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Morphological and antioxidant enzymatic activity responses of sapodilla rootstock to salinity stress

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Abstract:

Sapodilla is one of the important fruit crops in tropical area which has problem with seed germination. So *Manilkara hexandra* (kherol) is generally used as a rootstock for sapodilla. This experiment was performed to assess the physiological changes and antioxidant enzymatic activity of *Manilkara hexandra* seedling under salinity condition. A pot experiment was carried out in a greenhouse using different concentrations of seawater (3, 6, 9, 12 ds/m) on one-old age seedling and fresh water used as control. The experimental design was a Completely Randomized Design (CRD) experiment. Results showed that seawater irrigation had no significant effect on length and volume of roots. However, fresh weight of leaf, stem and root decreased significantly by increasing seawater concentration. POD and CAT antioxidant enzymes showed the similar trend under stress and their activities increased strongly. However, APX activities increased gradually by increasing salinity level. This dramatic increase might show that POD and CAT is a major enzyme among antioxidant enzymes examined in *Manilkara hexandra* (kherol) under salt stress. Thus, antioxidant defense system induced by salinity plays an important role in this plant under salinity stress.

Keywords: salinity, enzyme, pigment, sapodilla, seawater

Introduction

Manilkara hexandra is an important tree fruit crop growing under semi-arid conditions. *Manilkara hexandra* is mainly used as a rootstock for sapodilla because it has problem with seed germination. Sapota (*Manilkara archas* Forb.) also called Chickoo or Chiku belongs to sapotaceae family and is one of the most important fruit crops in tropical regions of world (Mohamed *et al*., 2003). In recent years, because of a lack of irrigation water, agricultural scientists encourage farmers to use of seawater (at least diluted) for crop irrigation (Liu *et al*., 2003). On the other hand, salinity is one of the most important abiotic stresses, limiting the growth of agricultural crops due to its both osmotic and toxic effects (Munns and Tester 2008). Salt stress increases the creation of reactive oxygen species (ROS) affecting membrane functions (Erdal *et al*., 2010). ROSs have potential to interact with many cellular components, causing significant damage to membranes and other cellular structures. However enzymatic defense system including peroxidase and catalase are responsible for maintaining the levels of ROS under tight control. These enzymes detoxify H_2O_2 (an active oxygen species) to H_2O (Borsani *et al*., 2011). Increased SOD, POD and CAT activities are related to salt tolerance of many plants as reported in various researches (Hishida *et al*., 2014; Unal *et al*., 2014). Walker et al. (2002) and Munns (2002) showed that salinity could reduce the photosynthetic rate through a decline in leaf growth, root, shoot vigor and yields and finally lead to plant death. Salinity affects photosynthesis by reducing stomatal conductance (Brugnoli and Lauteri, 1991), changing the plant's water status and pigments' concentration (Gebre and Tschaplinski, 2000) and by altering the chloroplast ultrastructure (Geissler *et al*., 2009). Ventura *et al*. (2011) reported that utilization of the seawater for irrigation resulted in the obvious increase of protein, carotene, soluble sugar, polyphenol, fatty acid. In addition, the activity of antioxidant enzyme such as superoxide dismutase was rapidly increased and then reduced slowly under high salinity stress. Previous research showed that salinity stress decreased vegetative growth and reduced minerals absorption by plant (Sekmen *et al*, 2012).

There is lack of evidence on the possibility of diluted seawater uses for irrigation of woody plants. In this research, we examined the effects of seawater

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irrigation on some biochemical and physiological characters of sapodilla rootstock seedling. Knowledge about physiological and biochemical bases of salinity alteration could help us to provide and to choose crops adapted or tolerant to salt stress. The results of this search reveal the effect of salinity on different characters of sapodilla rootstock seedling such as antioxidant enzymes, morphological characters and chlorophyll of leaf.

Materials and Methods:

Plant material and seawater dilution (DSW) management: A pot experiment was conducted in a greenhouse during two successive seasons of 2015 (from February 21st to May 21st). One year-old seedlings were prepared from research centre of Minab and transferred to Hormozgan University. In order to complete the establishment of seedlings, they were irrigated with purified water (drinking water) for two months. After this initial acclimation period, the samples were irrigated with different concentrations of diluted seawater (3, 6, 9, 12 ds/m) and control was irrigated with fresh water (1.3ds/m).To avoid osmotic shock, plants were irrigated step by step, by 3 ds/m per day, until the desired concentration was reached. The experiment was design as a Completely Randomized Design (CRD) with three replicates. The plant tissues were sampled three months after starting salinity treatments with appearance salinity symptoms. Leaves and roots of each sample were harvested separately at the end of the experiment. Fresh weight of leaf, stem and root, stem length, root length, root volume, root diameter, stem diameter and crown diameter were measured at harvest time. Some samples stored at -20°C for other analyses.

