EFFECT OF ORGANIC ACID TO MEDIATE SALT STRESS OF RED AMARANTH

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*This is to certify that thesis entitled, "**EFFECT OF ORGANIC ACID TO MEDIATE SALT STRESS OF RED AMARANTH**" submitted to the Faculty of Agriculture, Sher-e-Bangla Agricultural University, Dhaka, in partial fulfilment of the requirements for the degree of **MASTER OF SCIENCE (M.S.) in AGROFORESTRY AND ENVIRRONMENTAL SCIENCE**, embodies the result of a piece of bonafide research work carried out by **Shirmeen Sultana Jui**, Registration no.**13-05368** under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.*

I further certify that such help or source of information, as has been availed of during the course of this investigation has duly been acknowledged.

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Place: Dhaka, Bangladesh

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Associate Professor

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Dedicated To

My Beloved Parents

and Respected Teachers

Whose Prayers,

Efforts and

Wishes are an

Inspiration

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ABSTRACT

Amaranth *(Amaranthus* spp.) is a promising vegetable species often grown under semiarid conditions prone to both drought and salinity. This study was conducted to evaluate the effect of organic acid to mediate the salt stress of red amaranth. Both lab and pot experiment was conducted at Sher-e-Bangla Agricultural University, Dhaka to investigate the effect of organic acid to mediate the salt stress of red amaranth during the period from January to February 2020 in Rabi season. Lab experiment consisted of, red amaranth seed treated with eight salt concentrations: T_0 = 0 mM NaCl (control), T_1= 25 mM NaCl, T_2= 50 mM NaCl, T_3= 75 mM NaCl, T_4= 100 mM NaCl, T_5= 150 mM NaCl, T_6= 200 mM NaCl and T_7= 250 mM NaCl. The pot experiment was consisted of two factors (2) *viz.* Factor A: Three salt stress condition *viz.,* (3), S_0: Control (0 mM NaCl), S_1: Mild stress (50 mM NaCl) and S_2: Severe stress (100 mM NaCl) and Factor B: Different organic acid treatment *viz*. (3), A_0: (Control), A_1: Ascorbic acid, and A_2: Citric acid. Both lab and pot experiment was laid out in Completely Randomized Design (CRD) with three replications. Experiment result revealed that in lab experiment red amaranth seed treated with extreme salt concentration (250 mM NaCl) impacted negatively and recorded minimum germination (26.33 %), radical length (0.78 cm), plumule (0.27 cm) and total length (1.06 cm) compare to other treatments from the lab experiment. In pot experiment, different salt stress condition reduced yield which was minimum (120.02 g pot^{-1} and 12.09 t/ha) in severe salt stress condition. Among different organic acid, seeds treated with ascorbic acid performed best compare to citric acid or control treatment and recorded maximum yield pot^{-1} (211.11 g) and yield (21.26 t/ha) at 30 DAS. In case of combined effect, different salt stress condition along with ascorbic acid performed the best and increased 30.94 and 99.98 % yield pot^{-1}, and 30.92 and 99.98 % yield (t/ha) under mild and severe salt stress condition thus this treatment is suitable for saline thrives area for higher red amaranth vegetable production.

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LISTS OF ACRONYMS

Full word	Abbreviations	Full word	Abbreviations
Agriculture	Agric.	Milliliter	mL
Agro-Ecological Zone	AEZ	Milliequivalents	Meqs
And others	*et al.*	Triple super phosphate	TSP
Applied	App.	Milligram(s)	mg
Asian Journal of Biotechnology and Genetic Engineering	AJBGE	Millimeter	mm
Bangladesh Agricultural Research Institute	BARI	Mean sea level	MSL
Bangladesh Bureau of Statistics	BBS	Metric ton	MT
Biology	Biol.	North	N
Biotechnology	Biotechnol.	Nutrition	Nutr.
Botany	Bot.	Pakistan	Pak.
Centimeter	Cm	Negative logarithm of hydrogen ion concentration (-log[H+])	pH
Completely randomized design	CRD	Plant Genetic Resource Centre	PGRC
Days after sowing	DAS	Regulation	Regul.
Degree Celsius	°C	Research and Resource	Res.
Department	Dept.	Review	Rev.
Development	Dev.	Science	Sci.
Dry Flowables	DF	Society	Soc.
East	E	Soil plant analysis development	SPAD
Editors	Eds.	Soil Resource Development Institute	SRDI
Emulsifiable concentrate	EC	Technology	Technol.
Entomology	Entomol.	Tropical	Trop.
Environment	Environ.	Thailand	Thai.
Food and Agriculture Organization	FAO	United Kingdom	U.K.
Gram	g	University	Univ.
Horticulture	Hort.	United States of America	USA
International	Intl.	Wettable powder	WP
Journal	J.	Serial	Sl.
Kilogram	Kg	Percentage	%
Least Significant Difference	LSD	Number	No.
Liter	L	Microgram	µ

CHAPTER 1

INTRODUCTION

Red amaranth (*Amaranthus tricolor* L.) plays an important role in nutrition among the leafy vegetables grown in Bangladesh. The leafy amaranth is said to be the native of India (Shanmugavelu, 1989). Among the leafy types, *Amaranthus tricolor* L. is the most commonly cultivated species in Bangladesh. It is cultivated all over the country in any season due to its adaptability to a wide range of soil and climate (Alam *et al.*, 2007). However, during winter its growth and development is slower than summer and rainy season. The total production of Red amaranth was 48810 metric tons from an area of 33118 acres in Bangladesh (BBS, 2016). Red amaranth (*Amaranthus tricolor* L.) is the most widely grown commercial and dietary vegetables in Bangladesh and around the world because of their special nutritive value and widespread production. It contains an appreciable amount of iron, minerals, calcium and phosphorus. It is an excellent source of vitamin C. Among the vegetables of tropics Red amaranths are very easy to grow. Red amaranth is probably the most popular vegetable due to its short length, quick growing habit and riches in vitamins and minerals (Cole, 1979). It belongs to the Amaranthaceae family, which contains many salt-tolerant species, such as quinoa (*Chenopodium quinoa* Willd.) and saltbush (*Atriplex nummularia*). Quin *et al.* (2013) reported that 5 g L^{-1} of NaCl in the nutrient solution do not affect the *Amaranthus cruentus* L. yield, as well as its pigment content, photosynthesis, transpiration rate, stomatal conductance or antioxidant enzyme activities.

Salt stress is considered an alarming condition as it decreases the soil productivity which results in reduced crop yields (Hu *et al.*, 2006). More than 900 million hectares of land worldwide, 20% of the total agricultural land, are affected by salt, accounting for more than 6% of the world's total land area. NaCl is the predominant salt causing salinization and is unsurprising that plants have evolved mechanisms to regulate its accumulation. Salinity of soil and irrigation water is a continuing threat to economic crop production especially in arid and semiarid regions of the world (Kayani *et al.* 1990).

The ability of seed to germinate in saline environments, the cotyledons to break through a soil crust, emerging and seedlings to survive in saline conditions are crucial for crop production in saline soils (Maranon *et al.* 1989). Salt affected soils are distributed throughout the world and no continent is free from the problem (Brady and Weil, 2002). Salinization of soil is one of the major factors to limit crop production particularly in arid and semi-arid regions of the world. Globally, a total land area of 831 million hectares is salt affected. African countries like Kenya (8.2 Mha), Nigeria (5.6 Mha), Sudan (4.8 Mha), Tunisia (1.8 Mha), Tanzania (1.7Mha) and Ghana (0.79) are salt affected to various degrees (FAO, 2000). Salt stress is known to perturb a multitude of physiological processes (Ashraf, 2001). It exerts its undesirable effects through osmotic inhibition and ionic toxicity (Munns *et al*., 2006). Increased salinity caused a reduction in plant height, leaf length, leaf breadth, and number of leaf and average weight of plant (Jacoby, 1994).

Salinity of the soil relates to accumulation of salts. Salinity is known to induce stress in plants; hence the ability of plants to tolerate and thrive in saline soils is of great importance in agriculture. Since it indicates that the affected plants had genetic potential of salt tolerance, which is highly desirable trait (Francois and Mass, 1994; Makus, 2003). Sudhakar *et al*. (1993) attributes the lack of salinity cultivars to inadequate means of detecting and measuring plant response to salinity and ineffective selection methods. The salt-induced water deficit is one of the major constraints for plant growth in saline soil. Root zone salinity can rapidly inhibit root growth and in turn their capacity to uptake water and essential mineral nutrients from the soil (Ngigi, 2002), National Research Council (1990) studied effect of sodium chloride on growth, photosynthesis and respiration and found growth decreased with increasing salt concentration. Salt induced oxidative stress is one of the most important factors that affect plants. Proline plays a major role in the anti-oxidative stress as a hydroxyl radical scavenger (Smirnoff, 1993). Hydroxyl radical (OH-) are produced as a result of oxidative stress are harmful and can rapidly react with all types of biomolecules, such as DNA, proteins and lipids leading to radical chain processes, cross linking, peroxidation, membrane leakage, production of toxic compound and finally cell death (Hester *et al.,* 2001). For instance, it was found that increasing of salinity stress decreased almost all the growth parameters of red amaranth growth parameters and essential oil amount in Chamomile. Sohan *et al*.

(1999) reported that enhancing salinity treatments lead to growth reduction also reduces germination percentage and seedling weight of sunflower plants. Ashraf and Sharif (1997) reported that salinity treatment lead to reduction of growth and plant developments.

Recently, a great attention has been focused on the possibility of using natural and safety substances in order to improve plant growth, flowering and fruit setting. In this concern, organic acid have synergistic effects on growth, yield and yield quality of many plant species. These compounds have beneficial effects on catching the free radicals or the active oxygen (singlet oxygen, superoxide anion, hydrogen peroxide, hydroxyl radicals and ozone) produced during photosynthesis and respiration processes (Zhang and Klessing, 1997). One of the most familiar organic acid is ascorbic acid which is synthesized in higher plants and affects plant growth and development (Givan, 1979). The ability of ascorbate to lose or donate electrons to produce monodehydroascorbate (MDHA) is the basis of its biologically useful antioxidant capacity (Buettner and Schafer, 2004). Thus, high endogenous AsA (Ascorbic acid) in plants is necessary to counteract oxidative stress in addition in regulating other processes of plant metabolism. Endogenous AsA can be increased by exogenous application of AsA through the rooting medium (Chen and Gallie, 2004) as a foliar spray or as seed priming. Citric acid is among the inter mediate organic acids in Krebs cycle which produces cellular energy by oxidative phosphorylation (Wills *et al.*, 1981). Both play a critical role in the regulation of plant development and growth, as well as in regulation of both primary and specialized metabolic pathways, and are involved in the response to both abiotic and biotic stress. Moreover, they play roles as signalling molecules, not only as allosteric regulators of many key enzymes, but also as modulators of gene expression. (Drincovich *et al.,* 2021).

Therefore, the present investigation was undertaken to find out the effect of organic acid to mediated the salt stress of red amaranth with following objectives

i. To study the effects of different NaCl concentrations on germination and seedling growth behavior of red amaranth.

ii. To evaluate the effects of seed priming with ascorbic and citric acid on germination, growth and morpho-physiological parameters of red amaranth under different salt concentrations.

CHAPTER 2

REVIEW OF LITERATURE

An attempt was made in this section to collect and study relevant information available regarding the effect of organic acid mediated salt stress tolerance in red amaranth to gather knowledge helpful in conducting the present piece of work.

2.1 Red Amaranth (*Amaranthus tricolor*)

Red amaranth (*Amaranthus tricolor*) belonging to the family Amaranthaceae is a delicious vegetable with its considerable nutritional value around the globe and in particular, in parts of tropical and subtropical Asia, Africa and Central America. Amaranth is a fast-growing crop that is adaptable to a wide range of soils and climates. Chiefly grown during summer and rainy season, amaranth is an important and popular vegetable in Bangladesh because of its cheapest price, quick growing character and higher yield potential (Hossain, 1996). It is also one of the few C_4 crop species other than the grasses; thus, it performs well under adverse conditions, especially heat and drought. C_4 plants convert a higher ratio of atmospheric carbon to plant sugars per unit of water lost than those possessing the classical C_3 (Calvin cycle) pathway. In view of its tolerance to major abiotic stresses, amaranth has now emerged as a major climate resilient vegetable crop that not only fights climate change, but also fulfills the growing nutritional needs of human beings. Heat and salinity are known to be the two major abiotic stresses impacting agricultural production in Bangladesh and elsewhere. Amaranth is reported to be moderately salt tolerant and compares well with other vegetable crops such as cowpea and mustard (Omami *et al.* 2006). Therefore, in Bangladesh context, it is considered as a potential upcoming subsidiary food crop (Teutonic and Knorr, 1985). *Amaranthus tricolor* is usually much-branched, vigorous, erect or ascending annual plant with a stout stem, growing up to 125cm tall.The leaves contain about 3.5% protein, 0.25% fat, 6.6% carbohydrate, 3.1% ash, 24 mg iron per 100 g, 464 mg calcium per 100 g, they are rich in vitamin A and have a fair content of vitamins B1 and C. On zero moisture basis 100 g of the leaves contains up to 2441 mg calcium, 1008 mg phosphorus, 51 mg iron, 34 mg sodium, 4475 mg potassium, 37,623 micrograms beta-carotene equivalent, 0.68 mg thiamine, 2.37 mg riboflavin, 11.48 mg niacin and 730 mg ascorbic acid (Rahmatullah *et al*., 2013). However, in Bangladesh, its cultivation is increasing day by day (BBS, 2010) although its production is lower than other amaranth producing countries (Talukder, 1999).

2.2 Salt stress

Salinity coupled with low rainfall is one of the most serious factors in arid and semi arid regions of the world that adversely affect the productivity of present day agricultural crops (Munns and Tester, 2008). Worldwide, more than 45 million ha of irrigated land have been damaged by salt and 1.5 million ha are taken out of production each year as a result of high salinity levels in the soil (Munns and Tester, 2008). The total amount of salinity affected land in Bangladesh was 83.3 million hectares in 1973, which had been increased up to 102 million hectares in 2000 and the amount has risen to 105.6 million hectares in 2009 and continuing to increase, according to the country's Soil Resources Development Institute (SRDI). In the last 35 years, salinity increased around 26 percent in the country, spreading into non-coastal areas as well. The initial and primary effect of salinity, especially at low to moderate concentrations, is due to osmosis (Munns and Termaat, 1986). Most crops tolerate salinity up to a threshold level, above which yields decrease as salinity increases (Maas, 1986). Salinity stress causes extensive oxidative damage, affecting several physiological processes which results in significant reduction of different parameters such as germination capacity, radicle and plumule lengths, fresh and dry mass, yields, seed nutritional quality, productivity, chlorophyll, protein and sugar content, antioxidative enzymes activity as well as nodulation (Asaadi, 2009; Ghorbanpour *et al*., 2011; Tuncturk, 2011; Al-Saady *et al*., 2012; Talukdar, 2012; Kapoor *et al*., 2013; Pour *et al*., 2013).

2.3 Effect of different salt concentration

2.3.1 Germination percentage

Chowdhury *et al.* (2018) carried out an experiment in the Plant Physiology and Plant Biochemistry laboratory of Botany Department, Jahangirnagar University, Savar, Dhaka to know the effects of sodium chloride on germination and seedling growth of Sunflower (*Helianthus annuus* L.). Seeds of Sunflower, cultivar BARI Sunflower-2 (*Helianthus annuus* L.) were collected from Bangladesh Agricultural Research Institute (BARI) and were used as plant material. Eight different concentrations of (NaCl) solution viz. 25, 50, 75, 100, 125, 150, 175 and 200 mM and distilled water as control were used as treatment. In each treatment, 10 ml of NaCl solution was applied per petri dish. The experiment was arranged out in randomized block design with three replications. From the experiment result revealed that the highest germination percentage was recorded 90% in the control and the lowest value was 53.33% in 200 mM NaCl. The moderate germination percentages were recorded 76.67%, 73.34%, 70.00% and 66.67% in 25, 50, 75, 100 and 125 mM NaCl levels respectively.

Wouyou *et al*. (2016) conducted an experiment to study the effect of salinity resistance of six amaranth (*amaranthus sp*.) cultivars cultivated in benin at germination stage. The experiment was carried out in the University of Abomey-Calavi, Republic of Benin from September to October 2015. The experiment was laid out as a Randomized Complete Block Design (RCBD) with four replications. In this study, salt resistance level of six amaranth (Amaranthus sp.) cultivars including five from *Amaranthuscruentus* species (AA-04-028, AA-04-017, Locale, Rouge and Red-Sudan) and one from *Amaranthus* graecizans species (Stem2-Sat2) cultivated in Benin was evaluated at the germination stage. Seeds were submitted to treatment with five NaCl concentrations (0; 30; 60; 90 and 120 mM NaCl) in petri dishes. Seed germination was checked every day during the ten days incubation period. Germination percentage was determined within 10 days incubation. Four replicates of 50 seeds each were used. From the experiment result revealed that from day 2 to day 10, NaCl delayed seed germination rate proportionately to NaCl concentration. Salt stress reduced the rate of germination and the germination index in all cultivars. At the end of the 10th days, salt stress significantly decreased the rate of final germination for all cultivars investigated whatever the NaCl concentrations used, except for cultivar Red Sudan and Rouge which showed a slight stimulation of germination at 30 mM NaCl. However, the NaCl stress effects on seed germination of the six cultivars were significantly variable. The average reduction due to NaCl stress was 22.11%; 20.90%; 17.28%; 15.58%; 8.03% and 6.57% for AA-04-017, AA- 04-028, Rouge, Locale, Stem2-Sat2 and Red-Sudan, respectively. In absence of stress, the reaction of varieties were different: after 2 days, 66% of the seeds of Red-Sudan germinated, whereas for cultivars AA-04-028, AA-04-017 and Locale, respectively 92, 92 and 94% of seeds germinated during the same period; this rate was about 95.5 and 96% for Rouge and Stem2-Sat2. After 4 days, the percentages of seed germination were about 94.5, 95, 94, 95.5, 68.5 and 96%, respectively, for AA-04-

028, AA-04- 017, Locale, Rouge, Red-Sudan and Stem2- Sat2. No progress was observed in the rate of seed germination of control for the six cultivars after 4 days (from 4 days to the end of the experiment). NaCl stress effect resulted in a reduction of bud germination speed, visible for all varieties. Indeed, a reduction of the percentages of bud was observed for all varieties at the various NaCl concentrations used as well after 2, 4, 6, 8 and after 10 days. For AA-04- 028, the percentage of bud germination after 2 days shifts from 92% in absence of NaCl, to 78, 75, 15.5 and 1%, respectively at 30, 60, 90 and 120 mM of NaCl. Similar observations have been made concerning cultivars AA-04-017, Locale, Rouge, Red-Sudan and Stem2-Sat2 where seed germination percentage respectively shifts from 92, 94, 95.5, 66 and 96% on the control to 3, 0, 0, 1 and 1.5% at 120 mM of NaCl. Similar tendencies were observed for 4, 6 and 8 days. These observations indicated that salt stress delays seed germination for all cultivars.

