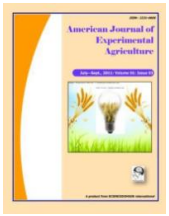

American Journal of Experimental Agriculture
7(2): 70-77, 2015, Article no.AJEA.2015.107
ISSN: 2231-0606

SCIENCEDOMAIN *international*
www.sciencedomain.org

Respiration and Antioxidant Enzymes Activity in Watermelon Seeds and Seedlings Subjected to Salt and Temperature Stresses

Bárbara França Dantas[1*], Rita de Cássia Barbosa da Silva[2], Renata Conduru Ribeiro[1] and Carlos Alberto Aragão[3]

[1]Brazilian Corporation of Agricultural Research, Embrapa Semi-Arid, Petrolina, Pernambuco State, Brazil.
[2]Federal Rural University of Pernambuco State- UFRPE, Serra Talhada Campus, Serra Talhada, Pernambuco State, Brazil.
[3]Department of Technology and Social Sciences- DTCS, Bahia State University, Juazeiro, Bahia State, Brazil.

Authors' contributions:

This work was carried out in collaboration between all authors. Authors BFD and CAA designed the study, advised the work, reviewed the experimental design, statistical analysis and reviewed and contributed in all drafts of the manuscript. Authors RCBS and RCR wrote the protocol, managed the analyses of the study and wrote the first draft of the manuscript. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/AJEA/2015/15749
Editor(s):
(1) Masayuki Fujita, Department of Plant Sciences, Kagawa University, Japan.
Reviewers:
(1) Anonymous, Turkey.
(2) Anonymous, Turkey.
Complete Peer review History: http://www.sciencedomain.org/review-history.php?iid=916&id=2&aid=7996

Original Research Article

Received16th December 2014
Accepted 19th January 2015
Published 2nd February 2015

ABSTRACT

This research aimed to evaluate the effect of salt and temperature stress on water uptake and respiration of watermelon seeds during germination process and to quantify changes in the activity of the antioxidant enzymes ascorbate peroxidase (APX), catalase (CAT) and glutathione-S transferase (GST) involved in protection against reactive oxygen species. The research was performed at the Seed Analysis Laboratory (LASESA) of Embrapa Semi-Arid, Petrolina, Pernambuco State, Brazil, from september to december 2011. The experimental design was completely randomized in a factorial 2x3 (cultivars x stress conditions) for respiration evaluation, 3x4

**Corresponding author: E-mail: Barbara.dantas@embrapa.br;*

(cultivars x electrical conductivities) for salt stress assays and 3x3 (cultivars x temperature) for temperature stress assays. The data were submitted to the mean test and evaluated using the standard errors of means. Respiration was measured by CO_2 releases by watermelon seeds cv. cv. Crimson Sweet and Charleston Gray evaluated by an infrared gas analyzer, from 0-120 hours of seed imbibition in different environmental conditions (0 dSm^{-1}/25°C, 0 dSm^{-1}/30°C, 4 dSm^{-1}/25°C). The antioxidant enzymes ascorbate peroxidase (APX), catalase (CAT) and glutathione-S transferase (GST) were evaluated in cvs. Crimson Sweet, Charleston Gray and Fairfax seeds and seedlings after five days imbibition in different electrical conductivities (0, 4 and 6 dSm^{-1}) or temperatures (20, 25, 30°C). Crimson Sweet seed respiration rate was increased with increasing temperature, salinity however did not influence the respiration of seeds until the radicle protrusion. The activities of APX and CAT enzymes were antagonistically influenced stresses. The activity of GST was not altered with increased electrical conductivity, however high temperatures led to increase of its activity in watermelon seedlings. The antioxidant detoxification system was activated when imposing temperature and salt stress in all studied watermelon cultivars. Different cultivars of watermelon show different adaptation to salt and temperature stress.

Keywords: Climate change; heat; NaCl; cucurbit; metabolism.

