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Germination and antioxidant defense system in onion (*Allium cepa*. L) cultivars under salt stress

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ABSTRACT

Salinity is one of the major environmental aspects, which affects on plant growth, development and productivity of the agricultural crops worldwide. The aim of this study was to evaluate germination, initial growth parameters, antioxidant enzyme (CAT, APX and GR) activities and proline accumulation, Na^+ / K^+ content in seedlings of four onion cultivars Agrifound Rose, Bellary, Prema-178 and Nasik Red exposed to salt stress with different concentrations of NaCl (0, 50, 100, 150 and 200 mM). In all cultivars, morphological parameters and relative water content decreased with increasing NaCl concentrations, whereas antioxidant enzyme activities, proline accumulation and Na^+ / K^+ content increased. Bellary cultivar showed highest salt tolerance than the Agrifound Rose, Prema-178 and Nasik Red. Decrease in RWC was least in Bellary as compared to other cultivars. The results suggest that high NaCl concentrations have a negative effect on the physiological quality of onion seedlings, resulting in lower seedling growth rates and increased antioxidant enzyme activity and among four cultivars Bellary was found more tolerant to salt stress.

Keywords: catalase, ascorbate peroxidase, glutathione reductase, morphological parameters, salt stress, proline.

INTRODUCTION

Salinity is a major problem in many parts of arid and semiarid regions of the world [1]. It also has an adverse effect on the growth and development of most salt sensitive plant species [2]. Nearly 20 % of world's cultivated area and 50 % of the world's irrigated lands are affected by salinity [3]. Salinity causes morphological, physiological and biochemical, molecular changes in the plants. Seed germination, seedling growth are adversely affected by high salt concentration, ultimately causing diminished economic yield, quality and productivity [4]. Successful seedling establishment depends on the frequency and ability of the seed species to germination. The decrease in germination race particularly under salt stress conditions may be due to the fact that seeds to prevent germination development and osmotically enforced dormancy under stress conditions. Plant growth is adversely affected by salinity from decreasing availability of water in the soil for plant consumption and toxicity of certain ions that contribute to salinity [5]. The mechanism of salt tolerance, cell turgor and depressed rates of root and shoot elongation, suggests that environmental salinity acts primarily on water uptake [6] Furthermore, high intracellular concentrations of both Na^+ and Cl^- can inhibit the metabolism of dividing and expanding seedlings, retarding germination and even leading to seed death [7].

Salt stress can also lead to excess intracellular production of reactive oxygen species (ROS) such as the superoxide radical ($O_2^{\bullet -}$), the hydroxyl radical (OH^-), hydrogen peroxide (H_2O_2) and singlet oxygen ($^1O_2$). To eliminate these

ROS, plants possess antioxidant defense systems which are an important first line of defense against free radicals under stress conditions [8]. This is the case of the antioxidant enzymes superoxide dismutase (SOD), which catalyzes the dismutation of $O_2^{\bullet -}$ into H_2O_2 and O_2; catalase (CAT) and ascorbate peroxidase (APX), which can cleave H_2O_2 into H_2O and O_2 [1]. However, this regulation can be ineffective if the stress is severe enough to considerably increase the production of ROS, which can cause a following of events such as peroxidation of lipids, degradation of membranes, and cell death. Therefore, the balance between the production of ROS and the ability to rapidly activate the antioxidant defense system reflects the ability of a plant to endure adverse conditions, indicating its adaptation or tolerance to the imposed stress [9].

Onion (*Allium cepa* L.) belonging to the family of Amaryllidaceae, is the second most important horticultural crop in the world after tomatoes, which is cultivated throughout the world and is extensively grown and widely consumed in India [10]. Onion seeds are convex one side and flattened on other side and are covered by a black seed coat. Kadapa district is a cafeteria for various horticultural crops, located at the heart of Rayalaseema region. With little or uneven rainfall it represents semiarid climate with hot summer. Onion is one of the major crops grown in Kadapa, so the present investigation is to exploit the genetic variability of the germ plasm of onion to identify the tolerant cultivars which may give reasonable yield on salt affected soils.