Kapoor and Pande (2015) carried out a research with the aim of identifying effect of salt stress on growth and physiological parameters of fenugreek (*Trigonella foenum-graecum* L.) variety RMt-1, a leafy vegetable. Experiments were carried out at Department of Biotechnology, Kumaun University, Bhimtal Campus, Bhimtal during rabi seasons of 2012-13 and 2013-14, respectively. Seeds of uniform size were selected and surface sterilized in 70% (v/v) ethanol for 2 min and 3% sodium hypochlorite solution for 20 min followed by washings for several times with distilled water. The seeds were then germinated on moist filter paper in petri dishes in the dark at a constant temperature of 25°C. The seeds were exposed to different concentrations (50, 100, 150 and 200 mM) of salt (NaCl). Comparisons of salt exposed plants were made with untreated (control) plants. The germination was recorded at 12 h interval for 5 days. The experiments were conducted with three replicates for each treatment. Irrigation was done with water and respective concentration of saline solution for control and treated plants respectively after 10th days. Various growth and physiological parameters were recorded at 15, 30, 45 and 60 Days After Sowing (DAS). From the experiment result revealed that maximum germination (100%) was observed in control as well as low (50 mM) salt concentration. Higher salt concentration i.e. 100, 150 and 200 mM enabled only 97, 41 and 30% germination, respectively. High salt concentration delayed the process of germination.

Panuccio *et al*. (2014) reported that the negative effect of salinity on seed germination might be happened due to salinity-induced ionic imbalance or toxicity.

Al-Seedi and Gatteh (2010) while studying the effects of salinity on seed germination, growth and organic compounds of Mung bean plant [*Vigna radiata* (L.) Wilczek] found that an increase in the salinity caused a corresponding decrease in germination rate, growth parameters and the carbohydrate content of the Mung bean plant.

Begum *et al*. (2010) suggested that germination of seed depends on the utilization of reserved food material of the seed. Salinity interferes with the process of water absorption by the seeds. This subsequently inhibits the hydrolysis of seed reserves which ultimately delays and decreases seed germination.

2.3.2 Root length

Hoang *et al*. (2019) conducted a study to know the effect of Physiological and phytochemical responses of red amaranth (*Amaranthus tricolor L.)* and green amaranth (*Amaranthus dubius L.)* to different salinity levels. The physiological and phytochemical responses of red amaranth (*Amaranthus tricolor* L.) and green amaranth (*Amaranthus dubius L*.) to different salinity levels were determined in two experiments conducted in Vietnam. Both experiments were performed in a net house involving pot experiments arranged in randomized complete block design (RCBD) with three replications. Two genotypes of amaranth were grown in garden soil, saline soil, 50% garden soil: 50% saline soil and 25, 50 and 100 mM NaCl. Salinization was imposed at 7, 14 and 21 days after transplanting. Results indicated that salinity stress reduced plant growth in both red and green amaranth at all treatments except for the 25 mM NaCl treatment. The most detrimental effect was noted in 100 mM NaCl treatment for all plant growth parameters. From the experiment result revealed that root length was reducing from (12.8, 8.6 and 5.9 cm for *Amaranthus tricolor* L. and 9.9, 8.1 and 6.1 cm for *Amaranthus dubius* L.) due to increasing salt concentration that is 25, 50 and 100 mM NaCl. These observations indicated that extreme salt stress showed negative impact on root length (cm) of the plant.

Kapoor and Pande (2015) carried out a research with the aim of identifying effect of salt stress on growth and physiological parameters of fenugreek (*Trigonella foenum-graecum* L.) variety RMt-1, a leafy vegetable. Experiments were carried out at Department of Biotechnology, Kumaun University, Bhimtal Campus, Bhimtal during rabi seasons of 2012-13 and 2013-14, respectively. Seeds of uniform size were selected and surface sterilized in 70% (v/v) ethanol for 2 min and 3% sodium hypochlorite solution for 20 min followed by washings for several times with distilled water. The seeds were then germinated on moist filter paper in petri dishes in the dark at a constant temperature of 25°C. The seeds were exposed to different concentrations (50, 100, 150 and 200 mM) of salt (NaCl). Comparisons of salt exposed plants were made with untreated (control) plants. The germination was recorded at 12 h interval for 5 days. The experiments were conducted with three replicates for each treatment. Irrigation was done with water and respective concentration of saline solution for control and treated plants respectively after 10th days. Various growth and physiological parameters were recorded at 15, 30, 45 and 60 Days After Sowing (DAS). From the experiment result revealed that the pattern of salt treated seeds shows gradual decrease in root length with increasing salt concentration as compared to control. The root length was increased from 2.34-7.48 and 0.89-2.44 cm in control and salt stressed plant respectively from 15-60 DAS. But significant reduction was observed in root length from 7.48 cm in control to 2.44 cm in 200 mM salt solution at 60 DAS.

Ratnakar and Rai (2013) observed while studying effects of NaCl salinity on seed germination and early seedling growth of *Trigonella foenum-Graecum* L. Var Peb and reported that increasing NaCl concentrations caused a gradual decrease in root length of the growing seedling.

2.3.3 Shoot length

Mahbubul *et al.* (2018) conducted a hydroponic experiment at Plant Physiology Laboratory, Department of Crop Botany, Bangladesh Agricultural University, Mymensingh, Bangladesh, during the period from December 2015 to July 2016 to investigate the effect of NaCl on morphological characters and growth of maize (*Zeamays* L.) seedlings. The experiment comprised two levels (0 dS m-1 and 8 dS m-1) of salinity for hydroponic experiment, designed in two factorials Complete Randomized Design (CRD) with three replications on four cultivars viz. Kaveri - 244+, BHARAT Hybrid Sultan 702, Getco seeds GP – 901, Essence –Platinum. Result indicated that effect of variety in combination with salinity on shoot length at 0 and 14 DAS was significant ($p < 0.05$). At 14 DAS, the highest shoot length was recorded in the treatment combination of Essence-Platinum and 0 dS m^{-1} NaCl (476 mm). The lowest shoot length was recorded in BHARAT Hybrid Sultan 702 with 8 dS m^{-1} NaCl (197 mm). Under stress condition, Getco seeds GP – 901 and Platinum-Essence had statistically similar shoot length. A 44% decrease in shoot length was observed in BHARAT Hybrid Sultan 702 followed by 20 % in Essence –Platinum with the increase in salinity level.

Hoque *et al.* (2015) suggested that the reduction in shoot length is due to excessive accumulation of salts in the cell wall elasticity. Additionally, there soon appears secondary cell with rigid cell walls which might results in decreased cell enlargement.

2.3.4 Plant height

Jahan *et al.* (2019) carried out a study at Horticulture Farm of Bangladesh Agricultural University, Mymensingh to evaluate the effects of different levels of NaCl salinity on plant growth and root yield of two carrot varieties during the period from November, 2016 to February, 2017. Four levels of NaCl salt concentration viz., 0 (Control), 50, 100 and 150 mM and two varieties of carrot namely Shundori and Kuruda were used for this pot experiment. The two-factor experiment was laid out in randomized complete block design with three replications. The yield and yield components varied significantly between two carrot varieties and intensity of salt concentration. The application of different salt concentrations significantly influenced the plant height of carrot at different stages of growth. Plant height of carrot increased with the advancement of time and was maximum in pots where no salt solution was applied attaining 36.30, 38.05, 39.24 and 40.02 cm at 82, 89, 96 and 103 days after sowing (DAS), respectively . The plant height was recorded minimum 26.33, 26.54, 26.97, 27.45, 27.65, 27.87 and 28.08 cm at 61, 68, 75, 82, 89, 96 and 103 DAS, respectively with highest salt concentration

Hoang *et al*. (2019) conducted a study to know the effect of Physiological and phytochemical responses of red amaranth (*Amaranthus tricolor* L.*)* and green amaranth (*Amaranthus dubius* L.*)* to different salinity levels. The physiological and phytochemical responses of red amaranth (*Amaranthus tricolor* L.) and green amaranth (*Amaranthus dubius* L.) to different salinity levels were determined in two experiments conducted in Vietnam. Both experiments were performed in a net house involving pot experiments arranged in randomized complete block design (RCBD) with three replications. Two genotypes of amaranth were grown in garden soil, saline soil, 50% garden soil: 50% saline soil and 25, 50 and 100 mM NaCl. Salinization was imposed at 7, 14 and 21 days after transplanting. Results indicated that salinity stress reduced plant growth in both red and green amaranth at all treatments except for the 25 mM NaCl treatment. The most detrimental effect was noted in 100 mM NaCl treatment for all plant growth parameters. From the experiment result revealed that Plant height was reducing from (35.8, 30.1 and 24.5 cm for *Amaranthus tricolor* L. and 25.5, 22.8 and 12.2 cm for *Amaranthus dubius* L.) due to increasing salt concentration that is 25, 50 and 100 mM NaCl. These observations indicated that extreme salt stress showed negative impact on plant height of the plant.

Omamt *et al.* (2006) reported that 100 mM NaCl caused a reduction in plant height (34%), leaf number (40%) and total leaf area (58%) in *A. tricolor.*

Misra *et al.* (1997) with their study on rice seedlings *Oryza sativa* L. vr. Damodar, Dantus *et al.* (2005) in their study on cowpea, *Vigna unguiculata* L., and finally by Memon *et al*. (2010) in their study on *Brassica campestris* L. where they indicated that the use of low concentrations of sodium chloride led to increases in plants lengths, whereas higher concentrations caused shortage.

2.3.5 Number of leaves

Hoang *et al*. (2019) conducted a study to know the effect of Physiological and phytochemical responses of red amaranth (*Amaranthus tricolor L.)* and green amaranth (*Amaranthus dubius L.)* to different salinity levels. The physiological and phytochemical responses of red amaranth (*Amaranthus tricolor* L.) and green amaranth (*Amaranthus dubius L.*) to different salinity levels were determined in two experiments conducted in Vietnam. Both experiments were performed in a net house involving pot experiments arranged in randomized complete block design (RCBD) with three replications. Two genotypes of amaranth were grown in garden soil, saline soil, 50% garden soil: 50% saline soil and 25, 50 and 100 mM NaCl. Salinization was imposed at 7, 14 and 21 days after transplanting. Results indicated that salinity stress reduced plant growth in both red and green amaranth at all treatments except for the 25 mM NaCl treatment. The most detrimental effect was noted in 100 mM NaCl treatment for all plant growth parameters. From the experiment result revealed that number of leaves was reducing from (24.2, 20 and 13.6 for *Amaranthus tricolor* L. and 14.7, 12.6 and 8.1 cm for *Amaranthus dubius* L.) due to increasing salt concentration that is 25, 50 and 100 mM NaCl. These observations indicated that extreme salt stress showed negative impact on number of leaves of the plant.

Mahbubul *et al.* (2018) conducted a hydroponic experiment at Plant Physiology Laboratory, Department of Crop Botany, Bangladesh Agricultural University, Mymensingh, Bangladesh, during the period from December 2015 to July 2016 to investigate the effect of NaCl on morphological characters and growth of maize (*Zeamays* L.) seedlings. The experiment comprised two levels (0 dS m^{-1} and 8 dS m^{-1}) of salinity for hydroponic experiment, designed in two factorials Complete Randomized Design (CRD) with three replications on four cultivars viz. Kaveri - 244+, BHARAT Hybrid Sultan 702, Getco seeds GP – 901, Essence –Platinum. Result indicated that Interaction effect of variety and NaCl level on no. of leaves plant-1 was significant. The highest no. of leaves per plantwas recorded in the treatment combination of Essence-Platinum with 0dSm-1 NaCl at 14 DAS (14.4). On the other hand, the lowest no. of leaves per plant was observed in BHARAT Hybrid Sultan 702 with 8 dS $m-^{1}$ NaCl (4.4) at 14 DAS. Leaf number was decreased with increasing in salt concentration in all maize genotypes except Getco seeds GP–901.The number of leaves of the studied cultivars was varied in response to salinity stress. With the increase in salinity level from 0 dsm^{-1} to $8dsm^{-1}$, number of leaves was highly affected in Kaveri–244+, though the lowest leaves was recorded with BHARAT Hybrid Sultan 702. BHARAT Hybrid Sultan 702 with no salinity also had lower leaf numbers at 14 DAS. Among other cultivars, Getco seeds GP–901 had increased numbers of leaves with 8ds m^{-1} than with no salinity condition. This behavior indicates a reduction in the appearance of new leaves except Getco seeds GP–901 could be associated with the osmotic stress.

Al-Maskri *et al*. (2010) conducted experiment to investigate the effects of salinity stress (NaCl) on growth of lettuce (*Lactuca sativa* L.) cv. Paris Islands Cos under closed-recycled nutrient film technique (NFT). Different salinity levels i.e. 50 mM and 100 mM along with control (0 mM) were used. The experiment was laid out at completely randomized design (CRD). It was observed that number of leaves was reduced (14.23, 12.65 and 12.08) significantly with increasing salinity levels (0 mM, 50 mM and 100 mM).

2.3.6 Leaf area

Hoang *et al*. (2019) conducted a study to know the effect of Physiological and phytochemical responses of red amaranth (*Amaranthus tricolor* L.*)* and green amaranth (*Amaranthus dubius* L.*)* to different salinity levels. The physiological and phytochemical responses of red amaranth (*Amaranthus tricolor* L.) and green amaranth (*Amaranthus dubius* L.) to different salinity levels were determined in two experiments conducted in Vietnam. Both experiments were performed in a net house involving pot experiments arranged in randomized complete block design (RCBD) with three replications. Two genotypes of amaranth were grown in garden soil, saline soil, 50% garden soil: 50% saline soil and 25, 50 and 100 mM NaCl. Salinization was imposed at 7, 14 and 21 days after transplanting. Results indicated that salinity stress reduced plant growth in both red and green amaranth at all treatments except for the 25 mM NaCl treatment. The most detrimental effect was noted in 100 mM NaCl treatment for all plant growth parameters. From the experiment result revealed that leaf area was reducing from (511.7, 392.5, 295.8 cm^2 for *Amaranthus tricolor* L. and 394.8, 246.7 and 195.3 cm^2 for *Amaranthus dubius* L.) due to increasing salt concentration that is 25, 50 and 100 mM NaCl. These observations indicated that extreme salt stress showed negative impact on leaf area of the plant.

Meneze *et al.* (2017) conducted an experiment to know the effect of growth and contents of organic and inorganic solutes in amaranth under salt stress. *Amaranthus cruentus* L. is a forage species, with grains that exhibit excellent nutritional characteristics, being the 'BRS Alegria' the first cultivar recommended for cultivation in Brazil. This study aimed at evaluating the effect of salt stress on the growth and concentrations of organic and inorganic solutes in *Amaranthus cruentus* L. ('BRS Alegria' cultivar). Height, stem diameter, number of leaves, leaf, stem and root dry mass, leaf area, relative water content and membrane integrity percentage, as well as soluble carbohydrate, free amino acid, soluble protein, free proline, Na^+, Cl^- and K^+ contents, were evaluated in different plant organs. Salinity significantly reduced the biomass yield and leaf area from the treatment with 25 mM of NaCl, indicating that the 'BRS Alegria' cultivar is sensitive to salt stress. Soluble carbohydrates in the leaves decreased by 59%, the other organic solutes showed no substantial increases. These results, coupled with the reduction in the relative water content and membrane integrity, suggest a low ability of this cultivar to adjust osmotically under salt stress.

The K^+/Na^+ ratio abruptly decreased in 25 mM of NaCl, suggesting an ionic imbalance, which may partially explain the salt-induced growth reduction. Mathur *et al.* (2006) reported, that the stress of the moth bean plant (*Vigna aconitifolia* L.) with increasing concentrations of sodium chloride, led to a decrease in leaf length and wide. This decrease was inversely proportional to the concentrations.

2.3.7 Dry matter weight

Hoang *et al*. (2019) conducted a study to know the effect of Physiological and phytochemical responses of red amaranth (*Amaranthus tricolor* L.) and green amaranth (*Amaranthus dubius* L.) to different salinity levels. The physiological and phytochemical responses of red amaranth (*Amaranthus tricolor* L.) and green amaranth (*Amaranthus dubius* L.) to different salinity levels were determined in two experiments conducted in Vietnam. Both experiments were performed in a net house involving pot experiments arranged in randomized complete block design (RCBD) with three replications. Two genotypes of amaranth were grown in garden soil, saline soil, 50% garden soil: 50% saline soil and 25, 50 and 100 mM NaCl. Salinization was imposed at 7, 14 and 21 days after transplanting. Results indicated that salinity stress reduced plant growth in both red and green amaranth at all treatments except for the 25 mM NaCl treatment. The most detrimental effect was noted in 100 mM NaCl treatment for all plant growth parameters. From the experiment result revealed that dry matter weight was reducing from (3.8, 2.3 and 1.7 g for *Amaranthus tricolor* L. and 2.1, 1.4 and 0.9 g for *Amaranthus dubius* L.) due to increasing salt concentration that is 25, 50 and 100 mM NaCl. These observations indicated that extreme salt stress showed negative impact on dry matter weight of the plant.

Mahbubul *et al.* (2018) conducted a hydroponic experiment at Plant Physiology Laboratory, Department of Crop Botany, Bangladesh Agricultural University, Mymensingh, Bangladesh, during the period from December 2015 to July 2016 to investigate the effect of NaCl on morphological characters and growth of maize (*Zeamays* L.) seedlings. The experiment comprised two levels (0 dS m^{-1} and 8 dS m^{-1}) of salinity for hydroponic experiment, designed in two factorials Complete Randomized Design (CRD) with three replications on four cultivars viz. Kaveri - 244+, BHARAT Hybrid Sultan 702, Getco seeds GP – 901, Essence –Platinum. Result indicated that interaction effect of variety and NaCl level on dry weight of plant with 0 dS m^{-1} and 8 dS m^{-1} was significant. The highest dry weight of plant (1.13 g) was recorded in the treatment combination of Essence – Platinum with 0 dS m^{-1}. On the other hand, the lowest dry weight was observed in BHARAT Hybrid Sultan 702 with 8 dS m^{-1} NaCl (0.38 g) respectively.

