



Received: 28 March 2022
Accepted: 19 April 2022
First Published: 30 April 2022

*Corresponding author: Jatin Kumar,
Department of Life Sciences, School
of Basic Sciences and Research
(SBSR), Sharda University, Greater
Noida - 201310, Uttar Pradesh, India
E-mail: jatin.kumar1@sharda.ac.in

Additional information is available at
the end of the article

ORIGINAL RESEARCH

Biochemical and physiological tolerance evaluation of the oil-yielding paradise tree (*Simarouba amara* Aubl.) under salt stress

Amit K Das¹, Amit Kumar Rai², Kaushal Bhargava³ and Jatin Kumar^{1*}

Abstract: *Simarouba amara* Aubl., a native of tropical regions of South and Central America was introduced in India for wasteland reclamation purposes. Present study was conducted to evaluate its ability to grow under salt stress conditions. *S. amara* seedlings were exposed to different concentrations of sodium chloride (50 mM, 100 mM, 150 mM, and 200 mM). Morphologically, at higher salt concentrations (150 and 200 mM NaCl), *S. amara* seedlings showed symptoms of leaf chlorosis, wilting, and drooping. A significant reduction in leaf relative water content, photosynthetic pigments and increase in lipid peroxidation was observed under high salt stress conditions. Under salt stress, levels of both enzymatic as well as non-enzymatic antioxidants increased majorly. At 200 mM NaCl, proline and cysteine content increased by 116% and 166% respectively. Among antioxidant enzymes, maximum increase in activity was observed in glutathione reductase (320%) followed by superoxide dismutase (129%), ascorbate peroxidase (80%), guaiacol peroxidase (80%) and catalase (59%) at 200 mM NaCl. A significant increase in the activities of both enzymatic and non-enzymatic antioxidants reveals that *S. amara* has better defensive mechanism against salt stress, but up to 100 mM concentration. At 150 and 200 mM NaCl, salt toxicity hampered growth of the plants.

Keywords: *Simarouba amara*; antioxidants; lipid peroxidation; Salt stress

1. Introduction

The world population is expected to be cross 9 billion by 2050 and with such fast growing numbers, there is an extreme stress on agriculture to satisfy the demands of food, fodder, fiber, etc. (Y. Ashraf et al., 2020). Agriculture is also getting affected due to various factors such as huge reduction in cultivable lands, urbanization, unusual weather patterns, etc. Among different environmental stress that have negative impact on agriculture, enhancement of salinity in cultivable lands (soil salinity) is the most prevalent one that hampers the crop productivity majorly. Soil salinity takes place due to various natural processes like weathering, sea water deposition, etc. or due to different human activities (irrigation with brackish ground water, improper drainage system, etc.) (Evelin et al., 2019). Such salt affected lands are not fertile and are not suitable for crop production/ agriculture. Due to salt stress, different phases of plant life are affected such as seed germination, vegetative growth and reproducibility, consequently leading to reduced growth and development in plants. Toxic effects of salt stress on plants include osmotic stress and oxidative stress due to reactive oxygen species (ROS) production which further lead to osmotic and ionic imbalance, damage to functional, structural proteins, DNA, and cell membrane, reduced photosynthesis, etc. (Shrivastava, Kumar, et al., 2015); (Tanveer, Yousaf, et al., 2020). Ion toxicity (due to high Na⁺ concentration) in plants affects various physiological activities which may lead to chlorosis and necrosis (Acosta-Motos et al., 2017). To mitigate the toxic effects of soil salinity, plants also have defensive mechanism which includes activation of enzymatic and non-enzymatic antioxidants, ion homeostasis, formation of com-



patible osmolytes, polyamines, nitric oxides, etc. Adaptive mechanism under salt stress conditions also include morphological changes. Thus, it is of high importance to explore some novel plants which have better defensive system and can tolerate salt stress conditions and can be grown/cultivated under high soil salinity conditions. Effect of salt stress has been recently investigated in various plants such as *Vigna unguiculata* (El-Taher et al., 2021), *Lactuca sativa* (Bres et al., 2022), *Phaseolus vulgaris* (Al-huraby and Bafeel, 2022), *Reaumuria soongorica* (Yan et al., 2022), *Capsicum annum* (Sarwar et al., 2022), etc. In plants, salt tolerant mechanism includes avoidance (salt exclusion, salt dilution, etc.) and tolerance (osmotic adjustment, hormone synthesis, etc.). Various heavy salt tolerant tree species are *Populus euphratica*, *Tamarix chinensis*, *Cocos nucifera*, *Avicennia marina*, *Pyrus betulaeifolia*, *Rhus Typhina*, etc. Moderate salt tolerant tree species include *Hippophae rhamnoides*, *Eucalyptus robusta*, *Pistacia chinensis*, *Gleditsia sinensis*, *Quercus rubra*, etc. Light salt tolerant tree species are *Jatropha curcas*, *Ginkgo biloba*, *Pistacia chinensis*, etc. Zhang et al. (2021).