MATERIALS AND METHODS

Collection, surface sterilization and growth conditions of onion cultivars

In the present study seeds of four onion cultivars, include Agrifound rose, Bellary, Prema-178 and Nasik red were collected from local farmers with reference to the YSR Horticultural University, Kadapa and National Horticulture Research and Development Federation (NHRDF) institute, Kurnool. Seeds of all four cultivars were first surface sterilized by using 70 % alcohol and then sterilized with 2 % sodium hypochlorite, then thoroughly washed several times with distilled water. The experiment was carried out using five salinity levels with NaCl (0, 50, 100, 150 and 200 mM). Healthy and homogenous seeds were transferred onto the wetted filter paper. The experiment was conducted in a germination chamber at 25^{o} C and the visual observations were recorded up to 10 days.

Morphological parameters

The seeds were considered germinated when the tip of the radicle had grown free of the seed coat. The Germination percentage was calculated by following Wiese and Binning equation: GP: Ni / N × 100 (Where Ni: No. of germinated seed till i^{th} day, N: Total no. of seeds) [11].

Germination race with Maguire equation: GR: N1 / D1 + N2 / D2 + … + Ni / Di (Where, GR: Germination Race, Ni: No. of germinated seeds in each numeration, Di: No. of days till n^{th} Numeration. N: No. of numeration times) [2].

Seed vigor was calculated with Abdul-baki and Anderson equation: Seed Vigor: {Germination percentage × means (RL + PL) mm} / 100, (Where, SV ·Seed Vigor, RL. Radicle Length, PL. Plumule Length) [12].

Relative water content

Relative water content was determined by the Yamasak et al (1999) [13]. Relative water content (RWC) was calculated according to the following formula,

Relative water content: (FW-DW) / (TW-DW) ×100 (Where, FW - Fresh Weight, DW - Dry Weight, TW-Turgid Weight).

Na^+ and K^+ ion measurement

Grind 500 mg of dry mass of tissue with 10 ml of 0.1% HNO_3, the extract were centrifuged at 3000 × g for 15 min, and supernatant was filtered through a filter paper. An aliquot of filtrate was used for Na^+ and K^+ measurement by flame photometer (*FP 8801*, Kruss Optanics, Germany) [14].

Antioxidant enzyme activities

Weigh 100 mg of fresh onion seedlings, were homogenized with 2 ml of extraction buffer containing 1 mM PMSF (Phenyl Methyl Sulphonyl Fluoride) and 50 mM sodium phosphate buffer (P^H 7.0), then centrifuged at 10,000 rpm for 10 min at 4 ^{o}C and the supernatants were collected and used for the enzyme assays of CAT, APX and GR.

Protein concentrations in the enzyme extract were determined by the method of Lowry et al. (1951) using defatted BSA as a standard [8].

CAT (E.C. 1.11.1.6) activity was measured spectrophotometrically (*Evolution – 201 UV-Visible spectrophotometer*, Thermo Scientific, India) by measuring the rate of H_2O_2 disappearance at 240 nm ($\Delta\varepsilon$ = 43.6 mM^{-1} cm^{-1}) according to the method Patterson et al. (1984). The activity was determined by the oxidation of H_2O_2 at 240 nm.

APX (E.C. 1.11.1.11) was assayed by the method of Nakano and Asada (1981). The activity of APX was recorded as decrease in absorbance at 290 nm for 1 min and the amount of ascorbate oxidized was calculated ($\Delta\varepsilon$ = 2.8 mM^{-1} cm^{-1}).

GR (E.C. 1.6.4.2) assay was performed according to Jiang and Zhang (2001) with little modifications. The recorded as the decrease in absorbance at 340 nm for 1 min ($\Delta\varepsilon$ = 3.4 mM^{-1} cm^{-1}).

