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Antioxidant activity of corn seeds after thermal stress

Atividade antioxidante de sementes de milho após estresse térmico

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ABSTRACT

The aim of this research was to analyze the effect of different temperatures on physiological quality and relate the responses with the antioxidant activity of three lots of corn seeds. The seeds from three lots were exposed for 24h at temperatures of 15, 25, 35 and 40°C and then evaluated for percentage, speed index and first germination count, as well as early growth and activity of antioxidant enzymes in the seedlings. The experimental design was completely randomized with four replications, with subsequent polynomial regression analysis. The results showed that the seeds from the three lots of corn exposed to temperatures of 15 and 40°C, were of better high physiological quality (vigor), which explains the low activity of enzymes of the antioxidant defense system in these same temperatures. The antioxidant defense system is related to the physiological quality of corn seeds exposed to thermal stress.

Key words: ***Zea mays***, *temperature, germination, growth.*

RESUMO

O objetivo neste trabalho foi analisar o efeito de diferentes temperaturas na qualidade fisiológica e relacionar com a atividade antioxidante de três lotes de sementes de milho, as quais foram expostas por 24h às temperaturas de 15, 25, 35 e 40°C e, após, avaliadas quanto à porcentagem, índice de velocidade e primeira contagem de germinação, como também crescimento inicial e atividade de enzimas antioxidantes nas plântulas. O delineamento experimental foi inteiramente casualizado, com quatro repetições, com posterior análise de regressão polinomial. Os resultados evidenciaram que as sementes dos três lotes de milho, expostas às temperaturas de 15 e 40°C, foram de melhor qualidade fisiológica (vigor), o que explica a baixa atividade das enzimas do sistema de defesa antioxidante nessas mesmas temperaturas. O sistema de defesa antioxidante tem relação com a qualidade fisiológica de sementes de milho expostas ao estresse térmico.

Palavras-chave: ***Zea mays***, *temperatura, germinação, crescimento.*

INTRODUCTION

Climatic conditions in different regions of Brazil are quite diverse and responsible for the most conspicuous environmental stresses, and can influence the natural distribution of plant populations, altering the biochemical and physiological processes in seeds, which can significantly decrease the physiological quality of seeds and, consequently the crop productivity (MARINI et al., 2012). Evidences suggest that the sudden and frequent variations in the climate of Brazilian tropical regions can cause the production of reactive oxygen species (ROS) in seedlings, such as the superoxide radical (O_2^-), hydroxyl radical (OH^-), hydrogen peroxide (H_2O_2) and singlet oxygen ($^1O_2$) (STANISAVLJEVIĆ et al., 2011). The balance between the production of these ROS and the ability to rapidly deploy the antioxidant defense system reflects in the plant response to stress and, consequently, in its adaptation and/or tolerance to adverse conditions which the plant is subject (MITTLER, 2002).

ROS are produced continuously in chloroplasts, mitochondria and peroxisomes during

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the normal metabolism of the plant. However, the production and removal of these ROS are highly controlled (APEL & HIRT, 2004), and this balance can be changed if there is uncontrolled increase of some kind of stress. Temperature is one of the most important factors that influence seed viability and seedling growth (MARINI et al., 2012). It determines the capacity and rate of germination (MEI & SONG, 2010). High or low temperature stresses can cause a series of events in cascade, starting with lipid peroxidation, advancing to the degradation of membranes and eventually, cell death.

Under these conditions, the plant growth is reduced and storage reserves are directed to maintain the active metabolism in organs with preferential growth, besides of activation of the enzymatic antioxidant system. There is an increase in the activity of enzymes superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase (APX) as a natural response during seed germination which characterizes a mechanism of stress tolerance (MEI & SONG, 2010) in order to reduce toxic products from the action of free radicals before damage can be established (CARNEIRO et al., 2011).