Simarouba amara Aubl., commonly known as paradise tree, belongs to Simaroubaceae family and is a native of tropical regions of South and Central America. It is a polygamodioecious, medium sized, evergreen, and multipurpose oil seed tree that is considered to adapt and grow well on wastelands, therefore useful for wasteland reclamation (Joshi, Joshi, et al., 2008). The tree has well-developed roots, prevents soil erosion and improves soil fertility. In addition, this plant was introduced in India from El Salvador by National Bureau of Plant Genetic Resources (NBPGR) especially for soil conservation and wasteland reclamation purposes. However its ability to grow under salt stress (soil salinity) has not been studied yet. In *S. amara*, effect of water stress and its amelioration through foliar application of salicylic acid have been investigated by Awate, Gaikwad, et al. (2014).

Thus, the aim of the present study was to determine the effect of salinity on *S. amara* plants. As seedlings growth stages of most of the crop plants are the most sensitive stages different stress (Cuartero et al., 2006), two-month-old seedlings were preferred for the stress study.

2. Materials and methods

2.1. Plant material and stress treatments

Seeds of *S. amara* were procured from University of Agriculture Sciences, Bangalore, India. Seeds were surface sterilized with 5% sodium hypochlorite solution for 20 minutes and then washed thoroughly with distilled water. Seeds were sown in clay pots containing autoclaved soil and were allowed to germinate under natural conditions of light, temperature and humidity. After 15-25 days of sowing, *S. amara* seeds started germination. Two-month-old uniform seedlings were transferred to other clay pots containing 4 kg of autoclaved sandy loam soil exposed to different concentrations of NaCl (0 mM, 50 mM, 100 mM, 150 mM, 200 mM). In each pot, three seedlings were planted and pots were placed in green house with $25\pm 2^{\circ}\text{C}$ and 55% relative humidity. Each of these treatments had three replicates and pots without the addition of NaCl served as control. The plants were harvested after 21 days of stress treatment Figure 1 and kept in -80°C for further experiments. All the fine chemicals used in the study were procured from Merck, Life Science, India.

2.2. Phenotypic assay

Morphological traits of all the *S. amara* plants after 21 days of stress treatment were analyzed. Leaf relative water content was determined following the method of Turner, Kramer, et al. (1980) and calculated by the equation: $\text{LRWC}(\%) = \frac{(\text{Dry weight of sample} - \text{Fresh weight of sample})}{(\text{Turbid weight of sample} - \text{Fresh weight of sample})} \times 100$.

2.3. MDA estimation

MDA concentration was determined following the method of Heath, Packer, et al. (1968). For each reaction, 250 mg of leaf material was homogenized in 5 mL of 1% trichloroacetic acid (TCA) and centrifuged at $8,500 \times g$ for 10 min at 4°C , after which 1 mL supernatant was collected

and was mixed with 4 mL of TCA/TBA reagent (0.5% TBA in 20% TCA) and then heated at 95°C for 30 min. After incubation, samples were centrifuged at 10,000 x g for 10 min at 4°C and specific and non-specific absorbance was measured spectrophotometrically at 535 and 600 nm, respectively.

2.4. Photosynthetic pigments

Leaf discs (100 mg) were soaked in 80% (v/v) acetone solution and kept in dark overnight. The absorbance of the acetone extracts was measured at 663, 645 and 470 nm. The concentrations of chlorophyll a and chlorophyll b were calculated using Arnon's equations (Arnon et al., 1949) and carotenoids content was measured following formula given by Lichtenthaler, Wellburn, et al. (1983).

2.5. Determination of sodium ions

Quantification of sodium ions in the aqueous extract of *S. amara* leaves was done by following protocol given by Weimberg (1987) with a flame photometer.

2.6. Non-enzymatic antioxidants

2.6.1. Proline estimation

Proline content was measured by following protocol given by Bates et al. (1973). Proline was extracted from 200 mg of leaves with 5 mL of 3% sulfosalicylic acid. The homogenate was centrifuged at 15,000 x g for 10 min at 4°C and 2 mL of supernatant obtained was reacted with 2 mL of glacial acetic acid and 2 mL of acid ninhydrin. The reaction mixture was heated at 100°C for 1 hr followed by cooling in ice. Then, 4mL of toluene was added with vortexing for diffusion of chromophore from aqueous to non-aqueous phase. Absorbance of aqueous phase was measured at 520 nm and proline concentration was determined from proline standard curve.

2.6.2. Cysteine estimation

Gaitonde method (1967) was followed for determination of cysteine content which was extracted from 250 mg of leaves with 5 mL of 5% chilled perchloric acid (v/v). The homogenate was centrifuged at 10,000 x g for 10 min at 4°C. Supernatant obtained (0.5 mL) was added to 0.5 mL of acid ninhydrin reagent II and 0.5 ml of glacial acetic acid. The reaction mixture was heated at 100°C for 15 min and pink color was developed. The absorbance was measured at 560 nm.

2.7. Antioxidant enzyme assays

S. amara leaves (1 g) were ground to powder in liquid nitrogen using pre-chilled mortar and pestle. The powder was homogenized with 4 mL of chilled 0.2 M phosphate buffer (pH 7.8) containing 0.1 mM EDTA. The homogenate was centrifuged at 13,000 x g at 4°C for 20 min. The supernatant was used for all the enzyme activity assays. Total soluble protein of the extract was measured following Bradford assay (Bradford et al., 1976) employing bovine serum albumin (BSA) as the standard. All the assays were performed thrice.

