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Physiological and phytochemical responses of red amaranth (*Amaranthus tricolor* L.) and green amaranth (*Amaranthus dubius* L.) to different salinity levels

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ABSTRACT

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 salt stress decreased growth parameters and biomass production in all treatments except for 25 mM NaCl. Na^+ and Cl^- content accumulated in both shoot and root, however, root had greater NaCl content than shoot. Total phenolics, total flavonoid content and antioxidant activity increased with increasing salinity levels from 25 mM to 50 mM NaCl; however, at 100 mM NaCl, all these parameters decreased. These results showed that red amaranth was more tolerant to salinity stress than green amaranth.

Key words: Antioxidant activity, NaCl content, Salt stress, Total flavonoid content, Total phenolics.

INTRODUCTION

Worldwide, soil salinity has adversely affected about 30% of the irrigated and 6% of total land area with a resultant monetary loss of USD 12 billion in agricultural production (Chaves *et al*., 2009). High salt content affects plant growth by modifying their morphological, anatomical (Hasan *et al*., 2018) and physiological traits (Khorshid *et al*., 2016). Such growth impairment is due to osmotic effects and ionic imbalances affecting plant metabolism (Greenway and Munns, 1980). The severity of salt damage has been found to be dependent on species, cultivar (Talukdar, 2011) and growth stages of the plant (Mahdi Dar *et al*., 2007).

A horticultural crop with potential for cultivation under saline condition is amaranth (*Amaranthus* spp.) (Omami *et al*., 2006). Amaranth is an edible plant that has been used by humans for over 4000 years. It is grown and consumed as grain or vegetable crop in Australia, Africa and Asia (Corke and Cai, 2016). In Vietnam, amaranth is commonly consumed as a leafy vegetable. It is a valuable food source of nutrient with high quality of proteins, vitamins, minerals and bioactive compounds such as phenolics (Gomes *et al*., 2016). The possible use of this plant as a nutritious green crop in semi-arid region has been proposed (Myers, 1996).

Phenolic compounds are well-described secondary plant metabolites, which can play an important role in absorbing and neutralizing free radicals, quenching singlet oxygen and decomposing peroxides (Ksouri *et al*., 2009). An enhancement of antioxidant activity and the amount of phenolic compounds can be observed under various environmental factors and stress conditions (Valifard *et al*., 2014). Hence, salt-stressed plants might represent potential sources of phenolic compounds for economical use.

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Despite a substantial amount of literature on responses of plants to salinity stress, little information is available on amaranth, especially the change in its phytochemical content in response to salt tolerance. The objectives of this study were to: (1) determine the growth and biomass production of amaranth under saline condition; (2) evaluate the NaCl uptake, phytochemical content and antioxidant activity of amaranth treated with varying levels of NaCl and (3) compare the response of red (*Amaranthus tricolor* L.) and green amaranth (*Amaranthus dubius* L.) to salinity stress.

MATERIALS AND METHODS

This study included two experiments: Experiment 1 with red amaranth and Experiment 2 with green amaranth. Both experiments were conducted in a net house at Huong So commune, Hue City, Thua Thien Hue province, Vietnam (16°29′28.6"N 107°34′05.1"E) from February to May 2017.

Seeds germinated in a tray (20×40 cm) containing a mixture of garden soil and coconut fiber. Ten days after sowing, the seedling was transferred at the stage of 2-fully-expanded leaves to a plastic pot (dimension 14×12×10 cm) filled with garden soil. The weight of the pot containing garden soil was 1.3 kg. No additional nutrient or fertilizer was given to plants.

The experiments were conducted using the following treatments: garden soil, saline soil, 50% garden soil: 50% saline soil and 25, 50 and 100 mM NaCl. The different treatments had electrical conductivities (EC) equivalent to 1.1, 7.4, 5.2, 4.1, 6.3 and 11.5 dS m^{-1}, respectively. Tribat garden soil, a production of Tribat company, was used in the experiment. Saline soil was collected at Quang Dien district, Thua Thien Hue province which belongs to North Central coastal area of Vietnam. The features of garden soil and saline soil were showed in Table 1. Application of NaCl was done 3 times at 7, 12 and 17 days after transplanting. The plants were irrigated by 200 mL NaCl per pot to prevent leaching.

A pot experiment was laid out in a randomized complete block design (RCBD) with three replicates. Each block includes 6 treatments. Each replicate comprised 5 plants. A total of 90 test plants were used.

