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Assessment of Antioxidant Activity, Grain and Oil Production of Amaranth (*Amaranthus retroflexus* L.) in Saline Conditions

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ABSTRACT

Salt stress is one of the major factors limiting crop productivity worldwide. Grain amaranth is new crop with high yield potential and good nutrition value which can be a good substitute for salt-sensitive crops in saline areas. This research was conducted to evaluate different level of salinity and applied salinity stress at several growth stages on some morphological and physiological traits of Amaranth via split plot experiment based on completely randomized with three replications. The main factor included five level of salinity (Control, 75, 150, 225 and 300 mM NaCl). The sub factor consisted applied salinity stress at four growth stages (Plant establishment, branching, flowering and grain filling period) under hydroponic system with Hoagland solution. According result of analysis of variance interaction applying different levels of salinity at different growth stages was significant at 1% probability level for all traits (instead hydrogen peroxide; H_2O_2, malon di aldehyde; MDA and total phenolic). Mean comparison results showed that application of 300 mM salinity concentration after plant establishment stage led to death of amaranth. Salinity application after establishment decreased significantly plant height, number of branches and panicle per plant as 44.9, 31.8 and 35.4%, respectively. Root volume only decreased after salinity 225 mM after plant establishment and 300 mM at the branching as 38 and 45%, respectively. Production of grain weight was not affected by 75 mM salinity, but at higher salinity showed significant decrease. The highest decrease in grain weight obtained by applying 225 mM salinity after the plant establishment and salinity at 300 mM after branching as 86.6 and 71.3%, respectively, resulting in a decrease in both 1000 kernel weight and grain number, respectively. Salinity application increased H_2O_2, MDA and total phenolics contents, severely. Most of characteristics were not affecting by 75 mM NaCl, but other concentrations had a negative effect on the growth and production of amaranth. In this study, the most sensitive application time to salinity was after plant establishment and the most tolerant was grain filling stage.

Keywords: *Biochemical traits, Morphology, Salinity stress.*

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INTRODUCTION

Salinity is an agricultural problem that decreases or restricts crop production in many areas. As concern about limited water resources continue to increase due to rapid expanding populations, there will be a greater need to use poor quality water in crop production. The increase in use of saline water for irrigation poses a potential hazard to the quality of agricultural soils. Appropriate management options are required to prevent and/or relieve salinity problems in crop production. Timing of salinity stress, i.e., initiation and termination of a salinization period at different growth stages, is one such option. This option considers crop sensitivity at different growth stages, which is one of the major issues in the utilization of saline water for crop production (Shalhevet, 1994). *Amaranthus*, collectively known as amaranth, is a cosmopolitan genus of annual, short-lived or the perennial plants. Some amaranth species are cultivated as leaf vegetables, pseudo cereals, and ornamental plants. Amaranth (*Amaranthus sp*.) is a promising crop for semi-arid regions. It exhibits a high nutritive value but also a fascinating ability to adapt to diverse harsh environments (Omami *et al*., 2006). Wouyou *et al*. (2017) reported NaCl salt stress reduced young plant growth in *A. cruentus* cultivars. It underlined, for the first time, variability of relative salt-stress resistance for some *A. cruentus* cultivars at young plant stage. Plant height, leaf number and root length appeared as the most suitable growth parameters for studying salt stress effect in *A.cruentus.* Omami *et al.* (2006) studied salinity impact on some Amaranth cultivars such as; *A. tricolor*, *A. hypochondriacus, A. cruentus* and showed that increasing salinity in the soil leads to significant reduction in crop height, leaf area, specific leaf area, and stomatal conductance rates. Dave and Patel (2011) examined effect of salinity on *Amaranthus lividus* and reported root and shoot length, number of leaves, fresh and dry weight of leaves; roots and stems significantly decreased with increasing salinity levels. In this study proline showed a significant increase with increasing salinity, while chlorophyll content reduced by salinity. Salinity reduced number of hairy roots and distal root growth and root cells were more resistant to water penetration (Wang and Li, 2008). Omami (2005) reported that the reduction in leaf area affected by salinity come from reducing leaf number and leaf size of amaranth. Malon di aldehyde (MDA) a product of lipild peroxidation, showed greater accumulation in plants under stress condition. Cell membrane stability has been widely used to differentiate stress tolerant and susceptible cultivars of some crops, and in some cases, lower MDA content could be correlated with the stress tolerance (Wang *et al*., 2009). As salt stress occur frequently plants have developed several strategies to cope with these challenges. One of the stress defense mechanisms is the antioxidant defense system, which includes the antioxidant enzymes. Antioxidant enzymes converts H_2O_2 into water and oxygen (Gaber, 2010). Increasing soil salinity loses leaf cells water, but this water losing from cell is temporary and can be recovering within hours (Cramer, 2002; Fricke and Peters, 2002). The increase levels of abscisic acid in shoots under salinity decreased the amount of gibberellin, cell elongation and development. In old leaves that do not grow, the solutes do not dilute by the lack of growing, so the toxic effects has seen, and even leads to the death of them (Munns and Tester, 2008). Odjegba and Chukwunwike (2012) by