2.7.1. SOD (EC 1 15 1 1 assay)

Superoxide dismutase activity was determined employing modified NBT method Beyer and Fridovich (1987). The reaction mixture with a total volume of 3 mL contained 60 mM phosphate buffer (pH 7.5), 2 μ M riboflavin, 0.1 mM Na₂EDTA, 13 mM L-methionine, 70 μ M NBT and 50 μ L of enzyme extract. Samples were incubated for 15 min under two 15 W fluorescent tubes. Absorbance was recorded spectrophotometrically for 3 min at 560 nm.

2.7.2. CAT (EC 1 11 1 6 assay)

Catalase activity was measured by the procedure described by Aebi et al. (1984). CAT activity was initiated by adding 50 μ L enzyme extract into the reaction mixture containing 90 mM phosphate buffer (pH 7.0) and H₂O₂ (18 mM). Decomposition of H₂O₂ was measured spectrophotometrically at 240 nm for 100 s at 25°C.

2.7.3. GPX (EC 1 11 1 7 assay)

Guaiacol peroxidase assay was conducted following the method of Thimmaiah et al. (1999). The reaction mixture (3 mL) contained 65 mM phosphate buffer (pH 6.0), 2.25 mM guaiacol, 11 mM H₂O₂ and 50 μ L enzyme extract. The increase in absorbance was monitored spectrophotometrically at 470 nm for 300 s at 25°C.

2.7.4. APX (EC 1 11 1 11 assay)

Ascorbate peroxidase assay was conducted employing modified method of Nakano and Asada (1981). The reaction mixture contained 80 mM phosphate buffer (pH 7.0), 0.5 mM ascorbic acid, 0.6 mM H₂O₂, 0.1 mM EDTA, and 0.2 mL enzyme extract. Absorbance changes were recorded spectrophotometrically for 500 s at 290 nm.

2.7.5. GR (EC 1 6 4 2 assay)

Glutathione reductase activity was measured following the protocol of Schaedle and Bassham (1977). Enzymatic reaction was initiated by adding 50 μ L enzyme extract to the reaction mixture containing 60 mM phosphate buffer (pH 7.5), 10 mM NADPH, 10 mM GSSG and 6.25 mM MgCl₂. Changes in absorbance were measured spectrophotometrically at 340 nm for 15 min.

2.8. Statistical analysis

All data in the present study was represented as mean \pm standard deviation of the mean (SD) and was analyzed employing one-way ANOVA through SPSS version 22.0 software. The differences between treatments were detected using Duncan's multiple range tests (P<0.05).

3. Results

3.1. Morphology analysis and leaf relative water content (LRWC determination)

Under salt stress conditions, best plant growth was observed at 50 mM NaCl followed by 100 mM NaCl concentration. At higher salt concentrations (150 and 200 mM NaCl), *S. amara* seedlings showed symptoms of leaf chlorosis, wilting, and drooping. Leaf relative water content (LRWC) of control seedlings was found to be 69.74% which further reduced when exposed to different concentrations of NaCl. NaCl treated seedlings exhibited 59.55%, 57.84%, 54.62% and 48.37% LRWC at 50 mM, 100 mM, 150 mM and 200 mM concentrations respectively Figure 2. Decrease in LRWC at maximum NaCl concentration (200 mM) was 30% Table 1.

3.2. MDA estimation

S. amara seedlings showed increase in MDA content when exposed to different NaCl concentrations. Control seedlings exhibited 13.12 μ mol g⁻¹ FW MDA content and with increase in stress concentrations, MDA content enhanced significantly (Figure 2D-F). MDA content was found to be 14.19 μ mol g⁻¹ FW, 21.07 μ mol g⁻¹ FW, 23.44 μ mol g⁻¹ FW and 24.73 μ mol g⁻¹ FW at 50 mM, 100 mM, 150 mM and 200 mM NaCl concentrations respectively (Figure 2D). Percentage increase in MDA content at maximum NaCl concentration (200 mM) was 88% (Table 1).

3.3. Photosynthetic pigments

NaCl stress resulted in decrease in photosynthetic pigments (chlorophyll a, chlorophyll b and carotenoids) in *S. amara* seedlings (Figure 2G-J). Control seedlings exhibited 1.43 mg g⁻¹ FW,

1.003 mg g⁻¹ FW and 10.44 mg g⁻¹ FW chlorophyll a, chlorophyll b and carotenoids contents respectively and with increase in stress concentrations, photosynthetic pigments reduced significantly. *S. amara* seedlings treated with different NaCl concentrations (50 mM, 100 mM, 150 mM and 200 mM), exhibited 0.67 mg g⁻¹ FW, 1.03 mg g⁻¹ FW, 0.88 mg g⁻¹ FW and 0.77 mg g⁻¹ FW chlorophyll a content respectively. At same NaCl range, chlorophyll b content was found to be 0.63 mg g⁻¹ FW, 0.47 mg g⁻¹ FW, 0.11 mg g⁻¹ FW and 0.07 mg g⁻¹ FW respectively. Carotenoids content was 9.32 mg g⁻¹ FW, 9.87 mg g⁻¹ FW, 5.27 mg g⁻¹ FW and 5.06 mg g⁻¹ FW respectively at similar NaCl range (Figure 2G). Decrease in chlorophyll a, chlorophyll b and carotenoids content at maximum NaCl concentration (200 mM) was 46%, 93% and 51% respectively (Table 1).