The experiments were terminated at 28 days after transplanting. Plant height, stem diameter, root length, number of leaves, total leaf area were recorded. Stem diameter was evaluated on the second internode above the ground with a measuring tape. Total leaf area per plant was measured by millimeter graph paper method (Pandley and Singh, 2011). Stem, leaves and root at harvest were dried in an oven 50°C for 60 h and weighed. At the end of harvest, the concentrations of Na^+ and Cl^- were measured in dried and ground tissue from shoot and root by atomic absorption spectrophotometry (Walinga *et al*. 1995). The shoots, which were dried in an oven at 50°C for 60 h, were used for determination of secondary metabolites.

Total phenolic content was determined by Folin Ciocalteau reagent (Velioglu *et al*., 1998). Colorimetric aluminum chloride method was used for flavonoid determination (Zhishen *et al*., 1999). The antioxidant activity was determined by DPPH radical scavenging assay following the procedure of Shimada *et al*. (1992).

The data gathered were subjected to Analysis of Variance (ANOVA) using Statistical Package for the Social Science (SPSS) software version 12. When F-test was found significant, mean comparison was done using Least Significant Difference (LSD) test at 5% level.

RESULTS AND DISCUSSION

Plant growth

The effect of salinity stress on amaranth vegetative growth and biomass production was presented in Table 2 and 3, respectively. 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. The root dry weight in red amaranth was affected greatly with up to 75% reduction; whereas in green amaranth, it was the leaf dry weight with up to 90% reduction. The salt-induced growth reduction has also been commonly observed in previous researches. Omami *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*. In

Table 1: Characteristics of the garden and saline soil used in this study.

Soil types	% N	% K_20	% P_20_5	$Na^+(Mgl^{-1})$	$Cl^-(Mgl^{-1})$	EC (dS m^{-1})	pH	Organic matter (%)
Tribat garden soil	0.45	0.73	0.35	0.6	0.97	1.1	6	24.91
Saline soil	0.5	0.62	0.2	38	57	7.4	7.5	0.3

Table 2: Vegetative growth of red (*Amaranthus tricolor* L.) and green amaranth (*Amaranthus dubius* L.) under different salinity levels.

Parameter	Genotype	Treatment					
		Garden soil	Saline soil	50% GS 50% SS	25	50 NaCl (mM)	100
Plant height (cm)	Red amaranth	34.1a*	28.3c	31.8b	35.8a	30.1c	24.5d
	Green amaranth	25.8A	20.6B	24.2A	25.5A	22.8B	12.2C
Stem diameter (mm)	Red amaranth	3.2a	2cd	2.8b	3.1ab	2.3c	1.7d
	Green amaranth	2.6AB	1.8C	2.2BC	2.4B	1.5D	1.2E
Root length (cm)	Red amaranth	13.2a	7.7c	11.3b	12.8a	8.6c	5.9d
	Green amaranth	9.9A	8.3B	9.4A	9.9A	8.1B	6.1C
Number of leaves	Red amaranth	23.6a	18.8c	21.9b	24.2a	20c	13.6d
	Green amaranth	15.5A	11.3D	13.6B	14.7A	12.6C	8.1E
Leaf area (cm^2)	Red amaranth	489.6a	409.8c	441b	511.7a	392.5c	295.8d
	Green amaranth	400A	290.7D	350.2B	394.8A	246.7C	195.3E

*Values with a common letter (lowercase for red amaranth; uppercase for green amaranth) in the same row are not significantly different using LSD at 5% level (GS: garden soil, 7 mM NaCl; SS: saline soil, 55 mM NaCl).

Table 3: Dry mass production of red (*Amaranthus tricolor* L.) and green amaranth (*Amaranthus dubius* L.) under different salinity levels.

Parameter	Genotype	Treatment					
		Garden soil	Saline soil	50% GS 50% SS	25	50 NaCl (mM)	100
Stem DW (g)	Red amaranth	1.3a	0.8c	1.1b	1.4a	0.8c	0.7c
	Green amaranth	1A	0.6B	0.8A	0.9A	0.6B	0.5B
Leaf DW (g)	Red amaranth	1.6a	1.1c	1.4b	1.7a	1.2c	0.8d
	Green amaranth	0.9A	0.5BC	0.7B	0.8A	0.6B	0.3D
Root DW (g)	Red amaranth	0.6a	0.3c	0.4b	0.7a	0.3c	0.2d
	Green amaranth	0.5A	0.2C	0.3B	0.4B	0.2C	0.1D

*Values with a common letter (lowercase for red amaranth; uppercase for green amaranth) in the same row are not significantly different using LSD at 5% level (GS: garden soil, 7 mM NaCl; SS: saline soil, 55 mM NaCl).

the present research work, red amaranth showed similar response with 42% reduction in leaf number and 40% in total leaf area. However, the reduction in plant height was lower compared to the previous study with only 28% decrease.