Estimation of proline

Proline was assayed according to the method described by Bates et al. (1973). Fresh seedlings of 500 mg were homogenized in 5 ml of 3 % (w/v) sulphosalicylic acid using motor and pestle 2.0 ml of extract, 2.0 ml of acid ninhydrin and 2.0 ml of glacial acetic acid were added and incubated for 1 h in a boiling water bath at 90 ^{o}C followed by an ice bath then add 4ml of toluene. The absorbance was measured at 560 nm against blank [14].

Visual observations of the morphological and quantitative measurement of biochemical parameters were done with minimum of three replicates involved in each experiment. The analysis of variance (ANOVA) was performed with Duncan's multiple range test using IBM SPSS software version 20.0. The p - value was significant ($p \leq 0.05$) of all cultivars (n=4).

RESULTS

In the present study, different levels of salinity have significant effect on all growth parameters of onion seeds germination percentage, germination race, seed vigor, fresh weight and dry weight, relative water content. Strong reductions in all traits were observed mainly at higher levels of salt concentration compared to control. According to the analysis of variance salt stress showed all the measured morphological parameters are significant ($p \leq 0.05$).

While in this experiment all the four cultivars of onion expressed different responses at four levels of salinity. In all cultivars the germination percentage was highest at control and started to decrease as the salt level increases. Among the four cultivars of onion seeds Bellary and prema -178 showed highest germination percentage, lowest was showed by Agrifound rose and Nasik red. Highest germination race was related to controls in all cultivars.

Under Salt stress revealed significant effect on germination race of all four cultivars of the onion seeds. The highest germination race was observed in Prema-178 and Bellary (9.76, 8.77, 6.58, 6.22 and 3.8) at all the salt concentrations (50,100, 150 and 200 mM). By increasing salt concentration, seed vigor declined (Table 1). The most seed vigor was related to control treatment and the least related to 200 mM in all cultivars. The highest seed vigor expressed cultivars were Bellary and prema - 178 in control treatment Bellary (2.45), prema - 178 (2.84) and the least was related to 200 mM Bellary (0.84), Prema - 178 (0.65). Fresh and dry weights of the seedlings were decreased with increasing salt levels but there was more than 50 % decrease in fresh weight at 200 mM salt concentrations. There was no significant decrease in fresh and dry weights of Bellary seedlings (Table 2).

The significant differences of Relative water content were observed in control and salt stressed onion seedlings (Table 2). Among four cultivars highest percentage of RWC was observed in controls of Bellary (93.88 %), under salinity levels, 87.63 at 50 mM, 80.9 at 100 mM, 79.08 at 150 mM and 63.13 % at 200 mM.

Na^+ content in onion seedlings increased linearly with increasing salt levels. Reaching the highest accumulation of Na^+ at 50, 100, 150 and 200 mM there was no change observed. When we compare all the four cultivars Na^+ content was less in Bellary seedlings at all the salinity levels (Fig.1A). Least amount of K^+ accumulation was observed when compared to Na^+ ion content (Fig. 1B). Na^+: K^+ was least at controls of the four varieties when compared to salt stress induced seedlings of onion (Fig. 1C).

Changes in antioxidant enzymes of activities of CAT, APX and GR were observed in onion seedlings. According to the analysis of variance salt stress showed all the measured antioxidant enzyme activities are significant ($p \leq 0.05$). The activities gradually increased in all four cultivars when compared to controls i.e. BL > PR > NR > AF (Fig. 2 A, B, C). The highest percentage (%) of CAT, APX and GR were observed in Bellary cultivar compared with a percentage of control (100 %). Changes in free proline content were measured in both control and stress induced onion seedlings. The results were depicted in figure 3, at increasing concentration of salinity more proline was accumulated in Bellary when compared to other cultivars.

