Antioxidative metabolism: a tool to detect small differences in the vigor of soybean seeds

Juliana de Magalhães Bandeira, Patrícia Marini, Isabel Cristina Gouvea de Borba, Andrea Bicca Noguez Martins, Luciano do Amarante & Dario Munt de Moraes

Universidade Federal de Pelotas, Departamento de Botânica, Programa de Pós-graduação em Fisiologia Vegetal. Campus Capão do Leão, s/n, caixa postal 354, CEP 96010-900, Capão do Leão, Rio Grande do Sul, Brazil. bandeira_jm@hotmail.com, marinipati@gmail.com, isabel.gouvea.borba@gmail.com, amartinsfv@hotmail.com, lucianodoamarante@yahoo.com.br, moraesdm@ufpel.tche.br.

Recebido em 23.IV.2013. Aceito em 25.VIII.2014

ABSTRACT – We aimed to differentiate the vigor of soybean seeds through antioxidant enzyme activity (superoxide dismutase-SOD, ascorbate peroxidase-APX, and catalase-CAT) and relate them to the viability and vigor standard tests to verify if these enzymes detect tenuous differences in seed vigor. The following tests were performed: germination, first germination count, germination speed index, initial growth of seedlings, electrical conductivity, and respiratory and enzymatic activity. It was only possible to differentiate just two lots of soybean seeds by vigor and germination test, considering that viability had higher germination in lot 2 (91%), which did not differ from lot 3 (87.17%) nor from lot 1 (85.17%), while SOD and APX enzymes activity, in the roots, differed amongst the three lots. Thus, it was concluded that the viability and vigor standard tests relate to antioxidant enzyme activity, characterizing this method as an efficient tool for detecting small differences in the vigor of soybean seeds.

Key words: ascorbate peroxidase, catalase, physiological quality, superoxide dismutase

RESUMO – **Metabolismo antioxidativo: uma ferramenta para detectar pequenas diferenças no vigor de sementes de soja**. Objetivou-se diferenciar o vigor de sementes de soja por meio da atividade das enzimas antioxidantes (superóxido dismutase-SOD, ascorbato peroxidase-APX e catalase-CAT) e relacioná-las aos testes padrão de viabilidade e vigor, tendo por finalidade verificar se estas enzimas detectam diferenças tênues no vigor das sementes. Foram realizados os seguintes testes: germinação, primeira contagem e índice de velocidade de germinação, crescimento inicial de plântulas, condutividade elétrica, atividade respiratória e enzimática. Foi possível diferenciar apenas dois lotes de sementes de soja pelos testes de vigor e germinação, sendo que a viabilidade apresentou maior germinação para o lote 2 (91%), não diferindo do lote 3 (87,17%), diferindo do lote 1 (85,17%), enquanto que a atividade das enzimas SOD e APX, nas raízes, diferenciou as sementes em três lotes. Portanto, conclui-se que os testes padrão de viabilidade e vigor têm relação com a atividade das enzimas antioxidantes, caracterizando este método como uma ferramenta eficiente para detectar pequenas diferenças no vigor de sementes de soja.

Palavras chave: ascorbato peroxidase, catalase, qualidade fisiológica, superóxido dismutase

INTRODUCTION

Germination is characterized by a sequence of events that results in the recovery of the metabolic activity of the embryonic axis. This process starts with the rehydration of the seed, where respiration is the first metabolic activity to be rapidly activated to high levels, a few hours after imbibition, accelerating

the metabolism and the activation of respiratory and hydrolytic enzymes (Höfs *et al*. 2004, Marenco & Lopes 2009), which are responsible for the degradation of seed reserves, which results in the growth and development of a new seedling.

Reactive oxygen species (ROS) are produced through the incomplete reduction of O_2 to H_2O consequent to the aerobic metabolic processes of plants, such as respiration and photosynthesis (Skutnik & Rychter 2009), resulting in a partial molecular reduction of oxygen (D'Autréaux & Toledano 2007, Stanisavljević *et al.* 2011). The radicals that are produced by many different enzymatic systems, such as plant NADPH oxidases, also known as respiratory burst oxidase homologues (RBOHs), are the most thoroughly studied enzymatic ROS-generating systems (Marino *et al.* 2012). The ROS produced by RBOHs play essential roles in diverse processes, such as root hair development, stomata closure, and signaling mechanisms in response to abiotic and biotic stimuli (Montiel *et al.* 2013).