The reduction in plant growth of green amaranth was greater than red amaranth at all salinity levels, except for root dry weight. Hence, on the basis of these parameters, *A. tricolor* was more tolerant than *A. dubius* at different salt levels. Omami *et al*. (2006) also stated that *A. hypochondriacus* and *A. cruentus* were more tolerant than *A. tricolor* at 100 mM NaCl based on the greater values in leaf area, plant height and shoot dry mass.

The current study showed that shoot biomass in red and green amaranth was higher than root biomass at all levels of NaCl. This is a particularly important characteristic for genotypes cultivated as leafy vegetable crops under salinity stress. A reduction in root growth with increasing root zone salinity (from 25 mM to 100 mM NaCl) was also observed on *A. cruentus*, *A. tricolor*, *A. hypchondriacus* and Accession' 83 by Omami *et al*. (2006) and in *Carthatmus tinctorius by* Bassil and Kaffka (2002).

NaCl content

Sodium chloride concentration in different plant parts increased with salinity stress except in the treatment with 25 mM NaCl. Concentrations of Na^+ in roots were higher than in shoots (Fig 1). In the red amaranth shoot, the highest increase in Na^+ content relative to the control was noted at 100 mM (5 times), followed by the treatment with 50 mM and saline soil (4 times) and then by 50% garden soil: 50% saline soil (2.8 times). A similar trend of Na^+ content was recorded in the root. The highest root Na^+ concentration was found in 100 mM NaCl treatment, yielding 8 times that of the control. Similar response to salt stress was observed in shoot and root Na^+ concentration of green amaranth except for root Na^+ content at the 25 mM treatment, yielding 2 times over the control.

The drastic increase in the concentration of Na^+ and Cl^- in the tissues of plants exposed to salinity led to toxicity as it was shown by a reduced plant growth. Na^+ disrupts the cell ion homeostasis by inducing an inhibition in the uptake of essential nutrients in a number of crops, e.g., in wheat (Raza *et al*., 2007) and in sunflower (Akram *et al*., 2007).

The accumulation of Na^+ and Cl^- in roots provides a mechanism for amaranth to cope with salinity in the rooting medium. In the present study, NaCl content in the red amaranth root was greater than that of green amaranth. This

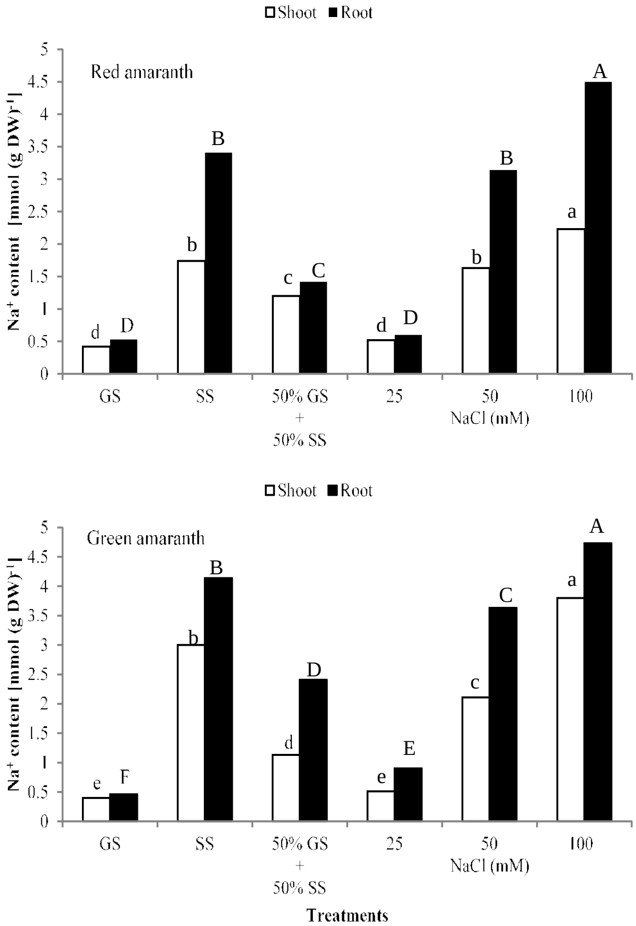

Fig 1: Na^+ content of red (*Amaranthus tricolor* L.) and green amaranth (*Amaranthus dubius* L.) under different salinity levels. Values with a common letter (lowercase for shoot; uppercase for root) are not significantly different using LSD at 5% level (GS: garden soil, 7 mM NaCl; SS: saline soil, 55 mM NaCl).

indicates the greater inhibition of transport of toxic ions to the leaf laminae which contributed to a better growth of *A. tricolor*. Similar mechanism was reported in previous studies in *A. cruentus* and *A. tricolor* (Omami, 2005), sweet pepper (Zandstra-Plom *et al*., 1998) and olive (Kchaou *et al*., 2010).

