normal seedlings was determined. The results were expressed as mg / seedlings.

Image of corn seedlings was then analyzed with the Groundeye software, using seeds sown in germitest paper as described. Four replicates of 20 seeds were used. The capture and initial analysis of the images of popcorn seedlings was performed at four days after sowing. The analysis was performed with automatic calibration, color thresholding using the RGB software with no further correction of its failures in identifying the parts of the seedlings. The images of the seedlings were assessed by the mean lengths of the hypocotyl and root; and by the indices of automatic vigor index, uniformity index and growth index provided by the Groundeye, following the criteria of Pinto et al. (2015).

The experimental design was completely randomized in a factorial scheme (4X5), using four hybrids of popcorn (P618, AP6002, AP8202, AP8203) and five temperatures (10, 13, 16, 19 and 25 °C). Data were statistically interpreted using analysis of variance and regression with the statistics program Sisvar (Ferreira, 2011). For the comparison of means, the Scott-Knott Test was used, at 5% probability.

To assess enzyme expression, we used two samples of 50 seeds from each treatment, maintained at the same germination test conditions for a period of 72 hours. Seeds were ground in a crucible with liquid nitrogen and PVP (polyvinylpyrrolidone), and stored at 86 °C. For the assessment of each isoenzyme, 100 mg of the macerated powder was added in 250 μL of extraction buffer (0.2 M Tris HCL pH 8,0 + 0,1% β-mercaptoethanol). Then, the samples were vortexed and kept in the refrigerator for 12 hours. After this period, the samples were again vortexed and centrifuged at 12,000 g for 30 minutes at 4 °C. The supernatant was poured into microtubes and the pellet was discarded.

The analysis were performed using the NATIVE-PAGE electrophoresis technique in polyacrylamide gels, in a lot system (7.5% gel separation gel and 4.5% concentration gel), by using the Tris-glycine pH 8.9 gel / electrode buffer system. Then, 60 μl of the supernatant of the samples were applied to the gel and the electrophoretic run performed at 120 V for 5 hours. At the end of the run, the gels were disclosed for the catalase enzymes (CAT - IUBMB: EC 1.11.1.6), alcohol dehydrogenase (ADH - IUBMB: EC 1.1.1.1), malate dehydrogenase (MDH -IUBMB: EC 1.1.1.37), and α-amylase (α-AMY - IUBMB: EC 3.2.1.1) in accordance with Alfenas (2006). The assessment of the isozyme patterns was made according to the intensity of the bands, using white light TFP-C / WL transilluminator (Biosystems, Curitiba, PR).

Results and Discussion

A significant interaction was noted between the temperatures of hybrids x popcorn with respect to the protrusion, germination, emergence, dry weight, hypocotyl and radicle length, growth, uniformity and seed quality. Similar results were reported by Vaz-de-Melo et al. (2012) when evaluating the effects of temperature in popcorn cultivars, however, they found that there was a significant interaction only with respect to germination, shoot length, fresh and dry weight. The water content of the seeds at the time of the test was 11.9%, 12.5%, 11.9%, 12.7% for the hybrids P618, AP6002, AP8202 and AP8203, respectively.

In this present work, the temperature of 10 °C hindered the feasibility and quality of seeds, as well as the development of popcorn seedlings. Silva-Neta et al. (2015) assessed the germination of maize strains (L63, L64 and L 91) at temperatures of 10, 15, 20, 25 °C, and reported that the germination of the strains was above 80% at 10 °C. Silva-Neta et al. (2015), also found that cold-tolerant strains have greater germination potential, as temperature reduces, proving the existing effect of the genotype.

With the data on radicular protrusion, it was possible to observe that only in the temperature of 13 °C, no statistical difference was found among the popcorn hybrids (Table 1 and Figure 1A). Hybrid AP8203 presented 59% of root protrusion, followed by AP6002 (53%), P618 (28%) and AP8202 (2%). At this temperature (13 °C) there was no development of normal seedlings of popcorn hybrids, thus compromising their quality (Table 1).