ROS have an essential role in several metabolic processes and are potentially dangerous when overproduced, especially when the plant is subjected to an environmental stress, which causes imbalance in cell redox state, commonly referred to as "oxidative stress" that can damage the membrane system leading to reduction of seed vigor (Apel & Hirt 2004, Mei & Song 2010, Carneiro *et al.* 2011) or lead to cell death (Marino *et al.* 2012).

In order to maintain a homeostatic balance, and eliminate these reactive species, plants present antioxidant enzymes 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) that breaks H_2O_2 into H_2O and O_2. These enzymes protect plant cells and their sub-cell divisions from the cytotoxic effects caused by ROS (Soares & Machado 2007).

Since changes in membrane level are not always detectable with germination and vigor analyses, and cell damage leads to loss of viability (Kibinza *et al.* 2006), one enriching alternative would be to assess the activity of antioxidant enzymes as a way to improve the responses of these tests, since these analyses could verify the beginning of possible degenerative changes in the seeds and seedlings through the destabilization of cell membranes, with higher sensitivity.

The study of the activity of enzymes from the antioxidative system has been widely used to assess biotic and abiotic stresses (Soares & Machado 2007) in several species, such as seeds and seedlings of alfalfa (*Medicago sativa* L.) (Cakmak *et al.* 2010), barley (*Hordeum vulgare* L.) (Mei & Song 2010), Jathropa (*Jatropa curcas* L.) (Cai *et al.* 2011) and *Picea omorika* (Pančić) Purk. (Prodanović *et al.* 2012). However, the relationship between the physiological quality of the seeds and the activity of these enzymes has been insufficiently studied.

Based on this, we aimed to differentiate the lots of soybean seeds through the activity of antioxidant enzymes and relate them to the viability and vigor standard tests to check if these enzymes detect tenuous differences in seed vigor.

MATERIALS AND METHODS

Three soybean seed lots, cultivar NA 4990RG obtained from a private company, were used. They were classified through germination standard test (GT %), which were performed according to the Seeds Analysis Rules (Brazil 2009). Four replicates of 200 seeds were used, composed by four subsamples of 50 seeds for each lot, using paper rolls as a special substrate for germination, which were previously humidified with distilled water in a proportion of 2.5 times its initial mass and kept in a germinator at 25ºC. The count was performed on the eighth day after sowing (DAS) and the results were expressed in germination percentage. The first germination count (FGC%) was conducted together with the germination test, being the first count for the soybean, performed on the fifth day after sowing, and the results were expressed in normal seedling percentage. The germination speed index (GSI) was performed according to Maguire (1962), using the germination test, through daily counts from radicle protrusion by the seed tegument, until the number of emerged seedlings remained constant.

The analysis of initial growth of seedlings was performed by measuring the length of the aerial part (APL) and of the roots (RL) of 40 seedlings for each replicate at the end of the germination test. The measurement was performed with a millimeter ruler and the results were expressed in centimeter seedling^{-1}. At the end of the germination test, the dry mass of the aerial part (APDM) and of the roots (RDM) were also measured, obtaining the dry mass of 40 seedlings for each replicate, gravimetrically, in a forced ventilation oven at 70 ± 1ºC, until constant mass with the results expressed in mg seedling^{-1}. For the electrical conductivity test (EC), four replicates for each lot were used with four subsamples of

25 seeds each, a total of 400 seeds per treatment. Initially, the mass of dry seeds was determined, which were placed in flasks with 75 mL of deionized water and kept in germinator at a constant temperature of 25ºC. After incubation periods of three, six and 24h readings in a Digimed CD-21 Condutivimeter were carried out, 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 100g of soybean seeds were placed in a storage flask, imbibed in 80 mL of water for 60 minutes to initiate 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 of CO_2 by milligram of seed per hour (mg CO_2 released mg of $seed^{-1}$ h^{-1}).