3.4. Determination of sodium ions (Na⁺)

Content of sodium ions (Na⁺) in the leaf samples of *S. amara* seedlings exposed to 0 mM, 50 mM, 100 mM, 150 mM and 200 mM NaCl concentrations was found to be 305 μmol g⁻¹ DW, 525 μmol g⁻¹ DW, 764 μmol g⁻¹ DW, 1156 μmol g⁻¹ DW, 1450 μmol g⁻¹ DW, respectively.

3.5. Non-enzymatic antioxidants

3.5.1. Proline estimation

NaCl stress resulted in increase in proline content. Control seedlings exhibited 26.19 μmol g⁻¹ FW proline content. Proline concentration significantly increased with increase in different stress concentrations (Figure 3A-C). *S. amara* seedlings exposed to increasing NaCl concentrations (50 mM, 100 mM, 150 mM and 200 mM) revealed proline content of 27.63 μmol g⁻¹ FW, 35.5 μmol g⁻¹ FW, 37.44 μmol g⁻¹ FW and 56.74 μmol g⁻¹ FW respectively (Figure 3A). Increase in proline content at 200 mM NaCl concentration was found to be 116% (Table 1).

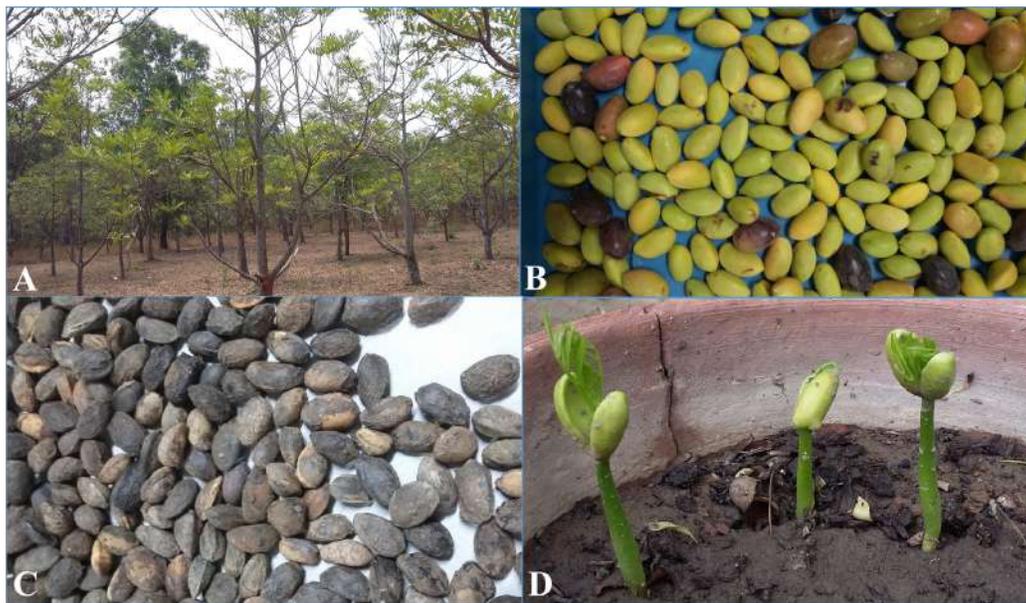


Figure 1. *S. amara* (A) trees growing at the University of Agricultural Sciences (U.A.S.), Bangalore, Karnataka, India, (B) fruits, (C) seeds, and (D) fifteen-day-old seedlings growing under controlled conditions.

Table 1. Effects of NaCl stress on various physiological and biochemical parameters in terms of percentage increase (↑) or decrease (↓) in their activities or contents compared to control.

	Control	NaCl	
		200 mM	% ↑ or ↓
LRWC (%)	69.74	48.37	↓ 30.64
MDA content ($\mu\text{mol g}^{-1}$ FW)	13.12	24.73	↑ 88.49
Chl. a content (mg g^{-1} FW)	1.43	0.77	↓ 46.15
Chl. b content (mg g^{-1} FW)	1.003	0.07	↓ 93.02
Carotenoids content (mg g^{-1} FW)	10.44	5.06	↓ 51.53
SOD activity (Unit $\text{mg}_{\text{protein}}^{-1}$)	6.25	14.35	↑ 129.60
CAT activity ($\text{mmol}_{\text{H}_2\text{O}_2}\text{min}^{-1} \text{mg}_{\text{prot}}^{-1}$)	0.62	0.99	↑ 59.68
APX activity ($\text{mmol}_{\text{Vit.C}}\text{min}^{-1} \text{mg}_{\text{prot}}^{-1}$)	1.57	2.84	↑ 80.89
GPX activity ($\text{mmol}_{\text{tetraguaiacol}}\text{min}^{-1} \text{mg}_{\text{prot}}^{-1}$)	0.05	0.09	↑ 80.00
GR activity ($\text{mmol}_{\text{NADPH}}\text{min}^{-1} \text{mg}_{\text{prot}}^{-1}$)	0.05	0.21	↑ 320.00
Proline content ($\mu\text{mol g}^{-1}$ FW)	26.19	56.74	↑ 116.65
Cysteine content ($\mu\text{mol g}^{-1}$ FW)	20.17	53.84	↑ 166.93



Figure 2. Effect of different concentrations of NaCl on two-month-old *Simarouba amara* seedlings.