At 16 and 19 °C, we observed that the germination was above 80% in all hybrids. The AP8203 and AP6002 hybrids were statistically superior to the other at these temperatures (Table 1 and Figure 1B). These results are contrary to those reported by Sbrussi and Zucareli (2014), who found that at 16 e 19 °C, there were high rates of non-germinated seeds, but it was not possible to confirm that the seeds were dead, since they looked intact with no release of exudates or presence of fungi. The same characteristics were observed in this present work, and it was not possible to confirm that the seeds that did not germinate at 10 and 13 °C were dead.

At the temperature of 25 °C, considered ideal for this species germination, the values of germination and root protrusion were above 96%. At 25 °C, it was also observed that all hybrids had the same behavior with respect to germination and root protrusion, whereas, when there is increase of temperature from 10 to 25 °C, there is an increase of these parameters (Figures 1A and B). Each species has a limiting and optimal temperature range. For the corn to germinate, it requires adequate moisture and soil temperature higher than 10 °C, and under favorable conditions (25 °C) it takes 4 to 7 days (Santos et al., 2015).

Regarding the emergence of seedlings in trays, at

Table 1. Protrusion (P), germination (G), emergence of seedlings (E) and dry mass of seedlings (MS) of popcorn submitted to different temperatures.

T (°C)	Hybrids	P (%)	G (%)	E (%)	MS (mg / seedlings)
10 °C	P618	0 A	0 A	0 A	0.000 A
	AP6002	0 A	0 A	0 A	0.000 A
	AP8202	0 A	0 A	0 A	0.000 A
	AP8203	0 A	0 A	0 A	0.000 A
13 °C	P618	28 C	0 A	72 B	0.000 A
	AP6002	53 B	0 A	83A	0.000 A
	AP8202	2 D	0 A	69 B	0.000 A
	AP8203	59 A	0 A	81A	0.000 A
16 °C	P618	93 A	84 B	84 B	0.115 D
	AP6002	98 A	96 A	97 A	0.189 A
	AP8202	92A	80 B	83 B	0.130 C
	AP8203	95 A	94 A	91 A	0.133 B
19 °C	P618	96 A	93 B	95 A	0.182 C
	AP6002	99 A	98 A	99 A	0.256 A
	AP8202	94 A	93 B	96 A	0.199 B
	AP8203	98 A	97 A	95 A	0.199 B
25 °C	P618	98 A	98 A	95 A	0.191 B
	AP6002	98 A	98 A	97 A	0.238 A
	AP8202	96 A	96 A	98 A	0.190 B
	AP8203	98 A	99 A	97 A	0.194 B
CV (%)		6.13	3.01	4.80	10.84

*Means followed by the same capital letter in the column within each temperature do not differ statistically from one another by the Scott-Knott test at 5% probability.

the temperatures 13 and 16 °C, the best seed quality was observed in the Hybrids AP8203 and AP6002 (Table 1). It is worth noting that, despite the greater seed quality of these hybrids, the emergence assessment occurred 20 days after test installation. However, as temperatures increased to 19 and 25 °C, all popcorn hybrids were highly vigorous, with no significant difference between cultivars (Table 1 and Figure 1C). According to Sans and Santana (2005), in low temperature conditions, the emergence of seedlings can take up to fourteen days. Low soil temperatures delay germination, decrease reserve mobilization, and consequently emergence speed (Cruz et al., 2007).

It was not possible to measure the dry mass of the hybrids of popcorn at temperatures 10 and 13 °C, since no germination occurred. At the other temperatures, there was a statistically significant difference with the AP6002 hybrid, especially with increased temperature (Table 1 and Figure 2A). Thus, exposure to low temperatures can reduce dry matter and also the height of seedlings (Guerra et al., 2014). Despite of the hybrid P618 having the lowest dry matter at temperatures 16 and 19 °C, it had high developing seedlings, which could be checked by the length of hypocotyl and root (Table 2 and Figures 2B and C). When corn seeds are germinated at low temperatures, it is necessary that, after radicle protrusion, vigorous seedlings are formed to withstand such stress (Silva-Neta et al., 2015). Thus, a strong selection pressure acts in response to germination, resulting in a number of strategies for survival and establishment of seedlings (Spindelbock et al., 2013). It is also worth mentioning that the initial growth of the corn seedlings is directly related to seed quality and consequently this will reflect on the performance of the seedlings (Mondo et al., 2013).