evaluate the physiological responses of *Amaranthus hybridus* L. under salinity stress reported salinity caused a significant decrease in whole plant dry weight, relative water content, total chlorophyll and protein content while an increase in malon di aldehyde content, catalase and ascorbate peroxidase activities were observed. The severity of these effects was concentration dependent. The biomass accumulation of the control plants was 11.67±0.39 g, while those that received 0.1 and 0.2 M NaCl had 9.22±0.28 and 6.94±0.07 g respectively. The increase in malon di aldehyde content and antioxidant enzymes activities were indications that salinity stress induced the production of reactive oxygen species (ROS) which caused oxidative damage to macromolecules in living cells. Amaranth is used for its grain and is also consumed as a cooked vegetable in many parts of the world. Owing to its high nutritive value and a wide adaptability to diverse environments, Amaranth has been considered a promising crop for semiarid regions (Cunningham *et al*., 1992; Allemann *et al*., 1996). The prospects for future cultivation of salt tolerant, high yielding genotypes of amaranth are very encouraging. However, despite a substantial amount of literature on responses of plants to salinity stress, little information is available on amaranth. Omami (2005) reported it is feasible to use saline water for growing amaranth with minimum yield losses if salt concentration and time of salinization can be managed. The objectives of study was to investigate the response of amaranth to different concentration of salinity and applied salinity stress at different growth stages on some morphological, physiological and biochemical traits of amaranth.

MATERIALS AND METHODS

Field and treatments information

This research was conducted in greenhouse of Islamic Azad University; Tabriz Branch to assessment the antioxidant activity, grain and oil production of Amaranth grain affected the different concentration of salinity and applied salinity stress at several growth stages via split plot experiment based on completely randomized design during 2013 with three replications. Place of research was located in Tabriz city at longitude 46°17'E and latitude 38°05'N in East Azerbaijan province (Northeast of Iran) with 1360 meters altitude. The main factor included five level of salinity (Control, 75, 150, 225 and 300 mili Molar; mM NaCl). The sub factor consisted applied salinity stress at four growth stages (Plant establishment, branching, flowering and grain filling period) under hydroponic system with Hoagland solution on grain amaranth (*A. hypochindriacus* × *A. hybridus*) as a new crop. 1100 gr. perlite medium grain size filled into pots. Grains randomly distributed on the surface of the perlite. Nutrition of crops was supplied by using nutrient solution after emergence.

Greenhouse management

***Plant Nutrition*:** Hoagland's A-Z solution was used to provide macro and micro nutrients (Table 1) (Nenova, 2008). Two weeks after emergence, grainlings thinned to five plants per pot and in third week after emergence were kept only three plants per pot. Every four days nutrient solution (1/2 fold in the early period of growth) was supplied to plants (Nenova, 2008). The most important factor to increase the availability of micro-nutrients was pH.

Table 1. The amount of nutrients need to produce Hoagland solution

Chemical compound	Concentration ($mg.l^{-1}$)	Chemical compound	Concentration ($mg.L^{-1}$)	Chemical compound	Concentration ($mg.l^{-1}$)
Micro elements	**18 L water (use in $ml.l^{-1}$ solution I)**	**Micro elements**	**18 L water (use in $ml.l^{-1}$ solution I)**	**Macro elements (solution I)**	
$NiSo_4.6H_2O$	1g	LiCl	0.5g	KNO_3	303.3
$Co(NO_3)_2.6H_2O$	1g	$CuSo_4.H_2O$	1g	$Ca(NO_3)_2.4H_2O$	472.2
KI	0.5g	$ZnSo_4$	1g	$NH_4H_2PO_4$	115.0
TiO_2	1g	H_3BO_3	11g	$MgSO_4.H_2O$	69.2
KBr	0.5g	$Al_2(SO_4)_3$	1g		
Iron ammonium citrate 0.5%	1 $ml.l^{-1}$	$SnCl_2.2H_2O$	0.5g		
		$MnCl_2.4H_2O$	7g		

The nutrient pH solution was reduced to 5 with adding nitric acid 95% (Kang and Van Iersel, 2004). Hoagland's solution electrical conductivity (EC) was the reference or control and salinity levels prepared by adding sodium chloride solution. Treatments were performed three weeks after seed emergence. The amount of used solution for treatment was determined based on available water in each pot. For this purpose, the weight of irrigated pearlite determined after 24 hours and the initial weight of pearlite before irrigation was fractioned. Then the amount of water turned to volume. The resulted number is between 550 to 600 mili liters (ml) of water for each pot. Accordingly, 550 mL of each solution was used for the treatments. No water leaching was permitted from pots. After 30 days, excess water used to leaching pots.

Traits measure

***Hydrogen peroxide (H_2O_2), Malon di aldehyde (MDA) and Total phenolics*:** H_2O_2 content in amaranth leaves at grain filling stage were determined according to Velikova *et al.* (2000) method. Leaf tissues (0.5 gr) were homogenized in an ice bath with 5 ml of 0.1% (w/v) trichloroacetic acid (TCA). The homogenate was centrifuged at 10,000g for 15 min and 0.5 ml of the supernatant was added to 0.5 mL of 10 mM potassium phosphate buffer (pH 7.0) and 1 mL of 1 M KI. The absorbance of the supernatant was measured at 390 nm. The level of lipid peroxidation (MDA) was determined in terms of thiobarbituric acid-reactive substances concentration as described by Noreen and Ashraf (2009). Fresh leaf (1.0 gr) was homogenized in 3 mL of 1.0% (w/v) TCA at 4°C. The homogenate was centrifuged at 20,000g for 15 min and 0.5 mL of the obtained supernatant was added to 3 mL of 0.5% (v/v) thiobarbituric acid (TBA) in 20% TCA. The mixture was incubated at 95°C in a shaking water bath for 50 minute, and the reaction was stopped by cooling the tubes in an ice water bath. Then the samples were centrifuged at 10,000g for 10 minute, and the absorbance of the supernatant was read at 532 nm. The value for nonspecific absorption at 600 nm was subtracted. The concentration of TBARS was calculated using the absorption coefficient, 155 mili $Molar.cm^{-1}$. Total phenolics were determined using Folin-Ciocalteau reagent (Noreen and Ashraf, 2009). Fresh leaf tissue (50 mg) was homogenized with 80% acetone and centrifuged at 10,000g for 10 minute. One-hundred µl of the supernatant were diluted with 2 mL of water and 1 mL of Folin–Ciocalteau's phenol reagent and shaken vigorously. Then 5 mL of 20%

sodium carbonate solution was added and the volume was made up to 10 ml with distilled water. The contents were mixed thoroughly and the absorbancem was read at 750 nm using a spectrophotometer (IRMECO U2020). The results were expressed as $mg.g^{-1}$ of fresh leaf.