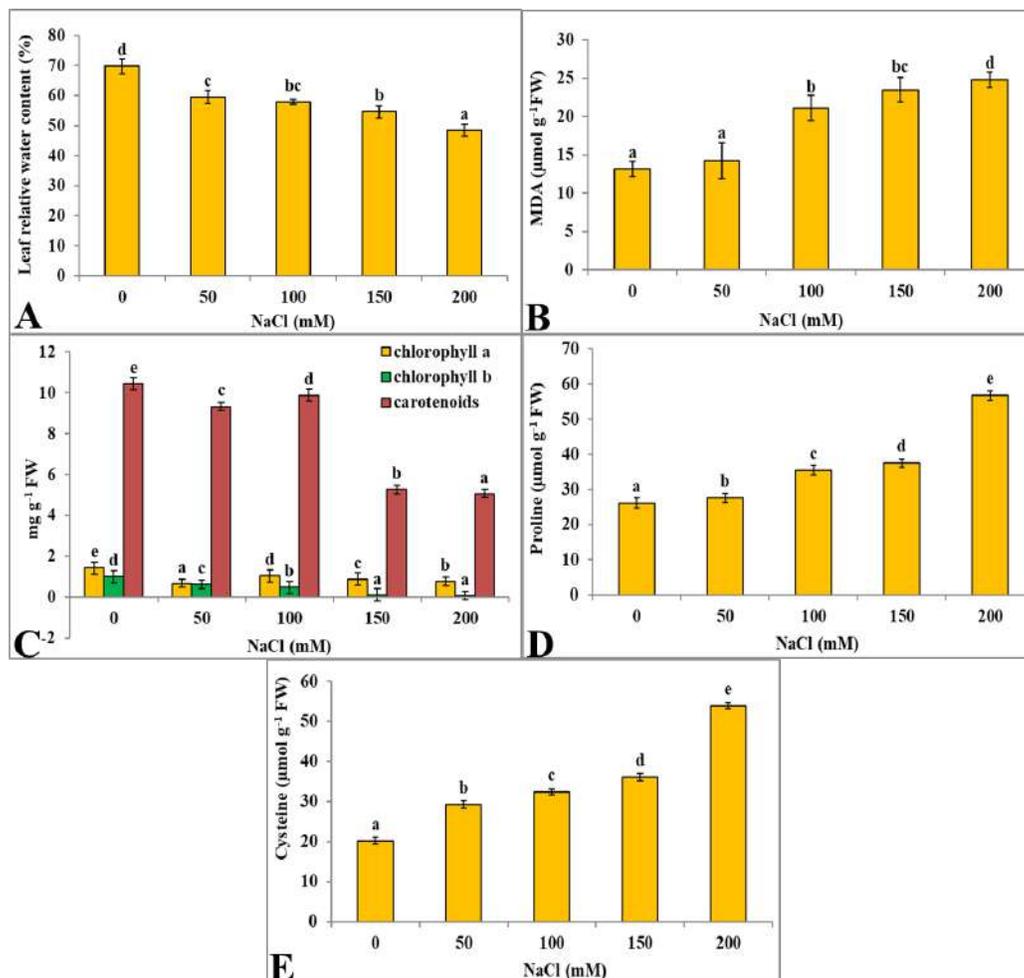


Figure 3. (A) Mean leaf relative water content (%), (B) MDA content, (C) photosynthetic pigments (chlorophyll a, chlorophyll b and carotenoids), (D) proline, and (E) cysteine contents in leaves of 2-month-old *S. amara* seedlings after 3 weeks in soil inoculated with different concentrations of NaCl. Values are means \pm SD (n = 9). Bars with the different letters represent significant differences between treatments at $p < 0.05$.

3.5.2. Cysteine estimation

S. amara seedlings exposed to NaCl stress resulted in increase in cysteine concentration. Control seedlings exhibited $20.17 \mu\text{mol g}^{-1}$ FW cysteine content and with increase in stress concentrations, cysteine content enhanced significantly (Figure 3D-F). Cysteine content was $29.28 \mu\text{mol g}^{-1}$ FW, $32.41 \mu\text{mol g}^{-1}$ FW, $36.07 \mu\text{mol g}^{-1}$ FW and $53.84 \mu\text{mol g}^{-1}$ FW in NaCl treated seedlings at 50 mM, 100 mM, 150 mM and 200 mM concentrations respectively (Figure 3D). Percentage increase in cysteine content at maximum NaCl concentration (200 mM) was 166% (Table 1).