DISCUSSION

Plants under salinity use a variety of strategies to neutralize the adverse effects of salt stress on the growth and development [15]. In the present investigation morphological parameters such as germination percentage, germination race, seed vigor, fresh weight, dry weight and relative water content were decreased when salt concentrations increased in the onion seedlings (Table1 and 2). Salinity inhibits the plant growth for two reasons one is water defect and the other is salt specific ion toxicity [12]. Different plants have different mechanisms to cope with this [14] Nonenzymatic antioxidants are more important to addition their dominant role in plant growth and development in addition to their antioxidant capacity. Comparison of morphological parameters under salinity conditions with control showed that Bellary is more tolerant cultivar than other onion cultivars of Prema-178, Nasik red and Agrifoundrose.

The fresh and dry weights of four onion seedlings were significantly modified in which the treatment proves that salt might be accumulated under the conditions of salt stress during germination process [15]. Relative water content in seedlings of Bellary reflects decreased water loss compared to other three cultivars may be through transpiration. Lowest Na^+ content in Bellary seedlings is due to lesser uptake of Na^+ (Fig. 1A). This is in good agreement with antioxidant defense mechanism under salt stress in wheat seedlings reported by Mandhania et al., 2006 [2].

It is well known that salt tolerance in most crop plants is associated with a major efficient antioxidant system including enzymatic and non-enzymatic antioxidants [7]. The results of present work under salt stress in onion cultivars suggest selecting a tolerant one among others.

The affinity of enzymes for their substrate is a very important factor to determine the antioxidant action. Considering that CAT has a low affinity to H_2O_2, this enzyme becomes active only when its substrate is accumulated, the activity of CAT increased with the increase in the saline concentration at Bellary cultivar (Fig. 2A) whereas for cultivars of Prema- 178, Nasik red and Agrifound rose, CAT activity was less when compared to Bellay at the concentrations of 50 to 200 mM NaCl, respectively. The increase in CAT activity in stressed plants might be an adaptation to eliminate H_2O_2 [17]. Bellary Cultivar showed its antioxidant defense system more strongly activated than Prema-178, Nasik red andAgrifound rose cultivars during the stress caused by the increasing salt concentrations.Plants with large ascorbate pools, accumulated by either ascorbate recycling or exogenous ascorbate supplementation, are able to maintain APX activity even under conditions of oxidative stress. Our results of APX increased activity in Bellary cultivar followed by Prema-178, Nasik red and Agrifound rose (Fig. 2B). The increased activities were compared with percentages of controls. GR is found in chloroplasts as well as in mitochondria and cytoplasm and has a role to scavenge H_2O_2 in plant cells. GR catalyzes the rate limiting step of ascorbate glutathione pathway. Increase in the glutathione reductase activity in plants resulted in the accumulation of glutathione (GSH) levels and ultimately confers the tolerance in plants [18]. In the present study with the greater increase in the activity of glutathione reductase in Bellary compared to other three cultivars (Fig. 2C).

Accumulation of free proline is a typical response to salt stress, which is a first manifestation of abiotic stress in plants. Apart from protection of macromolecules from denaturation and carbon and nitrogen reserve for stress relief, proline has several other functions during stress, e.g., osmotic adjustment, osmoprotectants, free radical scavenger and antioxidant activity [17]. In our study said that more proline was accumulated in Bellary followed by Prema-178, Nasik red and Agrifound rose cultivars.