Physiologic and morphologic traits: After harvest, traits such as plant height, number of branches, number of panicle, panicle length, leaf area, grain weight and grain weight per plant, shoot and root dry weight, and root length and volume was measured. Grain oil percentage were measured by the micro-souqksole method (Yaniv, 1999).

Statistical analysis

Analysis of variance (ANOVA) and mean comparisons were done by MSTAT-C software and Duncan multiple range test (DMART) at 5% probability level.

RESULT AND DISCUSSION

Plant height

Plant height was significantly affected by different level of salinity, application salinity stress at different growth stage and interaction effect of treatments at 1% probability level (Table 2). Salinity applying in the beginning stages of branching, flowering and grain filling had no significant effect on plant height, but salinity levels affected plant height. Salinity up to 150 mM did not effect on plant height at establishment stage but as it increased, significantly decreased plant height. Salinity level at 225 mM, decreased plant height to 38 cm which was 45% lower than the control treatments (Fig. 1).

Table 2. Analysis of variance effect of different salinity level and application salinity stress at different growth stage on measured traits

S.O.V	df	Plant height	Branch No.	Panicle length	Leaf area	Root volum	Root length
Different level of Salinity (A)	4	877.79**	4.23**	329.32**	36830.06**	124.475**	318.63**
Application salinity stress at different growth stage (B)	3	3141.02**	8.84**	388.93**	70866.19**	178.800**	67.4ns
A × B	12	492.09**	2.29**	76.18**	10701.07**	40.286**	188.41**
Error	40	70.35	0.45	11.517	1504.283	3.85	36.02
CV (%)	-	13.17	16.23	13.83	17.01	10.94	11.56

ns: non significant, * and ** significant at 5% and 1% levels, respectivly.

Continue of Table 2.

S.O.V	df	MDA	H_2O_2	Total phenol	1000 kernel weight	Grain No. per plant	Oil percentage
Different level of Salinity (A)	4	53.89**	21. 43*	4.54*	3.063**	15953.1**	163.88**
Application salinity stress at different growth stage (B)	3	6.23ns	4.41ns	0. 65ns	2.475**	86743.6**	52.79**
A × B	12	0.87ns	2.34ns	0.22ns	0.933**	30106.5**	4.35**
Error	40	18.57	3.84	0.88	0.017	35754.5	0.516
CV (%)	-	13.68	13.83	7.04	4.76	17.01	5.43

ns: non significant, * and ** significant at 5% and 1% levels, respectivly.

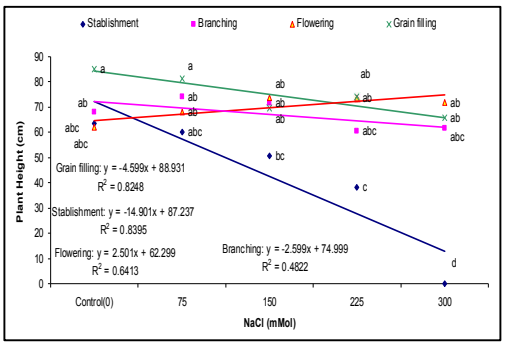

Fig. 1. Mean comparison interaction effect of different level of salinity and applying salinity stress at different growth stage on plant height via Duncan test at 5% probability level.

Applying 300 mM NaCl after establishment stage led to plant death. Simple linear regression equation showed that for each unit increase in salinity in the growth stage, plant height reduced 14.9 cm. While the reduction in the branching and grain filling was 4.6 and 2.6 units, respectively, which may not provide a significant effect (Fig. 1) However, salinity application after establishment of plant when cells and plant growth begins, coused a significant reduction in the amaranth height. Salinity by reducing water absorption decreased water potential and resulting reduced cell and plant growth (Yadav *et al.,* 2011). The beginning and end of branching in amaranth depends on the type of crop and environmental factors. In most crops branching ends by beginning of flowering (Beveridge *et al.,* 2003). Average salinity stopped growth of lateral branches for more weeks (Munns and Tester, 2008). Factors such as photoperiod active meristem numbers, crop hormones and assimilation rate are effective on branching of amaranth (Beveridge *et al.,* 2003). Omami (2005) studied effect of salinity on some varieties of grain amaranth (*A. tricolor, A. hypochondriacus* and *A. cruentus*) and announced that 200 mM salt decreased *A. hypochondriacus* height at a rate of 62% and *A. cruentus* by 59%. Should bear in mind that plant growth continue until the beginning of flowering stage, so apply salt after this point will not affect the height of amaranth. Abdel Aziz *et al.* (2011) reported similar results in *A. tricolor*.