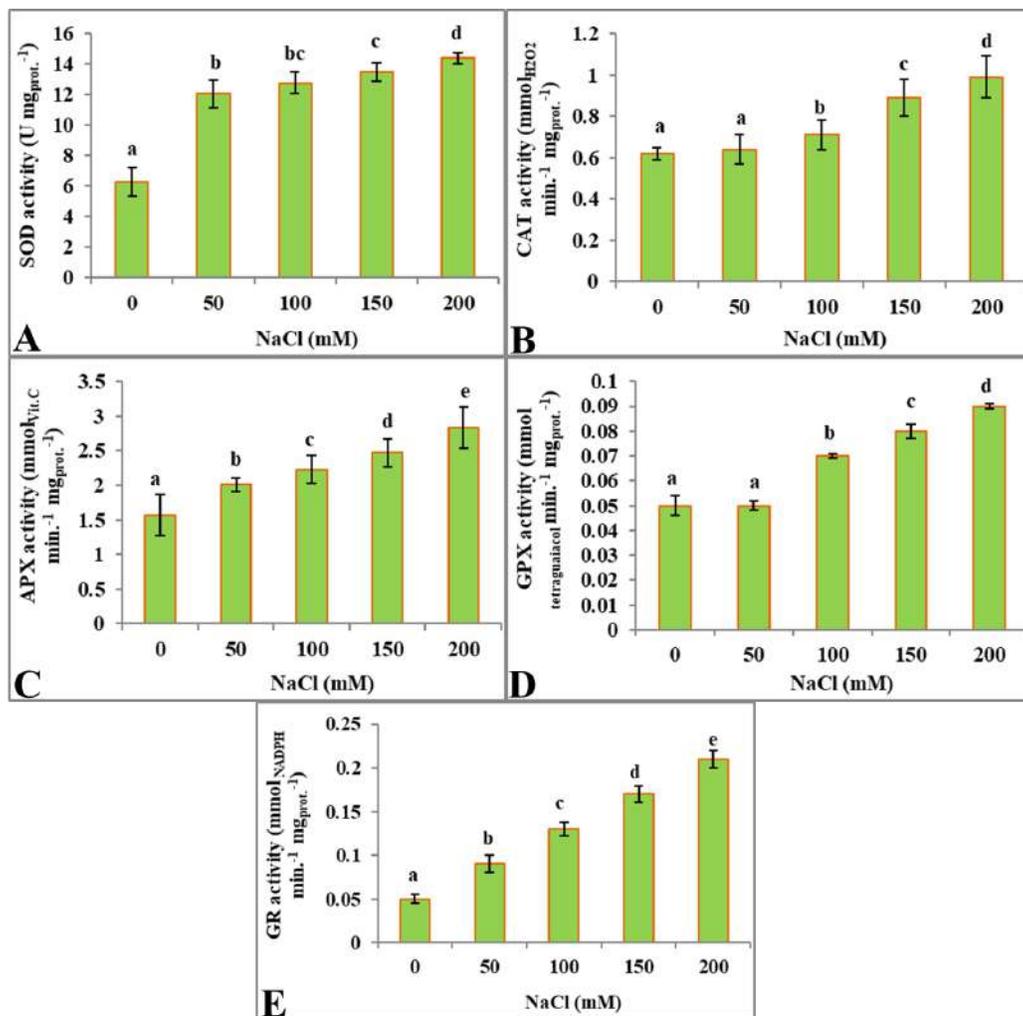


Figure 4. Effect of different concentrations of NaCl on SOD (A), CAT (B), APX (C), GPX (D), and GR (E) activities in leaves of 2-month-old *S. amara* seedlings exposed for three weeks in soil. Values are means \pm SD (n = 9). Bars with the different letters represent significant differences between treatments at $p < 0.05$.

3.6. Antioxidant enzyme assays

3.6.1. SOD assay:

In *S. amara* seedlings, NaCl stress resulted in increase in SOD activity. Control seedlings exhibited 6.25 Unit $\text{mg}_{\text{protein}}^{-1}$ SOD activity and with increase in different stresses concentrations, SOD activity increased significantly in each case (Figure 4A, F, K, P). SOD activity was 12.03 Unit $\text{mg}_{\text{protein}}^{-1}$, 12.74 Unit $\text{mg}_{\text{protein}}^{-1}$, 13.47 Unit $\text{mg}_{\text{protein}}^{-1}$ and 14.35 Unit $\text{mg}_{\text{protein}}^{-1}$ in *S. amara* seedlings exposed to 50 mM, 100 mM, 150 mM and 200 mM NaCl concentrations respectively (Figure 4A). SOD activity increased 129% at maximum NaCl concentration (200 mM) (Table 1).