The activity 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) was determined by using 400 mg of the aerial part and roots of soybean seedlings, which were collected, separately, at the end of the germination test, macerated with 10% of polyvinylpolypyrolidon (PVPP); enough to avoid oxidation of the material and homogenized in 1.5 mL of the extraction buffer (potassium phosphate 100 mM with pH 7.8, EDTA 0.1 mM and ascorbic acid 20 mM). The sample was centrifuged at 12.000g for 20 minutes at 4ºC and the supernatant was used to determine the enzyme activity based on its fresh mass (FM).

The assessment of the activity of SOD was based on the enzyme capacity to inhibit the photoreduction of tetranitroblue tetrazolium (NBT) (Giannopolitis & Ries 1997) through chemical reaction with potassium phosphate (50 mM, pH 7.8), methionine (14 mM), EDTA (0.1 μM), NBT (75 μM), and riboflavin (2 μM), added by 100 μL of basic 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 & Asada (1981), through the assessment of the ascorbate oxidation rate at 290 nm during two minutes. The potassium phosphate buffer (100 mM, pH 7.0) was incubated at 28-30ºC for 10 minutes and, later, with the addition of ascorbic acid (0.5 mM), H_2O_2 (0.1 mM) and 25 μL of enzymatic extract at the moment of the reading, completing the final volume to 2 mL with distilled water.

The CAT activity was determined according to Azevedo *et al.* (1998) estimated by the decrease in absorbance at 240 nm during two minutes in a chemical reaction containing potassium phosphate (100 mM, pH 7.0) incubated at 28-30ºC for 10 minutes, and, later, with addition of H_2O_2 (12.5 mM) and 25 μL of enzymatic extract at the moment the reading was performed, completing the final volume to 2 mL with distilled water.

The experimental delineation was fully randomized, with four repetitions for each of the analyses made. The data concerning the measured variables was submitted to analysis of variance and the means compared by the Tukey test ($p \leq 0.05$), through the software WinStat 2.0 (Machado & Conceição 2007).

RESULTS AND DISCUSSION

The analysis of variance showed a significant difference at 5% error probability level for the variables: germination percentage, first germination count, electrical conductivity after three, six and 24h of imbibition, respiratory activity and activity of the antioxidant enzymes superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase (APX) in soybean seeds, cv. NA 4990RG (Tab. 1).

The soybean seeds were classified in three lots by the germination test (Tab. 2), where lot 2 presented the highest germination percentage (91%), not differing statistically from lot 3 (87.17%) but differing from lot 1 (85.17%), whereas all were above the minimum Brazilian standard for production and commercialization of soybean seeds (Brazil 2005; Gianluppi *et al.* 2009). Concerning the vigor of the seeds, the lots were different and classified in two levels, one with more vigor (lot 2) and the other with less (lot 1), characterized by the first germination count test (FGC), germination speed index (GSI), aerial part length (APL), aerial part dry mass (APDM), corroborating to the germination test results (Tab. 2). Lot 3 remained in an intermediate level, since the variables APL and APDM did not statistically differ from the lot with higher vigor (lot 2), while according to the variables FGC, GSI and APDM did not differ from the lot with less vigor (lot 1). Root length (RL) and root dry mass (RDM) did not cause differentiation of any of the lots (Tab. 2).

Seed vigor is important as it enables identifying if seeds have the capacity to withstand climatic

Table 1. Analysis of variance of germination test (GT %), first germination count (FGC %), germination speed index (GSI), length and dry mass of aerial part (APL, APDM) and roots (RL, RDM), electrical conductivity (EC) after three, six and 24 hours of imbibition, seed respiratory activity (RA) and antioxidant enzyme SOD, CAT and APX activity in aerial part (AP) and roots (R) of soybean seedlings, cultivar NA 4990RG, at the end of the germination test.