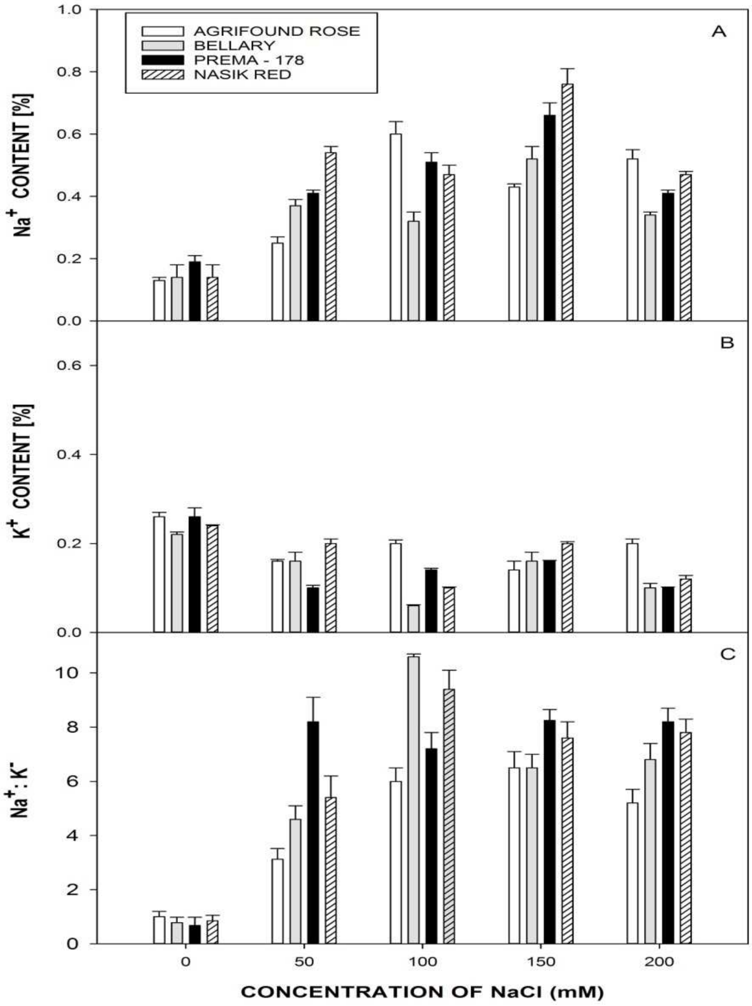

Fig. 1 Effect of salt stress on (A) Na^+ content, (B) K^+ and (C) Na^+: K^+ ratio in onion seedlings of Agrifound rose, Bellary, Prema - 178 and Nasik red, in response to varying concentration of NaCl (mM)