Cells survival depends on the degree of oxidative stress caused for the unbalance between the generation of ROS and the ROS detoxification. Monitoring of the activity of antioxidant enzymes as an indicator of this unbalance was observed in seeds and seedlings (RESENDE et al., 2003) of alfafa (***Medicago sativa***) (CAKMAK et al., 2010), barley (***Hordeum vulgare***) (MEI & SONG, 2010) and ***Jatropa curcas*** (CAI et al., 2011). According to some studies the decrease in antioxidant enzyme activity resulted in lower viability and vigor (DEMIRKAYA et al., 2010; PRODANOVIC et al., 2012; CHAUHAN et al., 2011).

Therefore, the aim of this research was to analyze the influence of different temperatures on the physiological quality of seeds and relate with the antioxidant activity of corn seeds.

MATERIALS AND METHODS

Three lots of corn seeds (***Zea mays*** L.), provided by the Pioneer Company, were used for the testes of viability and vigor according to the Rules of Seed Testing (BRASIL, 2009), these seeds were stressed at temperatures of 15, 25, 35 e 40°C for 24h. Germination tests were carried out on samples of 200 seeds (four sub-samples of 50 seeds) for each one of the four replicates. Paper rolls previously moistened with distilled water at a ratio of 2.5 times of its initial mass, were used as substrate for germination, and kept in a germinator at 25°C for seven days, these results were expressed in percentage of germination, considering the number of seedlings classified as normal. Evaluations of the first germination count were conducted jointly with the germination test, being the first count performed four days after sowing and expressed as percentage of normal seedlings. Analysis of speed index germination were conducted at the same moment with the germination test, considering daily counts of the radicle protrusion from the seed coat until the number of emerged seedlings remained constant. The last day of counting for this test was the same as indicated for the germination test after seven days of sowing and the calculation of speed index of germination was performed according to MAGUIRE (1962), using the following formula: speed index of germination = $G_1/N_1+G_2/N_2+...+G_n/N_n$ where G_1, G_2 and G_n refer to the number of seedlings emerged daily, and N_1, N_2, N_n time in days of sowing of the first, second and last count. The lengths and the dry mass of shoots and roots were measured in 40 seedlings per replication at the end of the germination test; the measurement of these lengths was obtained with the aid of a millimetered ruler and the results were expressed in mm seedlings^{-1} and mg seedlings^{-1}, respectively.

In addition to the growth characteristics, it was evaluated the activity of antioxidant enzymes superoxide dismutase (SOD, EC 1.15.1.1), ascorbate peroxidase (APX, EC 1.11.1.11) and catalase (CAT, EC 1.11.1.6) were evaluated in order to verify the effect of the stress agent on the antioxidant metabolism in corn seedlings. Enzymatic extracts were obtained from 400mg of plant material collected at the end of germination macerated with 10% of polyvinylpolypyrrolidone (PVPP), sufficient to prevent oxidation of material and homogenized in 1.5mL of 100mM potassium phosphate buffer pH 7.8 containing 0.1mM EDTA and 20mM ascorbic acid. The homogenate was centrifuged at 12.000g for 20 minutes at 4°C and the supernatant was assayed for enzymatic activity and quantification of protein by the BRADFORD method (1976).

The evaluation of the SOD activity was assayed spectrophotometrically with the inhibition of photochemical reduction of nitro-blue tetrazolium (NBT) at 560nm (GIANNOPOLITIS & RIES, 1977). The reaction medium contained 50mM of potassium phosphate pH 7.8, 14mM methionine, 0.1mM EDTA, 75µM NBT and 2mM riboflavin; where one unit of SOD was defined as the amount of enzyme that inhibits 50% NBT photoreduction under test conditions. APX activity was performed according to NAKANO & ASADA (1981), by evaluating the rate of oxidation of the ascorbate at 290nm. The reaction mixture incubated at 37°C was composed of 100mM potassium phosphate buffer pH 7.0, 0.5mM ascorbic acid and 0.1mM H_2O_2. The CAT activity was determined according to AZEVEDO et al. (1998), with modifications, estimated by measuring the initial rate of disappearance of H_2O_2 in a reaction medium containing 100mM potassium phosphate pH 7.0 and 12.5mM H_2O_2; the decrease in H_2O_2 was measured as a decline in optical density at 240nm, during 2 minutes.

The experimental design used was completely randomized, with four replications and the data related to the measured variables were subjected to the variance analysis with subsequent polynomial regression.