Meneze *et al.* (2017) conducted an experiment to know the effect of growth and contents of organic and inorganic solutes in amaranth under salt stress. *Amaranthus cruentus* L. is a forage species, with grains that exhibit excellent nutritional characteristics, being the 'BRS Alegria' the first cultivar recommended for cultivation in Brazil. This study aimed at evaluating the effect of salt stress on the growth and concentrations of organic and inorganic solutes in *Amaranthus cruentus* L. ('BRS Alegria' cultivar). Height, stem diameter, number of leaves, leaf, stem and root dry mass, leaf area, relative water content and membrane integrity percentage, as well as soluble carbohydrate, free amino acid, soluble protein, free proline, Na^{+}, Cl^{-} and K^{+} contents, were evaluated in different plant organs. Salinity significantly reduced the biomass yield and leaf area from the treatment with 25 mM of NaCl, indicating that the 'BRS Alegria' cultivar is sensitive to salt stress. Soluble carbohydrates in the leaves decreased by 59%, the other organic solutes showed no substantial increases. These results, coupled with the reduction in the relative water content and membrane integrity, suggest a low ability of this cultivar to adjust osmotically under salt stress. The K^{+}/Na^{+} ratio abruptly decreased in 25 mM of NaCl, suggesting an ionic imbalance, which may partially explain the salt-induced growth reduction.

2.4 Seed Priming

Seed priming is a pre-sowing treatment which leads to a physiological state that enables seed to germinate more efficiently. Seed priming has been shown to increase germination, with high levels of resistance to stress and productivity (Paparella *et al.*, 2015). In this procedure, the seeds are partially hydrated under the recommended temperature and time, allowing the initiation of the germination metabolic processes. Thus, the initial phases (phase I and II) occur, without phase III (primary root protrusion) (Bewley *et al.,* 2013). According to Paparella *et al.* (2015), seed priming can be performed with bioactive molecules, microorganisms or secondary metabolites, for example, salicylic or gibberellic acids. These compounds control key biochemical processes during maturation (germination) and plant development, while their exogenous application promotes antioxidant responses (Radhakrishnan *et al.,* 2013).

2.5 Organic acid contribution to plant

Organic acids play an integral role in plant primary metabolism, where they are involved in fundamental pathways such as, the tricarboxylic acid cycle, C_3, C_4 and CAM-photosynthesis and the glyoxylate cycle. They also arise as products of the degradation of more reduced compounds and are interconverted in many plant tissues. Organic acids, such as malate, fumarate, lactate, and citrate, have essential functions in many cellular processes such as stomatal function, phosphorous acquisition, aluminium tolerance, communication with microorganisms, CO_2 concentrating metabolism, temporary carbon storage, interchange of reductive power among sub-cellular compartments, and pH regulation. They also play a critical role in the regulation of plant development and growth, as well as in regulation of both primary and specialized metabolic pathways, some of which are involved in the response to both abiotic and biotic stress. Moreover, they play roles as signalling molecules, not only as allosteric regulators of many key enzymes, but also as modulators of gene expression (Drincovich *et al.,* 2021).

2.5.1 Fresh weight

Ghoohestani *et al.* (2012) conducted a study to know the effect of seed priming of tomato with salicylic acid, ascorbic acid and hydrogen peroxide on germination and plantlet growth in saline conditions. In order to improve the germination of tomato seeds in saline conditions, effect of seed priming by various concentrations of salicylic acid (SA) (0, 50, 100and150mg/L), ascorbic acid (ASA) (0, 50, 100 and 150mg/L) and hydrogen peroxide (HP) (0, 40, 80 and 120μM) was investigated. Saline solution of 10 dS/m was applied to impose salinity stress. The results showed that maximum fresh weight (0.139 g) of plantlet caused by seed priming in 150 mg/L ascorbic acid. Antioxidant nature and increase of plantlet water imbibitions ability in presence of ascorbic acid increased the plantlet fresh weight (Muller *et al.,* 2004). Salicylic acid and hydrogen peroxide 80 μM had favorable effects on fresh weight of tomato plantlet in salinity condition. No statistically significant differences in the levels of hydrogen peroxide with control in salinity condition.

2.5.2 Dry weight

Behairy *et al*. (2017) conducted a study to figure out the alleviation of salinity stress on Fenugreek seedling growth using salicylic acid, citric acid and proline. In this study, the effects of 100 ppm of salicylic acid, citric acid and proline on some of morphological traits, chlorophyll content, antioxidant enzymes activities and isozyme pattern were investigated in fenugreek seedling under salinity stress using different concentrations of NaCl (0, 1000, 2000 and 3000 ppm) during germination stage. The results showed that a significant decreasing of seedling dry weight was observed with the increasing of NaCl concentration. The pretreatment of seeds with salicylic acid, citric acid and proline caused to increase in dry weight compared to control. From the experiment result showed that salinity stress by NaCl caused a significant reduction in seedling dry weight. Pretreatment of seeds with salicylic acid, citric acid and proline under saline condition caused significant increase in seedling dry weight from 304 mg (for control) to 375mg (for salicylic acid), 319 mg (for citric acid), and 378 mg (for proline) cm at 3000 ppm.

Ghoohestani *et al.* (2012) conducted a study to know the effect of seed priming of tomato with salicylic acid, ascorbic acid and hydrogen peroxide on germination and plantlet growth in saline conditions. In order to improve the germination of tomato seeds in saline conditions, effect of seed priming by various concentrations of salicylic acid (SA) (0, 50, 100 and 150mg/L), ascorbic acid (ASA) (0, 50, 100 and 150mg/L) and hydrogen peroxide (HP) (0, 40, 80 and 120 µM) was investigated. Saline solution of 10 dS/m was applied to impose salinity stress. The results showed that maximum plantlet dry weight (0.0183 g) was achieved by salicylic acid 150 mg/L. The lowest value of dry weight was related to the seeds that had been done no pretreatment.

CHAPTER 3

MATERIALS AND METHODS

The experiment was conducted at Sher-e-Bangla Agricultural University, Dhaka to investigate the effect of organic acid to mediate the salt stress of red amaranth. Materials used and methodologies followed in the present investigation have been described in this chapter.

3.1 Experimental period

The experiment was conducted during the period from January to February 2020 (Rabi season).

3.2 Description of the experimental site

3.2.1 Geographical location

The experiment was conducted both in the Central laboratory and Agroforestry net house of Sher-e-Bangla Agricultural University (SAU). The experimental site is geographically situated at 23°77′ N latitude and 90°33′ E longitude at an altitude of 8.6 meter above sea level (Anon., 2004).

3.2.2 Agro-Ecological Zone

The experimental field belongs to the Agro-ecological zone (AEZ) of "The Madhupur Tract", AEZ-28 (Anon., 1988 a). This was a region of complex relief and soils developed over the Madhupur clay, where floodplain sediments buried the dissected edges of the Madhupur Tract leaving small hillocks of red soils as 'islands' surrounded by floodplain (Anon., 1988 b). For better understanding about the experimental site has been shown in the Map of AEZ of Bangladesh in Appendix- I.

3.2.3 Soil

The soil texture was silty clay with pH 6.1. The morphological, physical and chemical characteristics of the experimental soil have been presented in Appendix- II.

3.2.4 Climate and weather

The climate of the experimental site was subtropical, characterized by the winter season from November to February and the pre-monsoon period or hot season from March to April and the monsoon period from May to October (Edris *et al.*, 1979). Meteorological data related to the temperature, relative humidity and rainfall during the experiment period of was collected from Bangladesh Meteorological Department (Climate division), Sher-e-Bangla Nagar, Dhaka and has been presented in Appendix-III.

3.3 Experiment type

Both lab and pot experiment was conducted for this research

3.4 Laboratory condition

The temperature and relative humidity of the laboratory room were recorded daily basis during the study period with a digital thermo hygrometer (TERMO, TFA, Germany). The average minimum and maximum temperature during the study period of the culture room was 170C to 270C respectively and the average minimum and maximum relative humidity were recorded 54% and 75%, respectively.

3.5 Characteristics of test crop

Red amaranth was used for this experiment. Amaranth plants typically are annuals or short-lived perennials. The stems often are reddish in colour and sometimes are armed with spines; they bear simple, alternately arranged leaves and often feature a pinkish taproot. The plants can be monoecious (flowers of both sexes are on the same individual) or dioecious (each individual produces flowers of a single sex). The small flowers typically feature colourful bracts and are arranged in dense showy inflorescences; a single plant can produce hundreds or thousands of seeds, borne singly in dry capsule fruits. The plants utilize a photosynthetic pathway known as C4 carbon fixation, which largely prevents photorespiration and thus increases salt and drought tolerance.

3.6 Experimental materials

Different equipments such as 4-digit electric balance, petri dish, filter paper, micro pipette, forceps, magnetic stirrer, shelf for placing petri dish, plastic top etc. were used for this study.

3.7 Experimental design

Both lab and pot experiment was laid out in a Completely Randomized Design (CRD) with three replications.

3.8 Steps of the experiment

This experiment was completed in two steps. In the 1st step, Red amaranth salt tolerance ability was investigated and in 2nd step, investigated the effect of organic acid to mediate the salt stress of red amaranth.

3.9 Treatment of the 1st experiment

T_0 = 0 mM NaCl (Control)

T_1= 25 mM NaCl

T_2= 50 mM NaCl

T_3= 75 mM NaCl

T_4= 100 mM NaCl

T_5= 150 mM NaCl

T_6= 200 mM NaCl

T_7= 250 mM NaCl

3.10 Treatment of the 2nd experiment

Factor A: Salt stress condition *viz*. (3)

S_0: Control (0 mM NaCl),

S_1: Mild stress (50 mM NaCl) and

S_2: Severe stress (100 mM NaCl)

Factor B: Different organic acid *viz*. (3)

A_0: (Control),

A_1: Ascorbic acid, and

A_2: Citric acid

3.11 Lab experiment

3.11.1 Procedure of lab experiment

Twenty random seeds were selected from each of the treatment and it placed in 90 mm diameter petri dishes on whatman No.1 filter paper which was moist with distilled water. Different concentration of salt solution was added in the, whatman No.1 filter paper to observe the germination growth behavior of Red amaranth seeds. Experimental units (24 Petri dishes) were arranged in a completely randomized design with three replications. During the test, filter papers were kept moist condition with water in the Petri dishes. Seeds were kept at room temperature 25±1°C under normal light to help germination. Germination was considered to have occurred when radicle were 2 mm long (Akbari, Sanavy, and Yousefzadeh, 2007). Germination progress was inspected and data were collected at every 24h intervals and this process was continued up to 7 days. The seedlings which were short, thick and spiral formed hypocotyls and stunted primary root were considered as abnormally germinated seeds (ISTA, 2003). These types of abnormal or dead seedlings were rejected during counting. At the end of germination test (7 days), 5 random seedlings from each of the treatments were selected and their roots and shoots were cut from the cotyledons and length was measured and transferred to brown paper.

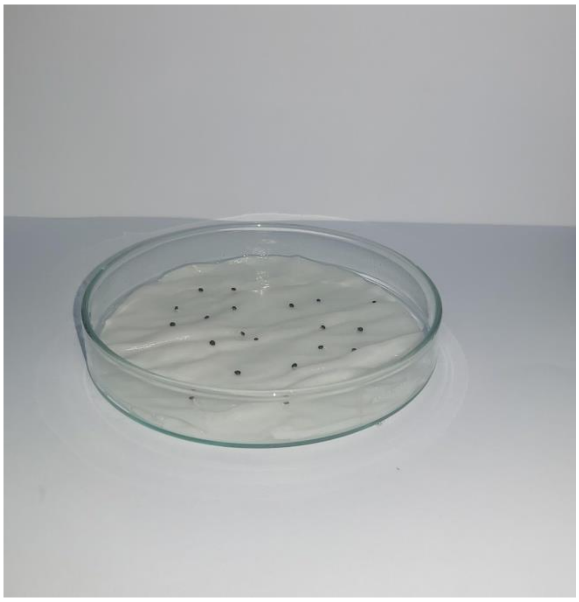

Plate 1. Picture showing placing seed in petri dishes on whatman No.1 filter paper.

3.12 Data collection

Data on seedling emergence of 5 red amaranths were collected from 1 to 7 days after seed placement. Normal seedlings were counted. The uprooted seedlings were cleaned with tap water and excess water was removed with tissue paper.

The following data were measured and weighted:

i. Germination(%)
ii. Radicle length (cm)
iii. Plumule (cm)
iv. Total length (cm)

3.13 Procedure of recording data

i) Germination percentage %

Germination percentage is an estimate of the viability of a population of seeds. The equation to calculate germination percentage (Abdel-Haleem, 2015).

$$\text{Germination \%} = \frac{\text{Seeds germinated in petri dish}}{\text{total seeds in petri dish}} \times 100$$

Plate 2: Picture showing germination percentage (%) of red amaranth seed

ii) Radicle length (cm)

The radicle length of five seedlings from each petri dish was measured using measuring scale at 7 days after seed placement. Measurement was done using a meter scale and mean data was expressed in millimeter (cm) for each treatment.

iii) Plumule length (cm)

The plumule length of five seedlings from each petri dish was measured using measuring scale at 7 days after seed placement. Measurement was done using a meter scale and mean data was expressed in millimeter (cm) for each treatment.

iv) Total length (cm)

The plumule length of five seedlings from each petri dish was measured using measuring scale at 7 days after seed placement. Measurement was done using a meter scale and mean data was expressed in millimeter (cm) for each treatment.

3.14 Field experiment

3.14.1 Selection and preparation of the pot

Plate 3: Picture showing pot filling and seed sowing of red amaranth

Earthen pots of having 12 inches diameter, 12 inches height with a hole at the centre of the bottom were used. Silt soil was used in the experiment. The upper edge diameter of the pots was 30 cm (r=15 cm). While filling with soil, the upper one inch of the pot was kept vacant so that irrigation can be provided using a hose pipe. As such the diameter of the upper soil surface was 15 inch (30 cm) and the area of the upper soil surface was (πr^2 = 3.14x 0.015x 0.015=0.07 m^2). The preparation of the pot was done in 6 January 2020.

3.15 Fertilizer application

In this experiment no chemical fertilizer were applied except cow dung and it's mixed properly during pot filling with soil.

3.16 Induced salt stress condition in the pot

By adding different salt concentration as par treatment requirement creating salt stress condition for experiment pots.

3.17 Sowing of seeds in the pot

Treated seeds of red amaranth were sown in the pot on 7 January having a depth of 1–2 cm. During seed sowing 0.5 g Bavistin were mixed with seeds. The seed was sowing in 1 g pot^{-1}.

3.18 Intercultural operations

i) Weeding and irrigation

Weeding was done during the experimental period to keep the pot free from hazardous effect of weed during the growth and development of vegetables. Irrigation was done as per necessity for sufficient moisture by watering cane.

Plate 4: Picture showing weeding and thinning in the experiment pots of red amaranth

ii) Thinning out

The emergence of Amaranth was started after 5 days of sowing respectively. Thinning was carried out three times. For red Amaranth first, second and third thinning was done after 10 days, 15 days and 20 days of sowing respectively.

iii) Pest and disease management

No pesticide and insecticide was applied as the crops were not infected by any pest and disease.

3.19 Methods of data collection

Red amaranth was harvested at 30 days after sowing (DAS) when the crop reached at edible size. Plant samples of red amaranth were collected randomly from each replication of the respective pots. A total of 54 (2 from each replication) plants of red amaranth were selected from each pot for data collection.

The data were recorded on the following parameters

i. Plant height (cm)
ii. Number of leaves per plant

iii. Leaf length (cm)
iv. Leaf width (cm)
v. Number of stem girt
vi. Plant fresh weight (g)
vii. Plant dry weight (g)
viii. Yield pot^{-1}
ix. Yield ha^{-1}

3.20 Procedure of recording data

i) Plant height (cm)

The height of the selected plant was measured from the ground level to the tip of the plant at 10, 20, 30 DAS. Mean plant height of red amaranth plant were calculated and expressed in cm.

ii) Number of leaves plant^{-1}

Total number of leaves plant^{-1} was counted at 10, 20 and 30 days after sowing. Randomly 2 plants were selected and measured and the sum of number of leaf was divided by 2 to record number of leaf per plant.

iii) Leaf length (cm) and leaf width (cm)

The leaf length and leaf width of the upper 3rd leaf of each plant was measured against a centimetre scale at 30 DAS.

iv) Number of stem girth

Total number stem girth plant^{-1} was counted at 30 days after sowing. Randomly 2 plants were selected and measured and the sum of number of stem girth was divided by 2 to record number of stem girth per plant.

v) Plant fresh weight (g)

Randomly 2 plants were selected from the each replication and the sum of the fresh weight of 2 plants was divided by 2, then it was recorded as weight of plant fresh weight (g) at 30 DAT.

vi) Plant dry weight (g)

The dry weight of the plants from each pot was measured at 30 days after sowing. Dry weight was recorded by drying the sample in an oven at 70°C until attained a constant weight.

vii) Yield pot^{-1} (g)

The yield (g) of red amaranth per pot was calculated by taking fresh weights of all the plants that grows in each pot at 30 DAS.

viii) Yield ha^{-1}

The yield (t) of red amaranth per hectare was calculated by converting the total yield (kg) of red amaranth per square meter.

3.21 Data analysis technique

The collected data were compiled and analyzed statistically using the analysis of variance (ANOVA) technique with the help of a computer package program name R-3.5.1 software (R Core Team, 2013) and the mean differences were adjusted by Least Significant Difference (LSD) test at 5% level of probability (Gomez and Gomez, 1984).

CHAPTER 4

RESULTS AND DISCUSSION

This section envelops the presentation and discussion of the results obtained from study to investigate the adaptation of salt tolerant capacity of red amaranth through organic acid treatment under salt stress condition. The results of the germination and growth parameters of red amaranth as influenced by different concentrations of priming agent (Salt concentrations) with organic acid in salt stress condition have been presented and discussed in this chapter.

4.1 First experiment: Study on the effect of different salt concentrations on the germination and growth behavior of red amaranth.

In this chapter, the results found from the first experiment regarding the effects of different salt concentrations on the germination percentage (%), radicle, plumule and total length of the red amaranth have been presented.

4.1.1 Germination percentage (%)

Germination percentage is an estimate of the viability of a population of seeds. There was significant variation in terms of germination percentage due to seed priming with salt at different concentration (Figure 1). Result shows that among all test samples, the highest germination percentage (87.33%) was observed in T_0 treatment which was statistically similar with T_1 (83%) treatment. After that the third and fourth highest germination percentage (77.33 and 74.67%) were observed in T_2 and T_3 treatment. The lowest germination percentage (26.33%) was observed in T_7 treatment. In case of germination inhibition percentage, T_1 (4.96%) T_2 (11.45) and T_3 (14.50%) treatment show the minimum germination inhibition percentage comparable to others treatment over control (Figure 2). While maximum germination inhibition percentage (69.85%) was observed in T_7 treatment over control. Chowdhury *et al.* (2018) reported that the highest germination percentage was recorded 90% in the control and the lowest value was 53.33% in 200 mM NaCl. The moderate germination percentages were recorded 76.67%, 73.34%, 70.00% and 66.67% in 25, 50, 75, 100 and 125 mM NaCl levels respectively. Increasing of salt concentration reduces germination percentage. Kapoor and Pande (2015) reported that the high salt concentration delayed the process of germination. Panuccio *et al*. (2014) reported that the negative effect of salinity on seed germination might be happened due to salinity-induced ionic imbalance or toxicity.

