



Physiological and Molecular Responses of Red Amaranth (*Amaranthus cruentus* L.) and Green Amaranth (*Amaranthus hypochondriacus* L.) to Salt Stress

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Abstract

Salinization, as a serious climate change phenomenon, continues to cause critical issues for soils and crops. Abundance of genetic diversity of plants could contribute in solving the impacts of salinity. However, the relations between physiological and molecular traits of plant species need deep investigation and interpretation. Pot experiments were conducted in the greenhouse to investigate the difference in behavior between two amaranth species when they were beforehand subjected to salinity-stress conditions, specifically on some specific physiological and molecular characteristics. After 45 days from transplanting, salt stress was induced using a 150 mM sodium chloride solution. Treatments were arranged in randomized complete block design using three replications. After 0, 2, 6, 10, and 24 h, samples were collected to evaluate physiological data as well as diversity of gene expression. The samples were immediately frozen in liquid nitrogen and stored in -80° C ultralow temperature refrigerators. Findings revealed that the red amaranth species (*Amaranthus cruentus*) was more tolerant to saline stress than the green amaranth species (*Amaranthus hypochondriacus*). Result data revealed that malondialdehyde (MDA) content in the red amaranth increased after 2 h then decreased (after 6 and 10 h) before rising again after 24 h. In the green amaranth, MDA content initially decreased after 2 h before increasing and later decreasing in a zigzag pattern ending in an increase after 24 h. There was an initial steep rise in proline content of green amaranth after 2 h which continued to still rise moderately till 24 h. In the red amaranth the initial rise in proline continued after 2, 6 and 10 h before it stopped. Results also showed that red amaranth gave higher value of superoxide dismutase (SOD) activity as compared with green amaranth in all tested salinity exposure times. The investigation on the expression of four genes assessed through quantitative PCR indicated the efficiency of red amaranth in increasing the expression of SOS1, HKT1, NHX1, and DGR2 genes, which encode adaptation-related proteins under salinity stress. In summary, the current work demonstrated that red amaranth could be an efficient genetic resource in improving salt-tolerant genotypes belonging to *Amaranthus* genus.

Keywords *Amaranthus* sp. · Defensive Performance · Ionic toxicity · Osmo-protectants · Salt stress-related gene expression

1 Introduction

All sectors in the agricultural field have been particularly affected by the issues related to climate change around the world. Unfavorable conditions arising from climate change create various eco-stresses such as drought, heating, nutritional deficiency and salinity which cause mischievous

impacts on crop growth and productivity (El-Bially et al. 2018; Saady et al. 2020a; Ramadan et al. 2023a). The negative effects of eco-stresses on crop growth are mainly attributed to defect in plant physiology and biology (Saady 2014; El-Bially et al. 2022a; El-Metwally et al. 2022; Saady et al. 2023a; Shaaban et al. 2023a; Shahin et al. 2023). Ultimately, these stresses certainly lead to yield losses and poor

quality (Saady 2015; Abd-Elrahman et al. 2022; Shaaban et al. 2023b; Hassan et al. 2024).

Salinity is a very harmful challenge to agriculture, as it poses a major threat to crop growth and development (Mukhopadhyay et al. 2021). Physiologically, oxidative pressure of salinity causes over-generation of hazardous molecules called reactive oxygen species (ROS), such as hydrogen peroxide hydroxyl radicals, and superoxide radicals, via disruption of electron transport systems in chloroplast and mitochondria (Sun et al. 2016; Analin et al. 2020). Accordingly, the functional imbalance of photosynthesis process and cellular severe oxidization are regarded as the major biochemical evidence of salinity (Vineeth et al. 2023). Molecularly, in addition to photosynthesis disturbance and lipid peroxidation, salinity increases the deterioration rate of DNA with cell death (Wang et al. 2019; El-Metwally and Saady 2021a; Doklega et al. 2023; Ali et al. 2024). Hence, there is a dire need to depend on sturdy plants that have the potential to thrive in stressed conditions, (Saady and El-Metwally 2019; Ramadan et al. 2023b; Saady et al. 2023b). It was found that plants' tolerance to salinity is owing to protecting growth and photosynthesis machinery, modifying antioxidant defense enzymes, and maintaining plant water status (Mubarak et al. 2021; Mansour et al. 2021). Salt stress tolerance is also molecularly linked to the expression of specific genes (Razzaq et al. 2021).

The genus *Amaranthus* as an individual of Amaranthaceae plant family comprises more than 50 herbaceous species (Stetter and Schmid 2017). *Amaranthus* species can be exploited as leafy vegetables or as cereals (Baraniak and Kania-Dobrowolska 2022). In last decades, there has been a great attention in numerous of amaranth species due to their nutritional value and stress resistance (Wouyou et al. 2017; Sarker et al. 2018; Pulvento et al. 2022). However, there was a significant variation among amaranth species in salinity tolerance (Luyckx et al. 2021; Tebini et al. 2022). Since amaranth belongs to C_4 photosynthetic pathway plants, it has greater ability to withstand the stressed conditions via better utilization of water compared to C_3 plants (Assad et al. 2017). Red amaranth (*A. cruentus* L.) and green amaranth (*A. hypochondriacus* L.) are hopeful as binary-use crops, because their leaves and seeds can be nutritionally used, which are richer than true cereals in minerals, vitamins, and proteins (Tang et al. 2014; Ngugi et al. 2017; Hoidal et al. 2020; Gómez et al. 2023).