RESULTS AND DISCUSSION

The percentage of germination from the three lots of corn seeds was not affected by the different temperatures (15, 25, 35 and 40°C), exceeding 90% in the three lots, however, the vigor determined by the first count and speed index of germination of corn seeds showed higher performance in extreme temperatures of 15 and 40°C, being higher in this last one (Figure 1A and B).

The results found for the length and dry mass of aerial part and roots of seedlings, showed a similar tendency to the observed at the first germination count and speed index of germination, expressing higher vigor in extreme temperatures (15 and 40°C) (Figures 1C, D, E and F). Extreme temperatures can induce degenerative changes that spurred the initial growth of seedlings leading to the rapid consumption of seed reserves, during the

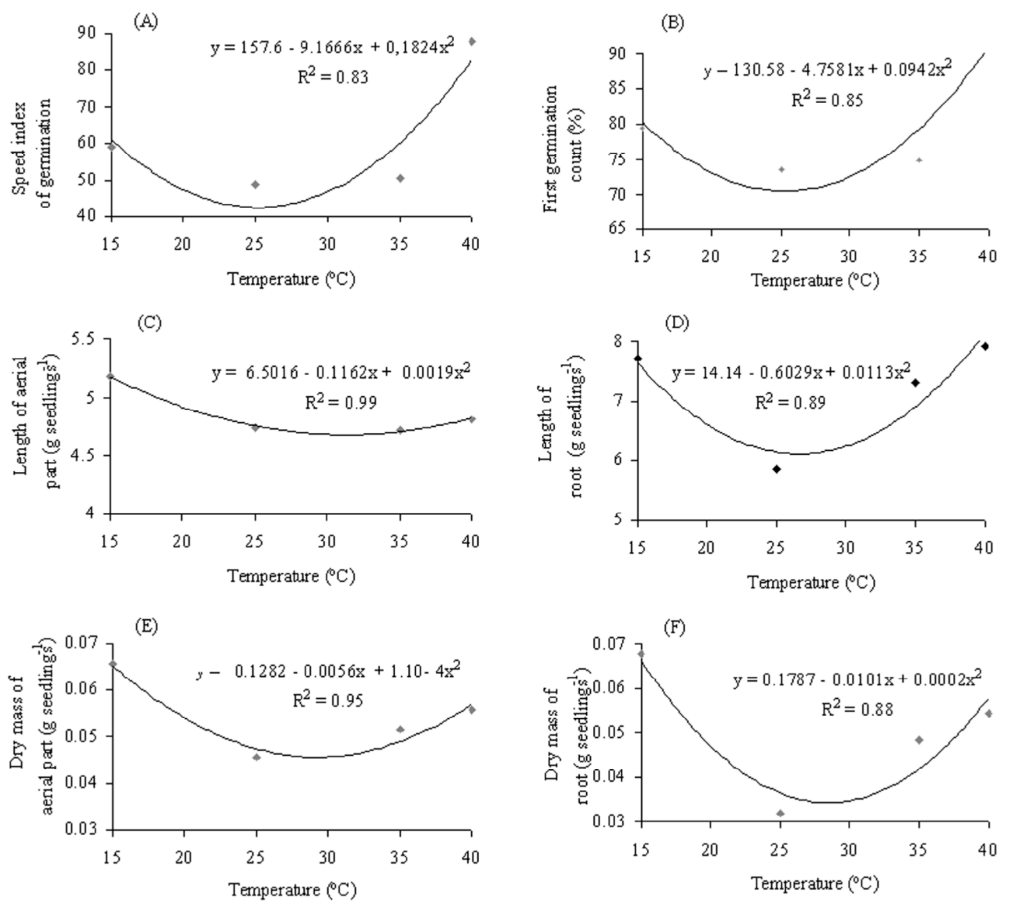

Figure 1 - First germination count (A), speed index of germination (B), length of aerial part (C) and root (D), dry mass of aerial part (E) and root (F) from three lots of corn seeds exposed to different temperatures (15, 25, 35 and 40°C) during 24h. * Significant at 5% by the F test.

early germination process (PATANÉ et al., 2006). There was a strong investment in the initial growth of seedlings in order to not impair the processes of synthesis and release of energy, as a way to continue the autotrophic germination process, characterizing a defense strategy of the seeds subjected to such temperatures. However, this answer does not mean that these seedlings would growth efficiently with high vigor, considering exposure to adverse environmental conditions.