Phytochemical content

In the current study, salinity stress increased the total phenolic content and total flavonoid content in both genotypes. The increase in total phenolic and flavonoid content resulted in an increase of antioxidant activity with up to 35% in red amaranth and 23% in green amaranth. The 50 mM NaCl treatment produced the greatest total phenolic content and flavonoid in red amaranth. However, high salt concentration (100 mM NaCl) reduced these parameters. *A. tricolor* produced a higher value of total phenolic content and total flavonoid content compared to that of *A. dubius* at all salinity treatments. Total phenolic content of red amaranth ranged from 19 to 27 mg GAE $(g\ DW)^{-1}$; while in green amaranth, from 13.9 to 20.1 mg GAE $(g\ DW)^{-1}$.

Salt tolerance seems to be favored by an increased antioxidant activity to detoxify ROS (Kibria *et al*., 2017). Increase in phenolic content in different tissues under increasing salinity has also been reported in a number of

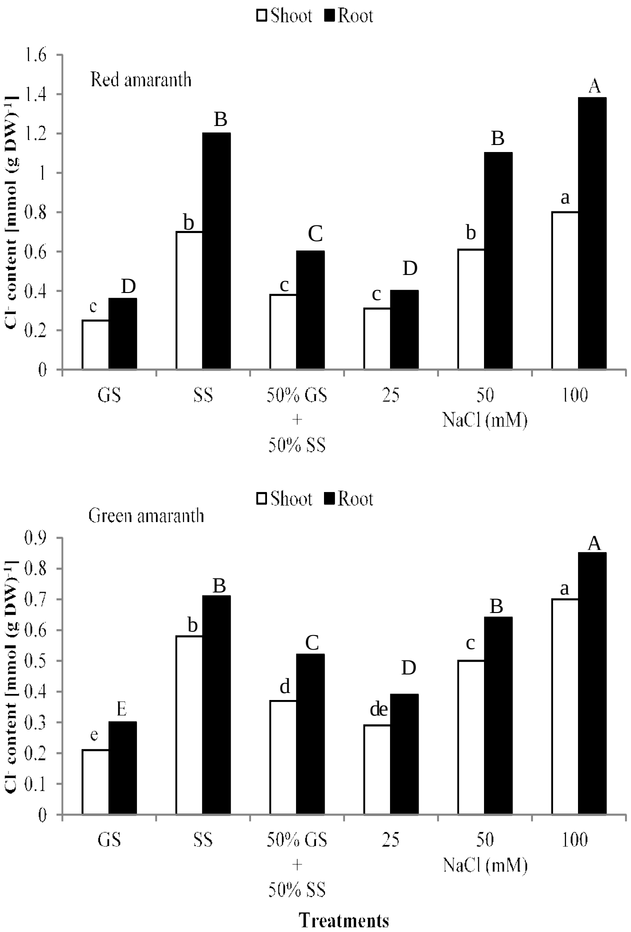

Fig 2: Cl^- content in the shoot and root of red (*Amaranthus tricolor*) and green amaranth (*Amaranthus dubius*) under different salinity levels.

Values with a common letter (lowercase for shoot; uppercase for root) are not significantly different using LSD at 5% level (GS: garden soil, 7 mM NaCl; SS: saline soil, 55 mM NaCl).

