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# THE EVALUATION OF GRAIN AND OIL PRODUCTION, SOME PHYSIOLOGICAL AND MORPHOLOGICAL TRAITS OF AMARANTH 'CV. KONIZ' AS INFLUENCED BY THE SALT STRESS IN HYDROPONIC CONDITIONS

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## Abstract
The purpose of this study was investigation of salinity effect on some traits of Amaranth. A split plot designed with three replications with two factors: 5 salinity levels (control, 75, 150, 225, 300 mM NaCl) and applied time at 4 levels (plant establishment, branching, flowering, grain filling) in a greenhouse under hydroponic system. Application of 300 mM salinity after plant establishment led to death of amaranth. Salinity application after establishment decreased significantly plant height and number of branches as 44.9 and 31.8, respectively. Production of grain weight was not affected by 75 mM salinity, but at higher salinity showed significantly decrease. The highest decrease in grain weight obtained by applying 225 mM salt after the plant establishment and salinity at 300 mM after branching as 86.6 and 71.3 percent 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 hadnot affect by 75 mM NaCl, but other concentrations had a negative effect on the growth and production of Amaranth and increasing salinity had more negative impact. In this study, the most sensitive to salinity was after plant establishment and grain filling stage was the most tolerant.
**Key words:** Amaranth, growth stage, salinity, yield.

## Introduction
Amaranth belongs to genus *Amaranthus*. The genus includes about 60 species of amaranth that the majority of them are wild. Some of them as edible crops and some of them are used as ornamental crop (Borneo & Aguirre, 2008; Pospisil *et al*., 2006). Based on the soil map published in recent years, the area of soils with low to moderate salinity is 25.5 million hectares and 8.5 million hectares of soils tolerate high salinity (Almodares *et al.,* 2008). Salinity leads to serious changes in the photosynthesis and photorespiration of plants (Vega *et al.,* 2006). High levels of sodium reduce potassium adsorption and its accumulation in the cytoplasm stopped the activity of many enzymes (Jaleel *et al.,* 2008), because, potassium had import role in various processes such as metabolism, growth and adaptation to stresses. The salinity prevents enzyme activity, cell division and development, and causes disorganization of the membrane and ion balance and eventually led to the growth stop (Mahajan *et al.,* 2008). Salinity had a significant reduction in growth and yield of Amaranthus family. Omami (2005) and Omami *et al.,* (2006) studied salinity impact on Amaranth cultivars) *A. tricolor ،Accession '83 ،A. hypochondriacus* and *A. cruentus* showed that increasing salinity in the soil leads to a significant reduction in crop height, leaf area, specific leaf area, and stomatal conductance rates. Dave and Patel (2011) examined effect of salinity 2.7, 5.5, 8.5, 11.1 and 13.8 ds/m in *A. lividus*. The root and shoot length, number of leaves, fresh and dry weight of leaves, roots and stems showed a significant 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 & Li, 2008). Malondialdehyde (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 stress tolerance (Wang et al., 2009). As salt stress occur frequently and can affect most habitats, plants have developed several strategies to cope with these challenges. One of the stress defense mechanisms is the antioxidant defense system, which includes antioxidant enzymes. antioxidant enzymes converts $H_2O_2$ into water and oxygen (Gaber, 2010). Soil salinity is one of the most important environmental and ecological tensions in many parts of the world, nowadays is one of the main reasons in reduction of agricultural products. Grain amaranth is new crop with high yield potential and good nutrition can be a good substitute for salt-sensitive crops in such areas. Growth study reactions and seed production in saline conditions and determining plant tolerance to salinity and the sensitivity of the different growth stages were important also.