1. INTRODUCTION

The Fifth Assessment Report of the Intergovernmental Panel on Climate Change [1] indicates that there is a very high confidence that anthropogenic greenhouse gas emissions have caused global warming. This warming causes greater atmospheric dynamics, accelerating the hydrologic and energy cycles in the atmosphere, which consequently can affect the frequency and intensity of extreme climatic events. These climate projections, released by the IPCC, have shown drought scenarios and extreme rainfall events in large areas of the planet. At Brazil, the most vulnerable region to climate change, from a social and agricultural point of view, is the countryside of Brazilian Northeast, where the climate is semi-arid, BSWh' [2] and vegetation is xerophytic corresponding to Caatinga biome. Rainfall reductions appear in most global IPCC models [3], as well as a warming of up to 3-4°C for the second half of the XXI century. This leads to 15-20% flow rate reductions of São Francisco River (main water resource in this region), as well as, dams level reduction and increased salinization of soils and wells [4,5]. This may result in plants temperature, water and salt stresses.

Abiotic stresses such as high temperatures, water deficit and salinity, individually or associated lead to a series of morphological, physiological, biochemical and molecular changes that adversely affect plant growth and productivity [6]. In seeds and seedlings of many species temperature and/or salt stresses may cause a decrease of water uptake by seeds [7], germination percentage and/or speed [8,9,10,11], changes in reserve mobilization [12], altered antioxidant enzymes activity [13] and different gene expression [14,15]

Drought, salinity, extreme temperatures and oxidative stress are often interrelated and can cause similar cellular damage. Stress in seeds and seedlings causes changes in growth conditions which affects homeostasis in cells metabolism. Thus cells require an adjustment of metabolic pathways, in order to acquire a new state of homeostasis, resulting in acclimation or tolerance [16,17]. Enzymes such as catalase (CAT, E.C. 1.11.1.6) and ascorbate peroxidase (APX, EC 1.11.1.11) and glutathione-S transferase (GST,EC 2.5.1.13) as well as non-enzymatic compounds comprise effective antioxidant systems to protect against oxidative stress [18].

This study aimed to evaluate the imbibition, respiration and changes in the activity of the antioxidant enzymes ascorbate peroxidase, catalase and glutathione-S-transferase involved in protection against reactive oxygen species (ROS) in salt stress and temperature.

2. MATERIALS AND METHODS

The research was performed at the Seed Analysis Laboratory (LASESA) of Embrapa Semi-Arid, Petrolina, Pernambuco State, Brazil. Three watermelon cultivars were studied Charleston Gray, Fairfax and Crimson Sweet of 2010/2011 harvest seeds.

2.1 Experimental Design and Treatments

Three assays were performed in a completely randomized experimental design and in factorial scheme. For seed respiration evaluation a factorial scheme 2x3 (cultivars x environmental conditions), with two replications of 50 seeds, was arranged with two watermelon cvs. Crimson Sweet and Charleston Gray subjected to three environmental conditions (EC), combining different electrical conductivities and temperatures, which were (1) 0 dSm^{-1} EC at 25°C, (2) 0 dSm^{-1} EC at 30°C, (3) 4 dSm^{-1} EC and 25°C.

Antioxidant enzyme activity was evaluated in three watermelon cvs. Crimson Sweet, Charleston Gray and Fairfax seeds and seedlings after five days imbibition in different electrical conductivities (0, 4 and 6 dSm^{-1})in a 3x4 (cultivars x electrical conductivities) factorial scheme for salt stress assay and in differenttemperatures (20, 25, 30°C) in a 3x3 (cultivars x temperature) factorial scheme for temperature stress assays. All assays were performed in triplicates of 50 seeds.

The data obtained in all three assays were submitted to the mean test and evaluated using the standard errors of means.

2.2 Seeds Imbibition and Respiration

Two replications of 50 seeds of watermelon cv. Charleston Gray and Crimson Sweet, were sowed onto rolls of germitest paper soaked in distilled water in a volume equivalent to 2,5 times the paper weight and incubated to germinate in different environmental conditions (0 dSm^{-1}/25°C, 0 dSm^{-1}/30°C, 4 dSm^{-1}/25°C). Quiescent seeds, as well as imbibed for 6, 24, 48, 72, 96 and 120 hours, were weighted evaluation of weight gain through water uptake. Respiration was measured by CO_2 releases by watermelon seeds in a 500 cm^3 volume chamber linked to a infrared gas analyzer, model IRGA LI-6200 (Li-Cor, Lincoln, Nebraska, USA). An average of 15 measurements, performed at each 5 minutes, was divided by the dry weight of the seeds.

