

Respiration and antioxidant enzymes activity in watermelon seeds and seedlings subjected to salt and temperature stresses

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 (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 CO₂ 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

27 increased salinization of soils and wells [4, 5]. This may result in plants temperature, water
28 and salt stresses.

29 Abiotic stresses such as high temperatures, water deficit and salinity, individually or
30 associated lead to a series of morphological, physiological, biochemical and molecular
31 changes that adversely affect plant growth and productivity [6]. In seeds and seedlings of
32 many species temperature and/or salt stresses may cause a decrease of water uptake by
33 seeds [7], germination percentage and/or speed [8, 9, 10, 11], changes in reserve
34 mobilization [12], altered antioxidant enzymes activity [13] and different gene expression [14,
35 15]

36 Drought, salinity, extreme temperatures and oxidative stress are often interrelated and can
37 cause similar cellular damage. Stress in seeds and seedlings causes changes in growth
38 conditions which affects homeostasis in cells metabolism. Thus cells require an adjustment
39 of metabolic pathways, in order to acquire a new state of homeostasis, resulting in
40 acclimation or tolerance [16,17]. Enzymes such as catalase (CAT, E.C. 1.11.1.6) and
41 ascorbate peroxidase (APX, EC 1.11.1.11) and glutathione-S transferase (GST, EC
42 2.5.1.13) as well as non-enzymatic compounds comprise effective antioxidant systems to
43 protect against oxidative stress [18].

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

47

48 **2. MATERIAL AND METHODS**

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

53 **2.1 Experimental Design and Treatments**

54 Three assays were performed in a completely randomized experimental design and in
55 factorial scheme. For seed respiration evaluation a factorial scheme 2x3 (cultivars x
56 environmental conditions), with two replications of 50 seeds, was arranged with two
57 watermelon cvs. Crimson Sweet and Charleston Gray subjected to three environmental
58 conditions (EC), combining different electrical conductivities and temperatures, which were
59 (1) 0 dSm⁻¹ EC at 25°C, (2) 0 dSm⁻¹ EC at 30°C, (3) 4 dSm⁻¹ EC and 25°C.

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

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

68 **2.2 Seeds imbibition and respiration**

69 Two replications of 50 seeds of watermelon cv. Charleston Gray and Crimson Sweet, were
70 sowed onto rolls of germitest paper soaked in distilled water in a volume equivalent to 2,5
71 times the paper weight and incubated to germinate in different environmental conditions (0
72 dSm⁻¹/25°C, 0 dSm⁻¹/30°C, 4 dSm⁻¹/25°C). Quiescent seeds, as well as imbibed for 6, 24,

73 48, 72, 96 and 120 hours, were weighted evaluation of weight gain through water uptake.
74 Respiration was measured by CO₂ releases by watermelon seeds in a 500 cm³ volume
75 chamber linked to a infrared gas analyzer, model IRGA LI-6200 (Li-Cor, Lincoln, Nebraska,
76 USA). An average of 15 measurements, performed at each 5 minutes, was divided by the
77 dry weight of the seeds.

78 2.3 Antioxidant Enzymes Assay

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

84 For determination of the antioxidant enzymes response to temperature stress, **triplicates of**
85 **50 seeds** were sowed **onto rolls of germitest paper soaked** in distilled water in a volume
86 equivalent to 2,5 times the substrate paper weight and incubated at 20, 25 and 30°C for
87 germination.