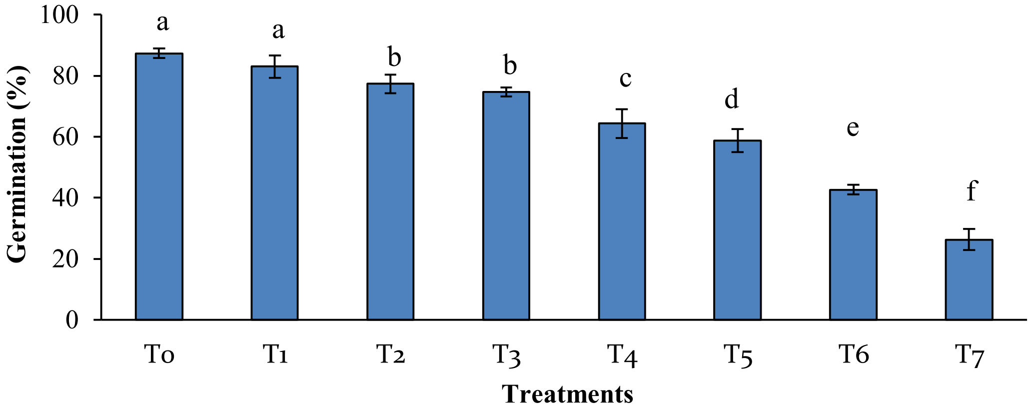

Figure 1. Effect of seeds priming on germination percentage (%) of red amaranth under different salt concentrations. Here, T_0: 0 mM NaCl (control), T_1: 25 mM NaCl, T_2: 50 mM NaCl, T_3: 75 mM NaCl, T_4: 100 mM NaCl, T_5: 150 mM NaCl, T_6: 200 mM NaCl and T_7: 250 mM NaCl

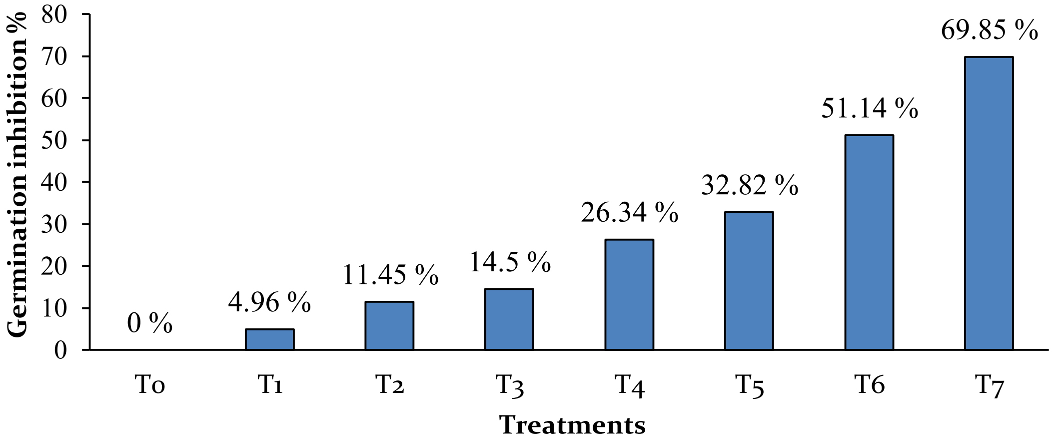

Figure 2. Effect of seeds priming on germination inhibition percentage (%) over control of red amaranth under different salt concentrations. Here, T_0: 0 mM NaCl (control), T_1: 25 mM NaCl, T_2: 50 mM NaCl, T_3: 75 mM NaCl, T_4: 100 mM NaCl, T_5: 150 mM NaCl, T_6: 200 mM NaCl and T_7: 250 mM NaCl

4.1.2 Radicle length (cm)

The radicle is the first part of a seedling (a growing plant embryo) to emerge from the seed during the process of germination. Significant variation was observed in radicle length due to the effect of seed priming with different salt concentration (Figure 3). From the experiment result revealed that the maximum radicle length (4.79cm) was observed in T_0 treatment. 2nd, 3rd and 4th maximum radicle length (4.54, 4.15 and 2.96cm) were observed in T_1, T_2 and T_3 treatment comparable to others treatment. The minimum radicle length (0.78cm) was observed in T_7 treatment. In case of radicle length inhibition percentage, minimum radicle length inhibition percentage (5.22, 13, 36 and 38.20%) was observed in T_1, T_2 and T_3 treatment comparable to others treatment over control (Figure 4). The maximum radicle length inhibition percentage (83.72%) was observed in T_7 treatment over control. The result obtained from the present study was similar with the findings of Hoang *et al*. (2019) who reported that root length was reducing due to increasing salt concentration. Ratnakar and Rai (2013) observed while studying effects of NaCl salinity on seed germination and early seedling growth of *Trigonella foenum-Graecum* L. Var. Peb and reported that increasing NaCl concentrations caused a gradual decrease in root length of the growing seedling.

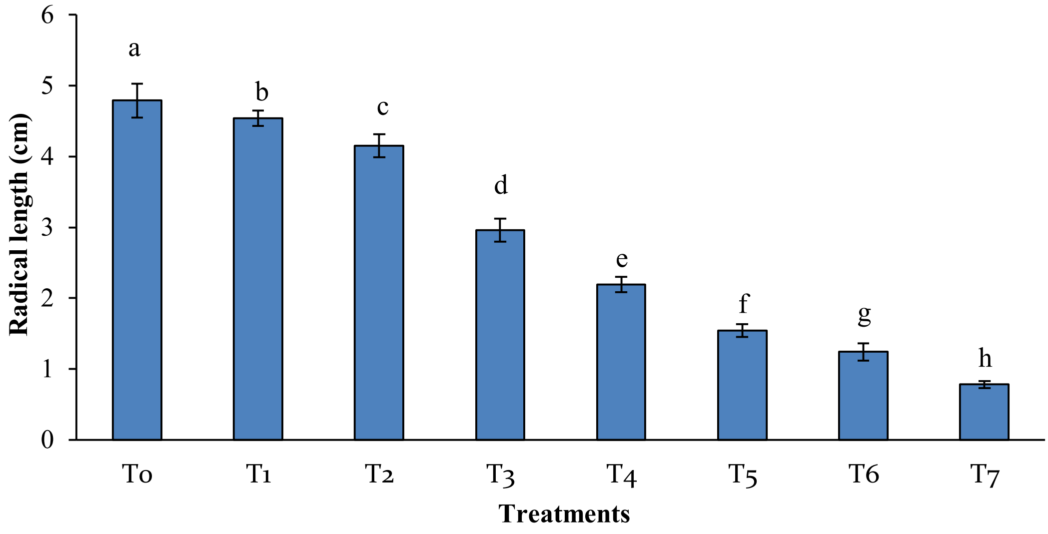

Figure 3. Effect of seeds priming on radical length (cm) of red amaranth under different salt concentrations. Here, T_0: 0 mM NaCl (control), T_1: 25 mM NaCl, T_2: 50 mM NaCl, T_3: 75 mM NaCl, T_4: 100 mM NaCl, T_5: 150 mM NaCl, T_6: 200 mM NaCl and T_7: 250 mM NaCl

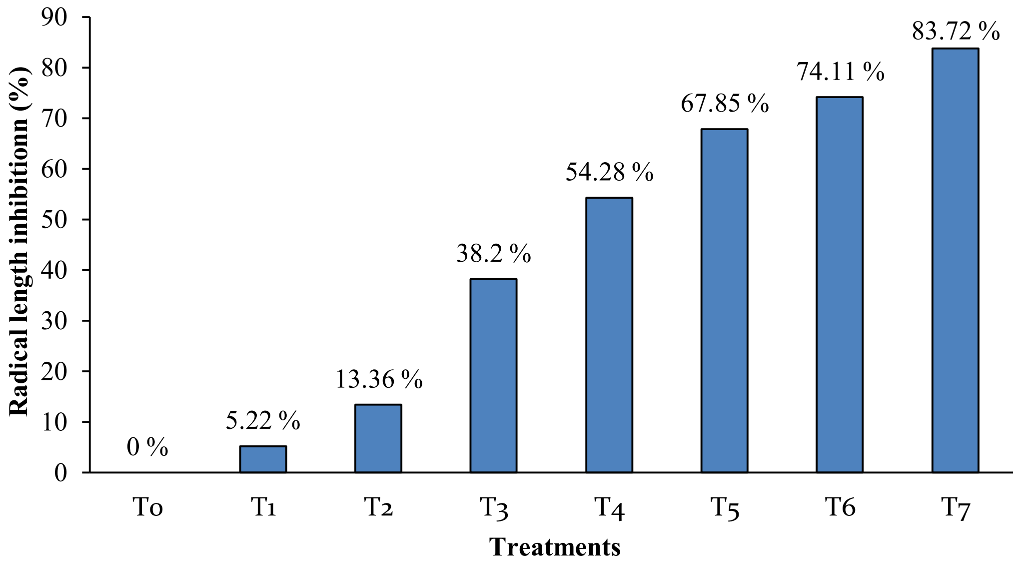

Figure 4. Effect of seeds priming on radical length inhibition (%) over control of red amaranth under different salt concentrations. Here, T_0: 0 mM NaCl (control), T_1: 25 mM NaCl, T_2: 50 mM NaCl, T_3: 75 mM NaCl, T_4: 100 mM NaCl, T_5: 150 mM NaCl, T_6: 200 mM NaCl and T_7: 250 mM NaCl

4.1.3 Plumule length (cm)

Plumule grows after the radicle. Plumule is the part of the embryo, which helps in the development of the shoot system, comprising of stem, leaves, flowers, seeds etc. (Figure 5). From the experiment result revealed that the maximum plumule length (3.14cm) was observed in T_0 treatment. 2^{nd}, 3^{rd} and 4^{th} maximum plumule length (2.79, 2.21 and 1.94cm) were observed in T_1, T_2 and T_3 treatment comparable to others treatment. The minimum plumule length (0.27cm) was observed in T_7 treatment. In case of plumule length inhibition percentage, minimum plumule length inhibition percentage (11.15, 29.69 and 38.22%) was observed in T_1, T_2 and T_3 treatment comparable to others treatment over control (Figure 6). The maximum plumule length inhibition percentage (91.40%) was observed inT_7 treatment over control. Mahbubul *et al.* (2018) also found similar result which supported the present finding and reported that shoot length of maize decreasing with the increase in salinity level.

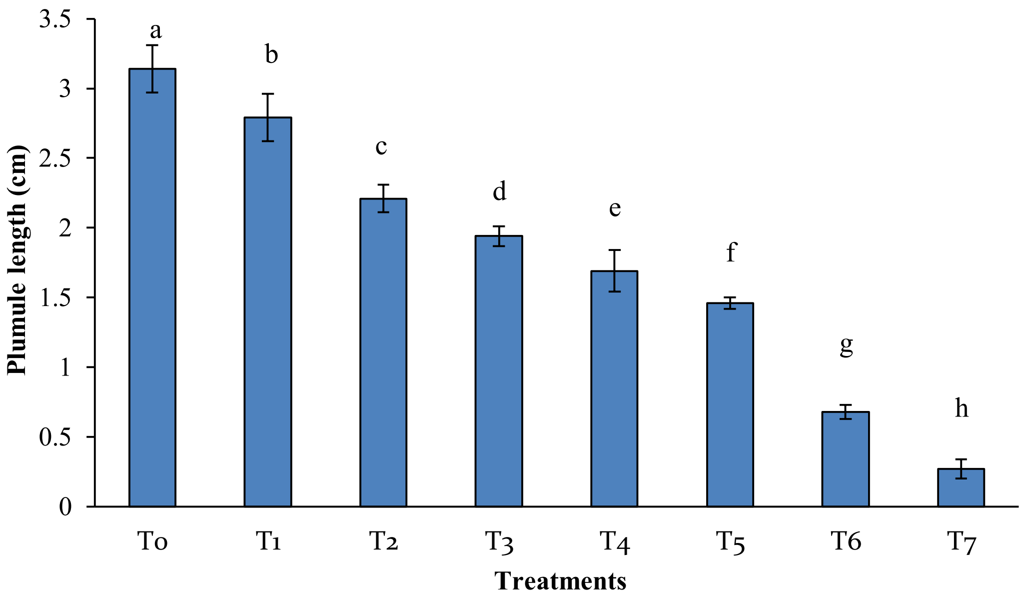

Figure 5. Effect of seeds priming on plumule length (cm) of red amaranth under different salt concentrations. Here, T_0: 0 mM NaCl (control), T_1: 25 mM NaCl, T_2: 50 mM NaCl, T_3: 75 mM NaCl, T_4: 100 mM NaCl, T_5: 150 mM NaCl, T_6: 200 mM NaCl and T_7: 250 mM NaCl

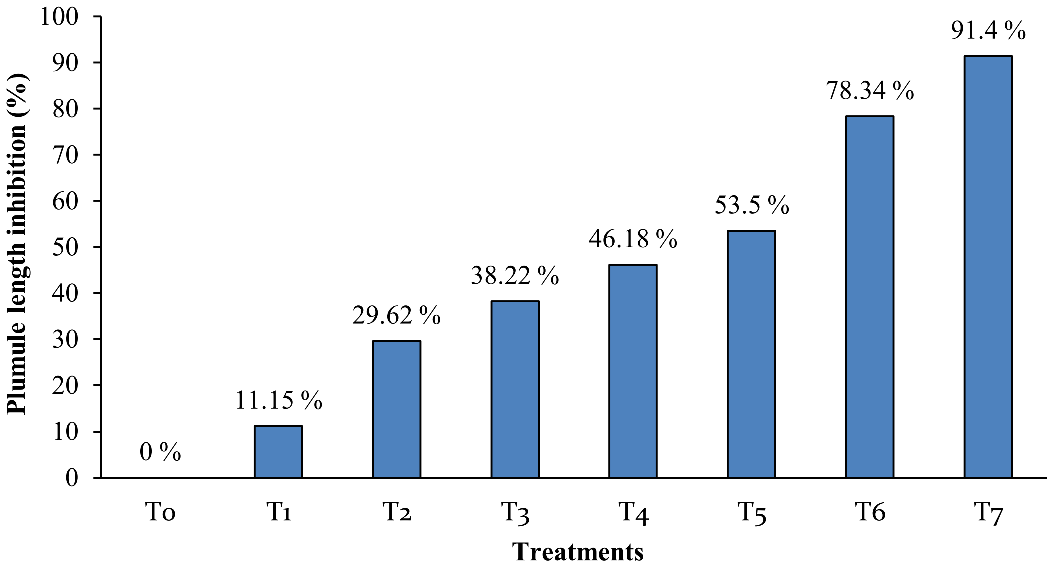

Figure 6. Effect of seeds priming on plumule length inhibition (%) over control of red amaranth under different salt concentrations. Here, T_0: 0 mM NaCl (control), T_1: 25 mM NaCl, T_2: 50 mM NaCl, T_3: 75 mM NaCl, T_4: 100 mM NaCl, T_5: 150 mM NaCl, T_6: 200 mM NaCl and T_7: 250 mM NaCl

4.1.4 Total length (cm)

Radicle plus plumule length was considered the total length. Figure 7. From the experiment result revealed that the maximum total length (7.93 cm) was observed in T_0 treatment. 2nd, 3rd and 4th maximum total length (7.33, 6.36 and 4.90cm) was observed in T_1, T_2 and T_3 treatment comparable to others treatment. The minimum total length (1.06cm) was observed in T_7 treatment. In case of total length inhibition percentage, minimum total length inhibition percentage (7.57, 19.80 and 38.21%) was observed in T_1, T_2 and T_3 treatment comparable to others treatment over control (Figure 8). The maximum total length inhibition percentage (86.63%) was observed in T_7 treatment compared to control.