2.3 Antioxidant Enzymes Assay

Antioxidant enzymes activity was assayed in seeds of watermelon cv. Crimson Sweet, Charleston Gray and Fairfax. For determination of seeds antioxidant enzymes response to salt stress, triplicates of 50 seeds were sowed onto rolls of germitest paper soaked with different NaCl solutions with electrical conductivity of 0, 4 and 6 $dS.m^{-1}$ [18,19] in a volume equivalent to 2,5 times the substrate paper weight and incubated at 25°C for germination.

For determination of the antioxidant enzymes response to temperature stress, triplicates of 50 seeds were sowed onto rolls of germitest paper soaked in distilled water in a volume equivalent to 2,5 times the substrate paper weight and incubated at 20, 25 and 30°C for germination.

A minimum of 20 seeds and seedlings per replication were collected in liquid nitrogen at the fifth day after sowing for subsequent extraction and quantification of enzyme activity. Catalase (CAT, E.C. 1.11.1.6) and ascorbate peroxidase (APX, EC 1.11.1.11) and glutathione-S transferase (GST,EC 2.5.1.13) were extracted and their activity in germinating watermelon seed was quantified [13]. All assays were performed in triplicates of 50 seeds.

3. RESULTS AND DISCUSSION

Watermelon seeds imbibition curve showed a rapid initial water uptake (phase I), that in few hours, reached germination phase II, also called lag phase, due to a plateau in water uptake. Seeds initial radicle protrusion (1%) started at 72 hours (arrow) and an increase in water uptake (phase III) started after 96 hours imbibition (Fig. 1A and 1B). Both cultivars showed a triphasic imbibition curve, as expected, but cv. Charleston Gray seeds showed higher amount of water uptake (Fig. 1B).

Water uptake was very little influenced by mild stresses imposed to watermelon seeds (Fig. 1). Although environmental stresses did affect radicle protrusion in watermelon cultivars Crimson Sweet, Charleston Gray nor Fairfax (data not shown), Crimson Sweet seeds showed a slight increase in water uptake when subjected to supra optimal temperature (Fig. 1A), as well as a high increase in CO_2 release, due to respiration (Fig. 1C).

Seeds respiration followed the imbibition curve, however cv. Crimson Sweet seeds respiration was widely affected by temperature increase, mainly during germination phase II (Fig. 1C). Charleston Gray seeds water uptake and respiration was less influenced by temperature and salt stress than Crimson Sweet seeds (Fig. 1).

Seeds of both cultivars subjected to stressful conditions during 0, 6, 24, 48, 72, 96 and 120 hours showed progress of respiratory activity. According to results, it can be estimated that respiration is almost zero when the quiescent seeds have low moisture content, such as 9,54% and 8,75% in Charleston Gray and Crimson Sweet seeds respectively. The seeds respiration enhances quickly reaching high values upon soaking.

Watermelon seeds of the three studied cultivars showed around 100% radicle protrusion in all temperatures to which they were submitted, however seedling development was hindered by 30°C (data not shown) due to a mild temperature stress. A major hydrogen peroxide detoxifying system in plant cells is the ascorbate-glutathione cycle, in which, ascorbate peroxidase (APX) enzymes play a key role catalyzing the conversion of H_2O_2 into H_2O, using ascorbate as a specific electron donor. The APX responses are directly involved in the protection of plant cells against adverse environmental conditions [20]. Electrical conductivity and temperature affected APX activity in five days old watermelon seedlings. It is noted that APX activity in cotyledons and embryonic axis, had decreased with increasing salinity levels for all cultivars studied (Figs. 2A and 2B). Cotyledons and embryonic axis of all watermelon cultivars showed the same response to temperature stress, with higher activity in optimum germination conditions and decrease according to stress imposition (Figs. 2C and 2D). These results demonstrate the existence of differential regulation in gene expression [14, 15] and correlated high levels of antioxidant enzymes, such as APX, with the increase of heat stress tolerance in cucumber plants [21].