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

93

94 3. RESULTS AND DISCUSSION

95

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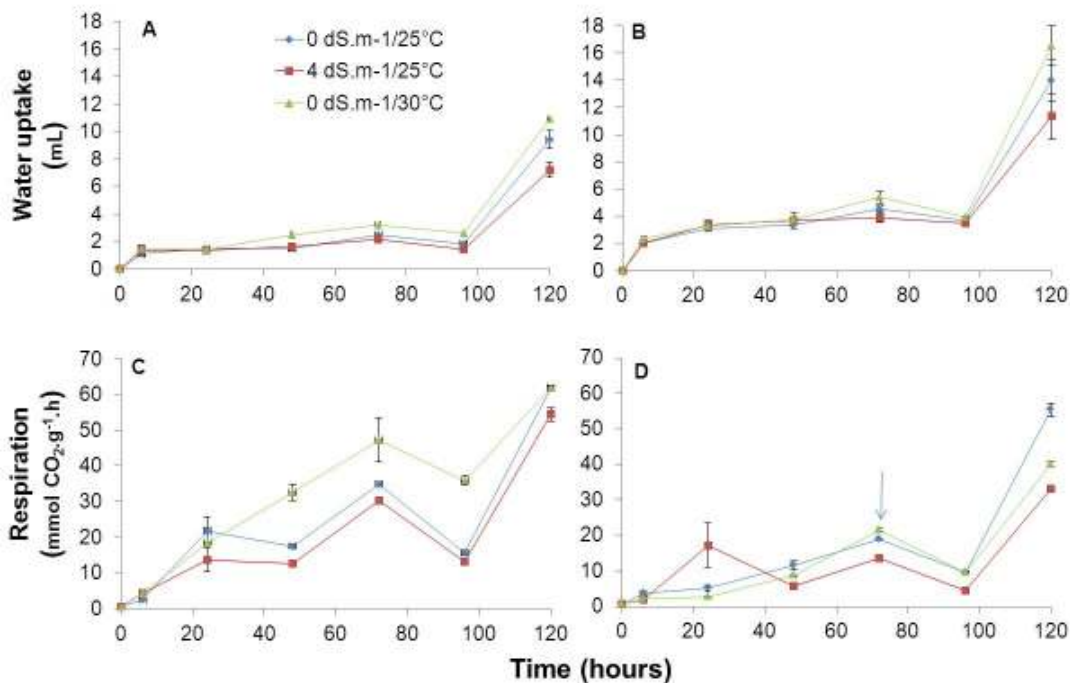


Fig. 1. Water uptake (A, B) and respiration (C,D) of watermelon seeds cultivars . Crimson Sweet (A, C) and Charleston Gray (B,D) subjected to different conditions of salt and temperature stress. Vertical bars represent the standard error of means.

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 CO₂ 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 H₂O₂ into H₂O, 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 (Fig. 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 (Fig. 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].