Source of variation	DF	F value								
		GT %	FGC%	GSI	APL	RL	APDM	RDM	EC 3h	EC 6h
Lots	2	7.84*	13.59*	10.24^{ns}	10.93^{ns}	3.65^{ns}	6.99^{ns}	3.39^{ns}	15.96*	61.98*
VC (%)	-	2.42	3.64	6.76	5.92	3.96	4.37	16.51	6.99	5.28

Source of variation	DF	F value							
		EC 24h	RA	SOD		APX		CAT	
				AP	Root	AP	Root	AP	Root
Lots	2	37.08*	24.82*	11.26^{ns}	165.82*	59.47^{ns}	145.68*	13.66*	0.34^{ns}
VC (%)	-	7.99	18.49	17.97	4.31	20.88	7.70	21.02	38.49

* Significant at 5%, by F-test ($p \leq 0.05$). ^{ns} Not significant at 5% level, by F-test ($p \leq 0.05$). DF = Degrees of freedom. CV = Coefficient of variation.

adversities, considering that the germination test alone does not assure success in the performance of seeds in the field (Franzin *et al.* 2004). Among these, the electrical conductivity test is very important to check the integrity of the cell membrane system (Lopes & Franke 2010, Tunes *et al.* 2011).

Electrical conductivity, in three hours of imbibition, enabled separating the soybean seeds in different lots, in which lot one had a lower vigor in relation to lots two and three (Fig. 1). However, after six and 24h of imbibition, it was possible to observe an increase in the amount of leachates released by the seeds, a result which enabled classifying the seeds in lots one and two with higher and lower vigor, respectively, besides separating lot three as intermediate compared to the other two (Fig. 1).

Respiratory activity also enabled distinguishing soybean seed lots in two levels, one with high vigor (lots 2 and three) and one with low vigor (lot 1) (Fig. 2A). These results match the ones found for soybean cv. 8000 (Mendes *et al.* 2009), sunflower (Dode *et al*. 2012) and cowpea (*Vigna unguiculata* L.) seeds

Table 2. Germination test (GT %), first germination count (FGC %), germination speed index (GSI), length and dry mass of aerial part and roots of three soybean lots.

Lot	GT (%)	FGC (%)	GSI	Length (cm)		Dry mass (mg)	
				Aerial part	Root	Aerial part	Root
1	[illegible]	69.77 (±0.24) b	50.22 (±0.59) b	7.16 (±0.23) b	8.16 (±0.12) a	123.1 (±0.40) b	11.3 (±0.80) a
2	91.00 (±1.76) a	78.80 (±2.30) a	60.68 (±3.09) a	8.60 (±0.23) a	8.75 (±0.22) a	137.5 (±4.40) a	15.4 (±1.0) a
3	87.17 (±0.24) ab	70.93 (±0.08) b	50.96 (±0.20) b	8.46 (±0.25) a	8.68 (±0.13) a	127.0 (±1.80.) ab	13.7 (±1.6) a

Means followed by standard error and same letter, in the column, do not differ by the Tukey test ($p \leq 0.05$).

(Aumonde *et al.* 2012), confirming the efficiency of the method in separating lots into different vigor levels.

The activity of the antioxidant enzymes was more sensitive than the other viability and vigor tests differing soybean seeds into three lots. The antioxidant enzymes SOD and APX presented higher activity in the roots than in the aerial part of the soybean seedlings at the end of the germination test (Figs. 2B and 2C, respectively), while the opposite was shown for the enzyme CAT (Fig. 2D). This shows that during the germination process higher accumulation of H_2O_2 occurs in the aerial part than in the roots of the seedlings, because CAT is responsible for detoxification of the hydrogen peroxide in excess in plant cells (Maia *et al.* 2012).

In the roots, the activity of SOD and APX was higher in lot 1, lower in lots 2 and 3 (Figs. 2B and

