academicJournals

Vol. 8(43), pp. 5368-5374, 7 November, 2013
DOI: 10.5897/AJAR2013.7833
ISSN 1991-637X ©2013 Academic Journals
http://www.academicjournals.org/AJAR

African Journal of Agricultural Research

Full Length Research Paper

Relationships between the physiological and biochemical modifications in soybean seeds under different temperatures

Juliana de Magalhães Bandeira[1]*, Patrícia Marini[1], Andrea Bicca Noguez Martins[1], Isabel Cristina Gouvea de Borba[2], Luciano do Amarante[1] and Dario Munt de Moraes[1]

[1]Department of Botany, Biology Institute, Federal University of Pelotas, Capão do Leão campus, P. O. Box 354, ZIP Code 96010-900, Pelotas, RS, Brazil.
[2]Department of Botany, Federal University of Rio Grande do Sul, Bento Gonçalves Avenue, number 9500, Vale campus, Neighbourhood Agronomy, building 43, number 433, ZIP Code 91501-970 - Porto Alegre, RS, Brazil.

Accepted 15 October, 2013

The aim was to analyze the effect of the stress caused by different temperatures, for a period of 24 h, on seed germination and initial growth of soybean seedlings to relate these variables with the antioxidant defense system. For this, three seed lots were exposed for 24 h at temperatures of 15, 25, 35 and 40°C, being evaluated for germination, first germination counting (FGC), germination speed index (GSI), length, dry mass of roots and shoots and electrical conductivity (after three, six and 24 h), respiratory activity, in addition to superoxide dismutase, catalase and ascorbate peroxidase activity. The lot two presented a higher germination (96.72%), followed by the lot three (83.25%) and one (73.25%). Similarly, the FGC (71.79%) and the GSI (50.50) showed highest average in the lot two. This lot also presented higher growth, biomass accumulation and lower loss of leachates during the imbibition period and different temperatures, as well as more constant respiratory activity compared to the other lots, characterizing lot two as more vigorous, which is related to the antioxidant defense system response that, in a general way, demonstrated lower activity of the antioxidant enzymes in the seedlings shoot above 35°C. Thus, the antioxidant defense system activity has relationship with the physiological quality of the soybean seeds exposed to different temperatures.

Key words: *Glycine max*, germination, vigor, growth, oxidative stress.

INTRODUCTION

The beginning of the seed germination process, from its rehydration and consequent respiratory process, marks the first metabolic activity to be quickly stimulated to very high levels, a few hours after imbibition, accelerating the metabolism and the activation of respiratory and hydrolytic enzymes, which are highly related to seeds physiological quality (Mendes et al., 2009; Aumonde et al., 2012).

However, during the maturation process or even during the storage of seeds quality may be prejudiced by several abiotic factors such as variations of temperature, that is directly related to the climate of different regions in Brazil and this directly influences in the physiological and metabolic processes during germination and initial growth of the seedlings, while the temperature variation is one of the main causes of seeds deterioration, reducing the

*Corresponding author. E-mail: bandeira_jm@hotmail.com

viability and the vigor of such seeds (Marini et al., 2012).

In this context, the changes of temperature may cause irreparable damage to the plant cells and tissues, as during the reactivation of the metabolic functions, the stress caused by changes of temperature can cause a small amount of electrons escape from the respiratory chain (Skutnik and Rychter, 2009), resulting in the partial reduction of molecular oxygen which leads to the production of reactive oxygen species (ROS), in the form of oxygen singleton ($^1O_2$), hydrogen peroxide (H_2O_2), hydroxyl radical (OH^-) and superoxide anion ($O_2^{\bullet -}$) (Stanisavljević et al., 2011). These are sub products of aerobic and photosynthetic metabolism, although under high stress conditions its production is accelerated and, when in excess, may damage the system of membranes or other molecules leading to the reduction of seeds vigor (Mei and Song, 2010; Carneiro et al., 2011), jeopardizing the development of the cultures (Forman et al., 2010).

Therefore, under adverse conditions, the activation of the antioxidant enzymatic system occurs. This activation is characterized by the increase in the activity of the superoxide dismutase enzymes (SOD), which catalyzes the dismutation of the superoxide radical in H_2O_2 and O_2, catalase (CAT) and ascorbate peroxidase (APX) which degrade the H_2O_2 in H_2O and O_2 (Deuner et al., 2011) as a natural answer during the germination of seeds, characterizing a tolerance mechanism to adverse condition (Mei and Song, 2010), in order to reduce the production of ROS before the damage happens (Carneiro et al., 2011). Based on this fact, the monitoring of the activity of these enzymes is used as an indicative to characterize the beginning of the deterioration process in seeds and seedlings.