Branch number

Evaluation result of analysis of variance showed effect of different level of salinity, application salinity stress at different growth stage and interaction effect of the treatments on branch number was significant at 1% probability level (Table 2). Salinity at the beginning of branching, early flowering and grain filling stages had not significant effect on number of branches per plant. Therefore, induction of branch buds must do before branching. Applying 300 mM salt after plant establishment had a significant effect on number of branches (Fig. 2).

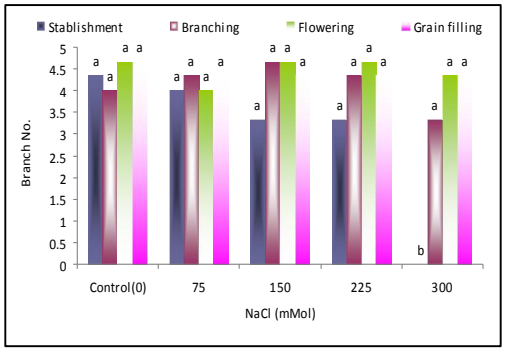

Fig. 2. Mean comparison interaction effect of different level of salinity and applying salinity stress at different growth stage on number of branches via Duncan test at 5% probability level.

Salinity reduced number of branches per plant and growth and development such as reducing number of florets and earliness flowering of plant affected by salinity (Muuns and Tester, 2008).

Panicle length

Result of analysis of variance revealed effect of different level of salinity, application salinity stress at different growth stage and interaction effect of the treatments on panicle length was significant at 1% probability level (Table 2). Low levels of salinity had not effect on amaranth panicle length but, 225 and 300 mM salt, showed a negative impact on it. None of the salt concentrations applied during flowering and panicle initiation, and grain filling duration had significant effect on panicle length. Panicle growth stops at grain initiation and grain filling stage. Salinity stress in the onset of flowering and panicle length had little effect on panicle length. Application of 225 mM salt in crop establishment reduced 46 % and application of 225 and 300 mM salt in branching reduced 33 and 64 % of the panicle length, respectively. Simple linear regression equation showed that for every unit increase in salinity after establishment, panicle length was reduced 6.5 units. While the loss in the branching stage is only 4 units. The drop in the panicle length at flowering and grain filling stage was not significant (Fig. 3).

Leaf area

According result of analysis of variance effect of different level of salinity, application salinity stress at different growth stage and interaction effect of treatments on leaf area was significant at 1% probability level (Table 2). Leaf area decreased by the salinity increasing. Maximum reduction in leaf area made by application of 300 mM at the beginning of branching to 66% of control treatments. However, if salinity applied at 300mM at flower initiation, leaf area decreased 32 %. So, the time of salinity stress had important role in decreasing traits.

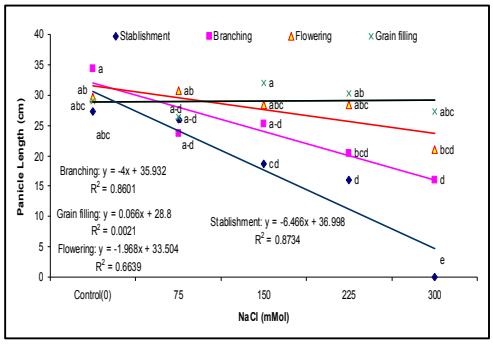

Fig. 3. Mean comparison interaction effect of different level of salinity and applying salinity stress at different growth stage on panicle length via Duncan test at 5% probability level.

Application of 225 mM salt in crop establishment, branching and early flowering reduced amaranth leaves rate as 62, 48 and 31%, respectively. Simple linear regression equations showed that for every unit increase in salinity after establisment 51 unit of leaf area reduced. Changes rate in salinity levels applied after flowering and grain filling was not significant on leaf area (Fig. 4). With increasing plant age, leaf area reduction was less affected by salinity. Applying 150 and 225 mM NaCl after plant establishment led to leaf burning and necrosis. While at 300 mM, the leaves of the plants died out and were suffering from root rot (Flowers, 2004). Applying 150 mM salinity after the establishment stage, branching and grain filling, decreased leaf area of amaranth to 54, 44 and 42% and 75 mM salinity on crop establishment and early stages of branching decreased 30 and 28%, respectively (Fig. 4). In their research, the salinity of 3000 ppm decreased the inflorescence length more than 36%. Should be kept in mind that the leaf growth in flowering stage reduced. Old leaves drying by salinity is the reason to decrease leaf area.

Salinity at early stage of plant growth reduced both number and leaf growth, and dried leaves. So in the early stages of growth, salinity can have a greater impact on leaf area. Makus (2003) reported that salinity made a significant decrease in Amaranth leaf area.

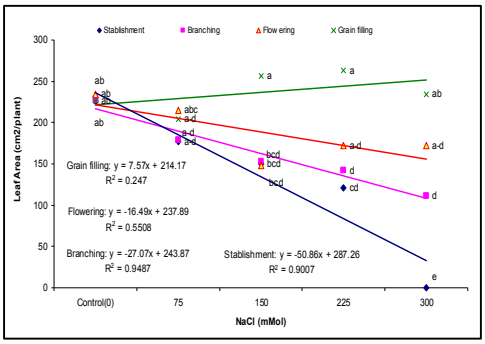

Fig. 4. Mean comparison interaction effect of different level of salinity and applying salinity stress at different growth stage on leaf area via Duncan test at 5% probability level.