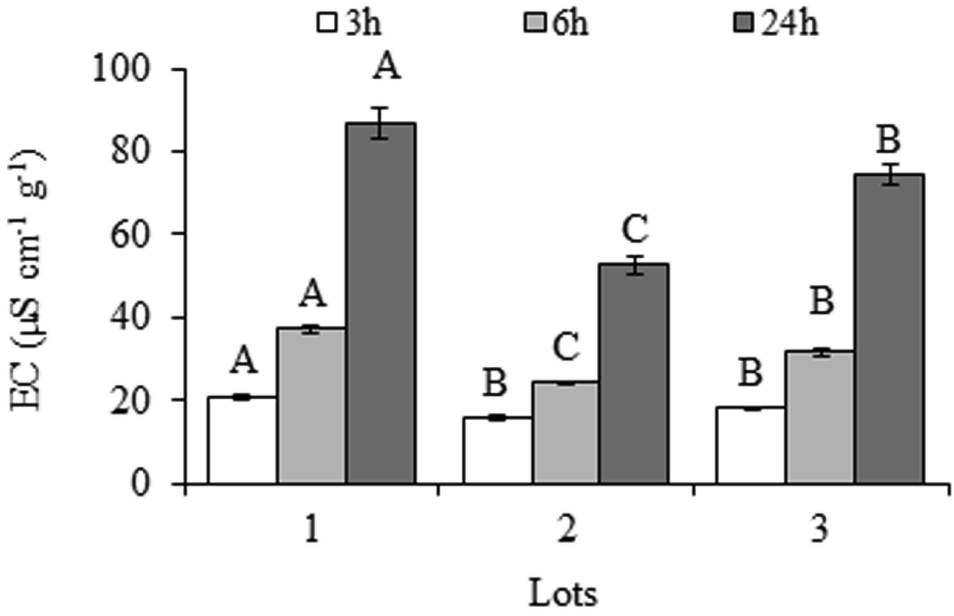

Fig. 1. Electrical conductivity (EC) after three, six and 24 hours of imbibition of the three lots of soybean seeds. Distinct letters differ from each other in the different imbibition periods by the Tukey test ($p \leq 0.05$). Bars represent mean standard error.

2C), enabling differentiating the soybean seeds in the three lots in terms of vigor. The CAT activity, on the other hand, did not show statistical difference (Fig. 2D). Concerning the activity of these enzymes in the aerial part of the seedlings, there was a higher activity in the lot with greater germination (lot 2) and lower activity in lot 1, while lot 3 was ranked in an intermediate position compared to the other two lots. According to Deuner *et al.* (2011), the balance between the production and removal of intracellular hydrogen peroxide is directly linked to the capacity of plants keeping high activity of SOD, CAT and APX, as well as in some related studies with abiotic stress, the decrease of antioxidant enzyme activity is related to seeds and seedlings with lower viability and vigor (Demirkaya *et al.* 2010, Chauhan *et al.* 2011, Prodanović *et al.* 2012).

Considering these results, we can infer that the lot with the best quality (lot two) (Fig. 2) showed higher activity of antioxidant enzymes in the aerial part and consequently greater ability to trigger the defense system, showing a better capacity to overcome adversities. Studies that have been developed analyzing the activity of these antioxidant enzymes, submitting different cultures to abiotic stress, have shown higher activity of SOD in more tolerant corn cultivars, both in their leaves (Hernández *et al.* 2000) and in their roots (Shalata *et al.* 2001). Nevertheless, Azevedo Neto *et al.* (2006) observed a decrease in the activity of SOD in the roots of a tolerant cultivar when submitted to saline stress, with a strong decrease of this enzyme in the sensitive cultivar. Besides this, in wheat seeds, it was noticed that the reduction of germination is associated with the accumulation of H_2O_2 concomitant to a progressive decrease of CAT and of SOD activity (Lehner *et al.* 2008).

The superoxide dismutase – SOD has the ability to catalyze the dismutation of the superoxide radical in H_2O_2 and O_2, and H_2O_2 is a regulator of a multitude of physiological processes like acquiring resistance, cell wall strengthening, senescence, phytoalexin production, photosynthesis, stomatal opening, and the cell cycle (Petrov & Breusegem 2012). Thus, we believe that the higher activity of SOD represents a higher capacity to tolerate biochemical and physiological changes that the seeds undergo during the beginning of the germination process, showing higher vigor to adverse conditions and then, a higher survival capacity.