**Material and methods**

The experiment was conducted in greenhouse of Tabriz Branch, Islamic Azad University, located at 15 km East of Tabriz in 2013. This place located at 46, 17 E and 38, 5 N degrees with 1360 meters altitude. Salinity factor levels in 5 different levels (control, salinity stress, 75, 150, 225 and 300 mM NaCl) and applied stress time at 4 levels (plant establishment, branching, flowering and grain filling) under hydroponic system with Hoagland solution arranged as split plot with three replications in grain amaranth CV. Koniz (*Amaranthus hypochindriacus* L.× *Amaranthus hybridus* L.) as a new crop. 1100 g. perlite medium grain size was filled into pots. Seeds randomly distributed on the surface of the perlite. Nutrition of crops was supplied by using nutrient solution after emergence. Hoagland's A-Z solution is used to provide macro and micro nutrients (Nenova, 2008). 2 weeks after emergence, seedlings have been thinned to 5 plants per pot and in third week after emergence were kept only 3 plants per pot. Every 4 days nutrient solution (1/2 fold in the early period of growth) was supplied to plants (Nenova, 2008). The amount of used solution for treatment was determined based on available water in each pot. For this purpose, the weight of irrigated perlite 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 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. Hydrogen peroxide content in amaranth leaves at grain filling stage were determined according to Velikova *et al.,* (2000). The level of lipid peroxidation (Malondialdehyde: MDA) was determined in terms of thiobarbituric acid-reactive substances (TBARS) concentration as described by Noreen and Ashraf (2009). After harvest, characteristics such as plant height, number of branches, seed weight and seed weight per plant was measured. Grain oil percentage were measured by micro-souqksole. Before statistical analysis, the normality test of data was performed. Data analyzed using MSTATC software. Mean comparisons done by Duncan's multiple range test at the 5 percent level.

**Results and discussion**

Applying different levels of salinity different growth stages and their interaction was significant for all traits (Table 1).

**Plant height:** 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 affect on plant height at establishment stage but increasing significantly decreased plant height. Salinity level at 225 mM, decreased plant height to 38 cm which was 44.9 percent lower than the control treatments mean (Fig. 1). Omami (2005) studied effect of salinity on some varieties of grain amaranth (*A. tricolor,* Accession '83, *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%. Apply 300 mM NaCl after establish stage led to plant death. Simple linear regression equation showed that for every unit increase in salinity in the growth stage, plant height reduced 14.9

units. 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).

**Branch number:** Salinity at beginning of branching, early flowering and grain filling stages had not significant effect on number of branches per Amaranth plant. In this study, 300 mM salt after plant establish had a significant effect on number of branches (Fig. 2). The beginning and end of branching in amarath depends on the type of crop and environmental factors. In most crops branching ends by beginning of flowering (Beveridge *et al.*, 2003).

**1000 Kernel Weight:** Salinity applying at 75, 150 and 225 mM in the establishment stage of Amaranth reduced 10, 13.3 and 23.3 percent of the 1000 kernel weight respectively (Fig. 3). Stress at different growth stage had different results. In this study, salinity applying 75 mM in the beginning stages of branching and flowering decreased 16.6 and 13.3 percent of 1000 kernel weight, respectively. Salinity application at 150 mM at the beginning of branching was not affected 1000 kernel weight and 150 mM salt in the flowering stages, increased 1000 kernel weight significantly. This increase was due to a decrease in the number of grain per plant (Fig. 4) which leads to more assimilate for grain filling. In the higher salinity of 150 mM was observed significantly reduction in 1000 kernel weight. Simple linear regression equations showed that for every unit increase in salinity after the establishment, 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 hadn't significant differences. Thus, the delay in applying salinity reduced the negative effects of salt stress on 1000 kernel weight (Fig. 3). Applying salinity stress in crop growth stage reduced growth. Similar results have been observed in other grain crops also (Omami, 2005). Research has shown that reproductive organs of plants are more sensitive to environmental stresses than grain filling period (Gelalcha & Hanchinal, 2013). This experiment showed a significant reduction in seed weight also. Application 225 and 300 mM salinity at the branching reduced 20% and 36%, respectively and application 300 mM salt in flowering stage decreased 1000 kernel weight as 16.6%.