Omami (2005) investigated effect of the salinity on leaf area of grain amaranth (*A. tricolor*, *A. hypochondriacus* and *A. cruentus*). 200 mM salt reduced leaf area of *A. hypochondriacus* and *A. cruentus* as 74% and 68% respectively. High salinity damage signs to plant detectable such as leaf burning by Na^{+}, and Cl^{-} ions (Wahome *et al.,* 2001). High salinity levels increased leaf senescence rate, and decreased photosynthesis (Carillo *et al.,* 2010). Decreasing Calcium absorption due to salinity caused a reduction in leaf growth (Lauchli and Grattan, 2007). Leaf growth rates decreasing could be due to osmotic effects of salts in the root zone (Carillo *et al.,* 2010).

Root length and volume

Root volume was significantly affected by different level of salinity, application salinity stress at different growth stage and interaction effect of treatments at 1% probability level. Also effect of different level of salinity and interaction effect of treatments on root length was significant at 1% probability level, but effect of application salinity stress at different growth stage was not significant (Table 2). Root length not affected by salinity of 75, 150 and 225 mM (Fig. 5). Root length affect in none of the concentrations of salt applying at flowering and grain filling stages. But salinity at 300 mM in branching decreased root length as 29%.

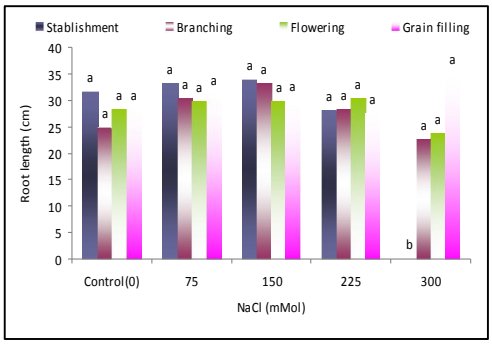

Fig. 5. Mean comparison interaction effect of different level of salinity and applying salinity stress at different growth stage on root length via Duncan test at 5% probability level.

The root volume was not affected by salinity of 75 and 150 mM, but applying higher salinity at early stages decreased root volume. Salinity at 225 mM application after establishment stage decreased amaranth roots volume, but root volume in 225 mM salt not changed, salinity at 225 mM application after establishment decreased root volume to 38%. Although only in the early stages of crop growth applying 225 mM salinity reduced amaranth roots volume, salinity applied at later stages of crop growth reduced significant the volume of amaranth roots also. So, salinity at 300 mM at the branching stage decreased 45% size of the amaranth roots. Simple linear regression equations showed that every unit increase in salin-

ity in establishment stage reduced 6.4 units root volume of amaranth. Changes in salinity levels after flowering and grain filling was not significant (Fig. 6). Other researchers have reported similar results in different crops (Huerta-Ocampo *et al*., 2014).

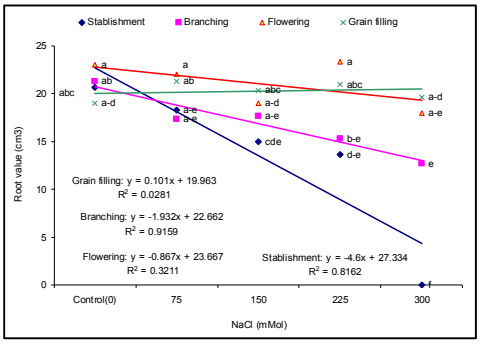

Fig. 6. Mean comparison interaction effect of different level of salinity and applying salinity stress at different growth stage on root volume via Duncan test at 5% probability level.

Munnes and Tester (2008) stated that root growth is rarely affected by salinity decreasing. Omami (2005) reported that the growth of roots in Amaranth more affected by salinity than shoot growth. Dave and Patal (2011) reported reducing root growth by salinity stress. Salinity stress reduced the rate of photosynthesis, growth and the transmission assimilate. So, decreasing of assimilate amount and transduction in the saline condition, decreased root and shoot growth. On the other hand, researches have shown that the amount of ABA increased in crop roots by salinity (Yadav *et al.,* 2011). ABA production in high salt concentrations in the roots, reduced root growth (Sharp and Le Noble, 2002).

MDA, H_2O_2 and total phenolic contents

Assessment result of analysis of variance indicated effect of different level of salinity on MDA content was significant at 1% probability level but effect of application salinity stress at different growth stage and interaction effect of treatments was not significant (Table 2). As well as effect of different level of salinity on H_2O_2 and total phenolic contents was significant at 5% probability level but effect of application salinity stress at different growth stage and interaction effect of treatments was not significant (Table 2). According mean comparison result increasing salinity level led to increase MDA, H_2O_2 and total phenolics contents in amaranth leaves (Table 3). Salinity levels of 75 mM had no significant effect on H_2O_2 and MDA content in amaranth leaves. However, enhancment of salinity to 150, 225 and 300 mM significantly increased H_2O_2 content as 35.9, 50.3 and 74.7%, respectively compared to non saline conditions; these increase for MDA amount were 62.9, 77.3 and 86.9 %, respectively. Total phenolics contents in the amaranth leaves significantly increased with enhancment of salinity from non salinity condition to 75, 150, 225 and 300 mM NaCl as 15.7, 24.2, 33.7 and 46.8%, respectively (Table 3). High increase content of H_2O_2 showed that amaranth in high salinity levels was sensitive; on the other hand the high increase content of MDA is Amaranth's appropriate response to the salinity. MDA is the decomposition product of the polyunsaturated fatty acids of the membranes under stress (Moosavi *et al*., 2009). The rate of lipid peroxidation level in terms of MDA can therefore be used as an indication to evaluate the tolerance of plants to oxidative stress as well as sensitivity of plants to salt stress. It is also known that the formation of the ROS enhances peroxidation at the cellular level and that the rate of such enhancement