Photosynthetic pigments: Fresh leaf tissues (0.1 g) were homogenized in chilled 80% (v/v) acetone. The homogenate was centrifuged at 8800 × g for 10 mins at 4 ˚C in dark. The absorbance of the acetone extract was measured at 663, 645 and 470 nm using a spectrometer (England UV-3200 model Cecil 2501). The contents of chlorophyll a, chlorophyll b and total chlorophyll were calculated according to Arnon (1949).

Enzyme activity assays: Frozen leaves (0.5 g) were homogenized in 50 mM potassium phosphate buffer (pH 7.8) containing 1 mM EDTA, 3 mM mercaptoethanol, and 2% (w/v) polyvinylpolypyrrolidone (PVPP). Then centrifuged at 16,000 × g for 30 mins at 4 ◦C and the supernatant was used for enzyme assays.

Peroxidase (POD) and catalase (CAT) activities were evaluated by Beyer and Fridovich (1987) protocol, with some modifications. 50 mM phosphate buffer (pH 7.8), 25 mM guaiacol, 200 mM H_2O_2, and 0.5 ml of enzyme extract was mixed for 3 ml solution. Then, the changes in absorbance of the solution were determined at 470 nm every 30 second. The CAT reaction solution (3 ml) contained 50 mM phosphate buffer (pH 7.0), 200 mM H_2O_2, and 50 ml of enzyme extract. Changes in absorbance of the solution at 240 nm were read every 60 s.

Ascorbate peroxidase (APX) activity was determined by Nakano and Asada (1981) method. After prepared solution, the changes in absorbance were read at 290 nm for 60 second.

Statistical analysis: Data were analyzed by variance (ANOVA) for a Completely Randomized Design (CRD) experiment and statistically significant differences between means were compared at $P \leq 0.05$ using Duncan's multiple range tests in SAS package program 9.1.

Results and Discussion:

Effect of salinity on plant growth: An analysis of variance indicated that the effect of salinity was not significant on root length and volume of root. However other characters showed significant differences (Table 1). Salinity caused a significant decline in the diameter of stem. However, compared with control, no significant differences were found in root volume and root length. Slightly increase in growth parameters occurred at the low salinity level as compared to the growth of the plants irrigated by fresh water (Table 2). The results indicated significant reduction in leaf fresh weight as salinity increased. The highest (2.9g per pot) and least (0.47g per pot) amount of fresh weight of leaf were related to control and 12 ds/m, 6 ds/m concentration of seawater treatment respectively. Fresh weight of stem and root decreased gradually with increasing intensity of stress. The highest (5.2g per pot) and least (1.4g per pot) level of stem fresh weight of stem were found in control and 12 ds/m respectively. Similarly, the fresh weight of root also showed the same trend and the control showed the lowest weight (Fig. 1).

These results agree with those given by Marcelis and Van Hooijdonk (1999) who showed that salinity limits aerial parts growth more than roots growth. contrary to our results Gadiri *et al*. (2006) reported that the use of sea water limited growth of the root of barley plants more than shoots or aerial parts.

Aldesuquy *et al*. (2001) reported that irrigation of wheat plants by seawater at 25% caused marked decrease in leaf area. Vigo *et al*. (2005) reported that irrigation with diluted seawater reduced leaf number, dry weight, leaf area and shoot number of different cultivars of olive. Di Baccio *et al*. (2004) also showed that, dry and fresh matter in leaves and roots, shoot and root lengths of sunflower decreased by increasing seawater concentration from 10 to 20%. Omoto *et al*. (2012) believe that salinity stress caused changes in mesophyll structure and closure of stomatal so these are attributed to the reduction of CO_2 diffusion to the chloroplast and reduction of photosynthesis. The reduction in dry matter also reported in avocado (Chirachint and Turner, 1988) and citrus rootstocks. Tozlu *et al*. (2000) and Verslues *et al*. (2006) also has been reported that inhibited plant growth is one of the most obvious results of salt stress. Some researchers

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Table 1. Results of ANOVA testing of some morphological characters of kherol under salinity stress.