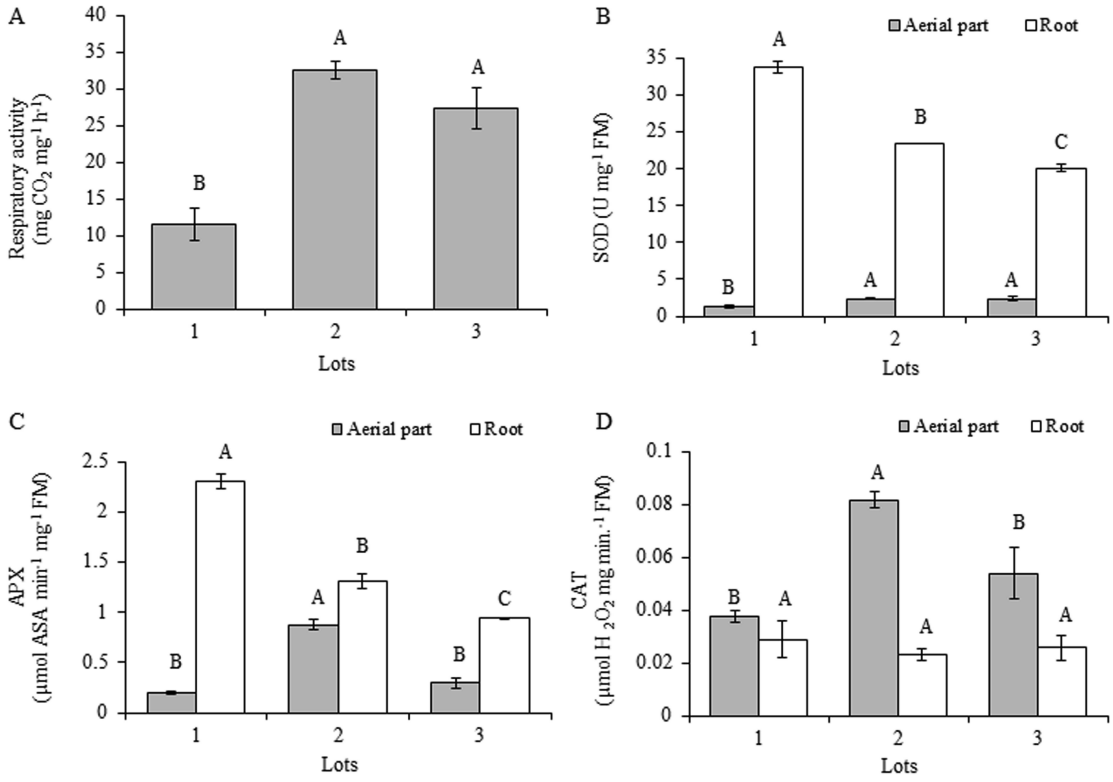

Fig. 2. Respiratory activity of seeds (A) and antioxidant enzymes SOD (B), APX (C) and CAT (D) activity based on fresh mass (FM) in the aerial part and roots of three lots of soybean seeds. Distinct letters differ from each other by Tukey test ($p \leq 0.05$). Bars represent means standard error.

CONCLUSION

The viability and vigor standard tests are related with antioxidant enzyme activity, characterizing this method as an efficient tool to detect small differences in the vigor of soybean seeds.

ACKNOWLEDGEMENTS

The authors thank the Programa Nacional de Pós-Doutorado–Coordenação de Aperfeiçoamento de Pessoal de Nível Superior /Fundação de Amparo à Pesquisa do Estado do Rio Grande do Sul for the financial support. We are grateful to Dr. H. D. Laughinghouse IV and R.M. Fischer for reviewing the English text.

REFERENCES

Apel, K. & Hirt, H. 2004. Reactive oxygen species: Metabolism, oxidative stress and signal transduction. Annual Review of Plant Biology 55:373-399.

Aumonde, T.Z., Marini, P., Moraes, D.M. de, Maia, M. de S., Pedó, T., Tilmann, M.A.A. & Villela, F.A. 2012. Classificação do vigor de sementes de feijão-miúdo pela atividade respiratória. Interciência 37(1):55-58.

Azevedo Neto, A.D., Prisco, J.T., Enéas-Filho J., Abreu, C.E.B. & Gomes Filho, E. 2006. Effect of salt stress on antioxidative enzymes and lipid peroxidation in leaves and roots of salt-tolerant and salt-sensitive maize genotypes. Environmental and Experimental Botany 56(1):87-94.

Azevedo, R.A., Alas, R.M., Smith, R.J. & Lea, P.J. 1998. Response from elevated carbon dioxide to air and ozone fumigation in leaves and roots of wild type and a catalase-deficient mutant of barley. Physiologia Plantarum 104:280-292.