relates to plant species and the severity of stress (Wang *et al.,* 2009). Variation in MDA contents were found in rice (Tijen and Ismail, 2005) and cotton (Meloni *et al.,* 2003) cultivars differing in salt tolerance. In amaranth leaves H_2O_2 remained unchanged due to salt stress. While, in contrast, it is generally known that salt stress enhances the production of singlet oxygen, superoxide anion, H_2O_2 and hydroxyl radical in the plants. However, regulation of these ROS depends on their rates of generation, their rate of reaction with other metabolites such as proteins, lipids and nucleic acids, their rate of degradation and rate of their neutralizing by enzymatic or the non enzymatic antioxidants. Generally, the dismutation of two superoxide anions either enzymatically or non enzymatically, give rise to the H_2O_2. H_2O_2 is also produced from the β-oxidation of fatty acids and the peroxisomal photorespiration reactions (Noreen and Ashraf, 2009). Of various secondary metabolites, terpenes and phenolics are more important to abiotic stress tolerance than the others due to the their structural properties. For example, the enhanced synthesis of soluble phenolics has been directly correlated with salt and heat tolerance of sugarcane (Wahid and Ghazanfar, 2006).

Table 3. Mean comparison effect of different level of salinity on H_2O_2, total phenolics and MDA content

Treatment	MDA (nmol.g^{-1} fw)	H_2O_2 (µmol.g^{-1} fw)	Total phenolic (mg.g^{-1} fw)
NaCl (mM)			
0	2.91*^{cd}	9.23^{d}	4.21 ^{d}
75	3.37 ^{c}	10.33 ^{dc}	4.87 ^{c}
150	4.74 ^{b}	12.54 ^{c}	5.23 ^{bc}
225	5.16 ^{ab}	13.87 ^{b}	5.63 ^{b}
300	5.44 ^{a}	16.12 ^{a}	6.18 ^{a}

*Treatments with the same letter(s) don't have significant difference via Duncan test at 5% probability level.

1000 Kernel Weight

Result of analysis of variance showed effect of different level of salinity, application salinity stress at different growth stage and interaction effect of treatments on 1000 kernel weight was significant at 1% probability level (Table 2). Salinity applying at 75, 150 and 225 mM in the establishment stage of amaranth reduced 1000 kernel weight as 10, 13.3 and 23.3%, respectively, (Fig. 7). In this study, salinity applying at 75 mM in the beginning stages of branching and flowering decreased 16.6 and 13.3% of 1000 kernel weight, respectively. Salinity application at 150 mM at the beginning of branching was not affected 1000 kernel weight and 150 mM in the flowering stages, increased 1000 kernel weight significantly. This increase was due to a decrease in number of grain per plant (Fig. 8) and high leaf area (Fig. 4), which leads to more assimilate for grain filling. In salinity more than 150 mM significantly reduction in 1000 kernel weight was observed. Simple linear regression equations showed that for every unit increase in salinity after the establishment stage, 1000 kernel weight was reduced 0.65 units. The reduction in the branching and flowering stages was 0.22 and 0.08 unit, respectively. Changes rate in salinity levels after grain filling had not significant differences.

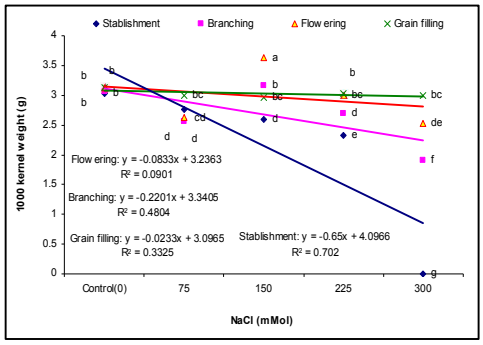

Fig. 7. Mean comparison interaction effect of different level of salinity and applying salinity stress at different growth stage on 1000 kernel weight via Duncan test at 5% probability level.

Thus delay in applying salinity reduced the negative effects of salt stress on 1000 kernel weight (Fig. 7). This experiment showed a significant reduction in grain weight also. Application of 225 and 300 mM salinity at the branching reduced 20% and 36%, respectively and application of 300 mM salt in flowering stage decreased 1000 kernel weight as 16.6%. Research has shown that reproductive organs of plants are more sensitive to the environmental stresses than grain filling period (Gelalcha and Hanchinal, 2013). The number of reproductive parts of plant during mild stress decreased, while the rate of decline in assimilates entered to reproductive parts of plant is more than decrease in reproductive parts of plant. Similar results have been reported by other researchers in wheat (Shah *et al.,* 2011).

Grain number per plant

According result of analysis of variance effect of different level of salinity, application salinity stress at different growth stage and interaction effect of treatments on grain number per plant was significant at 1% probability level (Table 2). Maximum number of grain per plant was 5524 in control. Salinity of 75 mM had not effect on grain per plant, however, higher salinity levels showed significantly negative effect on grain per plant. Decrease in grain per plant at 225 and 300 mM salinity was 38.5 and 56%, respectively. Application of 150, 225 and 300 mM, in the beginning stages of branching reduced the number of grain per plant 35.4, 38.5 and 35.5%, respectively. Application of 225 mM salinity after crop establishment reduced 81.2% of grain number per plant. Application of 150 mM salinity at crop establishment reduced 50.2% grain number per plant. So in three concentrations of 150, 225 and 300 mM, the maximum reduction in the number of grain per plant was affected by salinity imposed in the early stages of crop growth (Fig. 8).