**Seed number per plant:** The maximum number of grain per plant was 5524 in control. Salinity of 75 mM had not effect on seed per plant, however, higher salinity levels showed significantly negative effect on seed per plant. The decrease in seed per plant at salinity of 225 and 300 mM, was 38.5 and 56%, respectively. Application of 150, 225 and 300 mM, in the beginning stages of branching reduced 35.4, 38.5 and 35.5%, respectively, the number of seed per plant. Application of 225 mM salinity after crop establishment reduced 81.2% of seed number per plant. Application of 150 mM salinity at crop establishment reduced 50.2% seed number per plant. So in three concentrations of 150, 225 and 300 mM, the maximum reduction in the number of seed per plant was affected by salinity imposed in the early stages of crop growth (Figure 4). 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 & Tester, 2008).

**Seed weight per plant:** Maximum seed weight was 15 g in control treatment. 75 mM salinity had not effect on Amaranth seed production, but on the other salinity levels depending on the stage that salt stress was applied, the weight of the produced seed reduced. In all three levels of salinity, 150, 225 and 300 mM, the maximum reduction in yield was in the stage of crop establishment. Seed yield in both the 150 and 225 mM stress after crop establishment stage was 6 and 2 g per plant, respectively, which was 60 and 86.6%, respectively, less than control. Stress at the onset of branching in salinity levels of 150, 225 and 300 mM, a significant reduction in seed weight was obtained. In the three treatments, seed weight was 8.3, 7.3 and 4.7 g, which 38, 46.6 and 71.3 % respectively, less than control. With increasing salinity levels seed yield of amaranth decreased (Fig. 5). With increasing solute concentrations yield decreased and even plant dies at high concentrations. According to study, the low-salt (75 mM) concentration had not affected Amaranth seed yield, but higher salinity concentration showed significant decreasing yield. Application of 225 and 300 mM salt at the beginning of flowering decreased 40 and 66.6% of the seed weight. Simple linear regression equations showed that for every unit increase in salinity after crop establishment, seed production reduced 47.3 units. The reduction in branching and flowering stages were 1.9 and 1.7 units, respectively. Changes observed in morphological traits and yield components of produced seed after seed filling stages were not significant (Fig. 5).

**Oil Production:** As the stress is increased, oil percentage decreases. In addition, the 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 the establishment, 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. The study of linear regression equation showed that for every unit increase in salinity after the establishment ,branching, flowering and grain filling stages, 3.76, 2.89, 2.12 and 1.41 unit of oil percent were reduced. Thus, amaranth's oil production is more sensitive to salinity stress compared to its other characteristics (Fig. 6).

**$H_2O_2$, MDA and total phenolics contents:** Salinity was increased MDA, the $H_2O_2$ and total phenolics contents in amaranth leaves (Table 2). The 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. Result showed that total phenolics contents in 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 2). The high increase content of H2O2 showed that amarant in high salinity levels was sensitive; on the other hand the high increase content of MDA is Amaranth appropriate response to salinity. MDA is the decomposition product of polyunsaturated fatty acids of membranes under stress. 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 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 & Ismail, 2005) and cotton (Meloni *et al.*, 2003) cultivars differing in salt tolerance. In Amaranth leaves $H_2O_2$ remained changed 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 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 non-enzymatic antioxidants. Generally, the dismutation of two superoxide anions, either enzymatically or non-enzymatically, give rise to $H_2O_2$. $H_2O_2$ is also produced from the β oxidation of fatty acids and peroxisomal photorespiration reactions (Noreen & Ashraf, 2009).

## Conclusions

Seed yield reduction and its components within components of Amaranth growth indices 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 and yield components such as number and grain weight decreased with increasing salinity. Highest and lowest significant reduction in seed yield production was 86 % and 1000 kernel weight was 13 %. Salinity up to 75 mM had not significant effect on most morphological and physiological attributes. According to not 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 salt plant died in end of growth. Earlier salinity imposing increased salinity effect on plant, but extremely high salinity occurs at grain filling stage had no effect on seed production. The occurrence of 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 seed production in areas with low salinity and the most important limitation was high salinity of the soil in these areas in the entire developmental period.