Thus, the balance between the production of ROS and the capacity to quickly activate the antioxidant defense system reflects the plant response to the stress which it was exposed to and, consequently, to the adaptation and/or tolerance to adverse conditions (Soares and Machado, 2007), which may result in a series of metabolic events, starting with the lipid peroxidation, advancing to a degradation of membranes, and eventually, promote the cell death (Pacheco et al., 2007). Therefore, we focused on analyzing the influence of the stress caused by different temperatures, for a period of 24 h, on seed germination and initial growth of soybean (*Glycine max* L.) seedlings, as well as relate these variables with the antioxidant defense system.

MATERIALS AND METHODS

Three lots of soybean seeds (*G. max*), cultivar NA 4990RG, were exposed for 24 h at temperatures of 15, 25, 35 and 40°C to after that being evaluated in terms of viability, vigor and the activity of antioxidant enzymes. To do this, the germination test (G) was conducted according to the Seeds Analysis Rules (Brasil, 2009), with four repetitions of 200 seeds, composed by four subsamples of 50 seeds for each one, for each lot and each temperature, using as a substrate rolls of germination paper, which have been previously humidified with distilled water in a proportion 2.5 times its initial mass and kept all the treatments in germinators at 25°C. On the eighth day after sowing (DAS) the percentage of germination was analyzed, considering the number of seedling classified as normal. The first germination count (FGC) was conducted along the germination test, in which the first count for the soybean was performed on the fifth DAS and the results were expressed in percentage of normal seedlings (Brasil, 2009).

The germination speed index (GSI) was also performed with the germination test, through daily counts from the protrusion of the radicle by the tegument of the seed, until the number of emerged seedlings remained constant, and the calculation for this variable was performed according to Maguire (1962). The analysis of the seedlings initial growth was performed at the end of the germination test, through length and dry mass measures of the shoot (SL, SDM) and the roots (RL, RDM), of 40 seedlings for each repetition. The length measuring was performed with a millimeter ruler and the results expressed in mm $seedling^{-1}$ and the dry mass was obtained gravimetrically, in a forced ventilation oven 70 ± 1°C until reaching constant mass was obtained and the results were expressed in mg $seedling^{-1}$.

For the electrical conductivity test (EC) four repetitions of each lot were used with subsamples of 25 seeds, in a total of 400 seeds per treatment. Initially, the mass of dry seeds was determined, which were placed in bottles with 75 ml of deionized water and kept in germinator with a constant temperature of 25°C. After incubation periods of three, six and 24 h the readings in Digimed CD-21 Condutivimeter were made, with the results expressed in µS cm^{-1} g^{-1} of seeds according to the methodology described by Krzyzanowski (1991).

The respiratory activity (RA) was determined in the Pettenkofer apparatus, where 100 g of soybean seeds were placed in a storage bottle, imbibed in 80 ml of water for 60 min to accelerate the respiratory process and, after that, the release of CO_2 from the seeds was measured according to the methodology described by Mendes et al. (2009). The result was expressed in milligrams of released CO_2 by milligram of seed per hour (mg CO_2 released mg^{-1} of seed h^{-1}). Besides the parameters above, the activities of the 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 assessed to check the effect of the stressful agent on the antioxidant metabolism of the seedlings of the three lots of soybean seeds. Thus, at the end of the germination test 400 mg of the soybean seedlings shoots and roots were collected, separately, which were macerated with 10% polyvinylpolypyrolidone (PVPP) and homogenized in 1.5 ml of the extraction buffer, composed by potassium phosphate (100 mM, pH 7.8), EDTA (0.1 mM) and ascorbic acid (20 mM). The sample was centrifuged at 12.000 rpm for 20 min at 4°C and the supernatant was used to determine the enzymes activity (Deuner et al., 2011).

The assessment of the activity of SOD was determined based on the enzyme capacity to inhibit the photoreduction of the nitroblue tetrazolium (NBT) (Giannopolitis and Ries, 1977) through chemical reaction with potassium phosphate (50 mM, pH 7.8), methionine (14 mM), EDTA (0.1 µM), NBT (75 mM) and riboflavin (2 µM), added by 100 µl of enzymatic extract, completing the final volume of 2 ml with distilled water. The readings were performed at 560 nm, taking into account that one unit of SOD corresponds to the amount of enzyme able to inhibit in 50% the photoreduction of the NBT in test conditions.