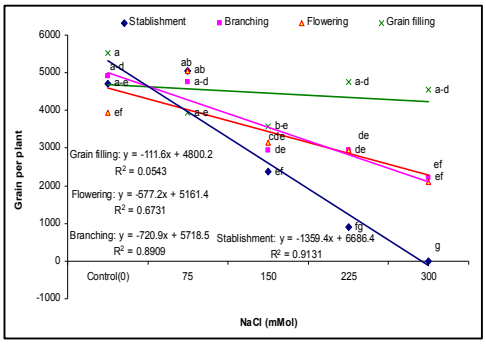

Fig. 8. Mean comparison interaction effect of different level of salinity and applying salinity stress at different growth stage on number of grain per plant via Duncan test at 5% probability level.

Oil production

Oil production was significantly affected by different level of salinity, application salinity stress at different growth stage and interaction effect of treatments at 1% probability level (Table 2). As salinity stress increased, oil percentage decreases (Fig. 9). In addition stress application in the early stages of growth had a greater impact on oil percentage. The highest reduction in oil

percentage was observed with applying salinity stress in the establishment stages. Applying salinity stress in establishment stage, branching, early flowering and grain filling stages respectively led to 80, 62, 51 and 30% decrease in amaranth's oil percentage compared to control condition.

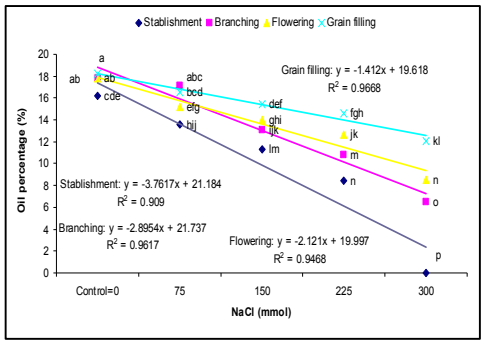

Fig. 9. Mean comparison interaction effect of different level of salinity and applying salinity stress at different growth stage on oil percentage via Duncan test at 5% probability level.

Linear regression equation showed that for every unit increase in salinity after the establishment, branching, flowering and grain filling stages, 3.7, 2.9, 2.1 and 1.4 unit of oil percent were reduced. Thus amaranth oil production is more sensitive to salinity stress compared to other traits (Fig. 9).

CONCLUSION

Grain yield reduction its components and growth indices of Amaranth affected by salinity were similar to most crops. Based on these results, the grain amaranth cultivar (cv. Koniz) growth factors such as crop height, productive branches, leaves and root development and yield components such as number and grain weight decreased with increasing salinity. The highest and the lowest significant reduction in grain yield production was 86 % and in 1000 kernel weight was 13 %. Salinity up to 75 mM had not significant effect on most morphological and physiological attributes. According to non-significant changes of imposing salinity on different characteristics at different stages of crop growth, it can be concluded that grain amaranth has a good tolerance to the environmental stresses ranging up to 75 mM NaCl extrusion. But with the increasing salinity, significant negative effects on the crop increased and in 300 mM salinity plant died in end of growth stage. Earlier salinity imposing increased salinity effect on plant, but extremely high salinity occurs at grain filling stage had no effect on grain production. Moderate salinities (150 and 225 mM) in the later stages of the plant life in the post-blooming stage did not cause a significant loss, but, rising tension in early period was not suitable for amaranth. Grain amaranth can produce suitable grain production in areas with low salinity and the most important limitation is high salinity of soil in these areas in the entire developmental period.

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REFERENCES

Abdel Aziz, N. G., M. H. Mahgoub. and H. S. Siam. 2011. Growth, flowering and chemical performance of *Amaranthus tricolor* L. plants as influenced by seaweed extract under salt stress conditions. J. Appl. Sci. Res. 7(11): 1472-1484.

Allemann, J., E. Heever. and A. Viljoen. 1996. Evaluation of *Amaranthus* as a possible vegetable crop. Appl. Plant Sci. J. 10: 1-4.

Beveridge, C. A., J. L. Weller, S. R. Singer. and J. M. I. Hofer. 2003. Auxillary meristem development, budding relationships between networks control-

ling flowering, branching, and photoperiod responsiveness. Plant Physiol. J. 131: 927-934.

Carillo, P., M. Grazia Annunziata, G. Pontecorvo, A. Fuggi. and P. Woodrow. 2010. Salinity stress and salt tolerance. *In*: Shanker, A. and B. Venkateswarlu, (Ed) Abiotic stress in plants-mechanisms and adaptations. Dep. Life Sci. Univ. Naples. Italy.

Cramer, G. R. 2002. Response of abscisic acid mutants of Arabidopsis to salinity. Funct. Plant Biol. J. 29(5): 561–567.

Cunningham, A. B., P. J. De Jager. and L. C. B. Hansen. 1992. The indigenous plant use program. Found. Res. Develop. Pretoria. South Africa.

Dave, D. S. and N. K. Patel. 2011. Salinity effect on *A. lividus* Linn. (*Amaranthaceae*) in relation of physiological and biochemical aspects. Life Sci. Leaflets. J. 21: 1018-1042.

Flowers, T. J. 2004. Improving crop salt tolerance. J. Exp. Bot. 55(396): 307-319.

Fricke, W. and W. S. Peters. 2002. The biophysics of leaf growth in salt-stressed barley, study at the cell level. Plant Physiol. J. 129: 374–388.

Gaber, M. A. 2010. Antioxidative defense under salt stress. Plant Signal Behav. J. 5(4): 369-374.

Gelalcha, S. and R. R. Hanchinal. 2013. Correlation and path analysis in yield and yield components in spring bread wheat genotypes under irrigated condition in Southern India. African J. Agric. Res. 8(24): 3186-3192.