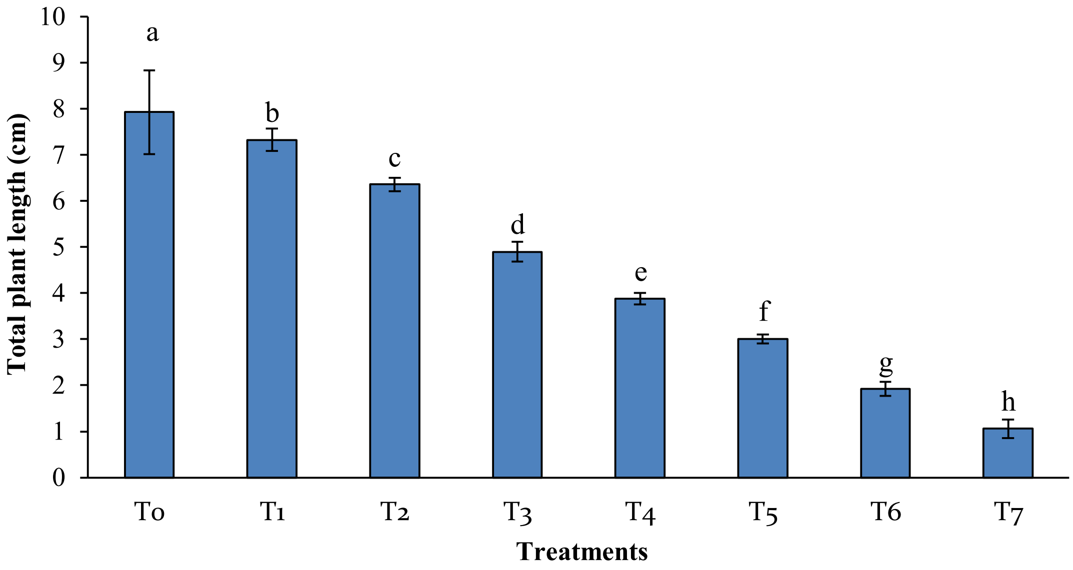

Figure 7. Effect of seeds priming on total plant length (cm) of red amaranth under different salt concentrations. Here, T_0: 0 mM NaCl (control), T_1: 25 mM NaCl, T_2: 50 mM NaCl, T_3: 75 mM NaCl, T_4: 100 mM NaCl, T_5: 150 mM NaCl, T_6: 200 mM NaCl and T_7: 250 mM NaCl

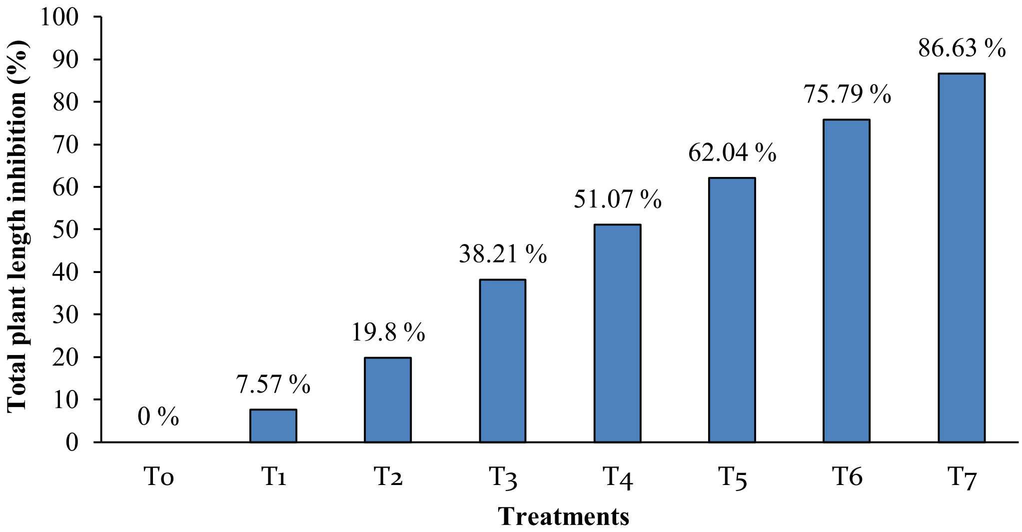

Figure 8. Effect of seeds priming on total plant length inhibition (%) over control of red amaranth under different salt concentrations. Here, T_0: 0 mM NaCl (control), T_1: 25 mM NaCl, T_2: 50 mM NaCl, T_3: 75 mM NaCl, T_4: 100 mM NaCl, T_5: 150 mM NaCl, T_6: 200 mM NaCl and T_7: 250 mM NaCl

4.2 Second experiment: Determination of growth behavior of red amaranth treated with organic acid under salt stress condition

This experiment was conducted under field condition. Red amaranth seed was treated in water, ascorbic acid and citric acid solution for 9 hours. water treated seed used as control and was exposed to 0, 50 and 100mM NaCl (salt) concentration to induced control, mild and severe salt stress conditions in each pot. The results have been presented and discussed under the following headings:

4.2.1 Plant height (cm)

Effect of salt stress condition

Plant height is an important morphological character that acts as a potential indicator of availability of growth resources and in its approaches. Significant variation was observed in plant height at various days after sowing due to the effect of different salt stress condition (Figure 9). The experiment result revealed that the maximum plant height (2.91, 12.89 and 27.79cm) at 10, 20 and 30 DAS was observed in S_0 (control) treatment. Whereas the minimum plant height (1.91, 8.04 and 17.78cm) was observed in S_2 (sever stress) treatment. In case of inhibition percentage over control, minimum plant height inhibition percentage (13.06, 20.33 and 17.24%) was observed in mild stress (S_1) condition while maximum (34.36, 37.63 and 36.02%) in severe stress condition (S_1) over control at 10, 20 and 30 DAS respectively. The result obtained from the present study was similar with the findings of Behairy *et al*. (2017) who observed that significant decreasing of seedling length was observed with the increasing of NaCl concentration. Omamt *et al.* (2006) reported that 100 mM NaCl caused a reduction in plant height (34%), leaf number (40%) and total leaf area (58%) in *A. tricolor.*

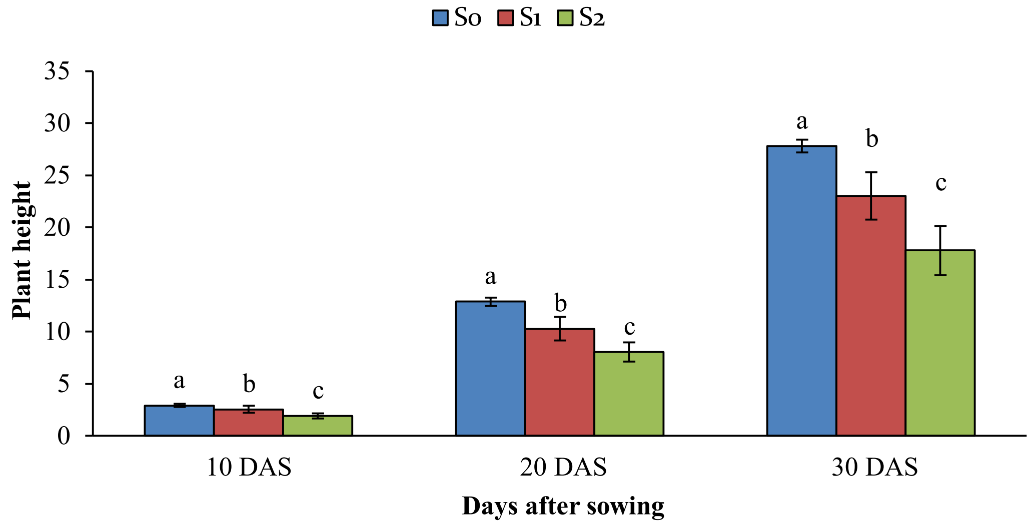

Figure 9. Effect of salt stress condition on plant height (cm) of red amaranth at different DAS. Here, S_0: control (0 mM NaCl), S_1: Mild stress (50 mM NaCl), and S_2: Severe stress (100 mM NaCl)

Effect of organic acid

Significant variation was observed in plant height at different days after sowing due to application of organic acid. From the experiment result revealed that plant height at different days were slightly increasing due to application of ascorbic and citric acid comparable to control treatment (Figure 10). The maximum plant height (2.67, 11.19 and 24.32 cm) at 10, 20 and 30 DAS respectively was observed in A_1 (Ascorbic acid) treatment. Whereas the minimum plant height (2.24, 9.69 and 20.89 cm) was observed in A_0 (control) treatment. Maximum plant height (19.20, 15.48 and 16.42%) increasing due to application of ascorbic acid while (8.93, 6.50 and 11.82%) in citric acid treatment over control at 10, 20 and 30 DAS respectively. Drincovich *et al.* (2021) reported that organic acid play a critical role in the regulation of plant development and growth, as well as in regulation of both primary and specialized metabolic pathways, some of which are involved in the response to both abiotic and biotic stress.

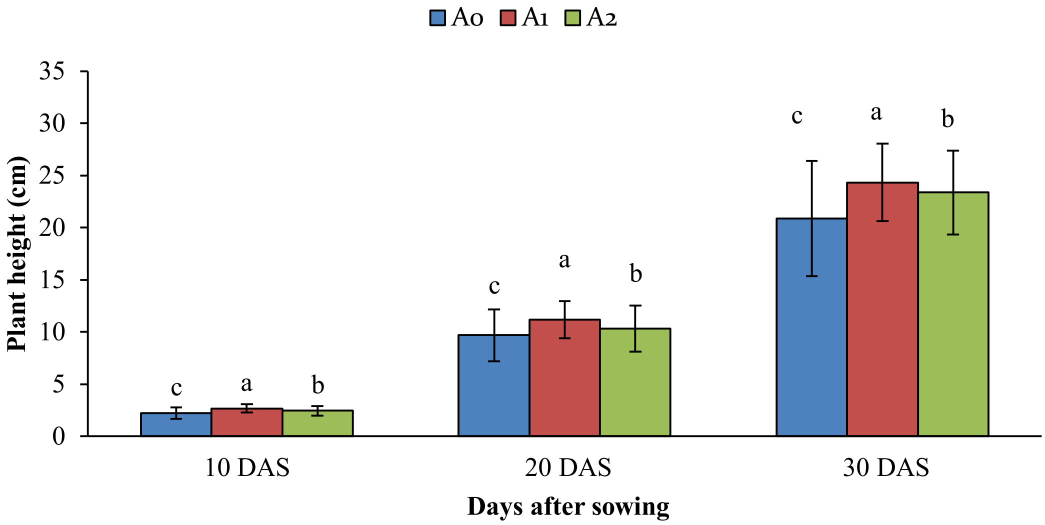

Figure 10. Effect of organic acid on plant height (cm) of red amaranth at different DAS. Here, A_0: (Control), A_1: Ascorbic acid and A_2: Citric acid

Combined effect of salt stress condition and organic acid

The plant height of red amaranth was studied to evaluate the salt-induced negative changes in the plants and the performance of AsA and CA against salt toxicity. Salinity severely affected the plant height of the plants. Plant height decreased by 23.3, 28.74, 26.65% and 41.8, 43.62, 45.45% at 10, 20 and 30 DAS respectively, under mild (50 mM NaCl) and severe stress (100 mM NaCl) condition, whereas both AsA and CA considerably recovered the plant height of the stressed plants (Table 1) at different days after sowing. In contrast to respective stress plant height recovery by AsA and CA was 28.64, 25.27, 23.80 and 16.82, 13.19, 18.34% under mild stress and 29.94, 26.39, 32 and (13.77, 8.75 23.53% under severe stress, respectively. So, it is very clear that both AsA and CA alleviated salt toxicity where AsA performed far better than the CA.

Table 1. Combined effect of salt stress condition and organic acid on plant height (cm) of red amaranth at different DAS

Treatment combinations	Plant height (cm) at 10 DAS	Plant height (cm) at 20 DAS	Plant height (cm) at 30 DAS
S_0A_0	2.87 a	12.77 a	27.50 a
S_0A_1	3.00 a	13.07 a	28.20 a
S_0A_2	2.87 a	12.83 a	27.67 a
S_1A_0	2.20 c	9.10d	20.17 c
S_1A_1	2.83 ab	11.40 b	24.97 b
S_1A_2	2.57 b	10.30 c	23.87 b
S_2A_0	1.67e	7.20e	15.00 e
S_2A_1	2.17cd	9.10 d	19.80 c
S_2A_2	1.90de	7.83e	18.53 d
LSD $_{(0.05)}$	0.27	0.82	1.21
CV (%)	6.44	4.54	3.06

Here, S_0: control (0 mM NaCl), S_1: Mild stress (50 mM NaCl), and S_2: Severe stress (100 mM NaCl), A_0: (Control), A_1: Ascorbic acid, and A_2: Citric acid.

4.2.2 Number of leaves

Effect of salt stress condition

A leaf is the principal lateral appendage of the vascular plant stem, usually borne above ground and specialized for photosynthesis. Significant variation was observed in number of leaves of red amaranth at various days after sowing due to the effect of different salt stress condition (Figure 11). From the experiment result revealed that the maximum number of leaves (4.32, 9.7 and 14.09) at 10, 20 and 30 DAS was observed in S_0 (control) treatment. Whereas the minimum number of leaves (3.32, 6.92 and 7.78) was observed in S_2 (sever stress) treatment. In case of inhibition percentage over control, minimum leaves inhibition percentage (8.80, 13.09 and 23.07%) was observed in mild stress (S_1) condition while maximum (23.15, 28.66 and 44.78%) in severe stress condition (S_1) over control at 10, 20 and 30 DAS respectively. Hoang *et al*. (2019) reported that extreme salt stress showed negative impact on number of leaves of the plant. Al-Maskri *et al.* (2010) also reported that number of leaves was reduced significantly with increasing salinity levels.

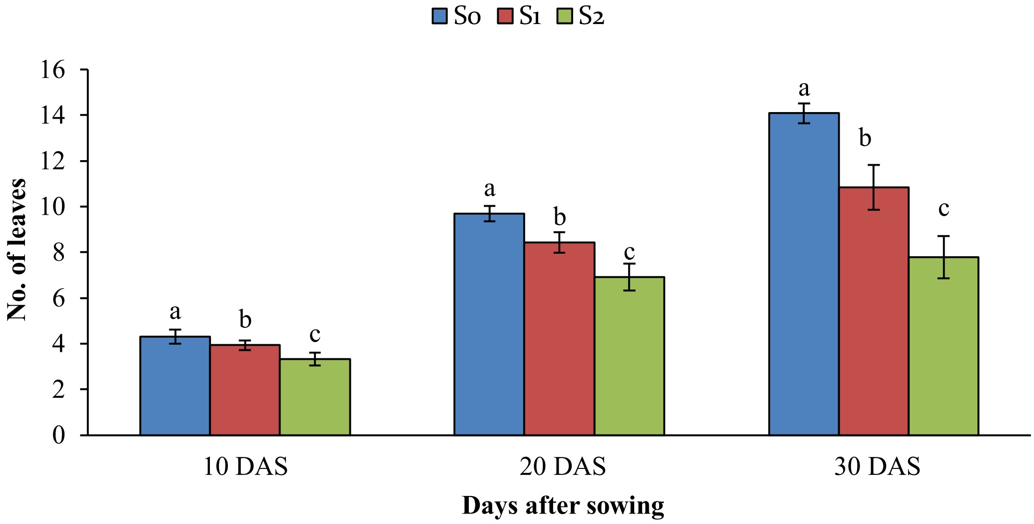

Figure 11. Effect of salt stress on number of leaf of red amaranth at different DAS. Here, S_0: control (0 mM NaCl), S_1: Mild stress (50 mM NaCl), and S_2: Severe stress (100 mM NaCl)

Effect of organic acid

Significant variation was observed in number of leaves of red amaranth at different days after sowing due to application of organic acid. From the experiment result revealed that number of leaves at different days were slightly increasing due to application of ascorbic and citric acid comparable to control treatment (Figure 12). The maximum number of leaves (4.01, 8.7 and 11.67) at 10, 20 and 30 DAS respectively was observed in A_1 (Ascorbic acid) treatment which was statistically similar with A_2 (3.87 and 8.41) treatment. Whereas the minimum number of leaf (3.71, 7.94 and 10.19) at 10, 20 and 30 DAS respectively was observed in A_0 (control) treatment. Maximum number of leaves (8.09, 9.57 and 14.52%) increasing due to application of ascorbic acid while (4.31, 5.92 and 6.48%) in citric acid treatment over control at 10, 20 and 30 DAS respectively.

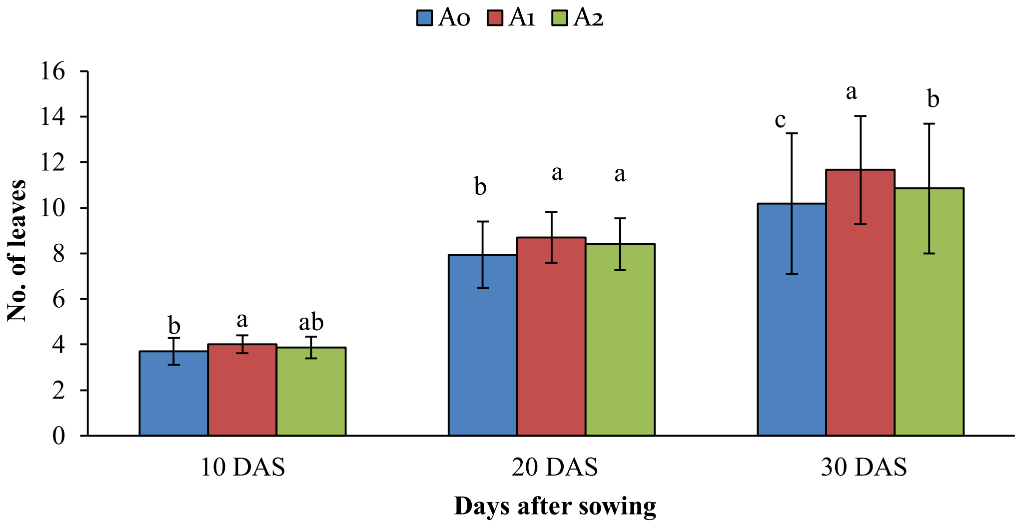

Figure12. Effect of organic acid on number of leaf of red amaranth at different DAS. Here, A_0: (Control), A_1: Ascorbic acid and A_2: Citric acid

Combined effect of salt stress condition and organic acid

Salinity severely affected the leaves of the plants. Leaves number decreased by 10.30, 16.72, 30.15 and 29.04, 34.17 50.47% at 10, 20 and 30 DAS respectively, under mild (50 mM NaCl) and severe stress (100 mM NaCl) condition, whereas both AsA and CA considerably helps in reduced the leaf loss of the stressed plants (Table 2) at different days after sowing. In contrast to respective stress plant leaf loss reduction by AsA and CA was 6.27, 10.79, 21.27 and 2.61, 6.65, 13.05% under mild stress and 17.82, 17.46, 28.99 and 11.22, 12.22, 9.13% under severe stress, respectively. So, it is very clear that both AsA and CA alleviated salt toxicity where AsA performed far better than the CA.

Table 2. Combined effect of salt stress and organic acid on number of leaf of red amaranth at different DAS

Treatment combinations	No. of leaves at 10 DAS	No. of leaves at 20 DAS	No. of leaves at 30 DAS
S_0A_0	4.27 ab	9.57 a	13.93 a
S_0A_1	4.40 a	9.87 a	14.30 a
S_0A_2	4.30 ab	9.67 a	14.03 a
S_1A_0	3.83 b-d	7.97 cd	9.73 d
S_1A_1	4.07 ab	8.83 b	11.80 b
S_1A_2	3.93 bc	8.50 bc	11.00 c
S_2A_0	3.03 e	6.30 f	6.90 f
S_2A_1	3.57 cd	7.40 de	8.90 e
S_2A_2	3.37 de	7.07 e	7.53 f
LSD $_{(0.05)}$	0.47	0.60	0.71
CV (%)	6.95	4.16	3.74

Here, S_0: control (0 mM NaCl), S_1: Mild stress (50 mM NaCl), and S_2: Severe stress (100 mM NaCl), A_0: (Control), A_1: Ascorbic acid, and A_2: Citric acid.

4.2.3 Leaf length (cm)

Effect of salt stress condition

Leaf size and shape influence a range of important physiological processes, including photosynthesis, transpiration and thermoregulation, and vary with a number of environmental factors. Salt stress significantly affects leaf length of red amaranth (Figure 13). From the experiment result revealed that the maximum leaf length (5.66 cm) was observed in control treatment (S_0) while the minimum at (3.04 cm) at severe salt stress condition (S_2 treatment) at 30 DAS. Due to salt stress condition minimum leaf length inhibition (13.07 %) was observed in mild salt stress condition (S_1 Treatment) while the maximum (46.29 %) in severe salt stress condition (S_2 Treatment) over control (S_0) at 30 DAS respectively. Mathur *et al.* (2006) reported, that the stress of the moth bean plant (*Vigna aconitifolia* L.) with increasing concentrations of sodium chloride, led to a decrease in leaf length and wide. This decrease was inversely proportional to the concentrations.