The APX activity was performed according to Nakano and Asada (1981), with some modifications, through the assessment of the ascorbate oxidation rate at 290 nm during two minutes. The reaction was calculated for a final volume of 2 ml where the potassium phosphate buffer (100 mM, pH 7.0) and ascorbic acid (0.5 mM) were incubated at 28 to 30°C for 10 min and, later, were added with H_2O_2 (0.1 mM) and 25 µl of enzymatic extract at the moment of the absorbance reading was performed.

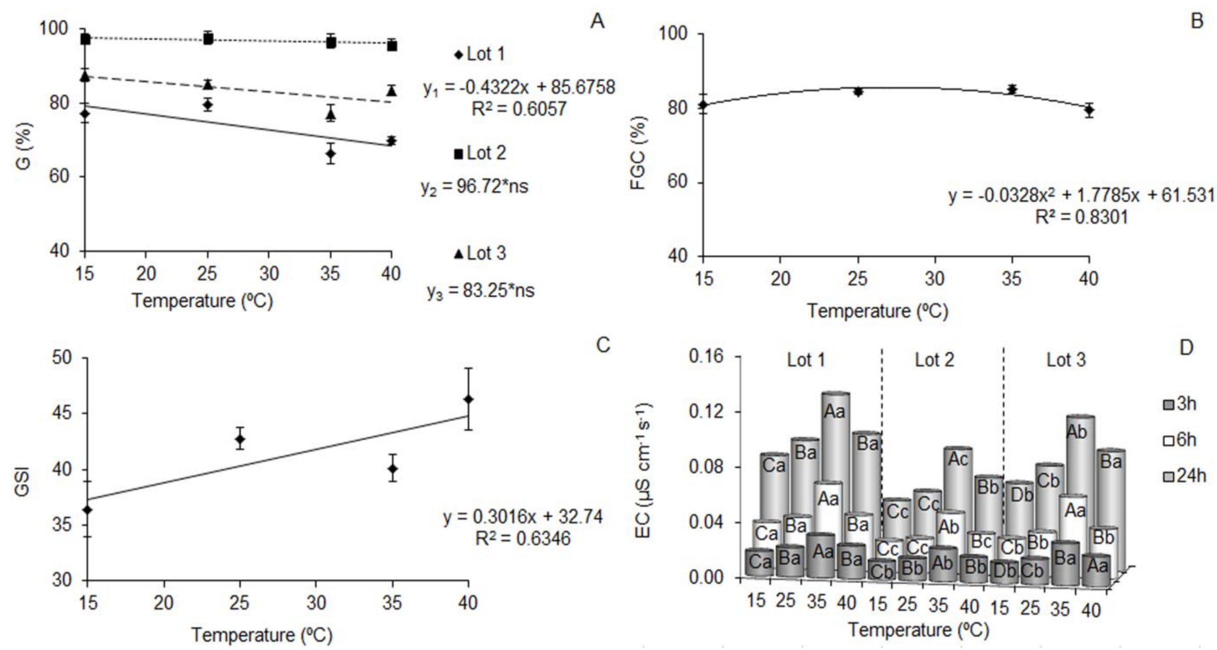

Figure 1. (A) Percentage of germination (G), (B) first germination count (FGC), (C) germination speed index (GSI) and (D) electrical conductivity (EC) after three, six and 24 hours of imbibition of three lots of soybean seeds (*Gycine max* L.), cv. IN 4990RG, exposed for 24 h at temperatures of 15, 25, 35, and 40°C. *ns = straight line equation is not significant at the 5% level of error probability. The bars represent the standard error of four replications, and uppercase letters differentiate different stress temperatures and the lowercase, show significant differences between lots by Tukey test (p ≤ 0.05).

The CAT activity was determined according to Azevedo et al. (1998), with some modifications, estimated by the decrease in absorbance at 240 nm during two minutes in a chemical reaction with a final volume of 2 ml containing potassium phosphate (100 mM, pH 7.0) incubated at 28 to 30°C for 10 min, and, later, was added with H_2O_2 (12.5 mM) and 25 µl of enzymatic extract at the moment of the absorbance reading was performed.