Similar results were reported in a study performed with rice seedlings subjected to different temperature stress (15, 25, 30 and 35°C) for 24h, where the more vigorous seedlings were those subjected to lower temperatures, between 15 and 25°C (MARINI et al., 2012). However, in the same experiment, higher temperatures (>25°C) did not affect early seedling growth, since growth and dry mass of shoots and roots decreased.

The higher vigor was expressed in corn seedlings subjected to extreme temperatures (15 and 40°C), which explains the low activity of enzymes of the antioxidant defense system in these temperatures and evaluation period (Figure 2).

Although the oxidative stress generated by biotic and abiotic factors stimulates the biosynthesis of

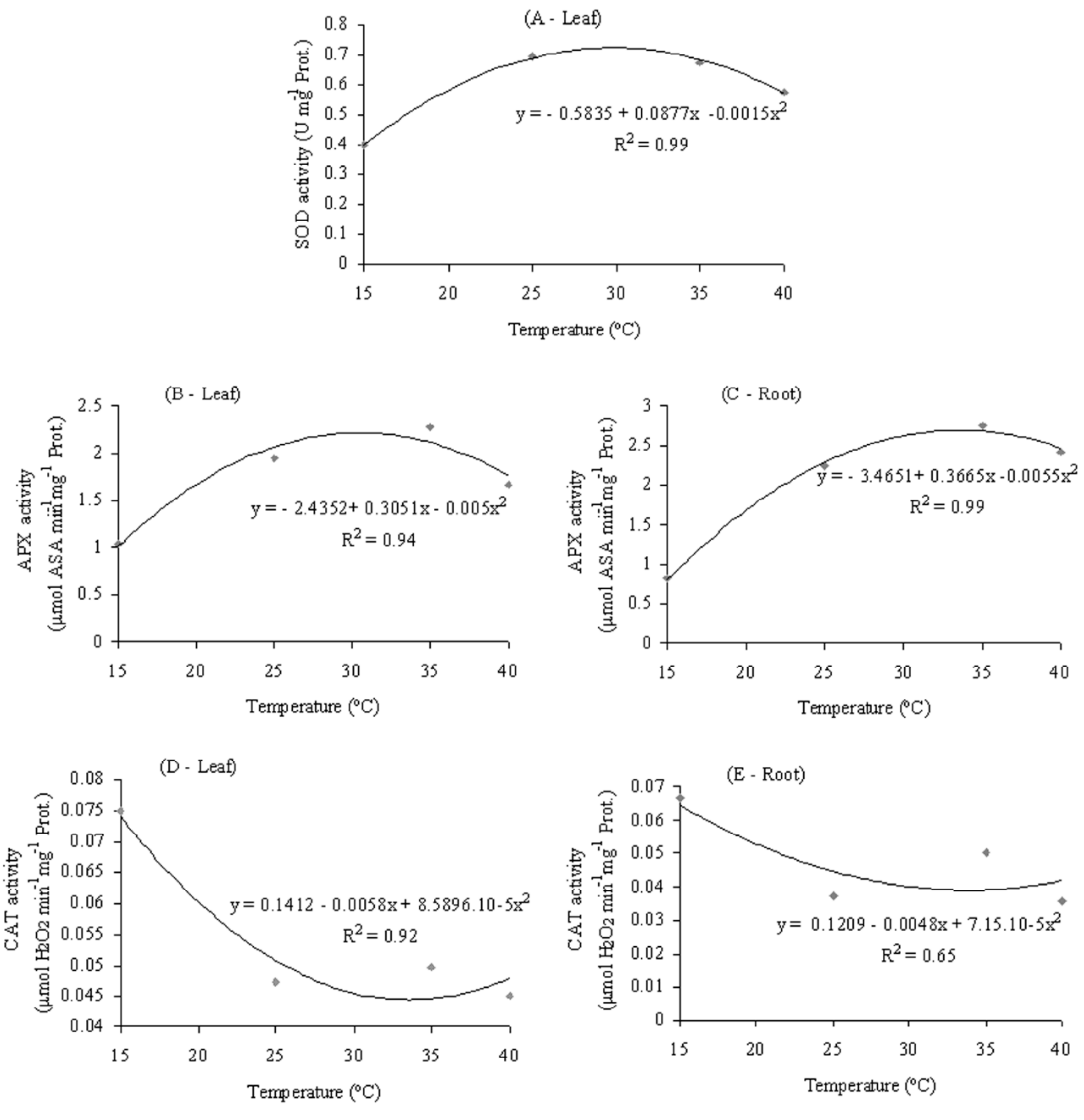

Figure 2 - Specific activity of the enzymes superoxide dismutase (SOD) (A), ascorbate peroxidase (APX) (B and C) and catalase (CAT) (D and E) in leaves and roots of corn seedlings from three lots of seeds previously subjected to temperatures of 15, 25, 35 and 40°C during 24h. * Significant at 5% by the F test.

antioxidants components and increase in antioxidant enzymes activity at the beginning of stress, there is a reduction of some antioxidant activities (SOARES & MACHADO, 2007). Therefore, there is an increase in reactive oxygen species (ROS) as by-product of respiratory metabolism (DAT et al., 2004), which was increased with temperature stress, resulting in rapid degradation of seed reserves and more vigorous seedlings as well as low activities of SOD, CAT and APX, as a survival strategy to the stress imposed.

The lower activity of the SOD in the seedlings obtained from seeds subjected to extreme temperatures (15 and 40°C), may be related to the increased levels of H_2O_2 because it is capable of inactivating enzymes by oxidizing thiol groups (BOWLER et al., 1994). In studies performed with two clover genotypes subjected to flooding stress, it was verified a low activity of this enzyme during the period of stress imposed (STOILOVA et al., 2012), which corroborates with the results found in this study, on the other hand, the activity of SOD at the root of the seedlings did not change significantly.