Despite its salt tolerance, the physiological mechanisms and molecular explanations in amaranth are still not fully understood. Herein, it has been hypothesized different amaranth species behave different physio-molecular performance under salinity stress. Therefore, the present study aimed to identify physiological and molecular changes explaining variability in salt tolerance between two amaranth species.

Salt stress was induced by 150 mM sodium chloride, and then physiological and molecular traits were assessed after 0, 2, 6, 10, and 24 h of a 24 h salinity-stress cycle.

2 Materials and methods

2.1 Experimental Site and Plant Materials

In greenhouses of the Horticulture Research Station, at El-Kanater El-Khairia, Qalubia Governorate, the current research was carried out during the two successive seasons of 2021 and 2022. The experiments included two different annual summer amaranth species, i.e. *Amaranthus cruentus* (red amaranth) and *Amaranthus hypochondriacus* (green amaranth). Seeds were obtained from a certified source, Osman Badawy Company for Importation of Ornamental Plant Seeds, Cairo, Egypt.

2.2 Trial Procedures and Sampling

Initially, seeds of the two amaranth species were planted on March 20th and 25th in the first and second growth seasons, respectively, in plastic trays, as a primary nursery, filled with a medium that contained a mixture of peat moss, sand, and vermiculite (2:1:1 by volume). Seeds were covered with a thin layer (0.5 cm) of media, irrigated carefully, then placed in the greenhouse. On April 20th and 25th of the first and second growing seasons, respectively, seedlings were pricked out and transplanted into 25-cm diameter plastic pots filled with aforementioned media, one seedling per pot.

The intended salt stress treatment was applied using 150 mM sodium chloride (NaCl) solution, equivalent to 13.70 dS m^{-1} , 45 days after transplantation. Control samples were taken before salt treatment (at 0 time), and treated samples were taken after 2, 6, 10, and 24 h. Treatments were arranged in randomized complete block design with three replicates. Leaf samples were immediately frozen in liquid nitrogen and stored in ultralow temperature refrigerators at $-80^{\circ}C$ to be used in analyzing the biochemical and molecular parameters.

2.3 Assessments

2.3.1 Plant Pigments

2.3.1.1 Chlorophyll Total chlorophyll (a+b) of the leaf samples was extracted with acetone (80%) and determined by monitoring the absorbance at wavelengths of 663 nm for

chlorophyll a and 645 nm for chlorophyll b according to the technique detailed by Wang (2006).

2.3.1.2 Anthocyanin Content of total anthocyanin was extracted using acidified methanol (1% HCl). The absorbance of the clear filtered anthocyanin solution was measured by a Spekol 11 spectrophotometer at 535 nm according to the method developed by Du and Francis (1973).

2.3.2 Defensive Performance

2.3.2.1 Lipid Peroxidation Lipid peroxidation was estimated by measuring the level of malondialdehyde (MDA) production using the thiobarbituric acid (TBA) method as described by Bailly et al. (1996).

2.3.2.2 Proline Content Samples weighing 100 mg of freshly powdered leaf tissue were extracted with 3% sulfosalicylic acid. After centrifugation, a ninhydrin solution containing acetic acid and 6 M H₃PO₄ (v/v, 3:2) was added to the supernatants, and the mixture was then heated at 100 °C for 60 min. Toluene was then added to the mixture, which was then let to sit at room temperature for 30 min. Utilizing a microplate reader (ACCURIS Smart Reader; Edison, NJ, USA) and L-proline (1–100 g), the absorbance was recorded at 520 nm as previously described by Khare et al. (2012).

2.3.2.3 Superoxide Dismutase Activity To determine superoxide dismutase (SOD) as an indicator for enzymatic antioxidant activity (u/g protein contents), the frozen shoots (about 0.5 g) were homogenized (1:9 w/v) in 0.1 M cooled

phosphate buffer (pH 7.8) or normal saline. The homogeneous mixture was centrifuged at 12,000 ×g for 20 min at 4°C and the supernatants were collected for determining SOD enzyme activity according to the procedure of Martínez et al. (2020).

2.3.3 Molecular Response and Gene Expression

2.3.3.1 RNA Extraction and cDNA Synthesis Total RNA was extracted from leaf tissues using EasyPure[®] RNA Kit (Ttrans, China). Extracted RNA was converted to cDNA using EasyScript[®] Frist-Strand cDNA synthesis superMix (trans, china), as described by Claros and Cánovas (1999).