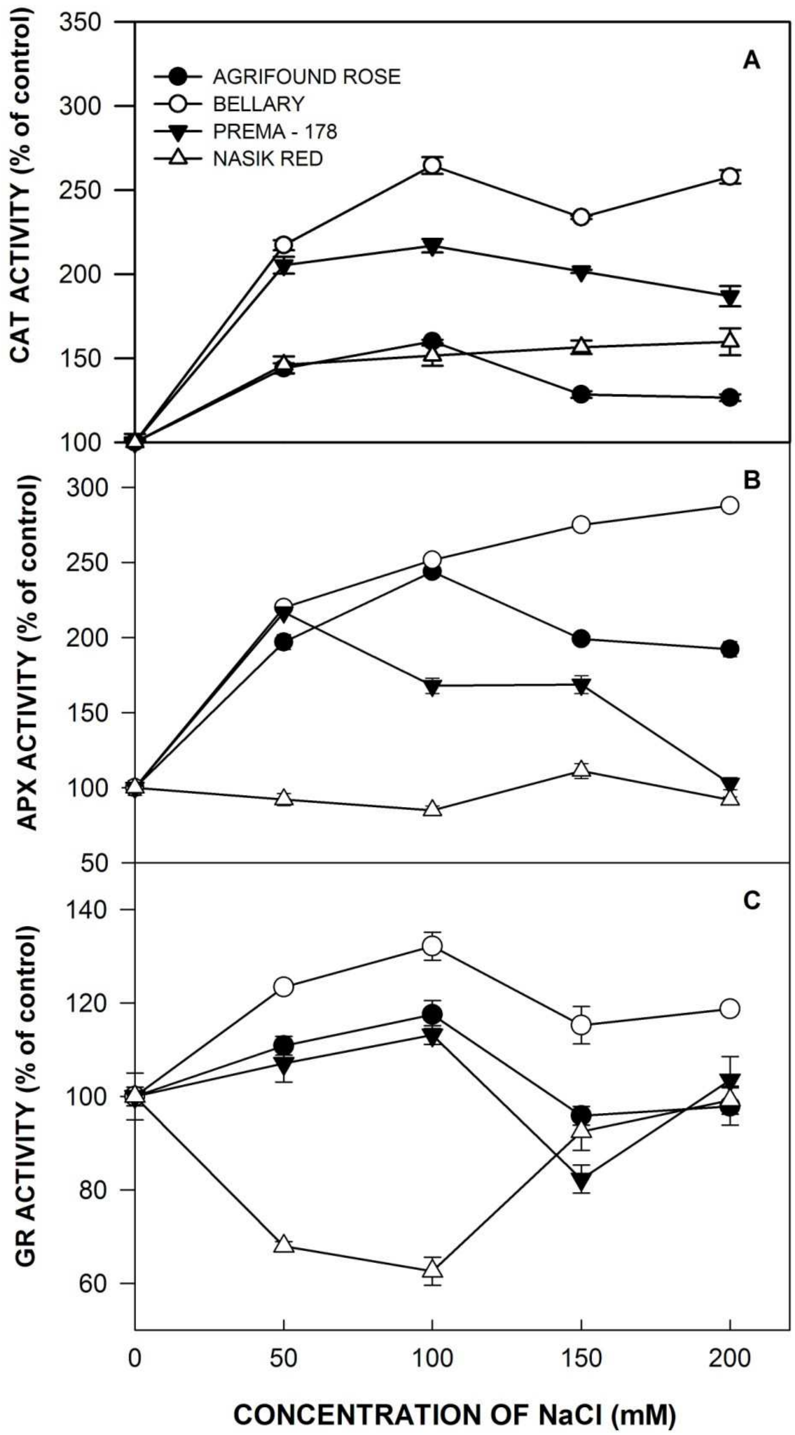

Fig. 2 Effect of salt stress on (A) catalase (CAT), (B) ascorbate peroxidase (APX) and (C) glutathione reductase (GR) in onion seedlings of Agrifound rose, Bellary, Prema-178 and Nasik red, in response to varying concentration of NaCl (mM)

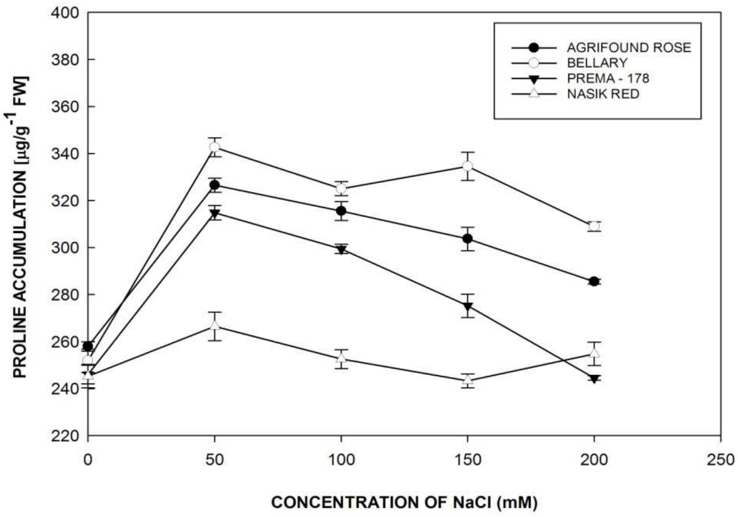

Fig. 3 Effect of salt stress on proline accumulation in onion seedlings of Agrifound rose, Bellary, Prema-178 and Nasik red, in response to varying concentration of NaCl (mM)

Table 1 Effect of salt stress on germination percentage, germination race and seed vigor of different onion cultivars in response to varying concentration of NaCl (mM). (*significant value of Duncan's multiple range test is p ≤ 0.05)