3.6.2. CAT assay:

A significant increase in CAT activity was revealed, when *S. amara* seedlings were exposed to different concentrations of NaCl. CAT activity of control seedlings was found to be $0.62 \text{ mmol}_{\text{H}_2\text{O}_2}\text{min}^{-1} \text{ mg}_{\text{prot}}^{-1}$ and it increased significantly due to each stress (Figure 4B,G, L,Q). NaCl treated seedlings resulted in CAT activity of $0.64 \text{ mmol}_{\text{H}_2\text{O}_2}\text{min}^{-1} \text{ mg}_{\text{prot}}^{-1}$, $0.71 \text{ mmol}_{\text{H}_2\text{O}_2}\text{min}^{-1} \text{ mg}_{\text{prot}}^{-1}$, $0.89 \text{ mmol}_{\text{H}_2\text{O}_2}\text{min}^{-1} \text{ mg}_{\text{prot}}^{-1}$ and $0.99 \text{ mmol}_{\text{H}_2\text{O}_2}\text{min}^{-1} \text{ mg}_{\text{prot}}^{-1}$ in at 50 mM, 100 mM, 150 mM and 200 mM concentrations respectively (Figure 4B). CAT activity increased by 59% at 200 mM NaCl concentration (Table 1).

3.6.3. APX assay:

APX activity increased significantly in *S. amara* seedlings treated with different concentrations of NaCl. Control seedlings exhibited $1.57 \text{ mmol}_{\text{Vit.C}}\text{min}^{-1} \text{ mg}_{\text{prot}}^{-1}$ of APX activity which increased significantly in each case (Figure 4C,H, M,R). APX activity was $2.01 \text{ mmol}_{\text{Vit.C}}\text{min}^{-1} \text{ mg}_{\text{prot}}^{-1}$, $2.23 \text{ mmol}_{\text{Vit.C}}\text{min}^{-1} \text{ mg}_{\text{prot}}^{-1}$, $2.47 \text{ mmol}_{\text{Vit.C}}\text{min}^{-1} \text{ mg}_{\text{prot}}^{-1}$ and $2.84 \text{ mmol}_{\text{Vit.C}}\text{min}^{-1} \text{ mg}_{\text{prot}}^{-1}$ in NaCl treated seedlings at 50 mM, 100 mM, 150 mM and 200 mM concentrations respectively (Figure 4C). APX activity increased by 80% at 200 mM NaCl concentration (Table 1).

3.6.4. GPX assay:

GPX activity of control seedlings was found to be $0.05 \text{ mmol}_{\text{tetraguaiacol}}\text{min}^{-1} \text{ mg}_{\text{prot}}^{-1}$. Compared to control seedlings, GPX activity was found higher in *S. amara* seedlings exposed to different NaCl concentrations. GPX activity increased significantly with increase in concentrations of each stress treatment (Figure 4D,I, N,S). In NaCl treated seedlings, GPX activity was $0.05 \text{ mmol}_{\text{tetraguaiacol}}\text{min}^{-1} \text{ mg}_{\text{prot}}^{-1}$, $0.07 \text{ mmol}_{\text{tetraguaiacol}}\text{min}^{-1} \text{ mg}_{\text{prot}}^{-1}$, $0.08 \text{ mmol}_{\text{tetraguaiacol}}\text{min}^{-1} \text{ mg}_{\text{prot}}^{-1}$ and $0.09 \text{ mmol}_{\text{tetraguaiacol}}\text{min}^{-1} \text{ mg}_{\text{prot}}^{-1}$ at 50 mM, 100 mM, 150 mM and 200 mM of NaCl concentrations respectively (Figure 4D). GPX activity increased by 80% at maximum NaCl concentration (200 mM) (Table 1).

3.6.5. GR assay:

GR activity significantly increased in *S. amara* seedlings exposed to different NaCl concentrations. Control seedlings exhibited $0.05 \text{ mmol}_{\text{NADPH}}\text{min}^{-1} \text{ mg}_{\text{prot}}^{-1}$ of GR activity, which significantly increased with increase in stress concentrations in each case (Figure 4E, J, O, T). In NaCl treated *S. amara* seedlings, GR activity was $0.09 \text{ mmol}_{\text{NADPH}}\text{min}^{-1} \text{ mg}_{\text{prot}}^{-1}$, $0.13 \text{ mmol}_{\text{NADPH}}\text{min}^{-1} \text{ mg}_{\text{prot}}^{-1}$, $0.17 \text{ mmol}_{\text{NADPH}}\text{min}^{-1} \text{ mg}_{\text{prot}}^{-1}$ and $0.21 \text{ mmol}_{\text{NADPH}}\text{min}^{-1} \text{ mg}_{\text{prot}}^{-1}$ at 50 mM, 100 mM, 150 mM and 200 mM of NaCl concentrations respectively (Figure 4E). GR activity increased by 320% at maximum NaCl concentration, i.e. at 200 mM (Table 1).

4. Discussion

Salinity stress is among the major abiotic stress affecting growth and development of plants. Various salt tolerant tree species have already been identified such as are *Populus euphratica*, *Tamarix chinensis*, *Cocos nucifera*, *Avicennia marina*, *Pyrus betulaefolia*, *Rhus Typhina*, etc are heavy salt tolerant tree species. Moderate salt tolerant tree species include *Hippophae rhamnoides*, *Eucalyptus robusta*, *Pistacia chinensis*, *Gleditsia sinensis*, *Quercus rubra*, etc. Light salt tolerant tree species are *Jatropha curcas*, *Ginkgo biloba*, *Pistacia chinensis*, etc. (Zhang et al., 2021). Identification of more salt-tolerant trees is an effective measure to increase the germplasm resources of salt-tolerant trees and improve saline soil management.