For RT-PCR optimization and amplification total mRNA was isolated from 0.5 g red amaranth (*A. cruentus*) and green amaranth (*A. hypochondriacus*) plant leaves of control and 150 mM salinity treatments after different time sequences (0, 2, 6, 10 and 24 h) of stress periods using the Plant RNA Kit (Sigma-Aldrich). Using spectrophotometer, the purified RNA was quantitated and analyzed on 1% agarose gel. 10 µg total RNA was treated with DNase RNase-free (Fermentas), 5 µg of which was reverse transcribed in a reaction mixture consists of oligo dT primer (10 pmL/µL), 2.5 µL 5X buffer, 2.5 µL MgCl₂, 2.5 µL 2.5 mM dNTPs, 4 µL from oligo (dT), 0.2 µL (5 Unit/µL) reverse transcriptase (Promega, Germany) and 2.5 µL RNA. RT-PCR amplification was performed in a thermal cycler PCR, programmed at 42 °C for 1 h and 72 °C for 20 min. Quantitative Real time PCR was carried out on 1 µL diluted cDNA by triplicate using the real time analysis using a Rotor-Gene 6000 (Germany) system. The primer sequences used in qRT-PCR are given in Table 1. Primers of four salinity responsive genes and two housekeeping genes as reference genes were utilized.

2.3.3.2 Gene Expression The method of SYBR[®] Green was employed to perform the analysis of the four gene expression. Reaction's mixture comprised 10 µL of SYBR Green Master Mix, 2 µL for each of template, forward primer and reverse primer, and sterile distilled water (total volume of 20 µL). PCR analyses were assessed via the conditions of 95 °C for 15 min followed by 40 cycles of 95 °C for 30s and 60 °C for 30s. The CT was used to compute ΔCT values (target gene CT subtracted from β-Actin gene CT). The relative

Table 1 Names of four salinity-responsive genes and two housekeeping genes (as reference genes) plus primer sets (direction and sequence)

Gene name		Sequence 5' → 3'
AhHKT1	F	5'- GGAGCTCTGGAGTTATGGAGG- 3'
	R	5'- ACAACCAAGATGGCCGAAC- 3'
AhSOS1	F	5'- TGGCCTGCACGAGAAAATTC- 3'
	R	5'- TCTCGAGGACTGTTCTGTGGA- 3'
AhDGR2	F	5'- CCCTTCCTGGTTGGATGGTC- 3'
	R	5'- CAACTTGAGCAATGGCGCTT- 3'
AhNHX1	F	5'- CCAATGTTCCAACGGCTCCA- 3'
	R	5'- GGTTTAGCCACCGAGTTGT- 3'
β-Actin	F	5'- ACTCCATCATGAAGTGCGAC - 3'
	R	5'- TTTCAGGAGGAGCAACAACC - 3'
GAPDH	F	5'- TTGGTTTCCACTGACTTCGTT - 3'
	R	5'-CTGTAGCCCCACTCGTTGT - 3'

F: Forward; R: Reverse

gene expression was identified using the 2- $\Delta\Delta C_t$ method (Livak and Schmittgen 2001).

2.4 Statistical Analyses

Through the software CoStat program, the analysis of variance (ANOVA) was utilized for statistical analysis of the results data based on procedures illustrated by Casella (2008). To compare among the means of treatments, Duncan's multiple range test ($p \leq 0.05$) was exploited (Steel and Torrie 1980). Furthermore, mRNA of salinity responsive genes was presented in showing hierarchical clustering-heat map diagram of transcriptional expression.

3 Results

3.1 Plant Pigments

Analysis of variance detected that total chlorophyll and total anthocyanin contents were significantly affected by amaranth species and time after initiating the salt-stress cycle (Table 2) and their interaction (Fig. 1). In this respect, chlorophyll content of green amaranth was greater than that of red amaranth by 37.9%. Time after initiating the salt-stress cycle showed reducing in total chlorophyll after 2 and 6 h and still with a further gradual reduction after 10 and 24 h. Results in Fig. 1 manifested quite clearly that time in each tested amaranth species had a significant effect on finally at the end reducing chlorophyll content (after 24 h). In green amaranth (Fig. 1a), there was a steady decline in total chlorophyll after 6 h and continued till 24 h. However, it was

Table 2 Effect of a 24-hour salinity-stress cycle (at 150 mM NaCl) on chlorophyll and anthocyanin contents in two *Amaranthus* species

Variable	Total chlorophyll (mg g ⁻¹ FW)	Total anthocyanin (mg g ⁻¹ FW)
Specie		
Red amaranth	4.01 ± 0.26 b	288.13 ± 15.05 a
Green amaranth	5.53 ± 0.35 a	59.65 ± 3.79 b
Time		
0 h	6.18 ± 0.50 a	146.9 ± 36.78 c
2 h	5.44 ± 0.47 b	205.65 ± 58.12 a
6 h	4.99 ± 0.38 b	206.82 ± 67.69 a
10 h	4.09 ± 0.30 c	168.87 ± 54.83 b
24 h	3.15 ± 0.31 d	141.22 ± 40.45 c

Values in the same column followed by the same letter are not statistically different according to Duncan's multiple range test ($p \leq 0.05$)

less apparent in the red amaranth which showed a steady decline after 10 h and continued till 24 h (Fig. 1a).