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Table 1. Analysis of variance of traits in Amaranth

| SOV | df | Plant height | Branch No | MDA | $H_2O_2$ | Total phenol |
|---|---|---|---|---|---|---|
| salinity levels | 4 | 877.79** | 4.23** | 53.89** | 21. 43 * | 4.54 * |
| salinity applying time | 3 | 3141.02** | 8.84** | 6.23 | 4.41 | 0. 65 |
| salinity level×time | 12 | 492.09** | 2.29** | 0.87 | 2.34 | 0.22 |
| error | 40 | 70.35 | 0.45 | 18.57 | 3.84 | 0.88 |
| CV (%) | | 13.17 | 16.23 | 13.68 | 13.83 | 7.04 |

and ** significant at 5% and 1% levels, respectivly

Table 1. Continue

| SOV | df | 1000 kernel weight | Seed No per plant | Seed W per plant | Oil percentage |
|---|---|---|---|---|---|
| salinity levels | 4 | 3.063** | 15953857.1** | 152.77** | 163.88** |
| salinity applying time | 3 | 2.475** | 8674358.6** | 99.66** | 52.79** |
| salinity level×time | 12 | 0.933** | 3010661.5** | 26.58** | 4.35** |
| error | 40 | 0.017 | 357547.5 | 3.5 | 0.516 |
| CV (%) | | 4.76 | 17.01 | 18.37 | 5.43 |

and ** significant at 5% and 1% levels, respectivly

Table 2. Mean of $H_2O_2$, Total phenolics and MDA content as affected by NaCl levels

| NaCl (mmol) | $H_2O_2$ (µmol/g fw) | Total phenolics ( mg/g fw) | MDA (nmol/g fw) |
|---|---|---|---|
| 0 | 9.23 d | 4.21 d | 2.91 cd |
| 75 | 10.33 dc | 4.87 c | 3.37 c |
| 150 | 12.54 c | 5.23 bc | 4.74 b |
| 225 | 13.87 b | 5.63 b | 5.16 ab |
| 300 | 16.12 a | 6.18 a | 5.44 a |