The Groundeye software has been efficient on the analysis of popcorn seedlings, on the assessment of the quality of seeds submitted to low temperatures. It is possible to find on Table 2 that the Groundeye software has even measured the 0.1 growth of roots in 13 °C, and 0.02 cm of hypocotyls at 16 °C. However, statistical difference for the development of roots and hypocotyls was only found for 19 °C and 25 °C. It was also possible to note better seed quality for Hybrid AP8203, through the analysis of their seedlings from 16 °C. Their root and hypocotyl lengths overcame those of all other hybrids

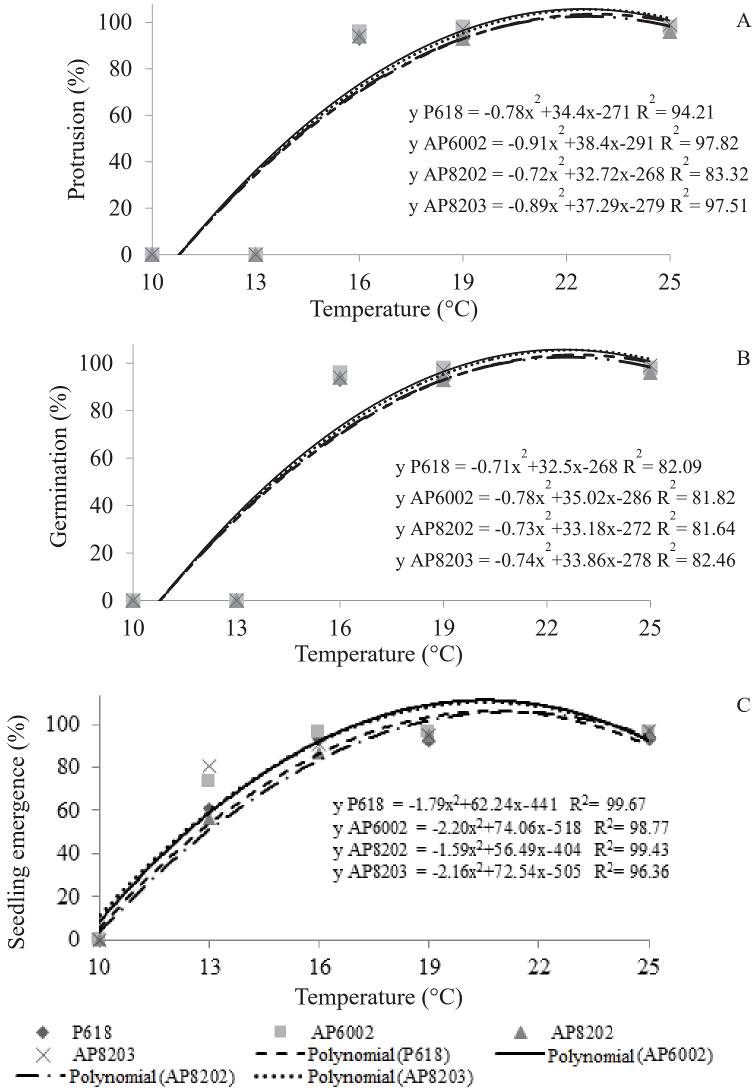

Figure 1. Protrusion (A), germination (B) of hybrid seeds and emergence (C) of popcorn seedlings submitted to different temperatures.

(Figures 2B and C). Using computerized image analysis with Groundeye, Pinto et al. (2015) have also shown consistency in results of seedling growth analysis.