Environmental stresses cause either enhancement or depletion of CAT activity, depending on the intensity, duration, and type of the stress [22,23,24]. In general, stresses that reduce the rate of protein turnover also reduce CAT activity. Stress analysis revealed increased susceptibility of CAT-deficient plants to paraquat, salt and ozone, but not to chilling [25]. In salt stress there is a maximum activity of catalase (CAT) at cotyledons and embryos at 4.0 $dS.m^{-1}$, in all watermelon cultivars studied. Higher salt concentrations inhibited CAT activity (Figs. 3A and 3B). Increasing temperature enhanced CAT activity in cotyledons and embryos of all watermelon cultivars, although Crimson sweet seedlings showed much higher CAT activity than other two cultivars (Figs. 3C, D).

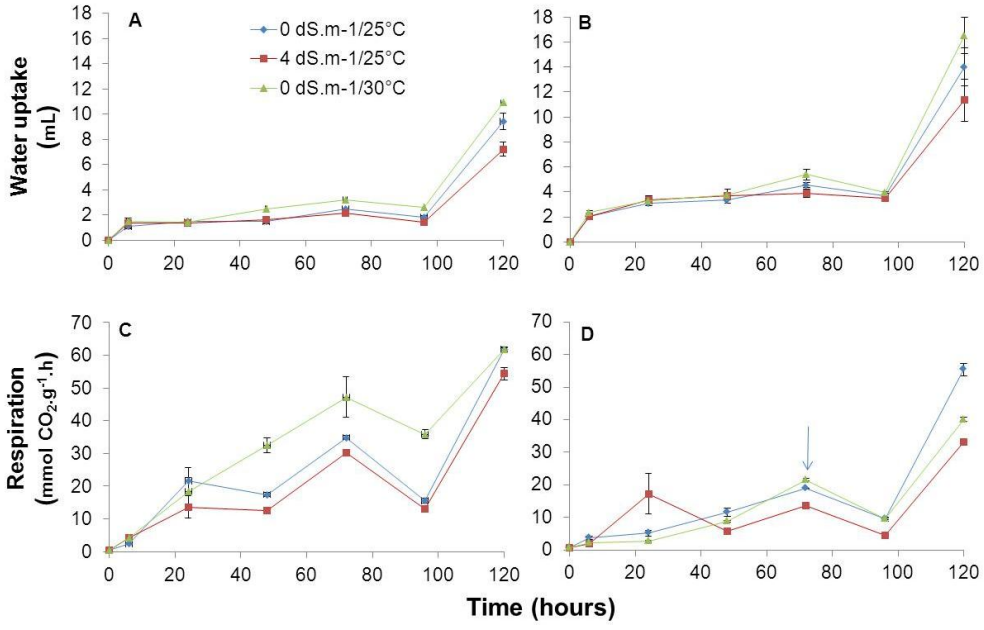

Fig. 1. Water uptake (A, B) and respiration (C,D) of watermelon seeds cultivars. Crimson Sweet (A, C) and Charleston Gray (B,D) subjected to different conditions of salt and temperature stress