As shown in Table 2, red amaranth had a 4.83-fold increase in total anthocyanin content of green amaranth. Also, time after initiating the salt-stress cycle had a main effect on increasing total anthocyanin (after 2 and 6 h) then decreased gradually (after 10 and 24 h). Concerning the interaction, anthocyanin content increased in red amaranth until 6 h after initiation of salt treatment then it decreased until the end of the 24 h (Fig. 1b). On the other hand, the change in anthocyanin content of green amaranth was not clear under different salt-stress cycles.

3.2 Defensive Performance

There were statistical variations between the two testes species of amaranth in malondialdehyde (MDA) content, proline content and superoxide dismutase (SOD) activity

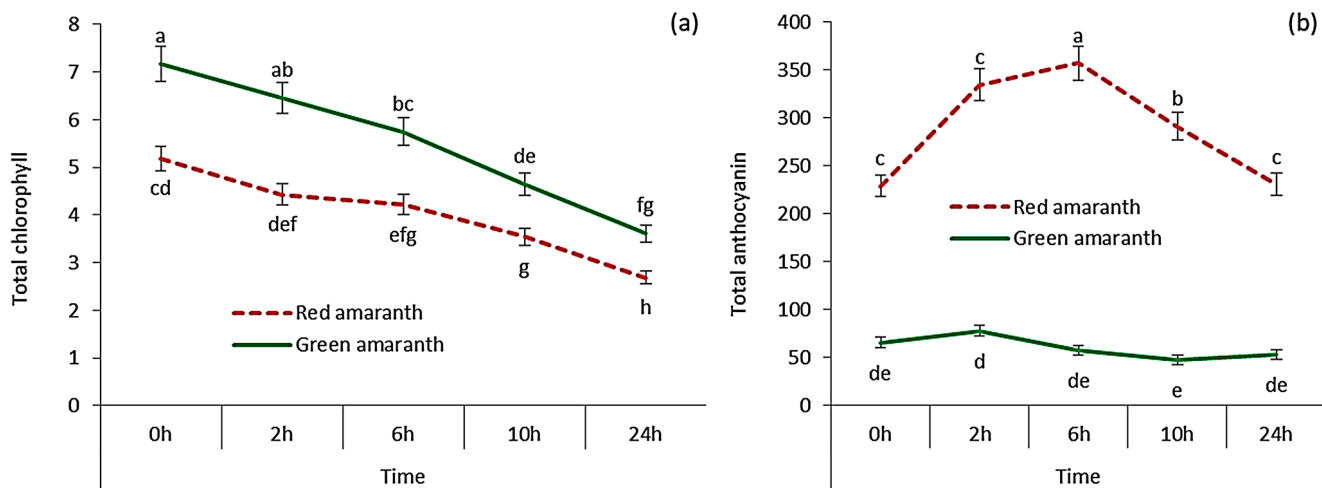


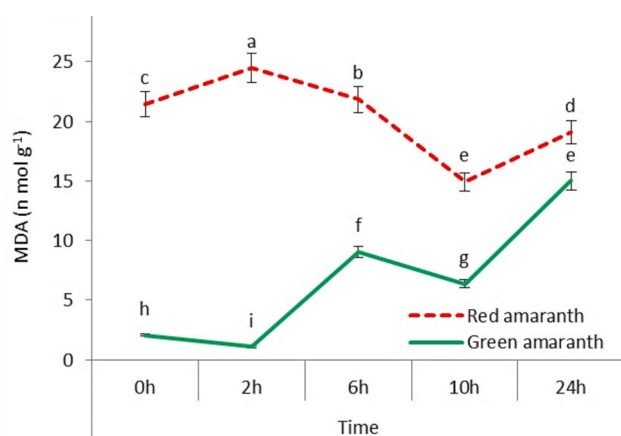
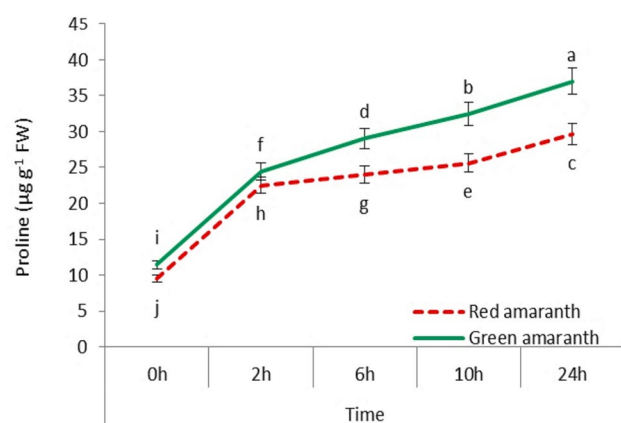
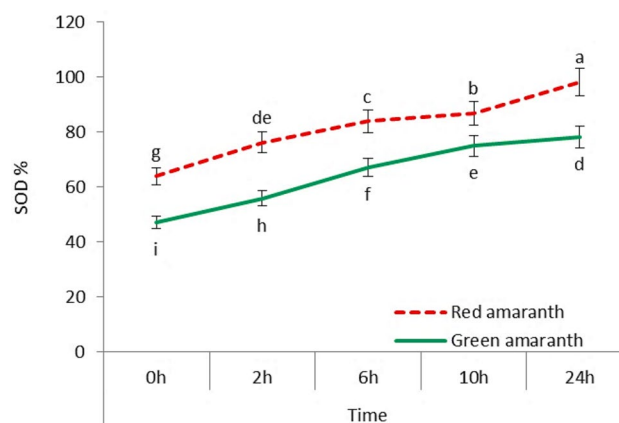
Fig. 1 Effect of interaction between *Amaranthus* species (red and green) and time (24-hour salinity stress-cycle) on total chlorophyll (a) and total anthocyanin (b) (mg g⁻¹ FW). Different letters imply that