The experimental delineation was fully randomized, with factorial 4 x 3 (four temperatures and three lots of soybean seeds), with four repetitions. The data concerning the measured variables of viability and vigor was submitted to variance analysis and when there was significant interaction, the data was submitted to polynomial regression. In case of not occurring significant interaction between the factors, the average test was performed using the Tukey test ($p \leq 0.05$), through the software WinStat 2.0 (Machado and Conceição, 2007). For the data concerning the activity of antioxidant enzymes, these were submitted to contrast orthogonal polynomials analysis (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

There was a significant interaction between the factors (lots and temperatures) for the germination percentage (Figure 1A), which presented a decline with the increase in temperature. Although the germination was higher in the seeds from lot two in all temperatures, with an average of 96.72% of germinated seeds, followed by lot three, with 83.25% and lot one, which was the only one with adjustment of regression equation, demonstrating to be sensitivity to higher temperatures, probably because it presented the lower germination percentage (73.25%) (Figure 1A). Marini et al. (2012) working with rice seeds cv. BRS 7 Taim under the influence of different temperatures (15, 25, 30 and 35°C) for 24 h, there was a reduction in the viability of the seeds which were exposed to temperatures above 25°C showing the damaging effect of seed exposed to different temperatures.

For the vigor results there was no significant interaction between the factors lots and temperatures. However, there was a statistical difference for the isolated factors, in which for the first germination count (FGC) and germination speed index (GSI), the lot two presented the highest averages, with 85.44% and 50.50, respectively, being statistically different from lots one and three (Table 1). Concerning the temperature factor, it was possible to estimate, according to the regression equation, the maximum value of 85.64% for the FGC at 27.11°C (Figure 1B), while the GSI presented growing linear response, ranging from 36.40 to 46.34, according to the increase of temperature (Figure 1C).

The responses from the growth and dry mass accumulation variables presented significant difference only between the lots, in which the length of the shoot and the roots, as well as the dry mass of the roots were higher in the lot two (Table 1) corroborating with the performance of seeds from this lot in the vigor tests represented by the FGC and GSI (Figure 1B and C). These vigor tests are extremely important as they enable

Table 1. First germination count (FGC), germination speed index (GSI), length and dry mass of shoot (S) and roots (R) of three lots of soybean (*Glycine max* L.), cv. IN 4990RG, exposed for 24 h at temperatures of 15, 25, 35, and 40ºC. * Different letters show significant differences between lots by Tukey test ($p \leq 0.05$).

Lot	FGC (%)	GSI	Length (mm)		Dry mass (mg)	
			S	R	S	R
1	81.47^{B}*	35.76^{B}	65.10^{C}	64.2^{C}	137.6^{A}	9.5^{B}
2	85.44^{A}	50.50^{A}	84.20^{A}	104.0^{A}	125.5^{B}	12.9^{A}
3	80.75^{B}	37.98^{B}	71.98^{B}	73.50^{B}	135.2^{A}	10.5^{B}

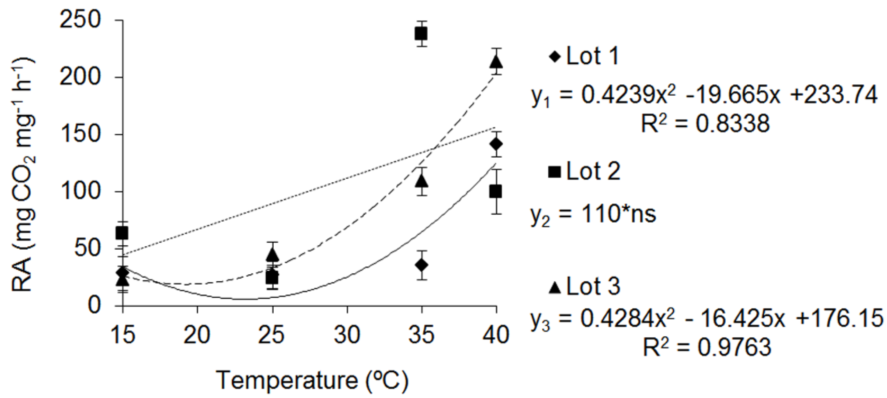

Figure 2. Respiratory activity (RA) of three lots of soybean seeds (*Gycine max* L.), cv. IN 4990RG, exposed for 24 h at temperatures of 15, 25, 35, and 40°C. *ns = straight line equation is not significant at the 5% level of error probability and the bars represent the standard error of four replications.

identifying if the analyzed seeds have the capacity to stand the climate adversities, considering that only the germination tests does not determine the success of performance of seeds in the field (Franzin et al., 2004).