By the index of growth, uniformity, and automatic seed quality, it was also possible to categorize popcorn hybrids within each assessment temperature (Table 2). At the temperature of 25 ºC, it is noteworthy the great seed quality of all hybrids by the analyzed indexes. However, it is important to observe that hybrids AP6002 and AP8203 at low temperatures (13, 16 and 19 ºC) overcome the other under the stress of cold temperature in growth rates, uniformity and automatic vigor. The use of parameters that allow the perception of reduced seed quality is very important in seed analysis, because this will aid on the removal of materials with low potential for plant emergence (Silva and Cícero, 2014), mainly under conditions of thermal stress. Thus, the indexes that are automatically analyzed by the Groundeye software allow us to accurately determine quality of seed and seedlings subjected to adverse temperature conditions. Thus, indexes used in the assessment of seed quality have achieved the purpose of such an assessment, which, according to Bennett (2002), are: greater sensitivity to assess the physiological aspect of seed quality than the germination test, and provide a consistent response with a lot categorization according to performance, it is objective, fast, simple and cheaper, repeatable and easy to interpret.

Several times low temperatures of the environment were noted to compromised the physiological quality of seeds, reducing their viability. Environmental stresses such as those caused by exposure of plants and seeds to cold temperature can also result in changes in the expression of

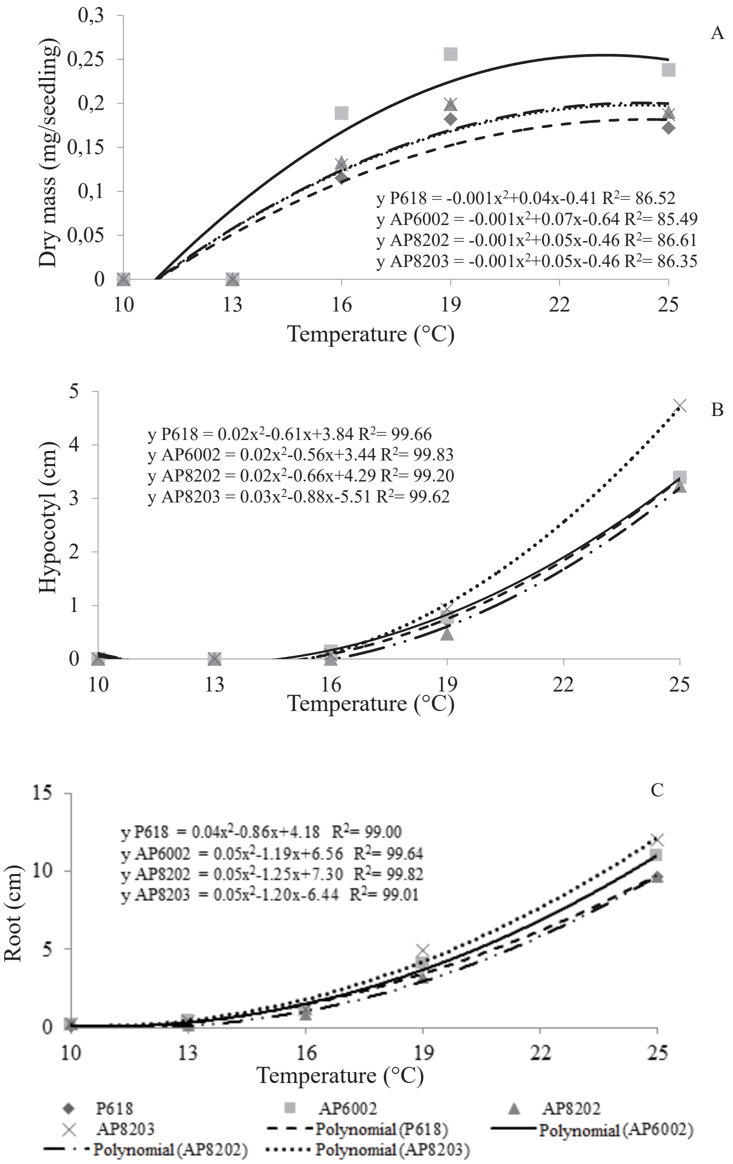

Figure 2. Dry mass (A); Length of the hypocotyl (B) and radicle (C) analyzed by the Groundeye of popcorn seedlings submitted to different temperatures.

enzymes (Mertz et al., 2009). Isoenzymes are products of gene expression and consequently highly influenced by the environment (Tunes et al., 2011).