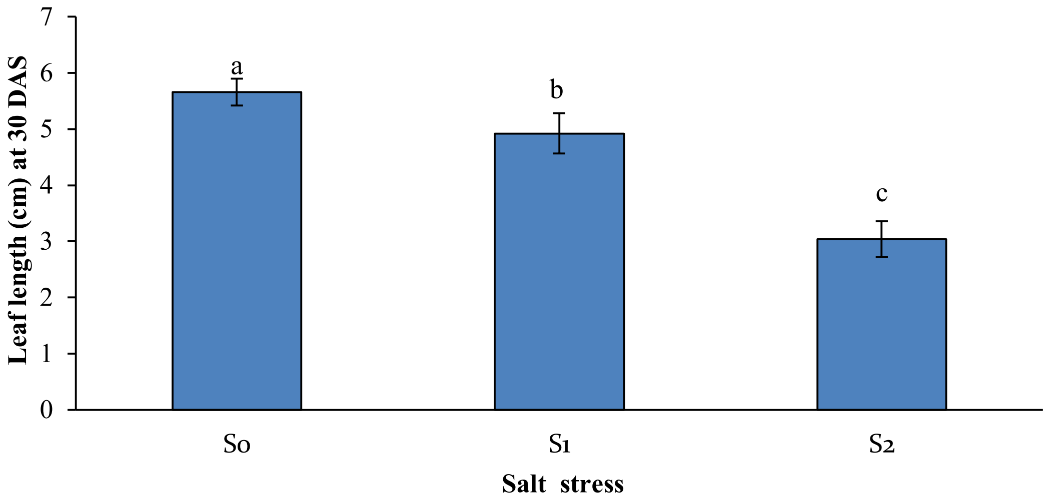

Figure 13. Effect of salt stress on leaf length (cm) of red amaranth at 30 DAS. Here, S_0: control (0 mM NaCl), S_1: Mild stress (50 mM NaCl), and S_2: Severe stress (100 mM NaCl)

Effect of organic acid

Different organic acid significantly affect leaf length of red amaranth (Figure 14). From the experiment result revealed that the maximum leaf length (4.83 cm) was observed in A_1 (Ascorbic acid) treatment while the minimum at (4.26 cm) at control (A_0) treatment at 30 DAS. Due to the effect of organic acid maximum leaf length increasing (13.38 %) was observed in A_1 Treatment(Ascorbic acid) while the maximum (6.34 %) in A_2 Treatment (Citric acid) over control (S_0) at 30 DAS respectively.

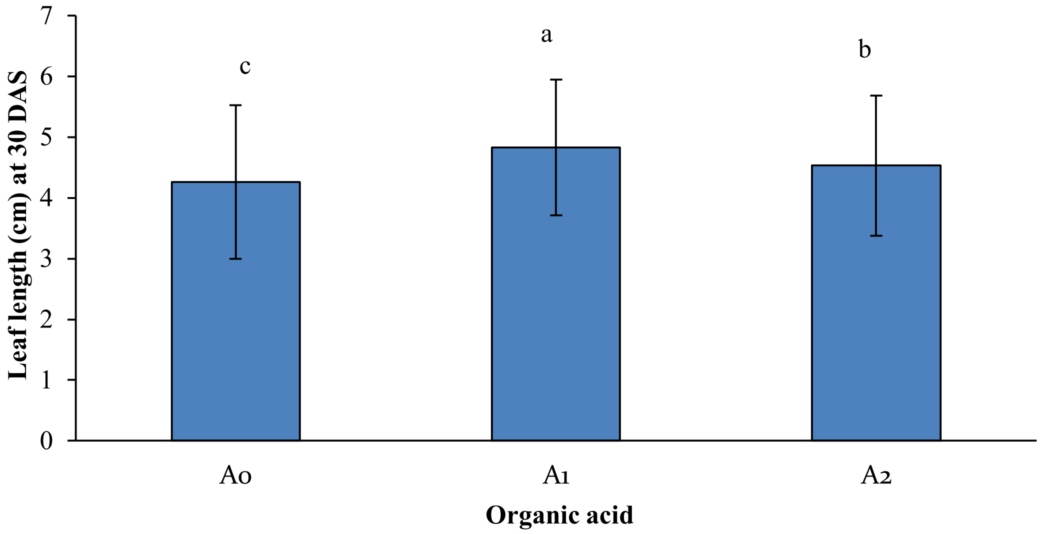

Figure 14. Effect of organic acid on leaf length (cm) of red amaranth at 30 DAS. Here, A_0: (Control), A_1: Ascorbic acid and A_2: Citric acid

Combined effect of salt stress condition and organic acid

Salinity affects almost all aspects of plant development including, germination, vegetative growth and reproductive development. Because many salts are also plant nutrients, high salt levels in the soil can upset the nutrient balance in the plant or interfere with the uptake of some nutrients as a result plants become weak which ultimately impact on the morphological feature of the plant. High salinity level severely affected the leaf length of the plants. Leaves length decreased by 18.08 and 51.18 % at 30 DAS respectively, under mild (50 mM NaCl) and severe stress (100 mM NaCl) condition, whereas both AsA and CA considerably helps in increasing leaf length of the stressed plants (Table 3). In contrast to respective stress plant, leaf length increasing due to AsA and CA was 17.00 and 8.83 % under mild stress and 24.81 and 13.70 % under severe stress condition respectively.

4.2.4 Leaf wide (cm)

Effect of salt stress condition

Salt stress significantly affects leaf wide of red amaranth (Figure 15). From the experiment result revealed that the maximum leaf wide (3.77 cm) was observed in control treatment (S_0) while the minimum at (1.99 cm) at severe salt stress condition (S_2 treatment) at 30 DAS. Due to salt stress condition minimum leaf wide inhibition (18.30 %) was observed in mild salt stress condition (S_1 Treatment) while the maximum (47.21 %) in severe salt stress condition (S_2 Treatment) over control (S_0) at 30 DAS respectively. The result obtained from the present study was similar with the findings of Mathur *et al.* (2006) who reported, that the stress of the moth bean plant (*Vigna aconitifolia* L.) with increasing concentrations of sodium chloride, led to a decrease in leaf length and wide. This decrease was inversely proportional to the concentrations.

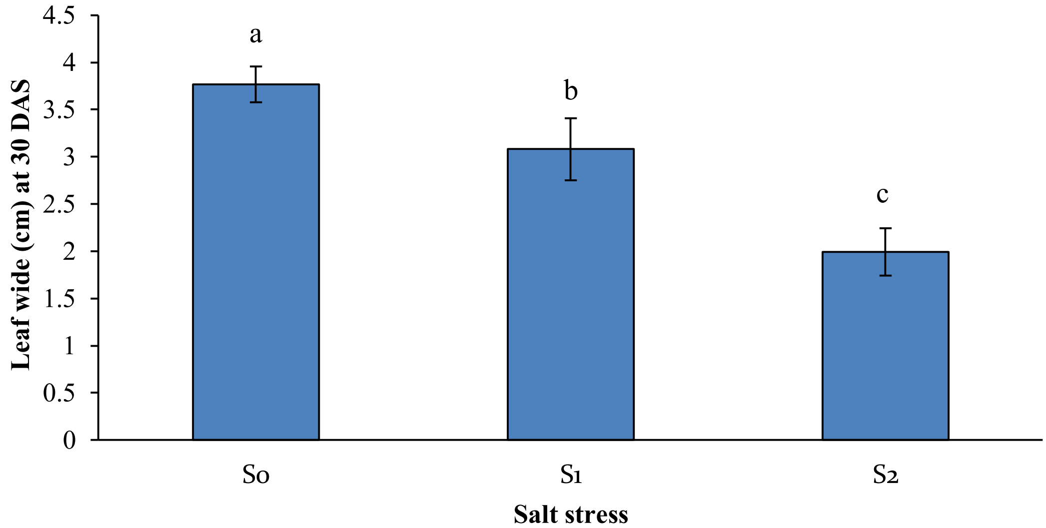

Figure 15. Effect of salt stress on leaf wide (cm) of red amaranth at 30 DAS. Here, S_0: control (0 mM NaCl), S_1: Mild stress (50 mM NaCl), and S_2: Severe stress (100 mM NaCl)

Effect of organic acid

Different organic acid significantly affect leaf wide of red amaranth (Figure 16). From the experiment result revealed that the maximum leaf wide (3.11 cm) was observed in A_1 (Ascorbic acid) treatment which was statistically similar with (3.01 cm) A_2 (Citric acid) treatment while the minimum at (2.71 cm) at control (A_0) treatment at 30 DAS. Due to the effect of organic acid maximum leaf wide increasing (14.76 %) was observed in A_1 Treatment(Ascorbic acid) while the maximum (11.07 %) in A_2 Treatment (Citric acid) over control (S_0) at 30 DAS respectively.

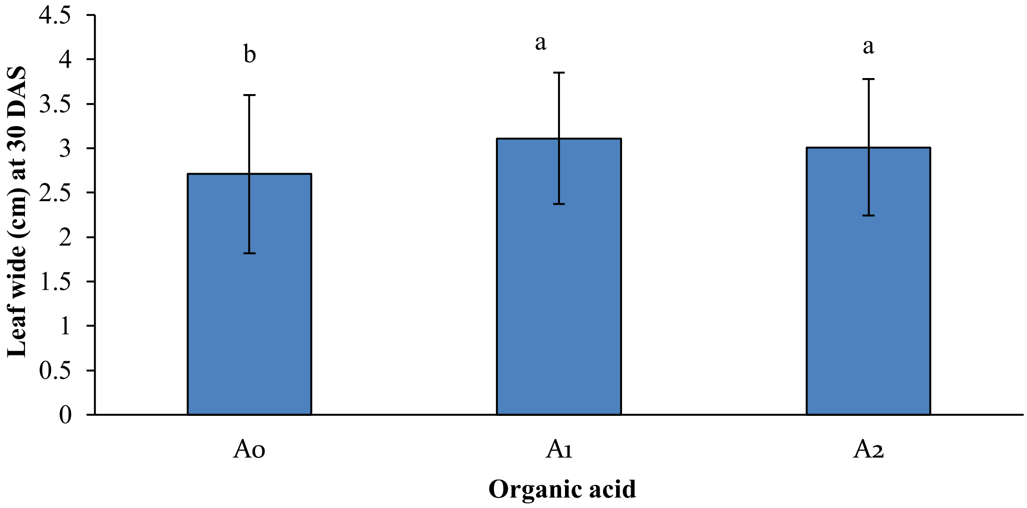

Figure 16. Effect of organic acid on leaf wide (cm) of red amaranth at 30 DAS. Here, A_0: (Control), A_1: Ascorbic acid and A_2: Citric acid

Combined effect of salt stress condition and organic acid

High salinity level severely affected the leaf wide of the plants. Leaves length decreased by 26.22 and 54.05% at 30 DAS respectively, under mild (50mM NaCl) and severe stress (100 mM NaCl) condition, whereas both AsA and CA considerably helps in increasing leaf wide of the stressed plants (Table 3). In contrast to respective stress plant, leaf wide increasing due to AsA and CA was 20.88 and 17.22 % under mild stress and 29.41 and 21.76 % under severe stress condition respectively.

4.2.5 Number of stem girth

Effect of salt stress condition

Salt stress significantly affects number of stem girth of red amaranth (Figure 17). From the experiment result revealed that the maximum number of stem girth (2.61) was observed in control treatment (S_0) while the minimum (1.21) at severe salt stress condition (S_2 treatment) at 30 DAS respectively. Due to salt stress condition minimum number of stem girth inhibition (33.72 %) was observed in mild salt stress condition (S_1 Treatment) while the maximum (53.6 %) in severe salt stress condition (S_2 Treatment) over control (S_0) at 30 DAS respectively.

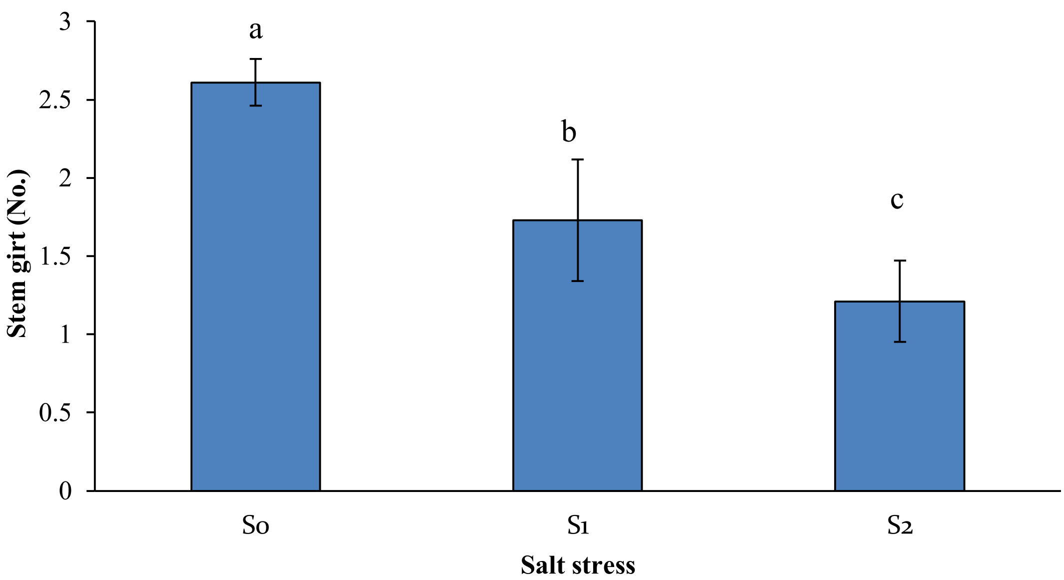

Figure 17. Effect of salt stress on number of stem girth of red amaranth at 30 DAS. Here, S_0: control (0 mM NaCl), S_1: Mild stress (50 mM NaCl), and S_2: Severe stress (100 mM NaCl)

Effect of organic acid

Different organic acid significantly affect number of stem girth of red amaranth (Figure 18). From the experiment result revealed that the maximum number of stem girth (2.09) was observed in A_1 (Ascorbic acid) treatment while the minimum at (1.62) at control (A_0) treatment at 30 DAS respectively. Due to the effect of organic acid maximum number of stem girth increasing (29.01 %) was observed in A_1

Treatment(Ascorbic acid) while the maximum (13.58 %) in A_2 Treatment (Citric acid) over control (S_0) at 30 DAS respectively.

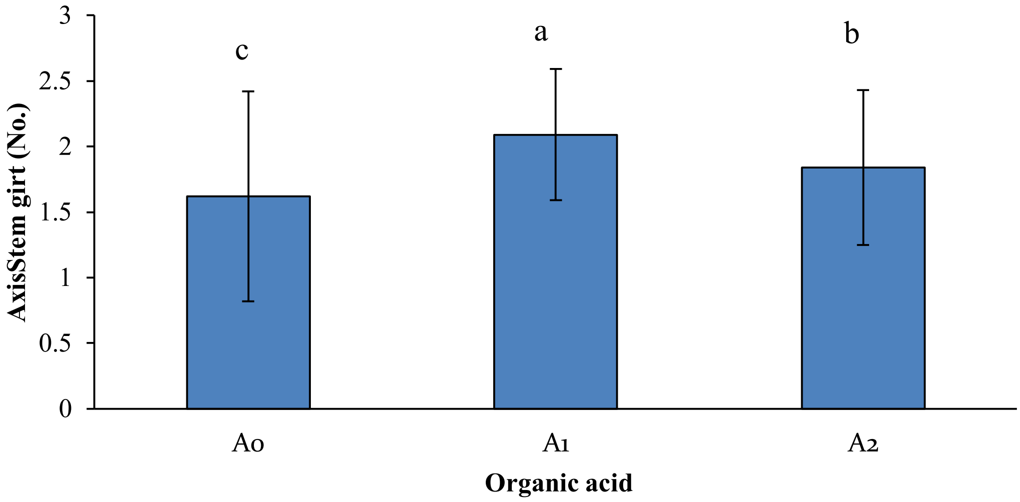

Figure 18. Effect of organic acid on number of stem girth of red amaranth at 30 DAS. Here, A_0: (Control), A_1: Ascorbic acid and A_2: Citric acid

Combined effect of salt stress condition and organic acid

Salinity level severely affected the number of stem girth of the plants. Number of stem girth of the red amaranth decreased by 52.43 and 65.17 % at 30 DAS respectively, under mild (50 mM NaCl) and severe stress (100 mM NaCl) condition, whereas both AsA and CA considerably helps in increasing the number of stem girth of the stressed plants (Table 3). In contrast to respective stress plant, number of stem girth of the red amaranth increasing by AsA and CA was 67.72 and 41.73 % under mild stress and 61.29 and 29.03% under severe stress condition respectively.

4.2.6 Plant fresh weight

Effect of salt stress condition

Plant fresh weight refers to the weight of plants just after the harvest. Salt stress significantly affects fresh weight of red amaranth (Figure 19). From the experiment result revealed that the maximum fresh weight (13.07 g) was observed in control treatment (S_0) while the minimum (6.00 g) at severe salt stress condition (S_2 treatment) at 30 DAS respectively. Due to salt stress condition minimum fresh weight inhibition (32.90 %) was observed in mild salt stress condition (S_1 Treatment) while the maximum (54.09 %) in severe salt stress condition (S_2 Treatment) over control (S_0) at 30 DAS respectively.

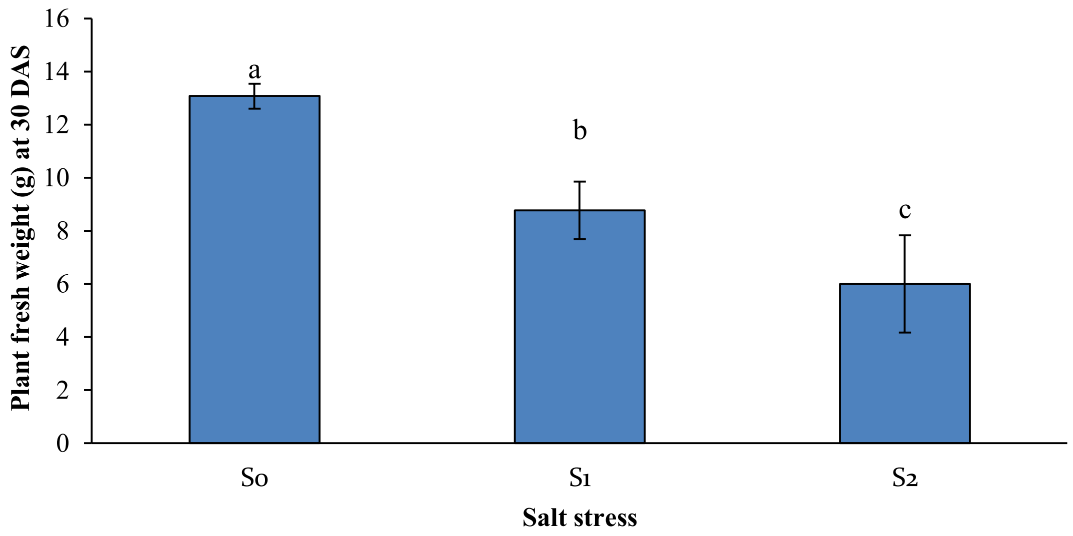

Figure 19. Effect of salt stress condition on plant fresh weight (g) of red amaranth at 30 DAS. Here S_0: control (0 mM NaCl), S_1: Mild stress (50 mM NaCl) and S_2: Severe stress (100mM NaCl)

Effect of organic acid

Different organic acid significantly affect fresh weight of red amaranth (Figure 20). From the experiment result revealed that the maximum fresh weight (10.56 g) was observed in A_1 (Ascorbic acid) treatment while the minimum at (8.19 g) at control (A_0) treatment at 30 DAS respectively. Due to the effect of organic acid maximum fresh weight increasing (28.94 %) was observed in A_1 Treatment(Ascorbic acid) while the maximum (11.11 %) in A_2 Treatment (Citric acid) over control (S_0) at 30 DAS respectively. Muller *et al.* (2004) reported that antioxidant nature and increase of plantlet water imbibitions ability in presence of ascorbic acid increased the plantlet fresh weight.