Concerning to electrical conductivity, there was an increase in the values of seeds imbibition water along the incubation periods (three, six and 24 h), as expected, considering that as longer is the imbibition period, greater should be the disruption of the system of membranes, resulting in greater loss of leachates, which was growing until it reached 35°C presenting significant fall when the three lots of seeds were exposed for 24 h in the highest temperatures (35 and 40°C) (Figure 1D).

As noticed, the lot two presented a lower loss of leachates along in all imbibition periods, as well as for the stress temperatures that the seeds were exposed to, demonstrating better vigor if compared to the others lots (Figure 1D) for presenting a better integrity of the cell membrane system and lower concentration of electrolytes released by the seeds during imbibition, showing less disruption of the membrane system (Lopes and Franke, 2010; Tunes et al., 2011), results which match with the previously presented viability and vigor tests (Table 1).

The vigor of the seeds, represented by the measuring of their respiratory activity when exposed to different temperatures presented significant interaction between such temperatures and the lots of soybean seeds, whereas lots one and three presented a decrease of respiratory activity until the temperature of 23.2 and 19.2°C, with 5.67 and 18.72 mg of CO_2 released mg^{-1} of seed h^{-1}, respectively, with growing activity in temperatures above these ones (Figure 2). Differently from these lots, lot two presented a more constant respiratory activity according to the increase of temperature, with average of 110 mg of CO_2 released mg^{-1} of seed h^{-1} (Figure 2). However, the higher respiratory activity evidenced above 25°C for lots one and three, in a general way, did not favor the initial growth of seedlings from these seeds lots (Table 1). Possibly some degenerative changes in seeds from lots one and three which resulted in a rapid consumption of their reserves right at the beginning of the germination process (Patané et al., 2006), which may have reduced the respiratory efficiency, leading to a lower performance in the synthesis process, inciting a decrease in the germination and vigor of the seeds, with direct consequences for the seedlings (Marini et al., 2012). Proving that the respiratory activity is directly related to the germination and vigor of the seeds, this fact was also noticed in other papers about soybean seeds cv. 8000 (Mendes et al., 2009), rice (*Oryza sativa* L.) cv. BRS 7