Low ambient temperatures caused changes in enzyme patterns, especially catalase (Figure 3A). The expression of this enzyme was high for all popcorn hybrids at low temperatures, except for hybrid AP6002, with expression reduced at 16 ºC. Reduced expression levels of catalase were also found at 25 ºC for AP8202 and AP8203 hybrids. The antioxidant enzyme systems of seeds are an important primary defense against free radicals generated under stress conditions, such as superoxide dismutase (SOD), which catalyzes the dismutation of the superoxide radical in H_2O_2 and $O_{2;}$ catalase (CAT) and ascorbate peroxidase (APX), which can break $H_2\,O_2$ in H_2O and O_2 (Carneiro et al., 2011). The regulation of these antioxidant enzymes can be lost if stress is too severe, greatly increasing the production of free radicals that can lead to a cascade of events that begins with lipid peroxidation, advancing towards membrane degradation and cell death (Greggains et al., 2000). Thus, increased expression of CAT is important to eliminate the accumulation of $H_2\,O_2$ resulting from lipid peroxidation in seeds (Eyidogan and Oz, 2007).

The alcohol dehydrogenase (ADH) enzyme operates in anaerobic metabolism, reducing acetaldehyde (primary

Table 2. Length of hypocotyl (H), of radicle (R), growth index, uniformity index and automatic vigor index obtained by analyzing images of seedlings provided by the Groundeye of popcorn seeds submitted to different germination temperatures.

Temperature	Hybrid	H (cm)	R (cm)	Growth	Uniformity	Vigor
10 ºC	P618	0.000 A	0.000 A	0.564 C	93.048 A	28.309 A
	AP6002	0.000 A	0.000 A	1.244 B	91.891 A	28.438 A
	AP8202	0.000 A	0.000 A	1.695 B	93.391 A	29.204 A
	AP8203	0.000 A	0.000 A	2.642 A	95.318 A	30.445 A
13 ° C	P618	0.000 A	0.132 A	1.321 B	93.423 B	28.951 B
	AP6002	0.000 A	0.421 A	3.790 A	97.395 A	31.872 A
	AP8202	0.000 A	0.106 A	1.013 B	92.456 B	28.446 B
	AP8203	0.000 A	0.401 A	3.613 A	96.816 A	31.574 A
16 ° C	P618	0.022 A	0.981 A	9.584 B	96.156 A	35.555 B
	AP6002	0.143 A	1.114 A	11.362 A	94.934 B	36.434 A
	AP8202	0.060 A	0.816 A	6.688 C	97.948 A	34.059 B
	AP8203	0.075 A	1.150 A	11.093 A	97.541 A	37.027 A
19 ° C	P618	0.723 A	4.045 B	40.538 B	76.864 B	52.954 B
	AP6002	0.787 A	4.054 B	42.553 B	81.926 A	57.545 A
	AP8202	0.470 B	3.212 C	33.298 C	77.656 B	46.368 C
	AP8203	0.926 A	4.931 A	49.994 A	82.526 A	58.292 A
25 ° C	P618	3.388 B	9.646 C	100.000 A	82.926 A	95.471 A
	AP6002	3.391 B	10.99 B	100.000 A	85.792 A	95.738 A
	AP8202	3.234 B	9.680 C	100.000 A	85.957 A	95.787 A
	AP8203	4.735 A	12.00 A	100.000 A	83.883 A	95.165 A
CV (%)		33.87	53.21	3.40	7.08	12.30

*Means followed by the same capital letter in the column, within each temperature, do not differ statistically from one another by the Scott-Knott test at 5% probability.