Source	DF	Stem length (cm)	Root length (cm)	Diameter of root (mm)	Diameter of stem (mm)	Volume of root	Diameter of crown	Leaf fresh weight	Stem fresh weight	Root fresh weight
Treatment	4	60.100**	7.73 ^{ns}	1.5**	1.8**	2.1^{ns}	2.3**	11.715**	26.982**	45.272**
Error	10	58.833	23.500	0.15	0.27	0.25	0.31	0.231	0.819	0.300
C*V (%)		11.42	11.82	19.3	12.6	17.8	14.4	11.10	9.45	4.71

Table 2.Effect of seawater irrigation on different characters of stem, root and crown of Kherol

Salinity(dsm^{-1})	Stem length(cm)	Root length(cm)	Root volume(cm^3)	Diameter of root(mm)	Diameter of stem(mm)	Diameter of crown (mm)
control	21.5^{ab}	12.5^{a}	3.0^{a}	2.0^{ab}	2.7^{a}	3.0^{a}
3.0	25.0^{a}	12.3^{a}	4.0^{a}	3.0^{a}	1.7^{b}	3.0^{a}
6.0	25.3^{a}	12.7^{a}	2.0^{a}	1.0^{b}	1.3^{b}	1.5^{b}
9.0	19.8^{b}	14.3^{a}	3.0^{a}	2.0^{ab}	1.0^{b}	2.0^{ab}
12	18.5^{b}	13.5^{a}	2.0^{a}	2.0^{ab}	0.8^{b}	3.0^{a}

The means with the same letter within column are not significantly different at P = 0.05 according to Duncan's multiple range tests.

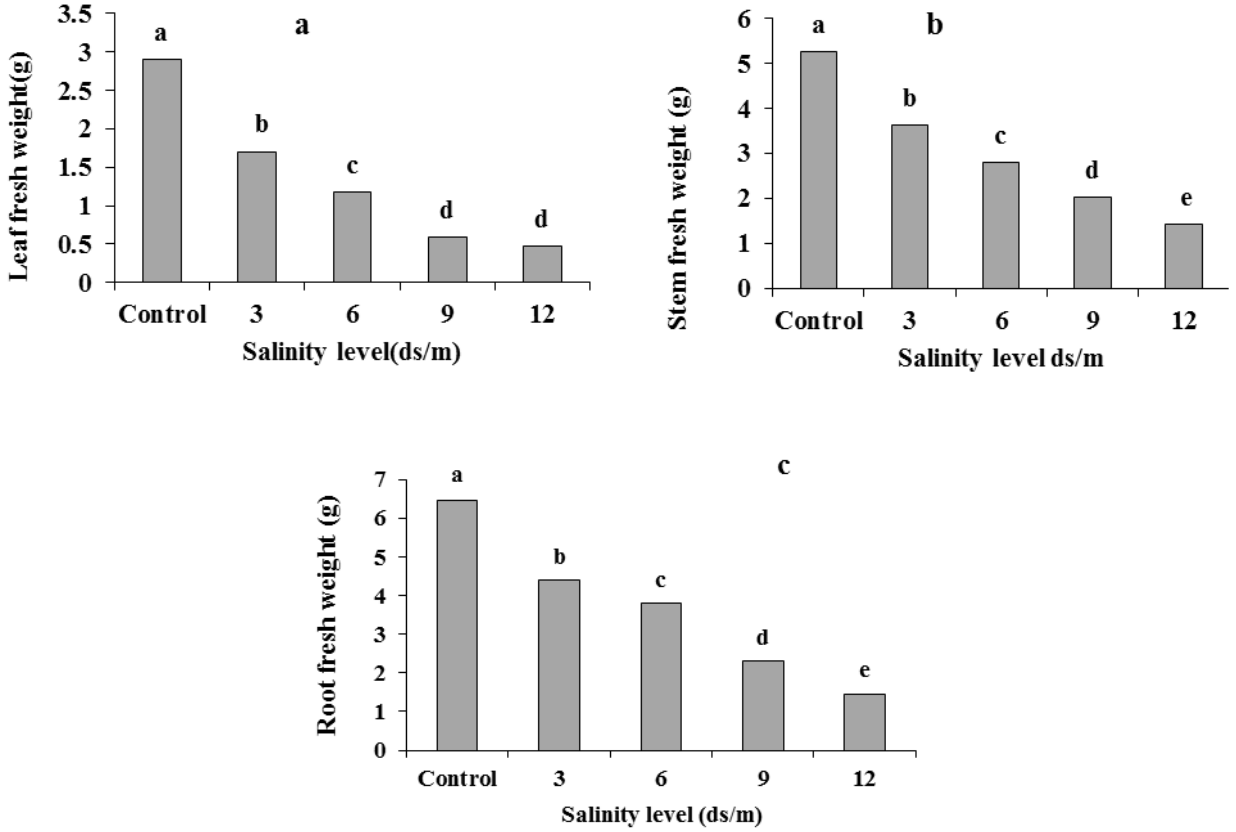

Fig 1. Effect of seawater irrigation on plant growth characters of Kherol seedlings.(a)leaf fresh weight,(b)stem fresh weight,(c) root fresh weight.