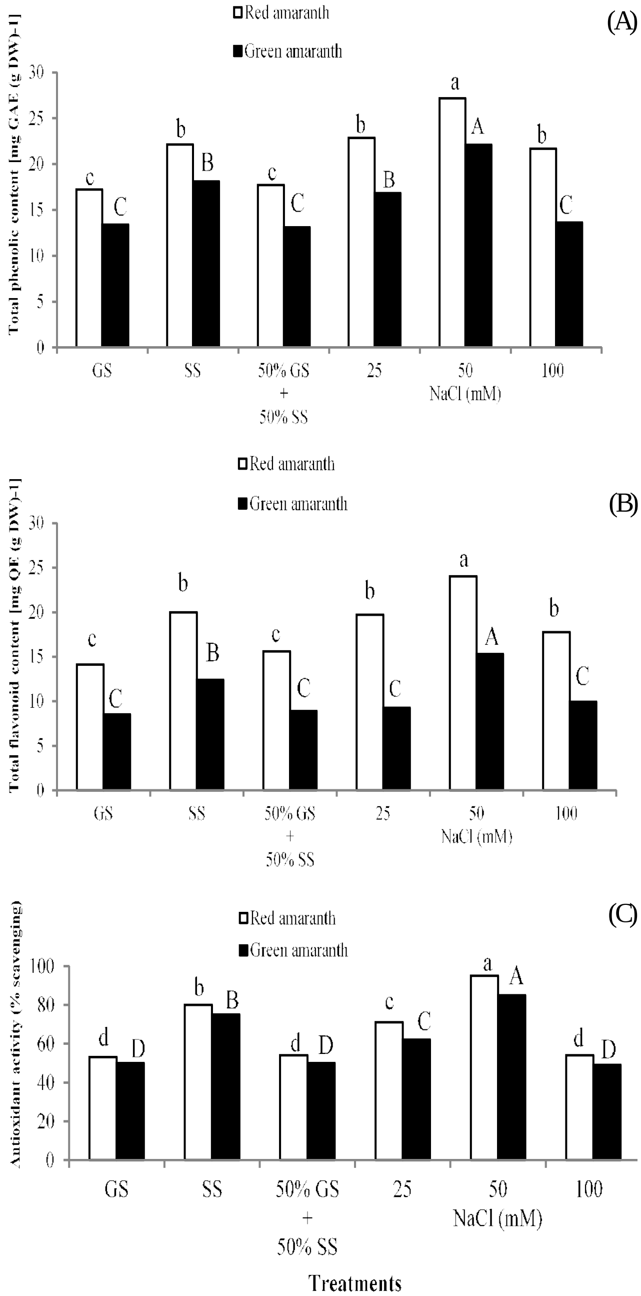

Fig 3: Total phenolic (A), flavonoid (B) and antioxidant activity (C) of red (*Amaranthus tricolor* L.) and green amaranth (*Amaranthus dubius* L.) under different salinity levels.

Values with a common letter (lowercase for red amaranth; uppercase for green amaranth) are not significantly different using LSD at 5% level (GS: garden soil, 7 mM NaCl; SS: saline soil, 55 mM NaCl; GAE: gallic acid; QE: quercetin).

plants such as pepper (Navarro *et al*., 2006) and tomato (Alves *et al*., 2018). Phenolic compounds exhibit antioxidant activity by inactivating lipid free radicals or preventing decomposition of hydroperoxides into free radicals (Pokomy *et al*., 2001). Several flavonoids act as potential inhibitor of the enzyme lipoxygenase, which converts polyunsaturated fatty acids to oxygen-containing derivatives (Nijveldt *et al*., 2001). These compounds which accumulate in plant tissues could help protect them from damaging effects of ROS and may help inhibit lipid peroxidation in stressed-plants (Potapovich and Kostyuk, 2003).

CONCLUSION

Salinity stress reduced plant growth of *A. tricolor* and *A. dubius* except for the 25 mM treatment. The sensitivity to stress differed with the level of salinity, the measured parameters and genotypes. Treatment with 100 mM NaCl caused the greatest reduction in all plant growth parameters.

Na^+ and Cl^- content in both red and green amaranth increased with increasing salinity level in all plant parts. The greatest increase was recorded in green amaranth with 8 times increase of Na^+ content in both shoot and root. NaCl content in the root was greater than in the shoot at all salinity treatments. This provides a mechanism for amaranth to cope with salinity stress by excluding most of the toxic ions from the root zone to the aerial parts. Total phenolics, flavonoid content and antioxidant activity increased with increasing salinity level up to 50 mM NaCl. All these parameters were reduced at 100 mM NaCl. Maximum increases in total phenolics content and flavonoid content were 28% and 54%, respectively, in red and green genotypes. In the current experiments, both *A. tricolor* and *A. dubius* growth were reduced under mild (from 25 to 55 mM NaCl) and moderate salinity (from 55 to 100 mM NaCl). According to the classification of crop tolerance to salinity (Mass and Hoffman, 1977), *A. tricolor* and *A. dubius* can be rated as moderately sensitivity to salinity stress, similar to other leafy vegetable crop. The decrease in growth of green amaranth was greater than red amaranth at all salinity levels, except for root dry weight. Hence, on the basis of these parameters, *A. tricolor* was more tolerant than *A. dubius*. *A. tricolor* also produced greater amount of phytochemical content than *A. dubius* under salinity stress which was linked to its free radical scavenging activities.

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