The activity of the APX enzyme was lower in aerial part and root of corn seedlings obtained from seeds exposed to temperatures of 15 and 40°C, and increased when the seeds were exposed to 25 and 35°C, which can be seen at the tendency curve adjusted for this enzyme (Figure 2B and C).

Activity of the CAT enzyme was low, both in the aerial part and in the root system of the seedlings, whose seeds were exposed to the temperature of 40°C (Figure 2D and E), indicating that exposure of seeds mainly at high temperatures result in an increasing of the H_2O_2 content in the seedlings, coinciding with the decrease in catalase activity due to its low affinity for H_2O_2. Similar results were reported in cucumber seedlings exposed to mercury, since that the higher stress imposed (high metal concentrations) inhibited the enzyme activity of the antioxidant system (CARGNELUTTI et al., 2006).

The decrease in CAT activity in the seed reduces the respiration capacity, reducing the energy supply (ATP) for germination of seed (DEMIRKAYA et al., 2010). The same happened in ***Picea omorika*** where high concentrations of heavy metal cadmium decreased the activity of CAT and SOD, as well as the germination (PRODANOVIC et al., 2012).

The decline in activities of the SOD, CAT and APX enzymes in seedlings whose seeds were exposed to 40°C suggests a delay in the removal of the O_2^-, H_2O_2 and the peroxides toxic to cells. This fact would become, according to HERNANDEZ & ALMANSA (2002), the lipid peroxidation and protein oxidationmore evident, due to the high capacity of free radical formation, promoting future reflections to the plants.

The ability to maintain SOD, CAT and APX activities at high levels, under stress conditions, is essential to the equilibrium between the formation and removal of the H_2O_2 from the intracellular environment (DEUNER et al., 2011). This was not possible to observe, since that in the stress situations imposed, the activities of antioxidant enzymes were low and the assessments were made during the initial growth of seedlings, which may reflect an insufficient period to demonstrate the effective detectable consequences of the stressful conditions.

CONCLUSION

The activity of the antioxidant defense system has relation with the physiological quality of corn seeds exposed to thermal stress.

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