metabolite of the ethanol cycle in the conversion route to acetic acid) to ethanol and oxidizing NADH to NAD^+ (Bray et al., 2000). Figure 3B shows that at temperatures 10, 13 and 16 °C, popcorn hybrids P618 and AP8202 have induced an increase in the expression of such enzyme compared to the temperature of 25 °C. Thus, on these temperatures, seeds become less susceptible to the deleterious effects of acetaldehyde with increased expression of ADH (Zhang et al., 1994). It is likely that the expression of ADH disclosed for treatments is associated with acetaldehyde accumulation. This activity may also result in an adequate supply of ATP via alcoholic fermentation. However, Taiz and Zeiger (2009) claim that more important than the ADH energy supply is its ability to convert toxic acetaldehyde into ethanol. As for hybrids AP6002 and AP8203, enzyme expression was only intensified at 10 and 13 ºC. From 16 °C on, there is a lower expression of the enzyme, which approximate from the patterns presented at 25 °C. Research studies on the expression of alcohol dehydrogenase in response to cold temperature suggest that ethanol would be responsible for maintaining the fluidity of the plasma membrane under cold stress conditions. Moreover, the accumulation process of ethanol involves the oxidation of NADH and results in the production of small amounts of ATP, which is key to the survival of several species under stress conditions and can guarantee seed germination (Kennedy et al., 1992).

Figure 3C shows increased expression of MDH enzyme for hybrids AP6002 and AP8203 from 16 ° C. Such results can be due to an increase in germination metabolism caused by increasing germination temperature. Thus, the expression of these enzymes has contributed to the maintenance of good physiological quality even when in adverse conditions.

The MDH enzyme expression of P618 and AP8202 have reduced with temperature decrease and this might explain their lower levels of germination, which can be confirmed in the physiological tests. MDH plays a significant role in the Krebs cycle, since it catalyzes the conversion of malate to oxaloacetate, producing NADH, which is a key to the production of ATP. This enzyme is linked to the generation of energy for important metabolic processes, such as germination (Taiz and Zeiger, 2009). Thus, major changes to this enzyme is associated with to a seed decay process, due to reduced breathing activity, resulting in the degradation and inactivation of other enzymes (Copeland and McDonald, 2001). The overexpression of this enzyme at 16 °C was also crucial for the seeds of AP6002 and AP8203 to achieve higher germination levels than the other popcorn hybrids.

Regarding the expression of α- amylase enzyme (α-AMY), major differences were observed with respect to lower temperatures (10 and 13 °C), with no germination of the hybrids. At 16 °C, it is possible to observe the start of expression of this enzyme, further increasing from 19 °C. In addition to antioxidant enzymes, the expression of the α and β amylases enzymes is extremely important for the seeds to germinate under low temperature conditions. The development of amylase expression is an important event, and may be noted during the start of germination, and its primary role is to provide substrates for seedling use until it becomes photosynthetically self-sufficient (Nedel et al., 1996).

In corn, the α-AMY enzyme, when promoting the hydrolysis of starch, makes the necessary carbohydrates available for the development of the embryo, thus enabling the germination process (Franco et al., 2002). However, the expression of α-AMY can only be found in soaked