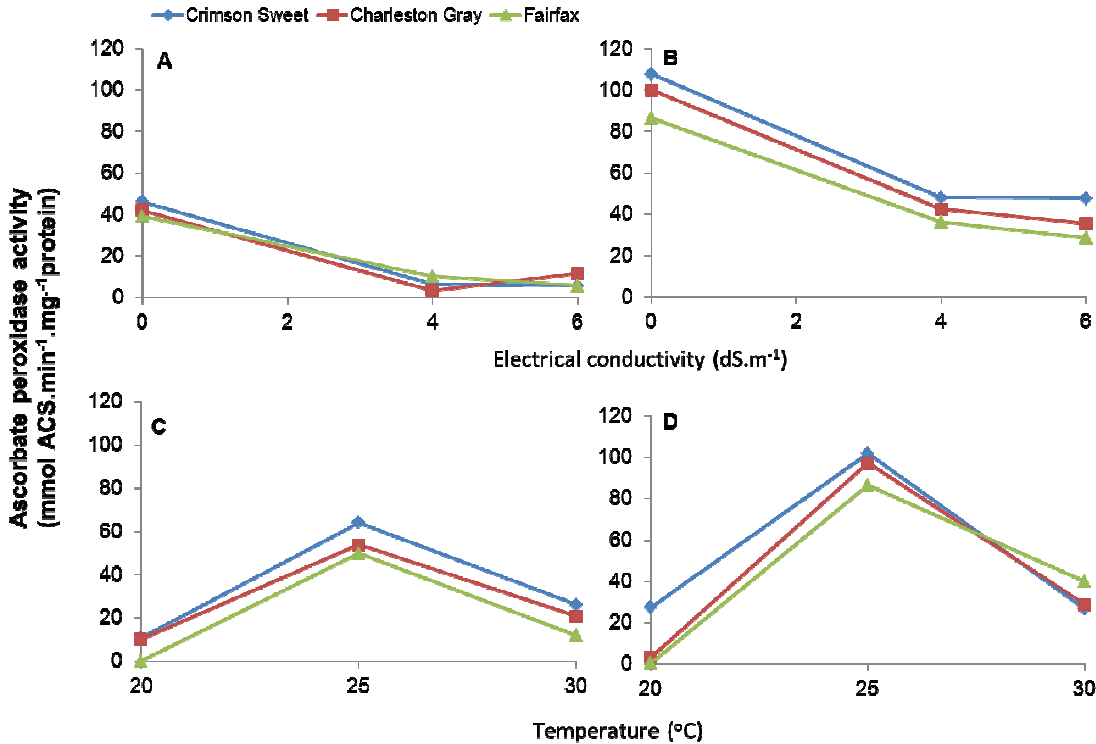


Fig. 2. Ascorbate peroxidase activity in cotyledons (A,C) and in embryonic axis (B,D) of five days germinated watermelon seeds cultivars *Crimson Sweet*, *Charleston Gray* and *Fairfax*, subjected to different conditions of salt and temperature stress. Vertical bars represent the standard error of means.

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 (Fig. 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 (Fig. 3C, D).

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 (Fig. 4C and 4D), however, none of the cultivars seemed to respond to salt stress by detoxifying mechanism by GST (Fig. 4A and 4B).