Parameter	NaCl (mM)	Agrifound rose	Bellary	Prema-178	Nasik red
	0	93.33 ±0.3*	81.67±0.2*	91.68±0.1**	81.67±0.2*
Germination percentage (%)	50	68.3 ±0.1**	73.34±0.1**	80±0.2*	68.34±0.1**
	100	26.6 ±0.2*	55±0.3*	71.67±0.3*	53±0.1*
	150	18.33 ±0.1**	52±0.4*	58.33±0.2*	43.34±0.1*
	200	11.66 ±0.4*	31.6±0.2*	46.67±0.1**	26.68±0.3*
	0	11.16±0.5*	9.76±0.2*	10.99±0.4*	9.77±0.1**
Germination Race	50	8.17 ±0.2*	8.77±0.2*	9.57±0.1*	8.15±0.2*
	100	3.18 ±0.1***	6.58±0.3*	8.57±0.2*	6.34±0.1**
	150	2.19 ±0.3*	6.22±0.1**	6.97±0.3*	5.18±0.1*
	200	1.39 ±0.2*	3.8±0.2*	5.38±0.1**	3.19±0.1*
	0	4.38±0.2*	2.45±0.3*	2.84±0.1*	2.57±0.2**
	50	3.3±0.1**	1.24±0.2*	1.87±0.2*	1.67±0.2*
Seed vigor	100	1.54 ±0.2*	0.57±0.1**	1.67±0.2*	1.21±0.3*
	150	0.91±0.2*	0.44±0.3*	0.75±0.3*	0.93±0.2*
	200	0.43 ±0.1*	0.84±0.1**	0.65±0.1*	0.45±0. 2**

Table 2 Effect of salt stress on fresh weight and dry weight and relative water content of different onion cultivars in response to varying concentration of NaCl (mM). (*significant value of Duncan's multiple range test is p ≤ 0.05)

Parameter	NaCl (mM)	Agrifound rose	Bellary	Prema-178	Nasik red
	0	475.3±0.2*	498.4±0.3*	573.3±0.2*	507.2±0.4 *
	50	441.7±0.3*	418.4±0.4*	476±0.1**	461.8±0.2**
Fresh weight(mg)	100	299.7±0.2*	341.8±0.2*	432.4±0.3*	484±0.3*
	150	247.6±0.2*	299.2±0.2*	262.9±0.2**	353.2±0.1**
	200	133.9±0.2*	279.6±0.3*	170.6±0.2*	159.8±0.2*
	0	23.4±0.1**	27.6±0.2**	28.6±0.2*	26.4±0.4*
	50	22.2±0.2*	24.8±0.3*	25.4±0.2*	21.8±0.2*
Dry weight(mg)	100	21.7±0.1***	23.2±0.1**	22.5±0.3*	19.8±0.1**
	150	20.2±0.1**	17.2±0.1***	18.1±0.1**	18.2±0.2*
	200	11.8±0.3*	13.8±0.2*	11.3±0.1*	13.2±0.2*
	0	81.89±0.1*	93.88±0.3**	92.43±0.2*	80.7±0.3*
	50	81.17±0.2*	87.63±0.2*	78.43±0.2*	74.97±0.4*
Relative water content(%)	100	70.22±0.2*	80.9±0.1**	67.45±0.3*	73.4±0.5*
	150	61.28±0.2**	79.08±0.1*	53.39±0.1**	70.57±0.2*
	200	53.22±0.3*	63.13±0.1**	51.55±0.2*	66.55±0.1**

CONCLUSION

In conclusion, there is no published data on morphological and expression levels of antioxidants in onions in seedling stages at in vitro conditions. High concentrations of salt exerted a negative effect in the physiological quality of onion seeds, causing lower growth rates and higher activity of the antioxidant enzymes, because of the increase in the activation of the antioxidant defense system where Bellary cultivar presented to be more tolerant to salt stress than other three cultivars.

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