there is significant variation between treatment means based on Duncan's multiple range test ($p \leq 0.05$)

Table 3 Effect of a 24-hour salinity-stress cycle (at 150 mM NaCl) on malondialdehyde (MDA) content, proline content and superoxide dismutase (SOD) activity in two *Amaranthus* species

Variable	MDA (n mol g ⁻¹)	Proline (μg g ⁻¹ FW)	SOD %
Specie			
Red amaranth	20.37 ± 0.96 a	22.23 ± 2.03 b	81.78 ± 3.42 a
Green amaranth	6.71 ± 1.51 b	26.86 ± 2.61 a	64.61 ± 3.50 b
Time			
0 h	11.76 ± 4.33 d	10.47 ± 0.46 e	55.46 ± 3.79 e
2 h	12.78 ± 5.24 c	23.47 ± 0.44 d	65.97 ± 4.59 d
6 h	15.46 ± 2.87 b	26.5 ± 1.13 c	75.51 ± 3.75 c
10 h	10.66 ± 1.92 e	29.01 ± 1.53 b	80.93 ± 2.71 b
24 h	17.04 ± 0.92 a	33.27 ± 1.65 a	88.12 ± 4.47 a

Values in the same column followed by the same letter are not statistically different according to Duncan's multiple range test ($p \leq 0.05$)

**Fig. 2** Effect of interaction between *Amaranthus* species (red and green) and time (24-hour salinity stress-cycle) on malondialdehyde (MDA) content. Different letters imply that there is significant variation between treatment means based on Duncan's multiple range test ($p \leq 0.05$)**Fig. 3** Effect of interaction between *Amaranthus* species (red and green) and time (24-hour salinity stress-cycle) on proline content. Different letters imply that there is significant variation between treatment means based on Duncan's multiple range test ($p \leq 0.05$)**Fig. 4** Effect of interaction between *Amaranthus* species (red and green) and time (24-hour salinity stress-cycle) on superoxide dismutase (SOD) activity. Different letters imply that there is significant variation between treatment means based on Duncan's multiple range test ($p \leq 0.05$)

(Table 3). In this concern, the values of MDA and SOD were higher (3.03 and 1.26 folds) and proline was lower (17.2% reduction) in red amaranth than that of green amaranth. Moreover, 24 h salinity-stress cycle (at 150 mM NaCl) had significant defensive performances. MDA content, SOD activity and proline content progressively increased with increasing exposure time to salinity, hence, 24-h showed the maximum values in this respect.

As for the interaction between each amaranth specie and time, result data in Fig. 2 revealed that MDA content in the red amaranth increased after 2 h then decreased (after 6 and 10 h) before rising again after 24 h. In the green amaranth, MDA content initially decreased after 2 h before increasing and later decreasing in a zigzag pattern ending with an increase after 24 h. Regarding the effect of interaction on proline, there was an initial steep rise in proline content of green amaranth after 2 h which continued to still rise moderately till 24 h (Fig. 3). In red amaranth the initial rise in proline continued after 2, 6 and 10 h before it stops. Also, the interaction effect showed that SOD activity was higher in salinity treated plants as compared with control (Fig. 4), where, there was a significant increase in SOD activity in both species. When comparing the two tested species in SOD activity, notably, results show that red amaranth gave higher values as compared with green amaranth in all tested times treatments.

3.3 Expression of some Salt Stress-Related Genes

To address the differences between both two amaranth species at the molecular level, the expression level of four salt stress marker genes (viz. SOS1, HKT1, NHX1 and DGR2) were studied by the real-time quantitative PCR (qPCR) approach including: the plasma membrane (PM) salt overly

sensitive 1 (SOS1), the high-affinity potassium transporter1 (HKT1), the vacuolar Na^+/H^+ antiporter (NHX1), and the abiotic stress-induced protein DUF642 (DGR2).