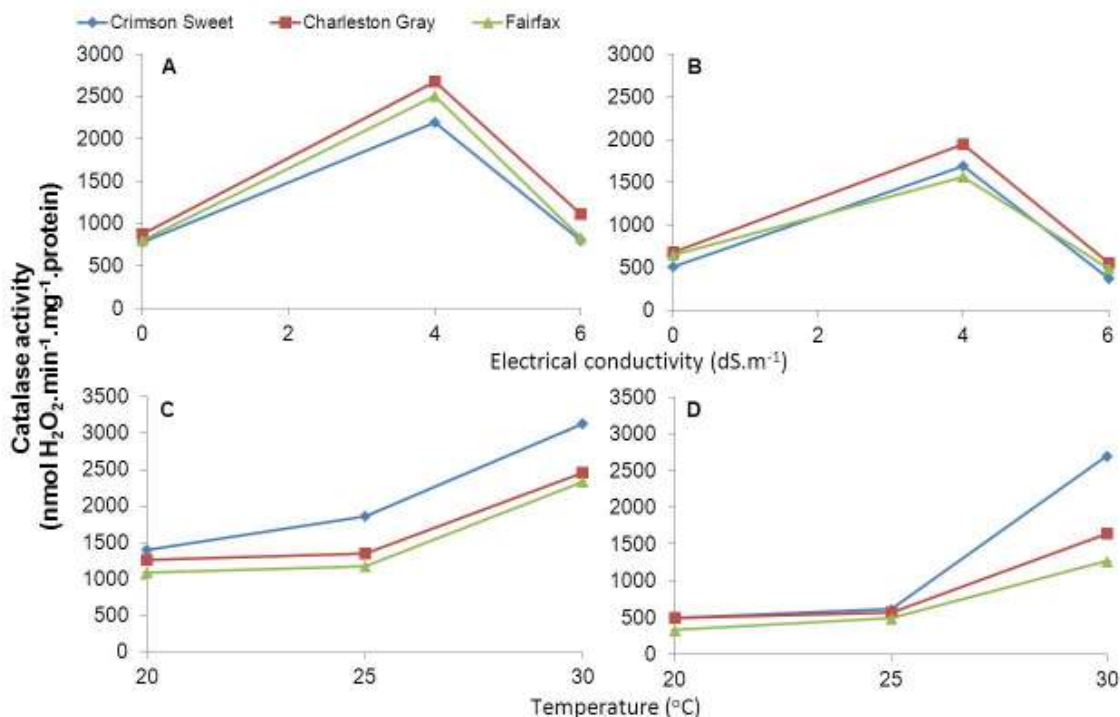
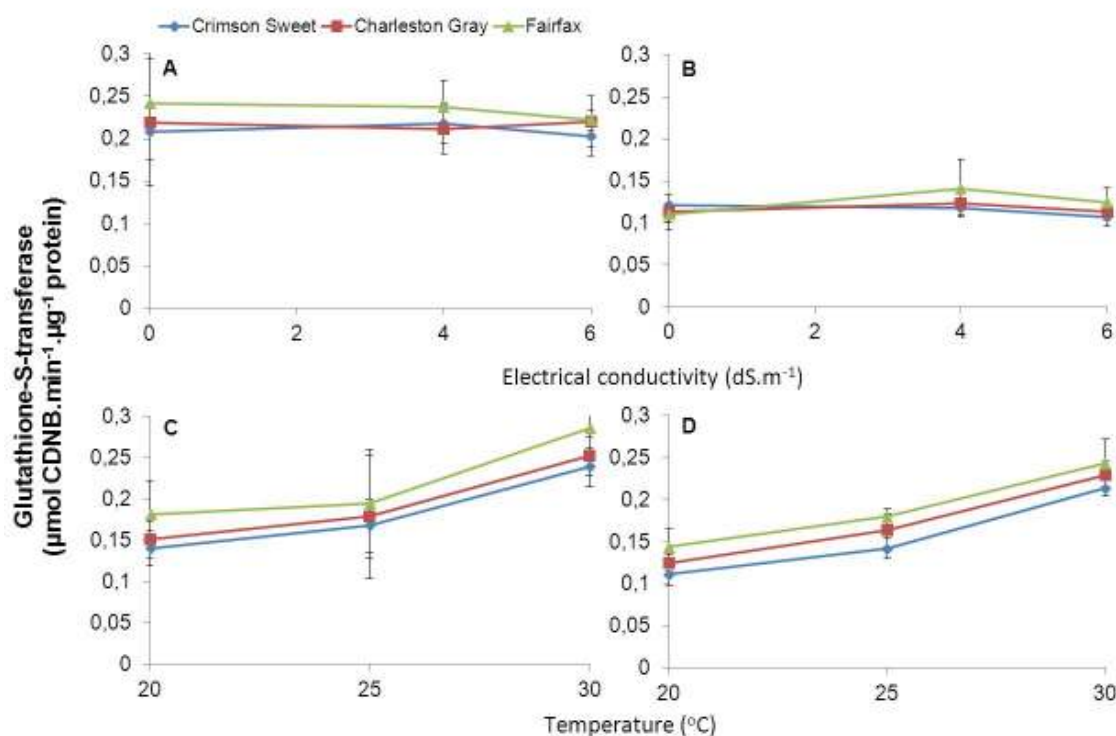


Fig. 3. Catalase (CAT) activity in cotyledons (A,C) and in embryonic axis (B,D) of five days germinated watermelon seeds cultivars *Crimson Sweet*, *Charleston Gray* and *Fairfax*, subjected to different conditions of salt and temperature stress. Vertical bars represent the standard error of means.

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 CO₂ 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 H₂O₂, suggesting that there is a differential regulation of ROS production and disposal mechanisms in different seed [32].

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



193 **Fig. 4. Glutathione-S-transferase activity in cotyledons (A,C) and in embryonic axis**
 194 **(B,D) of five days old watermelon seedlings of cultivars Crimson Sweet, Charleston**
 195 **Gray and Fairfax, subjected to different conditions of salt and temperature stress.**
 196 **Vertical bars represent the standard error of means.**
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198 4. CONCLUSIONS

199
 200 The antioxidant detoxification system was activated when imposing temperature and salt
 201 stress in watermelon cultivars Crimson Sweet, Charleston Gray and Fairfax, allowing
 202 adjustment of cell functions during mild stresses. On the other hand, higher stresses, such
 203 as 6 dS.m⁻¹, may have deleterious effects on these seedlings, especially regarding
 204 detoxification by CAT.

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

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