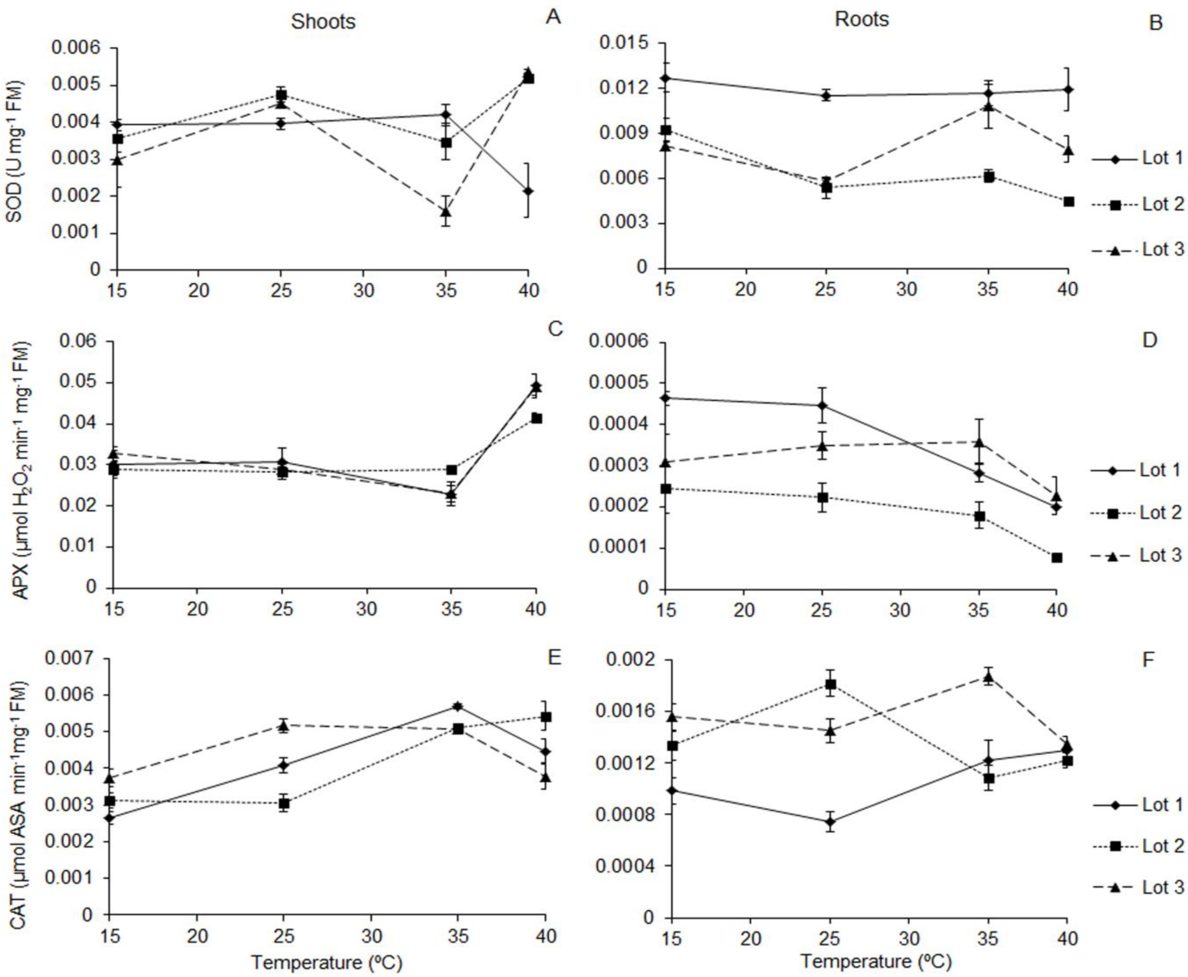

Figure 3. Antioxidant enzymes activity SOD (A and B), APX (C and D) and CAT (E and F) in the shoot and roots of three lots of soybean seeds (*Gycine max* L.), cv. IN 4990RG, exposed for 24 h at temperatures of 15, 25, 35, and 40°C. The bars represent the standard error of four replications. ASA means ascorbic acid and FM means fresh mass.

Taim (Marini et al., 2012) and cowpea (*Vigna unguiculata* L.) (Aumonde et al., 2012), which confirmed the relationship of the results found in this work. Among the enzymes involved in the removal of the ROS formed in adversity situations, the SOD is the first defense line against the oxidative stress (Pompeu et al., 2008; Pereira et al., 2012). The activity of this enzyme in the shoot of soybean seedlings did not show contrast of orthogonal polynomials ($p>0.5708$).

However, it was possible to observe that its most relevant activity for lots two and three after reaching 35°C while for lot one, when it reached such temperature, there was a decrease in the enzyme activity (Figure 3A), indicating that for this lot, probably above this temperature the thermal stress was so severe that the antioxidant defense system could not be activated, unlike in the lots that presented better viability and vigor (lots two and three) the increase of their activity above such temperature showed its ability to stimulated the activity of antioxidant defense system, representing a higher capacity to tolerate the biochemical and physiological changes which the seeds undergo during the beginning of the germination process, which probably reflected in better vigor to face adverse conditions and, thus, provide better survival capacity (Table 1).

In relation to the activity of the ascorbate peroxidase (APX) a quadratic tendency was showed ($p>0.0000001$), with a constant activity for the three lots until 35°C and steep increase from this temperature on (Figure 3C), which can be explained due to the increase of the enzyme activity SOD from that very same temperature, which provides a substrate for the activation of the enzyme APX. The catalase activity (CAT) did not present contrast of orthogonal polynomials ($p>0.02671$), but, in a general way, it was possible to observe a growing activity of the seedlings from the three lots up to temperature of 35°C with subsequent decrease for the lots one and three (Figure 3E), demonstrating that the temperatures above 35°C were so severe for these lots that their antioxidant system could not overcome the stress they were exposed to. The same was not observed for the lot two, in which it was showed that the CAT activity was growing from the temperature 35°C on, corroborating with the viability and vigor analysis that showed for most of the variables the lot two with better physiological performance in relation to lots one and three. Taking into consideration that the CAT has a lower affinity by the H_2O_2, it is responsible for the detoxification of this reactive oxygen species when this molecule is found in excess in the plant cells (Maia et al., 2012), therefore its higher activation in the seedlings from the lot two identifies that this lot has a better capacity to tolerate the stress exposed to.

In the roots, there was quadratic tendency ($p>0.000355$) for the SOD activity, presenting lower activity after reaching 35°C for lots two and three, whereas these results were different from the ones found in the aerial part. However, this differential response to the root part can be explained by the fact that this being the first organ to develop during the germination process and probably that is why it had a higher susceptibility to stress exposed in the seeds which produce these seedlings (Figure 3B).