Generally, all genes exhibited higher expression levels in the red amaranth species at one or more stress time-point compared to the green one. For example, within the short-term (2 h) of salinity, genes for HKT1, SOS1 and NHX1 increased by ~3-, ~5- and ~6-fold, respectively, in red amaranth compared to control, as well as by ~2-, ~2-, and ~4-fold, respectively, relative to the corresponding point of the green amaranth (Fig. 5a, b and d). In the mid-term of stress (6–10 h), red amaranth continued to accumulate higher transcripts of SOS1 that peaked at 6 h with up to 4-fold higher relative to analogous point in green species, before starting to decline at 10 h. Interestingly enough, the expression level of SOS at 24 h of salinity in red amaranth was roughly 1, 5 lower than in the green amaranth species (Fig. 5b). For HKT1 and NHX1, both genes exhibited roughly similar induction patterns that peaked at 10 h of stress before sharply declining at 24 h within each species (Fig. 5a and d). Nevertheless, only the expression level of HKT1 was higher at both 6 and 10 h in red amaranth

compared to green amaranth, while NHX1 showed its significant induction at 10 h only.

On the contrary, the expression level of DGR2 did not differ in both species in the short term (2 h of stress), although significant induction took place within each species by roughly ~6-fold (Fig. 5c). In the mid-term, (6–10 h of stress) however, DGR2 showed higher transcript abundance in red amaranth compared to green amaranth. Intriguingly and quite fascinating, unlike the SOS1, HKT1, and NHX1 in both species, the expression of DGR2 did not decrease in the long-term 24 h of salinity in red amaranth, where the transcript abundance was ~4-fold compared to green amaranth (Figs. 5 and 6).

4 Discussion

It is interesting to confirm that eco-stresses hugely impacted crop cultivations and production (Saady and Mubarak 2015; Saady et al. 2020b; El-Bially et al. 2022b). In this concern, salinity is one of the most critical abiotic problems which dramatically depress soil health and crop potentiality (Salem