Brasil. 2005. Ministério da Agricultura, Pecuária e Abastecimento. Sistema Integrado de Legislação. Instrução Normativa nº 243 de 20/12/2005, Anexo IX: Padrões para produção e comercialização de sementes de soja. Available in: http://www.agricultura.pr.gov.br/arquivos/File/PDF/padroes_soja.pdf. Access in 20.03.2013.

Brasil. 2009. Ministério da Agricultura, Pecuária e Abastecimento. Regras para análise de sementes. Ministério da Agricultura, Pecuária e Abastecimento. Secretaria de Defesa Agropecuária. Brasília, DF: Mapa/ACS. 395 p.

Cai, F., Lan-Ju, M., Xiao-Long A., Shun G., Lin, T. & Fang, C. 2011. Lipid peroxidation and antioxidant responses during seed germination of *Jatropa curcas*. Journal of Agriculture and Biology 13:25-30.

Cakmak, I., Atici O., Agar G. & Sunar, S. 2010. Natural aging-related biochemical changes in alfalfa (*Medicago*

sativa L.) seeds stored for 42 years. International Research Journal of Plant Science 1:1-6.

Carneiro, M.M.L.C., Deuner S., Oliveira, P.V. de, Teixeira S.B., Sousa C.P., Bacarin, M.A. & Moraes, D.M. de. 2011. Atividade antioxidante e viabilidade de sementes de girassol após estresse hídrico e salino. Revista Brasileira de Sementes 33:752-761.

Chauhan, D.S., Deswal, D.P., Dahiya, O.S. & Punia, R.C. 2011. Change in storage enzymes activities in natural and accelerated aged seed of wheat (*Triticum aestivum*). Indian Journal of Agricultural Sciences 81:1037-1040.

D'Autréaux, B. & Toledano, M.B. 2007. ROS as signalling molecules: mechanisms that generate specificity in ROS homeostasis. Nature Reviews Molecular Cell Biology 8:813-824.

Demirkaya, M., Dietz, K.J. & Sivritepe, Ö. 2010. Changes in antioxidant enzymes during ageing of onion seeds. Notulae Botanicae Horti Agrobotanici 38:49-52.

Deuner, C., Maia, M. de S., Deuner, S., Almeida, A. da S. & Meneghello, G.E. 2011. Viabilidade e atividade antioxidante de sementes de genótipos de feijão-miúdo submetidos ao estresse salino. Revista Brasileira de Sementes 33:711-720.

Dode, J.S.,Meneghello, G.E., Moraes, D.M. & Peske, S.T. 2012. Teste de respiração para avaliar a qualidade fisiológica de sementes de girassol. Revista Brasileira de Sementes 34:686-691.

Franzin, S.M., Menezes, N.L., Garcia, D.C. & Wrasse, C.F. 2004. Métodos para avaliação do potencial fisiológico de sementes de alface. Revista Brasileira de Sementes 26:63-69.

Gianluppi, V., Gianluppi, D., Marsaro Jr., A.L., Zilli, J.E., Nechet, K. de L., Barbosa, J.F. & Mattioni, J.A.M. 2009. Cultivo de Soja no Cerrado de Roraima: Tecnologia de sementes, Sistemas de Produção - 1**,** 1. ed., ISSN 2177-2169. Versão Eletrônica. Available in: http://sistemasdeproducao.cnptia.embrapa.br/FontesHTML/Soja/CultivodeSojanoCerradodeRoraima/semente.htm. Access in 20.03.2013.

Giannopolitis, C.N. & Ries, S.K. 1997. Superoxide dismutases. I. Occurrence in higher plants. Plant Physiology 59:309-314.

Hernández, J.A., Jiménez, A., Mullineaux, P. & Sevilla, F. Tolerance of pea (*Pisum sativum* L.) to long-term salt stress is associated with induction of antioxidant defense. 2000. Plant Cell Environment 23:853-862.

Höfs, A., Schuch, L.O.B., Peske, S.T. & Barros, A.C.S.A. 2004. Efeito da qualidade fisiológica das sementes e da densidade de semeadura sobre o rendimento de grãos e qualidade industrial em arroz. Revista Brasileira de Sementes 26:55-62.