Vertical bars represent the standard error of means

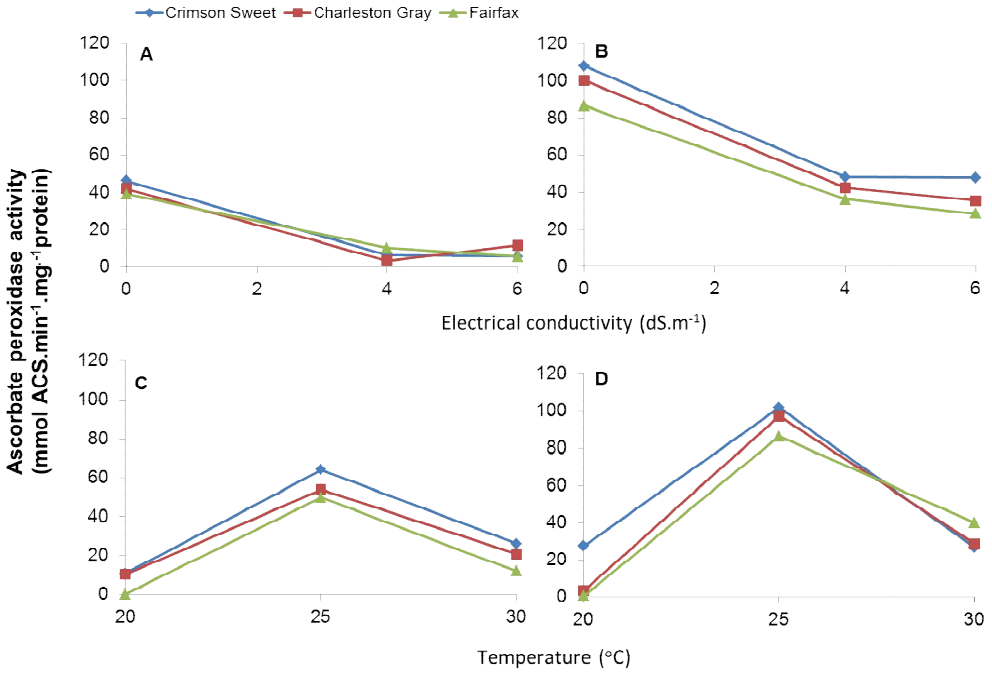

Fig. 2. Ascorbate peroxidase activity in cotyledons (A,C) and in embryonic axis (B,D) of five days germinated watermelon seeds cultivars Crimson Sweet, Charleston Gray and Fairfax, subjected to different conditions of salt and temperature stress

Vertical bars represent the standard error of means

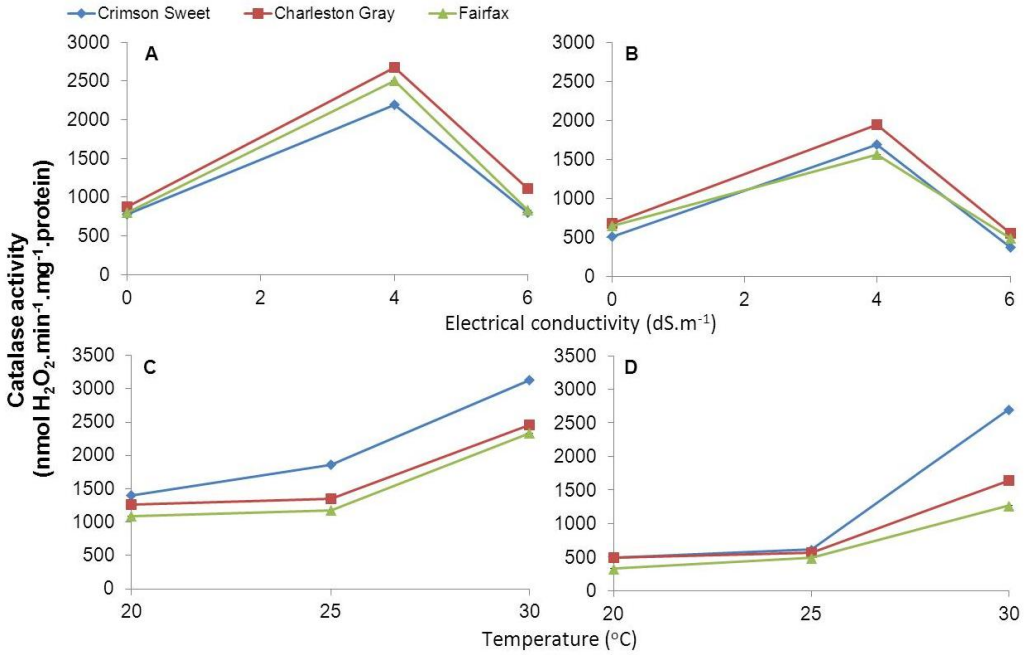

Fig. 3. Catalase (CAT) activity in cotyledons (A,C) and in embryonic axis (B,D) of five days germinated watermelon seeds cultivars Crimson Sweet, Charleston Gray and Fairfax, subjected to different conditions of salt and temperature stress

Vertical bars represent the standard error of means

GSTs are a group of soluble proteins found in the cytosol, are regulated in vivo by reactive oxygen species (ROS), whose main function is to catalyze the conjugation of reduced glutathione (GSH) with a wide variety of cytotoxic molecules produced as a result of oxidative stress [26]. In this study, temperature influenced GST activity, which showed similar behavior for all studied cultivars and organs and was increased at 30°C (Figs. 4C and 4D), however, none of the cultivars seemed to respond to salt stress by detoxifying mechanism by GST (Figs. 4A and 4B).