However, this differential response to the root part can be explained by the fact that this being the first organ to develop during the germination process and probably this is why showed higher susceptibility to stress exposed in the seeds giving rise these seedlings. These reports indicate that the enzymatic activity in seedlings whose seeds were exposed to a stressful factor should take into account the physiological stage and the organ in question.

There was no contrast of orthogonal polynomials ($p>0.076414$) for the activity of the enzyme APX, and it was showed a decrease of its activity in seedling roots which had their seeds exposed for 24 h to temperatures above 35°C for the lots two and three (Figure 3D), possibly due to the fact that the roots had presented higher sensitivity to the stress they were exposed to, jeopardizing the action of the antioxidant defense system in this organ. On the other hand, quadratic tendency was showed for the CAT activity ($p>0.006935$) with increase of its activation when reaching the temperature of 35°C for the lot two, probably due to the fact that this lot presented better physiological quality, being able to activate this enzyme, in order to bypass the stress exposed (Figure 3F).

According to what can be checked, there was a higher activation of the enzymes from the antioxidant defense system in the lot classified by physiological quality standard tests as of higher vigor (lot two) according to the increase of temperature. Similar to the results found, several studies have indicated a relationship between the responses obtained through the activation of the antioxidant defense system with the viability and vigor of seeds and seedlings (Demirkaya et al., 2010; Chauhan et al., 2011; Prodanović et al., 2012). In another work with wheat seeds, it was noticed that the reduction of viability (Lehner et al., 2008). What was also noticed by Maia et al. (2012), in papers about cowpea submitted to saline stress, where observed reduction of roots elongation is the fact that, according to these authors, it may be attributed, at least partially, to the reduction of catalase and ascorbate peroxidase activities.

The analyses of antioxidant enzymes activity enable confirming the results obtained by the viability and vigor tests, which indicated the lot two with higher physiological performance when exposed for only 24 h to different temperature. These results have a great value, since the relationship of the analysis of the activity of the antioxidant defense system with the physiological quality of soybean seeds enable to detecting subtle differences between the lots, enabling greater accuracy in making decisions concerning the discharge of low quality lots in beneficiation unit.

ACKNOWLEDGEMENTS

The authors thank the Programa Nacional de Pós-Doutorado (PNPD), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and Fundação de Amparo à Pesquisa do Estado do Rio Grande do Sul (FAPERGS) for the financial support.

REFERENCES

Aumonde TZ, Marini P, Moraes DM de, Maia M de S, Pedó T, Tilmann MAA, Villela FA (2012). Classificação do vigor de sementes de feijão-miúdo pela atividade respiratória. Interciência 37(1):55-58.

Azevedo RA, Alas RM, Smith RJ, Lea PJ (1998). Response of antioxidant enzymes to transfer from elevated carbon dioxide to air and ozone fumigation, in the leaves and roots of wild-type and a catalase-deficient mutant of barley. Physiol. Plant. 104(2):280-292.

Brasil (2009). Ministério da Agricultura, Pecuária e Abastecimento. Regras para análise de sementes/ Ministério da Agricultura, Pecuária e Abastecimento. Secretaria de Defesa Agropecuária. – Brasília: Mapa/ACS, P. 399.

Carneiro MMLC, Deuner S, Oliveira PV de, Teixeira SB, Sousa CP, Bacarin MA, Moraes, DM de (2011). Atividade antioxidante e viabilidade de sementes de girassol após estresse hídrico e salino. Rev. Bras. Sementes. 33:752-761.

Chauhan DS, Deswal DP, Dahiya OS, Punia RC (2011). Change in storage enzymes activities in natural and accelerated aged seed of wheat (*Triticum aestivum*). Indian J. Agric. Sci. 81:1037-1040.

Demirkaya M, Dietz KJ, Sivritepe Ö (2010). Changes in antioxidant enzymes during ageing of onion seeds. Not. Bot. Hort. Agrobo. 38:49-52.

Deuner C, Maia MS, Deuner S, Almeida A, Meneghello GE (2011). Viabilidade e atividade antioxidante de sementes de genótipos de feijão-miúdo submetidos ao estresse salino. Rev. Bras. Sementes 33(4):711-720.

Franzin SM, Menezes NL, Garcia DC, Wrasse CF (2004). Métodos para avaliação do potencial fisiológico de sementes de alface. Rev. Bras. Sementes 26:63-69.

Forman HJ, Maiorino M, Ursini F (2010). Signaling functions of reactive oxygen species. Biochemistry 49:835-842.