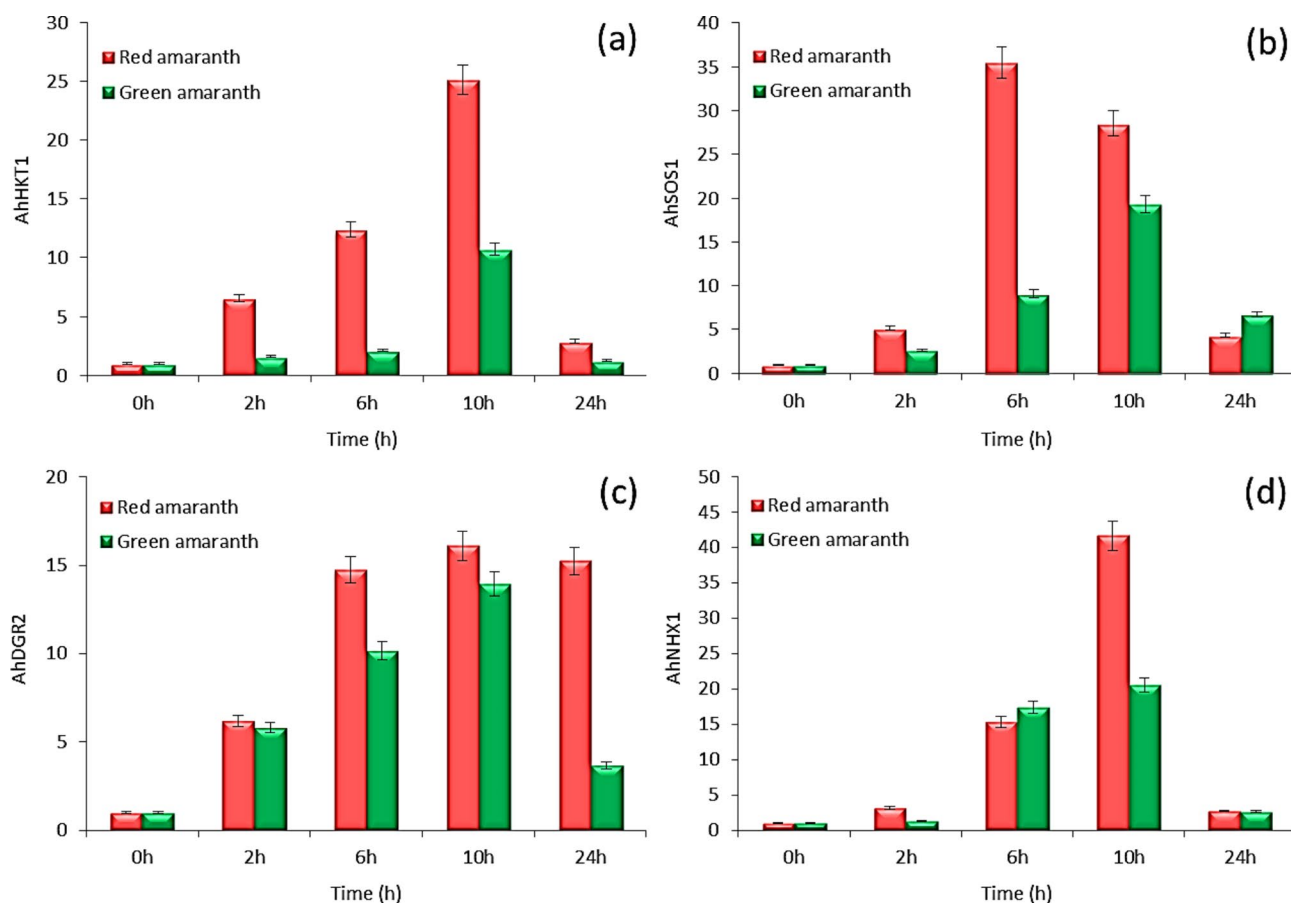
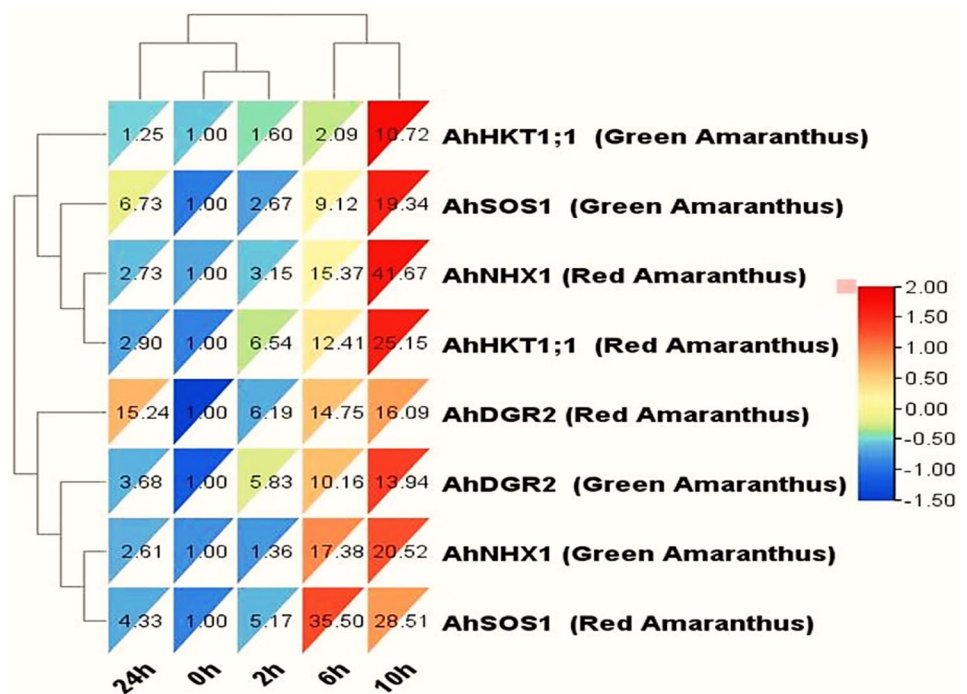


Fig. 5 Bar-charts of relative expression level (fold changes) in mRNA of the AhHKT1 (a), AhSOS1 (b), AhDGR2 (c) and AhNHX1 (d) genes in red and green *Amaranthus*. Plants were initially grown in control

and salt stress (150 mM) conditions for 24 h. Values are means $\pm$ SE of three biological replicates (each one of three plants, $n=3$)

Fig. 6 Diagram showing hierarchical clustering-heat map of transcriptional expression (fold changes) in mRNA of four salinity responsive genes; AhHKT1, AhSOS1, AhDGR2 and AhNHX1 genes in red and green *Amaranthus*. Plants were initially grown in control and salt stress (150 mM) condition for 24 h. Values are means $\pm$ SE of three biological replicates (each one of three plants, $n = 3$)



et al. 2021, 2022; Abd El-Mageed et al. 2022; Lasheen et al. 2023). Therefore, it is so significant to identify the plant performances toward salt stress in order to accurately address the stress-associated depression (Abdo et al. 2024). As obtained from the results of the current work, the longer the exposure periods to salinity, the lower the chlorophyll concentration. Salinity stress can induce the apparatus of photosynthesis to release excessive ROS, hastening disintegration rate of leaf pigments, causing cell death of leaves (Liang et al. 2015; Ramadan et al. 2024a). Despite *A. hypochondriacus* had greater chlorophyll content than *A. cruentus*, the decline rate in the former specie was higher with increased salt exposure time. Herein, with exposure times of 6, 10, and 24 h, the reductions in chlorophyll were 18.7, 31.79 and 48.2%, respectively for *A. cruentus*, while the corresponding reductions in *A. hypochondriacus* were 19.8, 35.1 and 49.7%, respectively, compared to zero time (0 h). Contrariwise, as expected, anthocyanin pigment content of the red specie (*A. cruentus*) was higher than that of green one. Furthermore, in red specie, the change in anthocyanin content was more evident with salt stress, where the minimal value was obtained at 0 h, while the maximum value was recorded at 6 h, then declined at 10 h and the 24 h. This could be referring to resilience of the red specie to counteract salt stress. To prove that the defensive performances, representing in of MDA content, proline content and SOD activity, were assessed.