It is well known that the respiratory process is the first metabolic activity rapidly activated after seed imbibition, initiating the germination process [27]. Thus, increased release of CO_2 characterizes integrity of cellular membranes, including mitochondrial and is indicative of higher seed and seedling ability of reorganization of cell systems and therefore greater vigor. There are many potential sources of ROS in plants, some are reactions involved in normal metabolism, such as photosynthesis and respiration. This makes ROS unavoidable byproducts of aerobic metabolism. Other sources of ROSs belong to pathways enhanced during abiotic stresses, such as drought, high temperature and salinity [16].

During the germination of seeds, several enzymes are involved in metabolic reactions of synthesis and degradation of molecules. Also a part of the germination process, during imbibition occurs the activity of free radical scavengers or antioxidant enzymes which are efficient in the detoxification defense mechanisms [28]. Under normal physiological conditions, a balance between production and elimination of ROS can be disturbed by adverse environmental factors. With increasing stress, ROS formation is enhanced and its elimination should occur steadily to prevent oxidative stress [29]. Thus, the synchronized action of enzymes responsible for the removal of ROS, such as APX, CAT and GST (Figs. 2, 3, 4), confers increased tolerance plants under stress conditions [30]. It has been reported that the production of ROS during seed germination is active and is in fact a beneficial biological activity associated with high germination capacity and development of vigorous seedlings [31]. The germination of seeds appears to be linked to the accumulation of a critical level of H_2O_2, suggesting that there is a differential regulation of ROS production and disposal mechanisms in different seed [32].

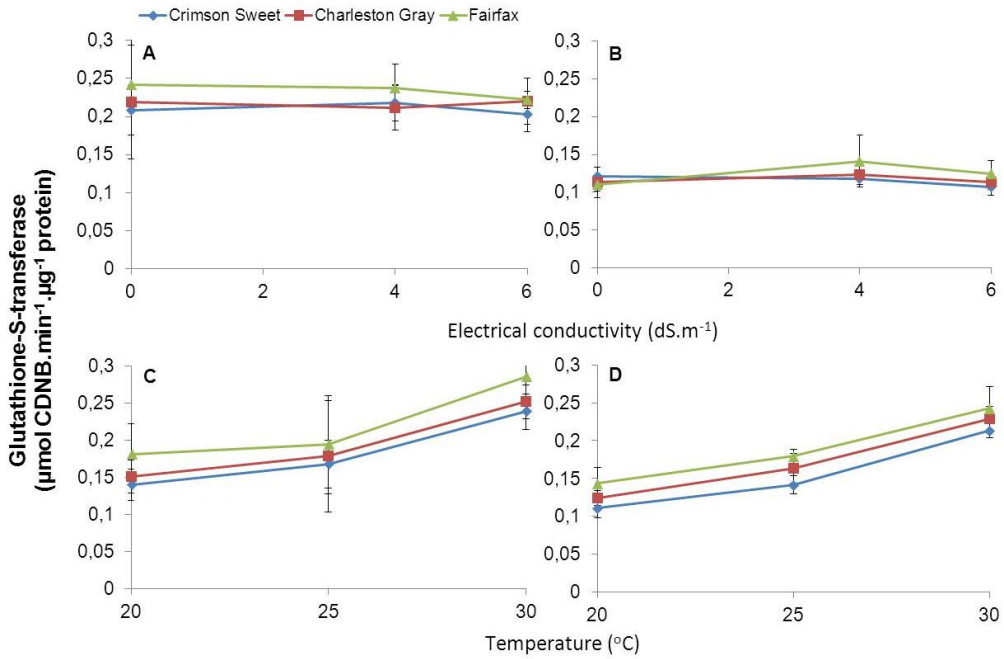

Fig. 4. Glutathione-S-transferase activity in cotyledons (A,C) and in embryonic axis (B,D) of five days old watermelon seedlings of cultivars Crimson Sweet, Charleston Gray and Fairfax, subjected to different conditions of salt and temperature stress

Vertical bars represent the standard error of means

Although the cultivars responded similarly to temperature and salt stress, Crimson Sweet seeds showed higher CAT activity as well as higher respiration rates than other cultivars (Figs. 1C and 3C). These different results suggest that the positive or negative effects of a particular stress combination could be dependent on the particular plant genotype, species, and/or timing and intensity of the different stresses involved [33].