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

Krzyzanowski FC, França-Neto JB, Henning AA (1991). Relato dos testes de vigor disponíveis para grandes culturas. Informativo Abrates 1:15-50.

Lehner A, Mamadou N, Poels P, Come D, Bailly C, Corbineau F (2008). Changes in soluble carbohydrates, lipid peroxidation and antioxidant enzyme activities in the embryo during ageing in wheat grains. J. Cereal Sci. 47:555-565.

Lopes RR, Franke LB (2010). Teste de condutividade elétrica para avaliação da qualidade fisiológica de sementes de azevém (*Lolium multiflorum* L.). Rev. Bras. Sementes 32:123-130.

Machado AA, Conceição AR (2007). WinStat – Sistema de Análise Estatística para Windows versão 2.0. Universidade Federal de Pelotas.

Maguire JD (1962). Speed of germination and in selection and evaluation for seedling emergence and vigor. Crop Sci. 2:176-177.

Marini P, Moraes CL, Marini N, Moraes DM de, Amarante L (2012). Alterações fisiológicas e bioquímicas em sementes de arroz submetidas ao estresse térmico. Rev. Ciênc. Agron. 43:722-730.

Maia JM, Ferreira-Silva SL, Voigt EL, Macedo CEC de, Ponte LFA, Silveira JAG (2012). Atividade de enzimas antioxidantes e inibição do crescimento radicular de feijão caupi sob diferentes níveis de salinidade. Acta Bot. Bras. 26(2):342-349.

Mei Y, Song S (2010). Response to temperature stress of reactive oxygen species scavenging enzymes in the cross-tolerance of barley seed germination. J. Zhejiang Univ. Sci. B 11:965-972.

Mendes CR, Moraes DM, Lima MGS, Lopes NF (2009). Respiratory activity for the differentiation of vigor on soybean seeds lots. Rev. Bras. Sementes 31(2):171-176.

Nakano Y, Asada K (1981). Hydrogen peroxide is scavenged by ascorbate specific peroxidase in spinach cloroplasts. Plant Cell Physiol. 22:867-880.

Pacheco AC, Custódio CC, Machado Neto NB, Carvalho PR, Pereira DN, Pacheco JGE (2007). Germinação de sementes de camomila [*Chamomilla recutita* (L.) Rauschert] e calêndula (*Calendula officinalis* L.) tratadas com ácido salicílico. Rev. Bras. Plantas Med. 9(1):61-67.

Patané C, Cavallaro V, Avola G, D'agosta G (2006). Seed respiration of sorghum [*Sorghum bicolor* (L.) Moench] during germination as affected by temperature and osmoconditioning. Seed Sci. Res. 16(4):251-260.

Pereira JWL, Filho PAM, Albuquerque MB, Nogueira RJMC, Santos RC (2012). Mudanças bioquímicas em genótipos de amendoim submetidos a déficit hídrico moderado. Rev. Ciênc. Agron. 43(4):766-773.

Pompeu GB, Gratão PL, Vitorello VA, Azevedo RA (2008). Antioxidant isoenzyme responses to nickel-induced stress in tobacco cell suspension culture. Sci. Agric. 65(5):548-552.

Prodanović O, Prodanović R, Anović Pristov JB, Mitrović A, Radotić K (2012). Effect of cadmium stress on antioxidative enzymes during the germination of Serbian spruce [*Picea omorika* (Panc.) Purkyne]. Afr. J. Biotechnol. 11:11377-11385.

Skutnik M, Rychter A (2009). Differential response of anti-oxidant systems in leaves and roots of barley subjected to anoxia and post-anoxia. J. Plant Physiol. 166(9):926-937.

Soares AM dos S, Machado OLT (2007). Defesa de plantas: sinalização química e espécies reativas de oxogênio. R. Tróp. Ci. Agr. Biol. 1(1):9-19.

Stanisavljević NS, Nikolić DB, Jovanović ŽS, Samardžić JT, Radović SR, Maksimović VR (2011). Antioxidative enzymes in the response of buckwheat (*Fagopyrum esculentum* Moench) to complete submergence. Arch. Biol. Sci. 63(2):399-405.

Steel RGD, Torrie JH (1980). Principles and Procedures of Statistics: A Biometrical Approach. Second edition Mc Graw-Hill Book Co., New York. P. 633.

Tunes LM de, Pedroso DC, Badinelli PG, Tavares LC, Rufino C de A, Barros ACSA, Muniz MFB (2011). Envelhecimento acelerado em sementes de azevém com e sem solução salina e saturada. Ciênc. Rural 41:33-37.