believe that reducing in plant growth is due to increasing Na^+ and Cl^- in tissues and disturbed ionic balances. Also it is well documented that salt tolerance is associated with the ability of plant to limit the uptake and/or transport of salt ions from root to shoot (Greenway and Munns, 1980; Tester and Davenport, 2003; Tester and Davenport, 2003). Ndayiragije and Lutts, 2006 believe that reduction of fresh and dry weights of root and leaves are due to reduction in physiological and biochemical activities like cell elongation, cell division and synthesis of auxins.

Chlorophyll: There was significant difference between salinity levels and control for Chlorophyll content of leaf. Fig. 4 reveals that chlorophyll *a* decreased considerably from 20 (mg/g FW) to 0.4 (mg/g FW) respectively in control and in 12 ds/m salinity concentration. Chlorophyll *b* content that was measured spectrophotometrically, also decreased significantly by increasing salinity level and minimum chlorophyll was found in 12 ds/m salinity level. In addition total

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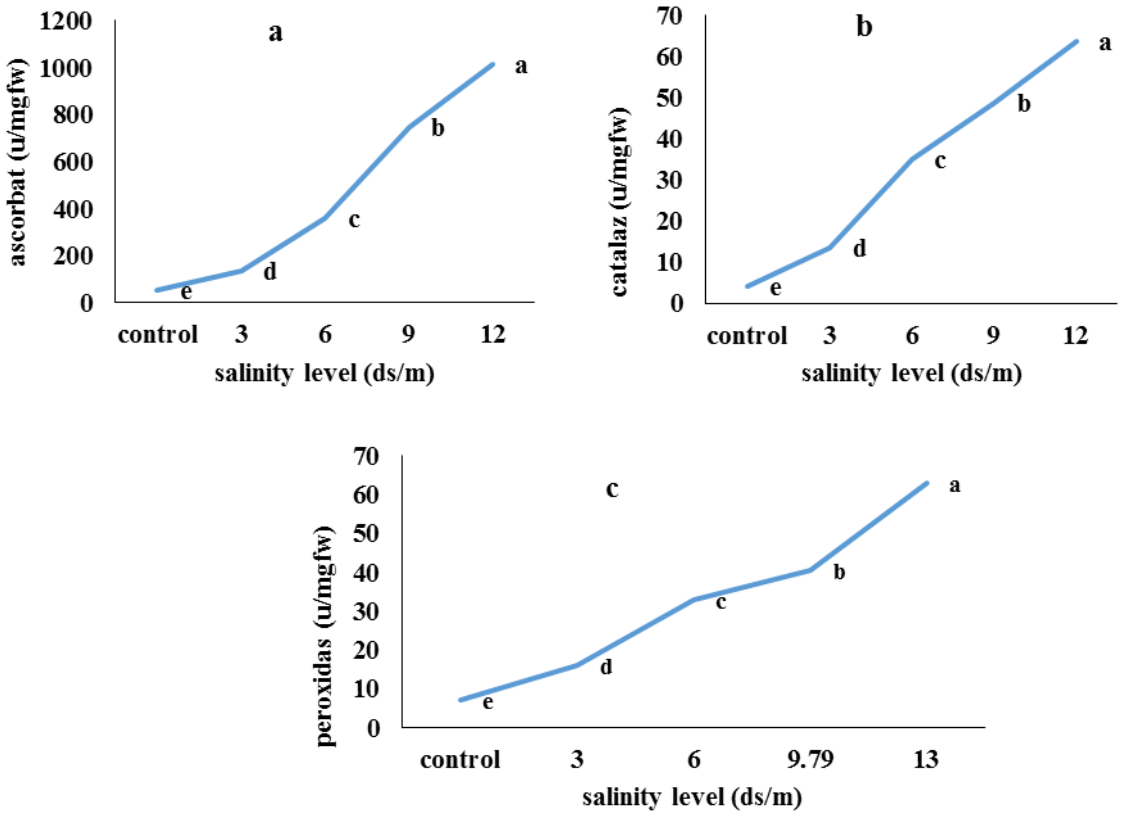