4. CONCLUSION

The antioxidant detoxification system was activated when imposing temperature and salt stress in watermelon cultivars Crimson Sweet, Charleston Gray and Fairfax, allowing adjustment of cell functions during mild stresses. One the other hand, higher stresses, such as 6 $dS.m^{-1}$, may have deleterious effects on these seedlings, especially regarding detoxification by CAT.

The different watermelon did not show same response to the stresses imposed, suggesting different mechanisms of adjustment and tolerance to abiotic stresses.

Further researches must be performed regarding other antioxidant enzymes and reactive oxygen species responses to each cultivar studied in this work, as well as other important watermelon cultivars.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Intergovernmental Panel on Climate Change (IPCC). Climate Change: Impacts, Adaptation, and Vulnerability, fifth assessment report; 2014. Accessed 10 December 2014.
   Available: http://www.ipcc.ch/index.htm
2. Köppen W. Climatology: A study of the climates of the earth. Fondo de Cultura Econômica. México; 1948. Spanish.
3. Angelotti F, Sá IB, Melo RF. Climate change and desertification in the Brazilian semiarid. In: Angelotti F, Sá IB, Menezes EA, Pellegrino GQ. Climate change and desertification in the Brazilian semiarid. Petrolina: Embrapa Semiárido, Campinas: Embrapa Informática Agropecuária; 2009. Portuguese.
4. Cavalcante LF. Salts and its problems in irrigated soils. Centro de Ciências Agrárias/ Universidade Federal da Paraíba. 2000;71. Portuguese.
5. Gondim TMS, Cavalcante LF, Beltrao NEM. Global warming: salinity and consequences on plant behavior. Rev. Bras. Oleag. Fibr. 2010;14(1):37-54. Portuguese.
6. Wang J, Zhang H, Allen RD. Overexpression of an Arabidopsis peroxisomal ascorbate peroxidases gene in tobacco increases protection against oxidative stress. Plant Cell Physiol. 1999;40(6):7225-732.
7. Nizam I. Effects of salinity stress on water uptake, germination and early seedling growth of perennial ryegrass, African J Biotechnol. 2011;10(51):10418-10424.
8. Dantas BF, Ribeiro RC, Matias JR, Araujo GGL. Germinative metabolism of Caatinga forest species in biosaline agriculture. J Seed Sci. 2014;36(2):194-203.
9. Silva FFS, Dantas BF. Effect of temperature on seed germination *Sideroxylon obtusifolium* (*Sapotaceae*) of different origins. Rev Sodebras. 2013; 8(90):40-43. Portuguese
10. Oliveira GM, Matias JR, Ribeiro RC, Barbosa LG, Silva JESB, Dantas BF. Seed germination of native tree species of the Caatinga at different temperatures. Scientia Plena. 2014;10(4):1-6. Portuguese.
11. Oliveira GM, Matias JR, Dantas BF. Optimum temperature for germination of native seeds of Caatinga.Informativo ABRATES. 2014;24(3):44-47. Portuguese.
12. Ribeiro RC, Dantas BF., Pelacani CR. Mobilization of reserves and germination of seeds of *Erythrina velutina* Willd. (*Leguminosae* - *Papilionoideae*) under different osmotic potentials. Rev Bras Sementes. 2012;34(4):580-588.
13. Ribeiro RC, Matias JR, Pelacani CR, Dantas BF. Activity of antioxidant enzymes and proline accumulation in *Erythrina velutina* Willd. seeds subjected to abiotic stresses during germination. J Seed Sci. 2014;36(2):231-239.
14. Xi DM, Liu WS, Yang GD, Wu CA, Zheng CC. Seed-specific overexpression of antioxidant genes in Arabidopsis enhances oxidative stress tolerance during