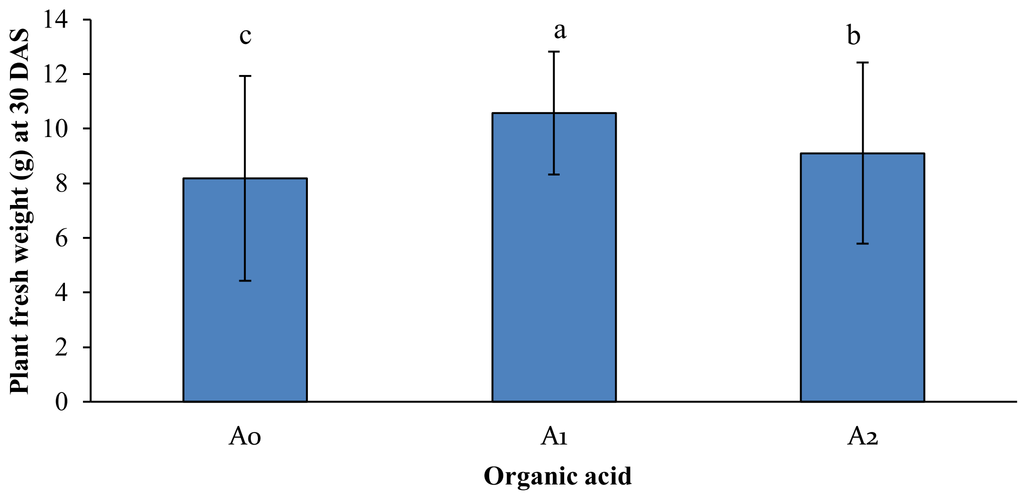

Figure 20. Effect of organic acid on plant fresh weight (g) of red amaranth at 30 DAS. Here, A_0: (Control), A_1: Ascorbic acid and A_2: Citric acid

Combined effect of salt stress condition and organic acid

High salinity stress causes detrimental effect on fresh weight production of the plant by limiting overall growth, plant height, number of leaves, leaf length and wide, number of stem girth, and so on. Fresh weight production of the red amaranth decreased by 39.64 and 67.50 % at 30 DAS respectively, under mild (50 mM NaCl) and severe stress (100 mM NaCl) condition, whereas both AsA and CA considerably helps in increasing the fresh weight by increasing a little bit some of the growth characters of the stressed plants (Table 3). In contrast to respective stress plant, fresh weight production of the red amaranth increasing by AsA and CA was 30.95 and 11.18% under mild stress and 100.24 and 34.54 % under severe stress condition respectively. Ghoohestani *et al.* (2012) reported that in salt stress condition maximum fresh weight (0.139 g) of plantlet caused by seed priming in 150mg/L ascorbic acid. The lowest value of fresh weight was related to the seeds that had been done no pretreatment.

4.2.7 Plant dry weight (g)

Effect of salt stress condition

Plant dry weight means the weight of the sample, excluding the weight of the water in the sample. Plant dry weight significantly affected due to different salt stress condition ((Figure 21).From the experiment result revealed that the maximum dry weight (0.94 g) was observed in control treatment (S_0) while the minimum (0.44 g) at severe salt stress condition (S_2 treatment) at 30 DAS respectively. Due to salt stress condition minimum dry weight inhibition (31.91 %) was observed in mild salt stress condition (S_1 Treatment) while the maximum (53.19 %) in severe salt stress condition (S_2 Treatment) over control (S_0) at 30 DAS respectively. Hoang *et al*. (2019) also found similar result which supported the present finding and reported that the extreme salt stress showed negative impact on dry matter weight of the plant.

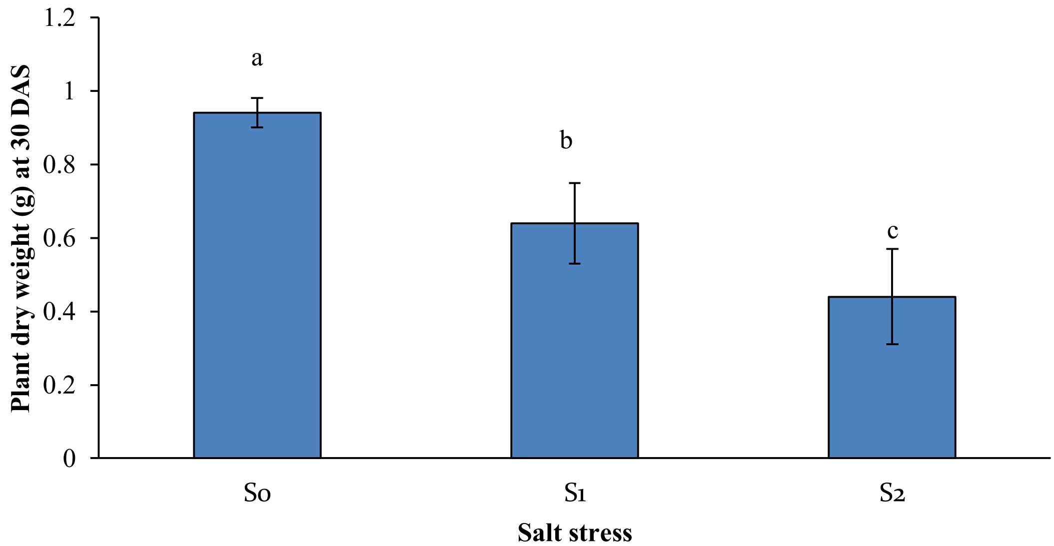

Figure 21. Effect of salt stress condition on plant dry matter weight (g) of red amaranth at 30 DAS. Here S_0: control (0 mM NaCl), S_1: Mild stress (50 mM NaCl), and S_2: Severe stress (100 mM NaCl)

Effect of organic acid

Different organic acid significantly affect dry weight of red amaranth (Figure 22). From the experiment result revealed that the maximum dry weight (0.78 g) was observed in A_1 (Ascorbic acid) treatment while the minimum at (0.60 g) at control (A_0) treatment at 30 DAS respectively. Due to the effect of organic acid maximum dry weight increasing (30.00 %) was observed in A_1 Treatment (Ascorbic acid) while the maximum (8.33 %) in A_2 Treatment (Citric acid) over control (S_0) at 30 DAS respectively.

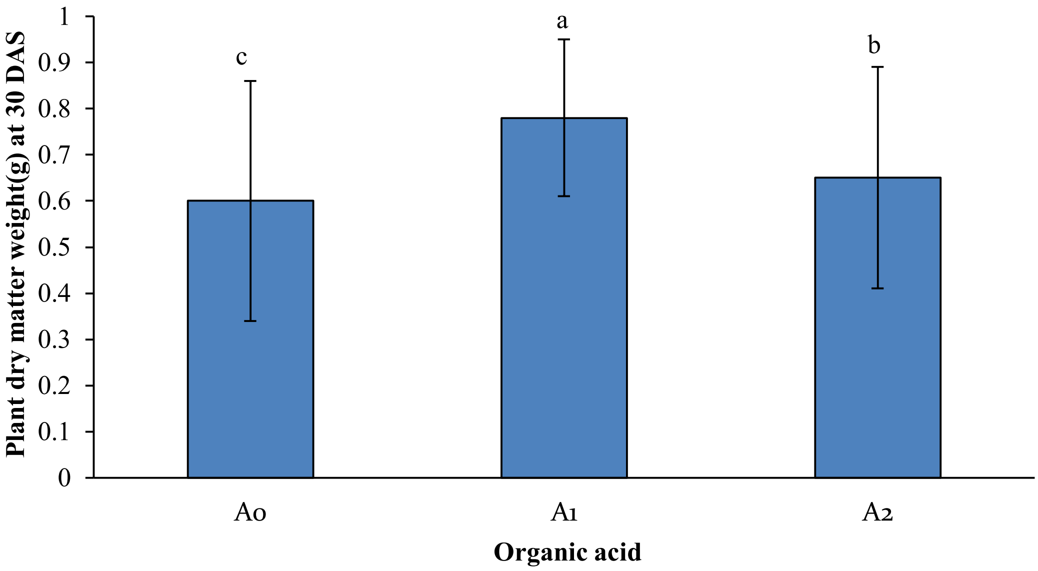

Figure 22. Effect of organic acid on plant dry matter weight (g) of red amaranth at 30 DAS. Here, A_0: (Control), A_1: Ascorbic acid and A_2: Citric acid.

Combined effect of salt stress condition and organic acid

At high levels salinity can have negative and potentially lethal effects on plants. Dry weight of the red amaranth decreased by 40.22 and 65.22 % at 30 DAS respectively, under mild (50 mM NaCl) and severe stress (100 mM NaCl) condition, whereas both AsA and CA considerably helps in increasing the dry weight by increasing a little bit some of the growth characters of the stressed plants (Table 3). In contrast to respective stress plant, dry weight production of the red amaranth increasing by AsA and CA was 40 and 10.91% under mild stress and 87.50 and 25 % under severe stress condition respectively. Behairy *et al.* (2017) also found similar result which supported the present finding and reported that salinity stress by NaCl caused a significant reduction in seedling dry weight. Pretreatment of seeds with salicylic acid, citric acid and proline under saline condition caused significant increase in seedling dry weight from 304 mg (for control) to 375 mg (for salicylic acid), 319 mg (for citric acid), and 378 mg (for proline) cm at 3000 ppm.

Table 3. Combined effect of salt concentration and organic acid on leaf length (cm), leaf wide (cm), stem girt (No.), plant fresh weight (g) and dry weight (g) at 30 DAS

Treatment combinations	**Leaf length (cm) at 30 DAS**	**Leaf wide (cm) at 30 DAS**	**Stem girt (No.) at 30 DAS**	**Plant fresh weight (g) at 30 DAS**	**Plant dry weight (g) at 30 DAS**
S_0A_0	5.53 bc	3.70 a	2.67 a	12.74 b	0.92 b
S_0A_1	5.83 a	3.83 a	2.63 a	13.31 a	0.97a
S_0A_2	5.60 ab	3.77 a	2.53 a	13.17a	0.94 ab
S_1A_0	4.53 e	2.73 c	1.27 e	7.69 e	0.55 e
S_1A_1	5.30 c	3.30 b	2.13 b	10.07 c	0.77 c
S_1A_2	4.93 d	3.20 b	1.80 c	8.55 d	0.61 d
S_2A_0	2.70 h	1.70 e	0.93 f	4.14 g	0.32 g
S_2A_1	3.37 f	2.20 d	1.50 d	8.29 d	0.60 d
S_2A_2	3.07 g	2.07 d	1.20 e	5.57 f	0.40 f
LSD $_{(0.05)}$	0.25	0.30	0.22	0.35	0.04
CV (%)	3.23	5.82	6.96	2.21	3.89

Here, S_0: control (0 mM NaCl), S_1: Mild stress (50 mM NaCl), and S_2: Severe stress (100 mM NaCl), A_0: (Control), A_1: Ascorbic acid, and A_2: Citric acid

4.2.8 Yield pot^{-1} (g)

Effect of salt stress condition

Yield is a return measure for an investment over a set period of time. From the experiment result revealed that the maximum yield pot^{-1} (261.42 g) was observed in control treatment (S_0) while the minimum (120.02 g) at severe salt stress condition (S_2 treatment) at harvest respectively (Figure 23). Due to salt stress condition minimum yield pot^{-1} inhibition (32.90 %) was observed in mild salt stress condition (S_1 Treatment) while the maximum (54.09 %) in severe salt stress condition (S_2 Treatment) over control (S_0) at harvest respectively.

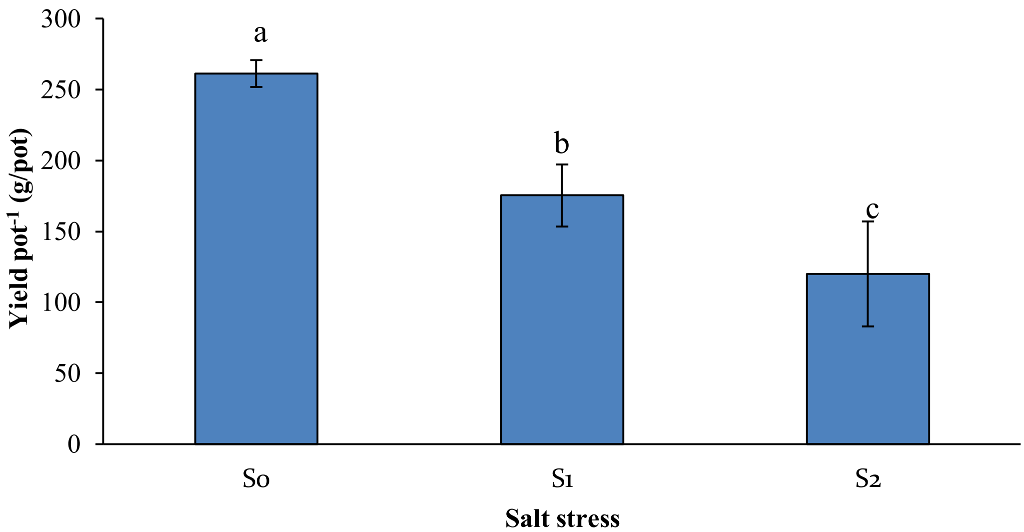

Figure 23. Effect of salt concentration on yield pot^{-1} (g/pot) of red amaranth. Here S_0: control (0 mM NaCl), S_1: Mild stress (50 mM NaCl), and S_2: Severe stress (100 mM NaCl)

Effect of organic acid

Different organic acid significantly affect yield pot^{-1} of red amaranth (Figure 24). From the experiment result revealed that the maximum yield pot^{-1} (211.11 g) was observed in A_1 (Ascorbic acid) treatment while the minimum at (163.80 g) at control (A_0) treatment at harvest respectively. Due to the effect of organic acid maximum yield pot^{-1} increasing (28.88 %) was observed in A_1 Treatment (Ascorbic acid) while the maximum (11.07 %) in A_2 Treatment (Citric acid) over control (S_0) at harvest respectively.

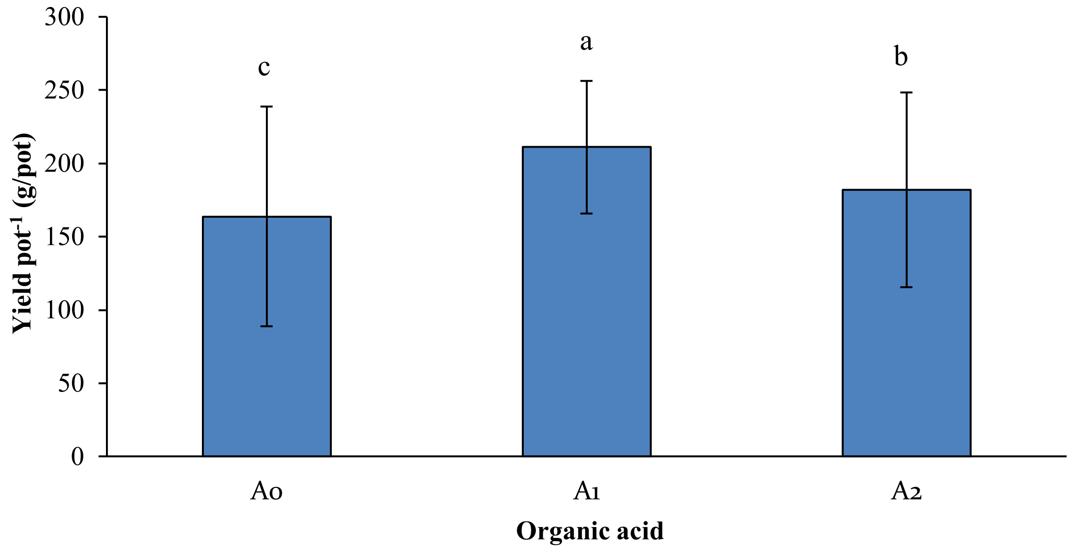

Figure 24. Effect of organic acid on yield pot^{-1} (g/pot) of red amaranth. Here, A_0: (Control), A_1: Ascorbic acid and A_2: Citric acid

Combined effect of salt stress condition and organic acid

Yield pot^{-1} of the red amaranth decreased by 39.62 and 67.47% at harvest respectively, under mild (50 mM NaCl) and severe stress (100 mM NaCl) condition, whereas both AsA and CA considerably helps in increasing yield pot^{-1} of the stressed plants (Table 4) of red amaranth. In contrast to respective stress plant, yield pot^{-1} of the red amaranth increasing by AsA and CA was 30.94 and 11 % under mild stress and 99.98 and 34.51 % under severe stress condition respectively.

4.2.9 Yield t ha^{-1}

Effect of salt stress condition

From the experiment result revealed that the maximum yield (26.32t/ha) was observed in control treatment (S_0) while the minimum (12.09 t/ha) at severe salt stress condition (S_2 treatment) at harvest respectively (Figure 25). Due to salt stress condition minimum yield inhibition (32.90 %) was observed in mild salt stress condition (S_1 Treatment) while the maximum (54.07 %) in severe salt stress condition (S_2 Treatment) over control (S_0) at harvest respectively.