Fig 3.Changes in antioxidant enzyme a (Catalas), b (Peroxidas) and c (Ascorbate Peroxidas) in the leaves of Kherol grown under seawater salinity

chlorophyll also decreased by increasing salinity seawater. Our results were in accordance with the findings of Aldesuquy and Ibrahim (2001) that showed, irrigation of wheat plants by seawater at 25% caused significantly decrease in pigment. Decrease in chlorophyll contents due to salinity has also been reported by Gu *et al*., (2004). Similar response was reported for melons (Kusvuran, 2010). Furthermore, Kaya *et al*., (2001) observed that chlorophyll content in spinach was reduced significantly by high salinity. Saha *et al*. (2010) also reported a steady decrease in the chlorophyll concentration with increasing in salt stress of different pumpkin genotypes. However, some studies (Evain *et al*., 2004; Paranychianakis and Chartzoulakis, 2005) have also reported an increase in chlorophyll contents in some cultivars of different plant species. Hussein et al. (2014) reported that chlorophyll *a* concentrations were higher in salt-treated onions in compared to the control plants. No negative effects of salinity on chlorophyll content in chick pea plants were stated (Beltagi, 2008). It has been explained that reduction of chlorophyll is due to the reduction of concentration of magnesium, which is a major component of leaf chlorophyll (Koyro, 2000). The reduction of photosynthesis rate in plants is due to chlorophyll reduction and also reduction of Co_2 absorption, lower values of stomatal conductance and relative water content (Fransisco, 2002; Naumann *et al*., 2007; 2008). Shah, (2007) believed that chlorophyll is membrane bound and depends upon the membrane stability thus it rarely remains intact under saline conditions. However, researchers exhibited that decline in chlorophyll may be due to suppression of specific enzymes that are responsible for its synthesis (Murkute *et al*., 2006; Keutgen and Pawelzik, 2007). Low to moderate salinity stress can rouse chlorophyll degradation, while higher salt concentrations affect chlorophyll synthesis more extremely (Santos, 2004).

Antioxidative enzyme activity: Fig 4. Shows the effect of seawater salinity on Peroxidase (POD), Catalase (CAT) and Ascorbate peroxidase (APX) activities in seedling of *Manilkara hexandra*. Peroxidase (POD) and Catalase (CAT) showed the same trend during stress and their activities increase strongly by increasing level of salinity. However Ascorbate peroxidase (APX) showed the different pattern of activity. Its activity increased gradually by increasing salinity levels. Similar to our results, the activity of antioxidant enzymes (CAT, POD, APX) also increased when bean seedling irrigated by 25% seawater (Azooz *et al*., 2011). In other research, Peroxidase (POD) activities in Faba bean leaf gradually increases with increasing salinity levels (Dawood *et al*, 2014). El-Bassiouny and Bekheta (2005) and Khattab (2007) also reported similar result. Anti-oxidative enzymes activities are important in the assessment of tolerance

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mechanisms because their activities are the first response against stresses. In agreement with our findings, Sreenivasulu *et al*, (1999) stated that in tolerant plants, POD activity was increased to defend plants against oxidative stresses. SOD enzyme was localized in chloroplast, mitochondrion, cytoplasm and peroxisome and acts as the first line to protect tissue against ROS by changing O_2 to H_2O_2 (Liu et al., 2007). Srivastav *et al*., (2010) showed that the salt treated seedlings of mango rootstock had higher CAT, SOD and POX activities than control plants.

Vaseva *et al*. (2012) believe that the recognition of physiological and biochemical mechanisms of the oxidative defense system could be necessary to determine salt-tolerant plants. Bian and Jiang (2009) and Dolatabadian and Jouneghani (2009) suggested that, plants can improve their protective mechanism to salinity stress by reducing oxidative damage through increasing activities of different antioxidant enzymes. In fact, some factors such as duration and intensity of the stress, variety in species could change the activities of antioxidant enzymes and plant tolerance (Dogan, 2013).

Conclusions:

Results of the current study indicated that seawater irrigation showed no significant effects on morphological characters such as length of stems and roots. However, fresh weight of stem, leaf and root were reduced significantly by increasing seawater concentration. Generally, *Manilkara hexandra* have very slow growth rate therefore didn't show any significant morphological changes during stress period. Antioxidant enzymes increased strongly under salinity condition. POD and CAT activities increased strongly during salinity stress meanwhile, APX increased gradually. To the best of our knowledge, there is little available data on studying the effects of seawater irrigation on woody crop such as *Manilkara hexandra.*

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