germination and early seedling growth. Plant Biotech J. 2010;8:796-506.
15. Yamaguchi-Shinozaki K, Shinozaki K. Gene networks involved in drought stress response and tolerance. J Exp Bot. 2007;58(2):221–227.
16. Mittler R. Abiotic stress, the field environment and stress combination. Trends Plant Sci. 2006;11(1):15–19.
17. Suzuki N, Mittler R. Reactive oxygen species and temperature stresses: A delicate balance between signaling and destruction. Physiol Plant. 2006;126(1): 45–51.
18. Perl-Treves R, Perl A. Oxidative stress: An introduction. In: Inzé D, Van Montagu M (eds). Oxidative Stress in Plants. London: Taylor & Francis; 2002.
19. Richards LA. Saline and sodic soils. México: Instituto Nacional de Investigaciones Agrícolas; 1980.
20. Caverzan A, Passaia G, Rosa SB, Ribeiro CW, Lazzarotto F, Margis-Pinheiro M. Plant responses to stresses: Role of ascorbate peroxidase in the antioxidant protection. Genet Mol Biol. 2012;35(4 Suppl):1011-1019.
21. Kang MH, Saltveit EM. Effect of chilling on antioxidant enzymes and DPPH-radical scavenging activity of high and low-vigour cucumber seedling radicles. Plant Cell Environ. 2002;25:1233-1238.
22. Chao H, Qing L, Yan Y. Short-term effects of experimental warming and enhanced ultraviolet-B radiation on photosynthesis and antioxidant defense of *Piceaasperata* seedlings. Plant Growth Regulation. 2009;58(2):153-62.
23. Sharma P, Jha AB, Dubey RS, Pessarakli, M. Reactive Oxygen Species, Oxidative Damage, and Antioxidative Defense Mechanism in Plants under Stressful Conditions. Journal of Botany. 2012:1-26. DOI:10.1155/2012/217037.
24. RagabMoussa H, Abdel-Aziz SM. Comparative response of drought tolerant and drought sensitive maize genotypes to water stress. Aust. J. Crop Sci. 2008;1(1): 31-36.
25. Willekens H, Chamnongpol S, Davey M, Schraudner M, Langebartels C, van Montagu M, et al. Catalase is a sink for H_2O_2 and is indispensable for stress defence in C-3 plants. EMBO J. 1997;16(16):4806–16.
26. Bibi N, Yuan S, Zhu Y, Wang X. Improvements of fertility restoration in cytoplasmic male sterile cotton by enhanced expression of glutathione S-transferase (GST) gene. J. Plant Growth Reg. 2014;33(2):420-429.
27. Höffs A, Schuch LOB, Peske ST, Barros ACSA. Effect of seed quality and seeding rate on yield and industrial quality rice. Rev. Bras.Sementes. 2004;26:55-62. Portuguese.
28. McDONALD MB. Seed deterioration: physiology, repair and assessment. Seed Science and Technology. 1999;27(1):177-237.
29. Scandalios JG. Oxidative stress: molecular perception and transduction of signals triggering antioxidant gene defenses. Brazilian Journal of Medical and Biological Research. 2005;38:995-1014.
30. Deuner C, Maia MS, Almeida AS, Meneghello GE. Viability and antioxidant activity in seeds of cowpea genotypes submitted to salt stress. Rev. Bras. Sementes. 2011;33(4):711-720.
31. Liu X, Xing D, LI L, ZHANG L. Rapid determination of seed vigor based on the level of superoxide generation during early imbibition. Photochemical &Photobiological Sciences. 2007;6:767–774.
32. Bailly C. Active oxygen species and antioxidants in seed biology. Seed Science Research. 2004;14(2):93-107.
33. Suzuki N, Rivero RM, Shulaev V, Blumwald E, Mittler R. Abiotic and biotic stress combinations. New Phytol. 2014;203:32–43. DOI: 10.1111/nph.12797.

© 2015 Dantas et al.; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history.php?iid=916&id=2&aid=7996