Regarding the defensive performance, although MDA was increased and proline decreased in *A. cruentus* compared to *A. hypochondriacus*, the SOD value was higher in

the former specie than the later one. Also, MDA, proline and SOD values progressively increased as salt exposure time elevated for both *A. cruentus* and *A. hypochondriacus*. MDA is produced via lipid peroxidation, caused by ROS, which are resulted in response to salt stress (Hasanuzzaman et al. 2021). Due to sodium chloride stress distinctive increases in H_2O_2 , lipid MDA and decrease in stability of cell membrane were obtained (El-Sayed et al. 2022; Helal et al. 2024). However, under such stress conditions, plants have evolved different mechanisms to tolerate the ecological stressors and maintain better growth and development (Noureldin et al. 2013; Saady et al. 2018; El-Metwally and Saady 2021b). Moreover, proline as an osmo-protectant accumulates in plants to be more adapted to stress conditions (Ali et al. 2021; Ramadan et al. 2024b). Under adverse conditions, proline can regulate water uptake, preventing the dehydration of plant tissues via readjustment the osmotic potential (Batista-Silva et al. 2019; Dong and Beckles 2019; Hadid et al. 2023, 2024). Based on the plant species, tolerance mechanism systems could involve the synthesis of osmolytes for osmotic regulation (Maathuis 2014; Munns et al. 2020b; Saady et al. 2021). On the other site, for quenching the ROS and their oxidative stress damage, enzymatic antioxidants are substantially produced in stressed plants (Makhlouf et al. 2022; Saady and El-Metwally 2023). In the present work, the activity of SOD was significantly increased under salinity stress. The increased activity of SOD enhances the mechanism of tolerance in plants by eliminating ROS generated via salinity stress (Chen et al. 2018; El-Metwally et al. 2021; Nie et al. 2023). Certainly, plant species with these

mechanisms of action can survive and grow well in stressful environments.

Despite the significance of tolerance mechanisms, more energy is consumed, impeding the plant growth and development (Munns et al. 2020a). Therefore, plant breeding programs should be had genetic diversity to improve the salt tolerance (Munns et al. 2000). In this connection, the current study reported a remarkable variability not only in physiological responses but also in molecular performance between the red and green species of amaranth, affording genetic materials for gaining salt-tolerant genotypes. Herein, our data demonstrated the efficacy of red amaranth in accumulating considerable levels of the four water-related marker genes (SOS1, HKT1, NHX1, and DGR2) that encoding adaptation-related proteins under salt stress. As documented by several salinity studies, salinity stress sorely influenced the ionic homeostasis (El Nahhas et al. 2021) which consequently affects the fastness of cell membrane with toxicity of the amassed Na^+ ions on the action of membrane transporter proteins (Alsamadany et al. 2022). In salinity-resistant strains, compartmentalization of Na^+ in plant cell vacuole, preventing the interference with cytosolic enzyme action is one of the efficient approaches to tolerating ion toxicity (Chinnusamy et al. 2005). Diversified NHX transporters with activation of PPi-ATPase or V-ATPase are responsible for transferring Na^+ to vacuole (Joshi et al. 2022; Zhang et al. 2022). Na^+ exclusion is another way to tolerate the toxic ions (Yang and Guo 2018; Flowers et al. 2019). The ejaculation of Na^+ out of plant root correlates with SOS1 transporter. Mutation occurring in genes coding for SOS2 and SOS3 proteins (regulate the activity of SOS1) may have a favorable influence on the ejaculation potency (Malakar and Chattopadhyay 2021). It has been recorded that some salt-tolerant genotypes of amaranth had lower amounts of Na^+ than the sensitive ones (Atou et al. 2023). HKT proteins included in recapture of Na^+ from xylem sap considered another molecular target that plainly impacts Na^+ transport to leaves (Gandonou et al. 2011).

5 Conclusions

Eventually, the present work has demonstrated the importance of red amaranth as a promising gene cluster that can be exploited in improving salt stress tolerance of genotypes of the *Amaranthus* genus. Herein, compared to green amaranth, red amaranth had greater activity of superoxide dismutase and higher efficiency to increase the expression of genes which encode adaptation-related proteins under salinity stress. However, the high chlorophyll and proline content with low malondialdehyde activity in green amaranth should be taken into account in breeding programs for

salt tolerance. Thus, the correlation between physiological parameter and gene expression together is great of significant to evaluate and screen which genotype and plant species are more tolerant to salt stress.

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Data availability The datasets used and/or analyzed during the current study available from the corresponding author on reasonable request.

Declarations

Conflict of interest The authors declare no conflict of interest.

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