Huerta-Ocampo, J. A., A. Barrera-Pacheco, Ch. S. Mendoza-Hernández, E. Espitia-Rangel, H. P. Mock. and A. P. Barba. 2014. Salt Stress-Induced Alterations in the Root Proteome of *A. cruentus*. J. Proteome Res. 13(8): 3607–3627.

Kang, J. G. and M. W. Van Iersel. 2004. Nutrient solution concentration affects shoot: root ratio, leaf area ratio, and growth of subirrigated salvia. Hort. Sci. J. 39(1): 49-54.

Lauchli, A. and S. R. Grattan. 2007. Plant growth and development under salinity stress. *In*: Jenks, M. A., P. M. Hasegawa, S. M. Jain (Ed) Advances in molecular breeding toward drought and salt tolerant crops. Springer Pub. Netherlands.

Makus, D. J. 2003. Salinity and nitrogen level affect agronomic performance, leaf color and leaf mineral nutrients of vegetable amaranth. Subtropical Plant Sci. J. 55: 1-6.

Meloni, D. A., M. A. Oliva, C. A. Martinez. and J. Cambraria. 2003. Photosynthesis and activity of superoxide dismutase peroxidase and glutathione reductase in cotton under salt stress. J. Environ. Exp. Bot. 49: 69-76.

Moosavi, A., R. Tavakkol Afshari, F. Sharif-Zadeh. and A. Aynehband. 2009. Seed priming to increase salt and drought stress tolerance during germination in cultivated species of Amaranth. Seed Sci. Technol. J. 37(3): 781-785.
DOI: 10.15258/sst.2009.37.3.26.

Munns, R. and M. Tester. 2008. Mechanisms of salinity tolerance. Annual Rev. Plant Biol. J. 59: 651 681.

Nenova, V. 2008. Growth and mineral concentrations of pea plants under different salinity levels and iron supply. Genetic Appl. Plant Physiol. J. 34(3-4): 189-202.

Noreen, Z. and M. Ashraf. 2009. Assessment of variation in antioxidative defense system in salt-treated pea (*Pisum sativum*) cultivars and its putative use as salinity tolerance markers. J. Plant Physiol. 166(16): 1764-1774.

Odjegba, V. J. and I. C. Chukwunwike. 2012. Physiological responses of *A. hybridus* L. under salinity stress. In-

dian J. Innovations Develop. 1(10): 742-748.

Omami, E. N. 2005. Response of amaranth to salinity stress. Dep. Prod. Soil Sci. PhD. Thesis. Faculty Natur. Agric. Sci. University of Pretoria. South Africa. 203 pp.

Omami, E. N., P. S. Hammes. and P. J. Robbertse. 2006. Differences in salinity tolerance for growth and water use efficiency in some amaranth (*Amaranthus spp*.) genotypes. New Zealand J. Crop Hort. Sci. 34(1): 11-22. DOI: 10.1080/01140671.2006.9514382.

Shah, W. A., H. Ullah Khan, S. Anwar. and K. Nawab. 2011. Yield and yield components of wheat as affected by different grain rates and nitrogen levels. Sarhad J. Agric. 27(1): 17-25.

Shalhevet, J. 1994. Using water of marginal quality for crop production: major issues. Agric. Water Manage. J. 25: 233-269.

Sharp, R. E. and M. E. Le Noble. 2002. ABA, ethylene and control of shoot and root growth under water stress. J. Exp. Bot. 53(366): 33-37.

Tijen, D. and T. Ismail. 2005. Comparative lipid peroxidation, the antioxidant defense systems and proline content in roots of two rice cultivar differing in salt tolerance. Env. Exp. Bot. J. 53(3): 247-257.

Velikova, V., I. Yordanov. and A. Edreva. 2000. Oxidative stress and some antioxidant systems in acid rain treated bean plants, protective role of exogenous polyamines. Plant Sci. J. 151: 59-66.

Wahid, A. and A. Ghazanfar. 2006. Possible involvement of some secondary metabolites in salt tolerance of sugarcane. J. Plant Physiol. 163(7): 723-730.

Wahome, P. K., H. H. Jesch. and I. Grittner. 2001. Mechanisms of salt stress tolerance in two rose rootstocks: *Rosa chinensis* 'Major' and *R. rubiginosa*. Scientia Hort. J. 87(13): 207-216.

Wang, Y. and X. Li. 2008. Salt stress-induced cell reprogramming, cell fate switch and adaptive plasticity during root hair development in Arabidopsis. Plant Signaling and Behavior. J. 3(7): 436-438.

Wang, X., G. Zhao. and H. Gu. 2009. Physiological and antioxidant responses of three leguminous species to saline environment during grain germination stage. African J. Bio-Tech. 8(21): 5773-5779.

Wouyou, A., Ch. B. Gandonou, F. A. Komlan, D. Montcho, A. S. Zanklan, S. Lutts. and S. L. Gnancadja. 2017. Salinity resistance of five amaranth (*A. cruentus* L.) cultivars at young plants stage. Intl. J. Plant Soil Sci. 14(3): 1-11.

Yadav, S., M. Irfan, A. Ahmad. and S. Hayat. 2011. Causes of salinity and plant manifestations to salt stress: A review. J. Environ. Biol. 32: 667-685.

Yaniv, Z., E. Shabelsky. and D. Schafferman. 1999. Colocynt h: Potential arid land oil grain from on ancient cucurbit. ASHS Press. Alexandria. pp: 257-261.