Kibinza, S., Vinel, D., Come, D., Bailly, C. & Corbineau, F. 2006. Sunflower seed deterioration as related to moisture content during ageing, energy metabolism and active oxygen species scavenging. Physiologia Plantarum 12:496-506.

Krzyzanowski, F.C., França-Neto, J.B. & Henning, A.A. 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. Journal of Cereal Science 47:555-565.

Lopes, R.R. & Franke, L.B. 2010. Teste de condutividade elétrica para avaliação da qualidade fisiológica de sementes de azevém (*Lolium multiflorum* L.). *Revista Brasileira de Sementes 32:123-130.*

Maguire, J.D. 1962. Speed of germination-aid in selection aid evolution for seedling emergence and vigor. Crop Science 2(2):176-177.

Machado, A.A. & Conceição, A.R. 2007. WinStat - Sistema de Análise Estatística para Windows. Versão 2.0. Universidade Federa de Pelotas, RS.

Maia, J.M., Ferreira-Silva, S.L., Voigt, E.L., Macedo, C.E.C. de, Pontes, L.F.A & Silveira, J.A.G. 2012. Atividade de enzimas antioxidantes e inibição do crescimento radicular de feijão caupi sob diferentes níveis de salinidade. Acta Botanica Brasilica 26(2):342-349.

Mendes, C.R., Moraes, D.M., Lima, M.G.S. & Lopes, N.F. 2009. Respiratory activity for the differentiation of vigor on soybean seeds lots. Revista Brasileira de Sementes 31(2):171-176.

Montiel, J., Arthikala, M-K & Quinto, C. 2013. *Phaseolus vulgaris* RbohB functions in lateral root Development. Plant Signaling & Behavior 8(1):144-146.

Marenco, R.A. & Lopes, N.F. 2009. Fisiologia Vegetal: Fotossíntese, Respiração, Relações Hídricas e Nutrição Mineral. 3ed. Universidade Federal de Viçosa: Ed. UFV. 486 p.

Marino, D., Dunand, C., Puppo, A. & Pauly, N. 2012. A burst of plant NADPH oxidases. Trends in Plant Science 17(1):9-15.

Mei, Y. & Song, S. 2010. Response to temperature stress of reactive oxygen species scavenging enzymes in the cross-tolerance of barley seed germination. Journal of Zhejiang University-SCIENCE B (Biomedicine & Biotechnology) 11:965-972.

Mendes, C.R., Moraes, D.M., Lima, M.G.S. & Lopes, N.F. 2009. Respiratory activity for the differentiation of vigor on soybean seeds lots. Revista Brasileira de Sementes 31(2):171-176.

Nakano, Y. & Asada, K. 1981. Hydrogen peroxide is scavenged by ascorbate-specific peroxidases in spinach chloroplast. Plant Cell Physiology 22:867-880.

Petrov, V.D. & Breusegem, F.V. 2012. Hydrogen peroxide - a central hub for information flow in plant cells. AoB Plants 4:1-13.

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

Shalata, A., Mittova, V., Volokita, M., Guy, M. & Tal, M. 2001. Response of the cultivated tomato and its

wild sal-tolerant relative *Lycopersicon pennellii* to salt-dependent oxidative stress: the root antioxidative system. Physiologia Plantarum 112:487-494.

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

Soares, A.M.S. & Machado, O.L.T. 2007. Defesa de plantas: Sinalização química e espécies reativas de oxigênio. Revista Trópica Ciências Agrárias e Biológicas 1(1):9-19.

Stanisavljević, N.S., Nikolić, D.B., Jovanović, Ž.S., Samardžić, J.T., Radović, S.R. & Maksimović, V.R. 2011. Antioxidative enzymes in the response of buckwheat (*Fagopyrum esculentum* Moench) to complete submergence. Archives of Biological Sciences 63(2):399-405.

Tunes, L.M. de, Pedroso, D.C., Badinelli, P.G., Tavares, L.C., Rufino, C. de A., Barros, A.C.S.A. & Muniz, M.F.B. 2011. Envelhecimento acelerado em sementes de azevém com e sem solução salina e saturada. Ciência Rural 41:33-37.