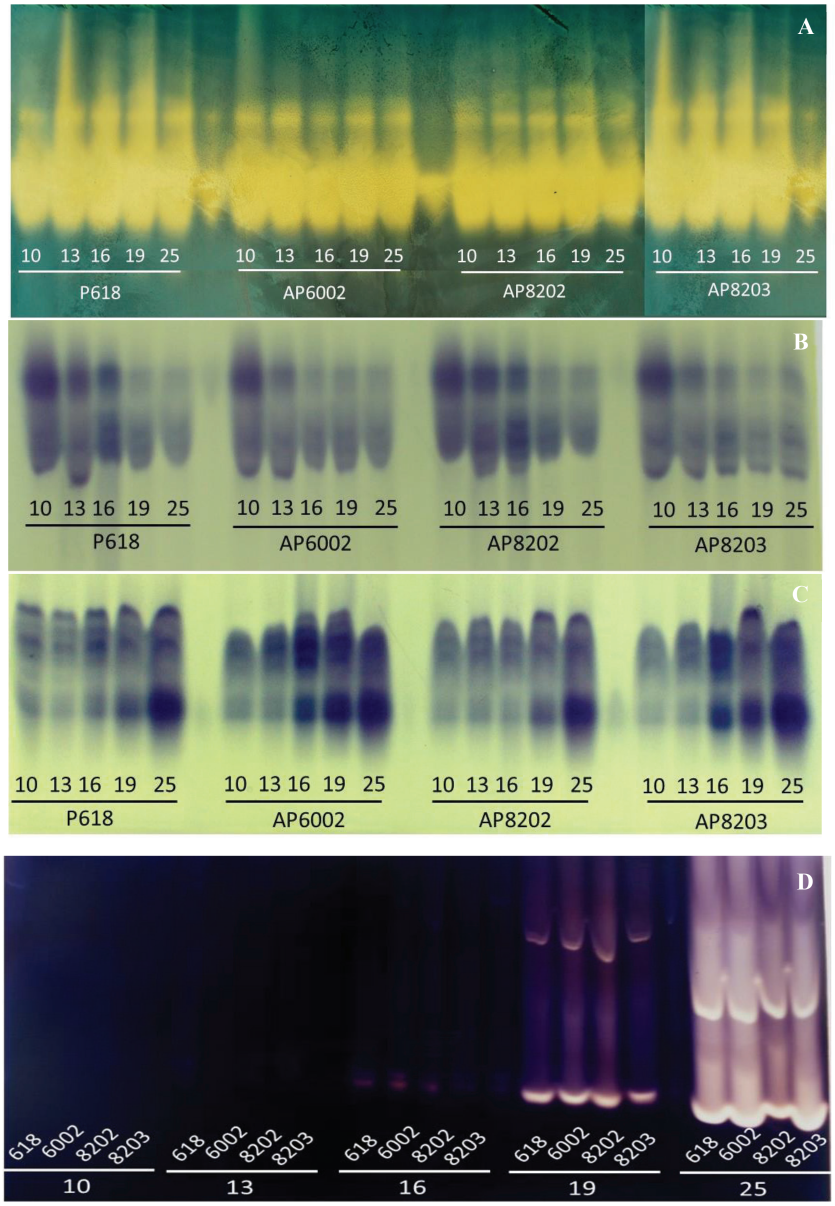

Figure 3. Isoenzymatic expressions of catalase (CAT) (A), alcohol dehydrogenase (ADH) (B), malate dehydrogenase (MDH) (C) and α-amylase (α-AMY) (D) of hybrid seeds of popcorn submitted to different temperatures.

seeds. Low temperatures promote the reduction of water absorption and consequently the reduction of the expression of this enzyme. Therefore, different levels of soaking can be found for treatments and different expressions of α-AMY (Figure 3D). According to Silva-Neta et al. (2015), seeds soaked at 10 °C for 72 h, have lower expression of α-amylase, suggesting that soaking at low temperatures may be related to reduced synthesis of enzymes necessary for the breakdown of starch for the growth of the embryo, which justifies delayed germination process.

The physiological, biochemical, and study results of images that we have presented and discussed prove that low temperatures compromise the quality of popcorn seeds, because they act on their viability, seed quality, biochemical reactions, and seedling development. Another aspect to be considered is the use of image analysis through the Groundeye software, to check for seed quality when subjected to stress conditions. Enzymes CAT, MDH, and α-AMY are useful biochemical markers in studies that focus on the physiological quality of popcorn seeds subjected to low temperatures.

Conclusions

Low temperatures can adversely affect plant physiological quality, seed isoenzymatic expression, and performance of popcorn seedlings. Such changes compromise seed quality. Hybrids AP6002 and AP8203 can be recommended for sowing at up to 16 °C. The Groundeye software was effective for the analysis of popcorn, on the assessment of the quality of seedling and seed subjected to low temperatures.

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