Treatments with the same letter(s) don't have significant difference

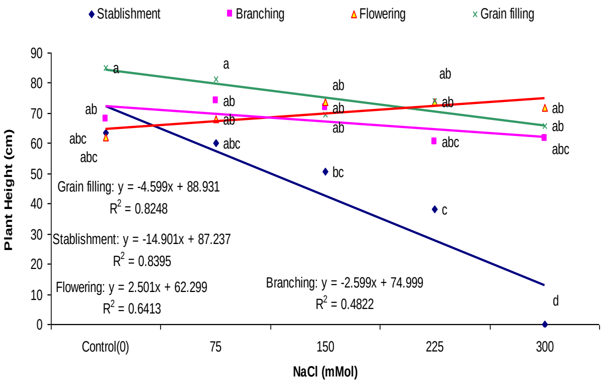


**Fig. 1.** Comparison means of plant height affected by salinity and applying time

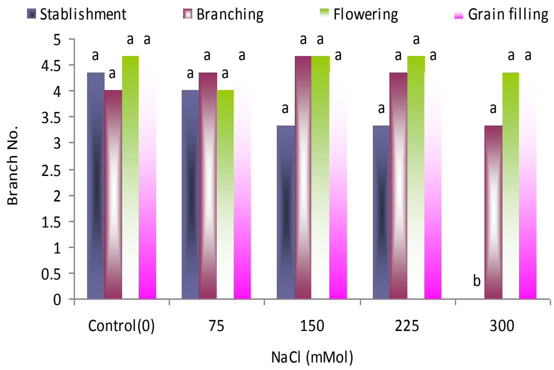


**Fig. 2.** Comparison of number of branches affected by salinity and apply time

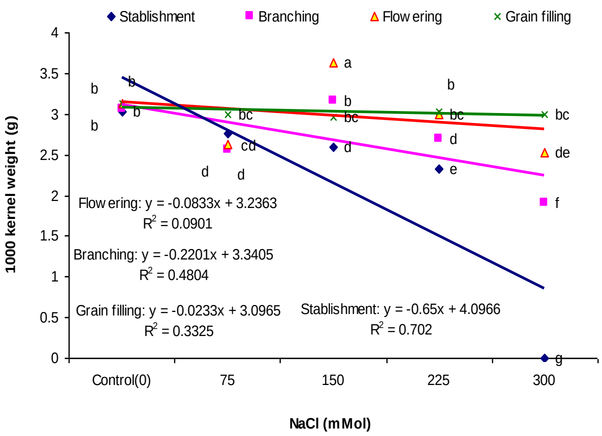


**Fig. 3.** Comparison of 1000 kernel weight under influence of salinity and application times

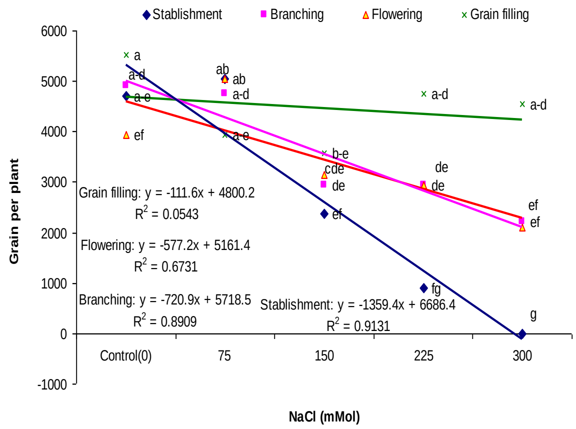


**Fig. 4.** Comparison of number of seed per plant under influence of salinity and application times

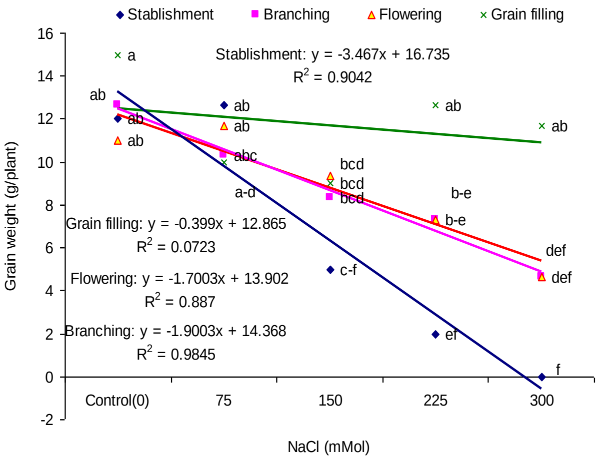


**Fig. 5.** Comparison of grain yield production in crop affected by salinity and time application

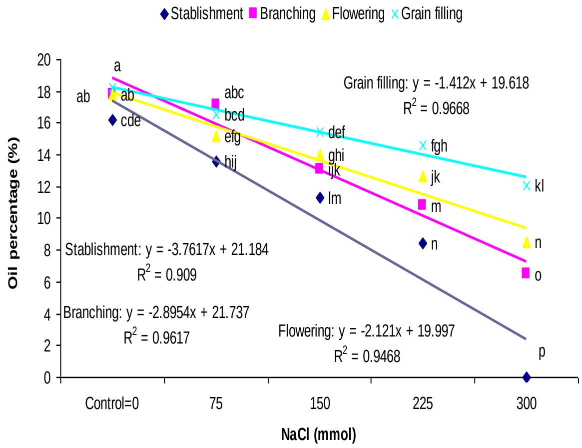


**Fig. 6.** Comparison of oil percentage under influence of salinity and application times