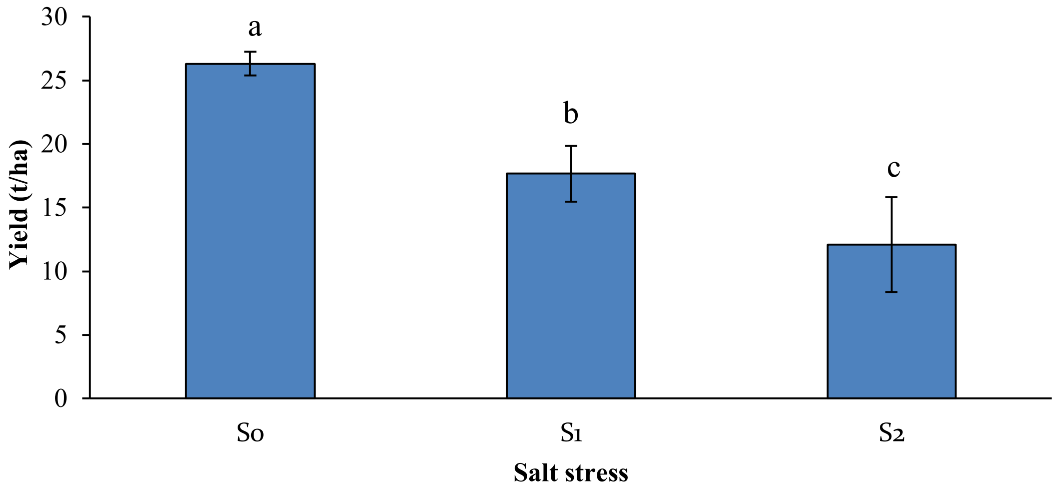

Figure 25. Effect of salt concentration on yield (t/ha) of red amaranth. Here, S_0: control (0 mM NaCl), S_1: Mild stress (50 mM NaCl) and S_2: Severe stress (100 mM NaCl)

Effect of organic acid

Different organic acid significantly affect yield of red amaranth (Figure 26). From the experiment result revealed that the maximum yield (21.26 t ha^{-1}) was observed in A_1 (Ascorbic acid) treatment while the minimum at (16.49 t/ha) at control (A_0) treatment at harvest respectively. Due to the effect of organic acid maximum yield increasing (28.93 %) was observed in A_1 Treatment (Ascorbic acid) while the maximum (11.10 %) in A_2 Treatment (Citric acid) over control (S_0) at harvest respectively.

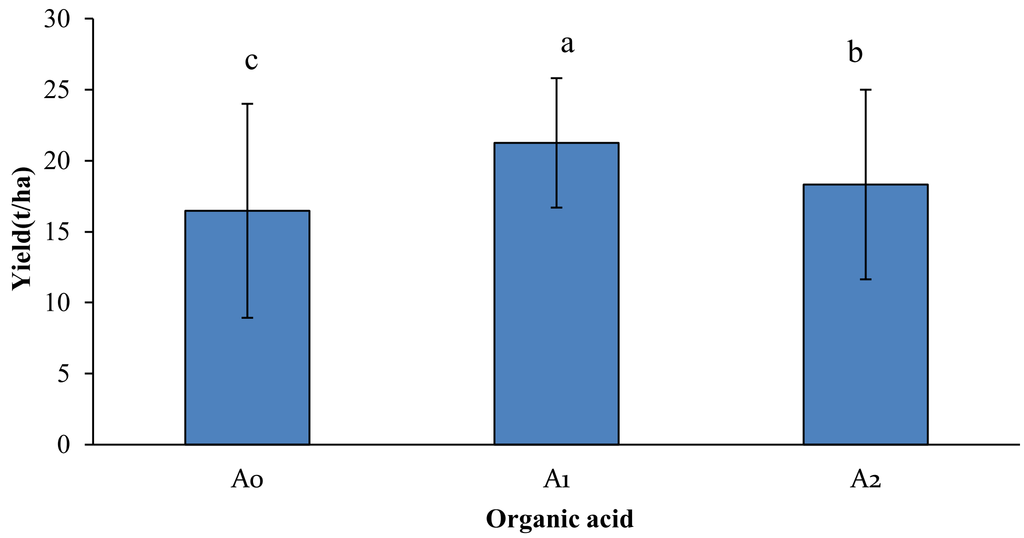

Figure 26. Effect of organic acid on yield (t/ha) of red amaranth. Here, A_0: (Control), A_1: Ascorbic acid and A_2: Citric acid

Combined effect of salt stress condition and organic acid

Yield tha^{-1} of the red amaranth decreased by 39.61 and 67.45% at harvest respectively, under mild (50 mM NaCl) and severe stress (100 mM NaCl) condition, whereas both AsA and CA considerably helps in increasing yield t ha^{-1} of the stressed plants (Table 4) of red amaranth. In contrast to respective stress plant, yield t ha^{-1} of the red amaranth increasing by AsA and CA was 30.92 and 11.17% under mild stress and 99.88 and 34.37 % under severe stress condition respectively. It is clear that AsA performed far better than the CA in mild and severe salt stress condition in case of yield performance by mitigating the negative impact of the salt stress to the plant.

Table 4. Combined effect of salt stress condition and organic acid on yield (g/pot) and yield (t/ha) of red amaranth at harvest respectively

Treatment combinations	Yield (g/pot)	Yield (t/ha)
S_0A_0	254.73 b	25.65 b
S_0A_1	266.20 a	26.80 a
S_0A_2	263.33 a	26.52 a
S_1A_0	153.80 e	15.49 e
S_1A_1	201.40 c	20.28 c
S_1A_2	171.00 d	17.22 d
S_2A_0	82.87 g	8.35 g
S_2A_1	165.73 d	16.69 d
S_2A_2	111.47 f	11.22 f
LSD $_{(0.05)}$	7.14	0.71
CV (%)	2.22	2.21

Here, S_0: control (0 mM NaCl), S_1: Mild stress (50 mM NaCl), and S_2: Severe stress (100 mM NaCl), A_0: (Control), A_1: Ascorbic acid, and A_2: Citric acid

CHAPTER 5

SUMMARY AND CONCLUSION

Summary

Salinity is one of the most important environmental constraints that limit plant productivity, particularly in arid and semi-arid climates. Excess of saline ions in soils generates an elevated osmotic pressure and an accumulation of toxic ions in plant tissues, notably Na^+, and consequently induces a decrease in growth and crop yield due to a disruption of several physiological processes. Soil salinity is a worldwide problem. Bangladesh is no exception to it. In Bangladesh, Salinization is one of the major natural hazards hampering crop production. Red Amaranth belongs to the Amaranthaceae family is one of the important vegetable crops of Bangladesh and sensitive to salt stress. To overcome the salinity problem seeds were treated with various organic acids to increasing plant physiological ability for tolerance and increasing production in saline soil condition.

Both lab and pot experiment was conducted at Sher-e-Bangla Agricultural University, Dhaka to investigate the effect of organic acid to mediated the salt stress of red amaranth during the period from January to February 2020 in Rabi season. Lab experiment consisted of single factors *viz;* Red amaranth seed treated with eight salt concentrations : T_0 = 0 mM NaCl (control), T_1= 25 mM NaCl, T_2= 50 mM NaCl, T_3= 75 mM NaCl, T_4= 100 mM NaCl, T_5= 150 mM NaCl, T_6= 200 mM NaCl and T_7= 250 mM NaCl. In pot the experiment was consisted of two factors (2) *viz;* Factor A: Salt stress condition (3): S_0: Control (0 mM NaCl), S_1: Mild stress (50 mM NaCl) and S_2: Severe stress (100 mM NaCl) and Factor A: Different organic acid treatment *viz*; (3) A_0: (Control), A_1: Ascorbic acid, and A_2: Citric acid. Both lab and pot experiment was laid out in a Completely Randomized Design (CRD) with three replications. Different parameters such as germination(%), radicle length (cm), plumule (cm), total length (cm) on laboratory-based and plant height (cm), number of leaves per plant , leaf length (cm) , leaf width (cm), number of stem girt, plant fresh weight (g), plant dry weight (g), yield pot^{-1} and yield ha^{-1} on pot based parameters were used for assessing results for this experiment.

In lab experiment result revealed that different salt concentration significantly impact on germination (%), radical length (cm), plumule (cm) and total length (cm) of red amaranth seedling. Control treatment (0 mM NaCl) had no impact on red amaranth while increasing salt concentration gradually decreasing germination(%), radicle length (cm), plumule (cm), total length (cm) and impact more under extreme salt concentration (250 mM NaCl) level recorded minimum germination(26.33%), radical length (0.78 cm), plumule (0.27 cm) and total length (1.06 cm) over control treatment comparable to others treatment.

In pot experiment different salt stress condition, different organic acid treatment and their combination had significant effect on red amaranth.

In case of salt stress condition maximum plant height (2.91, 12.89 and 27.79cm at 10, 20 and 30 DAS), number of leaves (4.32, 9.7 and 14.09 at 10, 20 and 30 DAS), leaf length (5.66 cm), leaf wide (3.77 cm), stem girth (2.61), fresh weight (13.07 g), dry weight (0.94 g), yield pot^{-1} (261.42 g) and yield (26.32 t/ha) at 30 DAS were recorded under control treatment while above parameters were gradually decreasing in mild and severe salt stress condition and increasing inhabitation percentage and it was higher in severe salt stress condition over control treatment comparable to mild salt stress condition.

In case of different organic acid treatment result reveled that different organic acid significantly affect on growth and development of red amaranth, and among different organic acid ascorbic acid perform best and recorded maximum plant height (2.67, 11.19 and 24.32 cm at 10, 20 and 30 DAS), number of leaves (4.01, 8.7 and 11.67 at 10, 20 and 30 DAS), leaf length (4.83cm), leaf wide (3.11 cm), stem girth (2.09), fresh weight (10.56 g), dry weight (0.78 g), yield pot^{-1} (211.11 g) and yield (21.26 t/ha) at 30 DAS. While the corresponding above parameters recorded minimum in control treatment.

In case of combined effect result revealed that different salt stress condition along with different organic acid treatment significantly effect on growth and development of red amaranth. Experiment result revealed that in contrast to respective stress plant, maximum plant height recovery 28.64, 25.27, 23.80 and 29.94, 26.39, 32 % at 10, 20 and 30 DAS, leaf loss reduction 6.27, 10.79, 21.27 and17.82, 17.46, 28.99% at 10, 20 and 30 DAS, increasing leaf length of the stressed plants 17.00 and 24.81 %, leaf wide increasing 20.88 and 29.41 %, number of stem girth of the red amaranth increasing 67.72 and 61.29 %, fresh weight production of the red amaranth increasing 30.95 and 100.24 % , dry weight production of the red amaranth increasing 40 and 87.50%, increasing yield pot^{-1} of the red amaranth 30.94 and 99.98 % and increasing yield t ha^{-1} of the red amaranth 30.92 and 99.98 % at 30 DAS by ascorbic acid treatment under mild and severe salt stress condition comparable to control treatment.

Conclusions

i. Based on the results of the present experiment, the following conclusion can be drawn:

ii. In lab experiment, red amaranth seed treated with extreme salt concentration (250mM NaCl) level negatively impact on germination (26.33 %), radicle length (0.78 cm), plumule (0.27 cm) and total length (1.06 cm) over control treatment comparable to others treatment

iii. In pot experiment, severe salt stress recorded minimum yield (120.02 g pot^{-1} and 12.09 t ha^{-1}) of red amaranth and maximum in control treatment.

iv. Seed treated with ascorbic acid performed the best comparable to citric acid or control treatment and recorded maximum yield pot^{-1} (211.11 g) and yield (21.26 t ha^{-1}) at 30 DAS.

v. In case of combined effect different salt stress condition along with ascorbic acid treatment performed the best and increased 30.94 and 99.98 % yield pot^{-1} and 30.92 and 99.98 % under mild and severe salt stress condition hence this treatment is suitable for saline thrives area.

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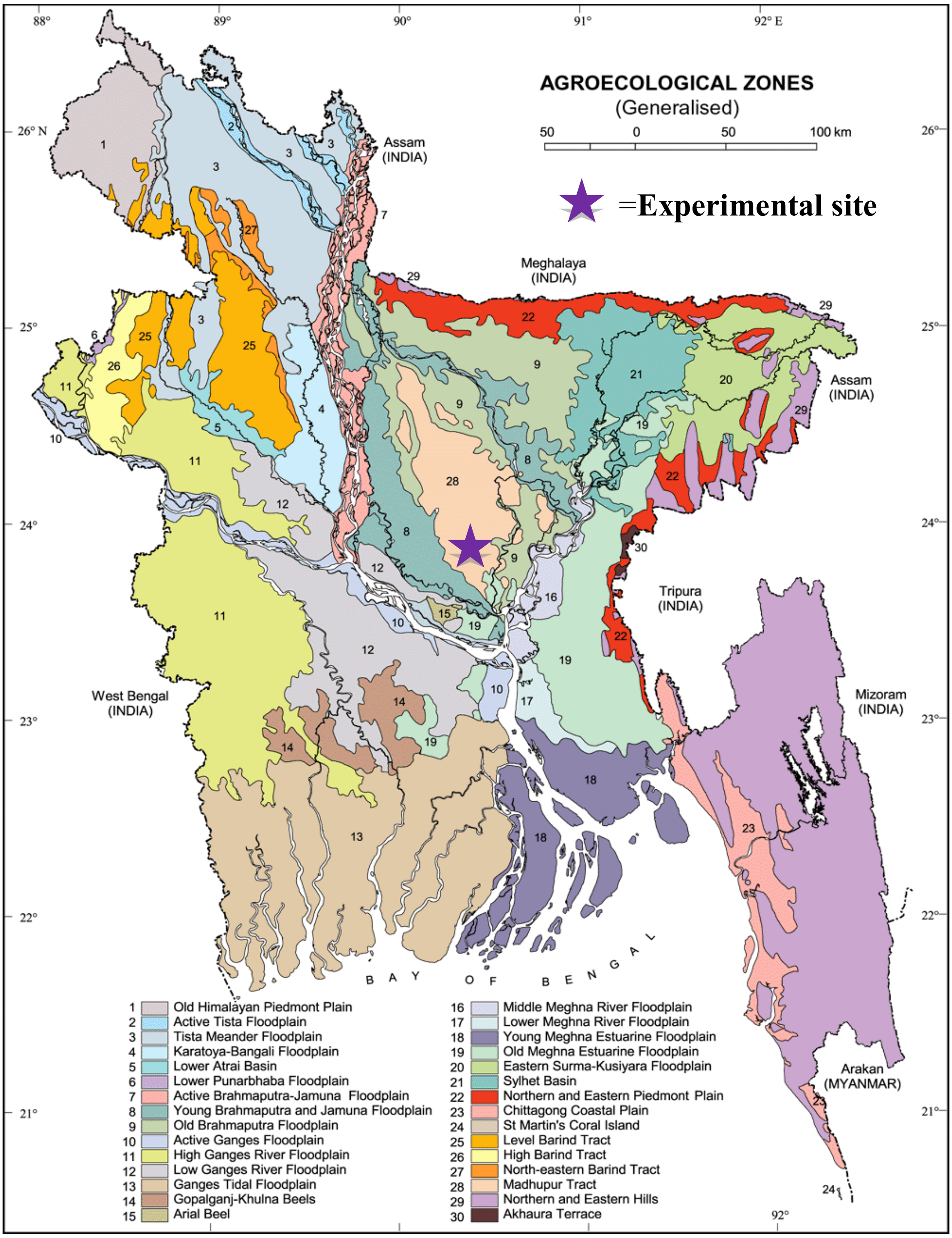
APPENDICES

Appendix I. Map showing the experimental location under study



Appendix II. Characteristics of soil of experimental field

A. Morphological characteristics of the experimental field

Morphological features	Characteristics
Location	Sher-e-Bangla Agricultural University Agronomy research field, Dhaka
AEZ	AEZ-28, Madhupur Tract
General Soil Type	Shallow Red Brown Terrace Soil
Land type	High land
Soil series	Tejgaon
Topography	Fairly leveled

B. The initial physical and chemical characteristics of soil of the experimental site (0 - 15 cm depth)

Physical characteristics	
Constituents	**Percent**
Sand	26 %
Silt	45 %
Clay	29 %
Textural class	Silty clay
Chemical characteristics	
Soil characteristics	**Value**
pH	5.6
Organic carbon (%)	0.45
Organic matter (%)	0.78
Total nitrogen (%)	0.03
Available P (ppm)	20.54
Exchangeable K (mg/100 g soil)	0.10

Appendix III. Monthly meteorological information during the period from January to February, 2020.

Year	Month	Air temperature (^{0}C)		Relative humidity (%)	Total rainfall (mm)
		Maximum	Minimum		
2020	January	25.5	13.1	41	00
	February	25.9	14	34	7.7

Source: Meteorological Centre, Agargaon, Dhaka (Climate Division)

Appendix IV. Analysis of variance of the data of germination (%), radicle length, plumule length and total length of red amaranth seedling treated with different salt concentration.

Mean square of					
Source	DF	Germination (%)	Radical length (cm)	Plumule length (cm)	Total length (cm)
Replication	2	10.29	0.0251	0.05533	0.0958
Treatment	7	1328.04*	7.4067*	2.849*	19.056*
Error	14	9.72	0.0190	0.0958	0.025

*: Significant at 0.05 level of probability

Appendix V. Analysis of variance of the data of plant height of red amaranth at different DAS treated with different salt stress condition along with different organic acid.

Mean square of plant height at				
Source	DF	10 DAS	20 DAS	30 DAS
Replication	2	0.08037	0.521	2.174
Salt stress(S)	2	2.29481*	52.924*	225.641*
Organic acid(O)	2	0.40148*	5.103*	28.210*
S×O	4	0.05370*	0.874*	4.863*
Error	16	0.02495	0.223	0.490

*: Significant at 0.05 level of probability

Appendix VI. Analysis of variance of the data of number of leaves of red amaranth at different das treated with different salt stress condition along with different organic acid.

Mean square of number of leaves at				
Source	DF	10 DAS	20 DAS	30 DAS
Replication	2	0.01593	0.0137	0.197
Salt stress(S)	2	2.29481*	17.4059*	89.641*
Organic acid(O)	2	0.20259*	1.3081*	4.929*
S×O	4	0.3537*	0.1448*	0.785*
Error	16	0.07218	0.1208	0.166

*: Significant at 0.05 level of probability

Appendix VII. Analysis of variance of the data of leaf length, leaf width and number of stem girt of red amaranth at different das treated with different stress condition along with different organic acid.

Mean square of				
Source	DF	Leaf length	Leaf width	Number of stem girt
Replication	2	0.1481	0.1078	0.0070
Salt stress(S)	2	16.3226*	7.2311*	4.5048*
Organic acid(O)	2	0.7515*	0.3900*	0.4904*
S×O	4	0.0493*	0.0494*	0.1693*
Error	16	0.0215	0.0294	0.0166

*: Significant at 0.05 level of probability

Appendix VIII. Analysis of variance of the data of fresh weight, dry weight, yield per pot and yield (t/ha) red amaranth at different das treated with different salt stress condition along with different organic acid.

Mean square of					
Source	DF	Fresh weight	Dry weight	Yield per pot	Yield (t/ha)
Replication	2	0.927	0.00652	371	3.76
Salt stress(S)	2	114.227*	0.57633*	45691*	463.24*
Organic acid(O)	2	12.819*	0.08145*	5128*	51.95*
S×O	4	2.546*	0.01070*	1018*	10.32*
Error	16	0.042	0.00069	17	0.17

*: Significant at 0.05 level of probability