Simarouba amara is a multipurpose tree that is considered to grow well under different agro climatic conditions and on wastelands Kumar, Agrawal, et al. (2015). However, its ability to grow under salt stress conditions has not been studied yet. Present study was aimed to evaluate the effects of salt stress (NaCl) on morphology, antioxidants, lipid peroxidation and physiological activities in *Simarouba amara* DC. seedlings. In the current study, higher salt conditions (150

and 200 mM NaCl) proved to be toxic to *S. amara* seedlings in terms of morphology, leaf relative water content (LRWC), lipid peroxidation, and photosynthetic pigments. At higher NaCl concentrations, *S. amara* seedlings showed symptoms of leaf chlorosis, wilting, and drooping, which could be due to the accumulation of sodium ions in leaves in higher amount, affecting different physiological processes and causing ion toxicity. Presence of high toxic Na⁺ ions in the plants hampers the uptake of K⁺ ions, affecting stomatal conductivity, consequently leading to severe water loss, necrosis, etc. In the present study too, significant decrease in LRWC in *S. amara* seedlings was also observed under NaCl stress due to osmotic stress induction. Similarly, a decrease in LRWC was observed in *Rosmarinus officinalis* L. exposed to 50 and 100 mM NaCl concentrations (Mehrizi et al., 2012). Omami and Hammes (2006) also observed a decrease in LRWC in *A. tricolor* and *A. cruentus* plants exposed to PEG and NaCl treatments (separately and combined). Both of these stresses also resulted in decrease in leaf relative water potential in other studies (Kaydan & Yagmur, 2008) ; (Hassan et al., 2015).

Salt stress results in oxidative stress due to the formation of reactive oxygen species (ROS), which can affect the formation of important biomolecules like DNA, lipids, etc. Membrane lipid peroxidation is the major consequence of ROS, which is measured by calculating the malondialdehyde (MDA) content (?). In our study, high lipid peroxidation was observed and MDA content significantly increased in response to increasing NaCl concentrations, clearly indicating ROS induced oxidative damage. Increase in lipid peroxidation with increase in the concentrations of NaCl has been observed in different studies (Rakhmankulova et al., 2014); (Amirjani, 2015) ; Srivastava et al. (2015) ; Mohamed et al. (2015) ; (Farhoudi et al., 2015) ; (Vromman et al., 2016).

Salinity stress also hampers the photosynthesis process and reduction in chlorophyll levels is generally observed due to oxidative stress. In the present study, photosynthetic pigments (chlorophyll a, chlorophyll b and carotenoids) decreased with increase in concentration of NaCl stress. Salinity stress reduces stomatal conductance and photosynthesis rate of plants (Shabani et al., 2013) by changing various pigments concentrations (Eshghizaden et al., 2012) altering the organelles ultrastructure, stomata opening (Ashraf & Harris, 2013) and modifying different enzymes involved in photosynthesis process (Eshghizaden et al., 2012). Similar to our result, NaCl stress resulted in decrease in photosynthetic pigments in other studies also (Qados et al., 2011) ; (Rakhmankulova et al., 2014) ; (Vromman et al., 2016) ; (Bres et al., 2016) ; (Akcin & Yalcin, 2016).

To mitigate the harmful effects of ROS, plants have active defensive system, comprising of enzymatic and non-enzymatic antioxidants. In the present study, amount of proline and cysteine increased significantly in NaCl treated seedlings, which reveals their protective role under stress conditions. Proline is an amino acid, osmolyte, and antioxidant molecule that is crucial for plants under high salinity stress conditions (radical scavenger). In the present study, proline content increased by 116% at maximum NaCl concentration (200 mM), showing its activity under stress conditions. Similarly, Vijayalakshmi et al. (2016) observed percentage increase of 72.7% and 51.1% in proline content in *Carthamus tinctorius* L. cultivars, TSF1 and SM respectively, at maximum NaCl concentration, i.e. 2%. Ghosh et al. (2011) also observed 3.2 and 3.5 times increase in proline content in two rice varieties (Nonabokra and Pokkali respectively) at 200 mM NaCl concentration. Increase in proline content has also been observed in other study under NaCl stress conducted by Razavizadeh and Ehsanpour (2009). Cysteine is a sulfur containing amino acid and an antioxidant that has ability to trap reactive oxygen species. In the present study, cysteine content increased significantly with an increase in NaCl concentrations. The role of cysteine in conferring protection against ROS damage has also been observed in different studies (Romero et al., 2001) ; (Fediucca et al., 2005) ; (Vijendra et al., 2016) .

Superoxide Dismutase (SOD: EC.1.15.1.1) is a metalloenzyme, that acts firstly to mitigate ROS toxic effects by breaking superoxide radicals into hydrogen peroxide. In the current study, activities of all the antioxidant enzymes significantly increased in response to NaCl stress. In the present study, SOD activity was found to be significantly increased with increasing salt (NaCl) concentrations. Our results are in corroboration with other studies in which SOD activity significantly increased in response to salt stress. In *Lycopersicon esculentum*, Mohamed et al. (2015) observed SOD activity of 25.56 U g⁻¹ dm and 103.03 U g⁻¹ dm in control plants and NaCl treated

(100 mM) plants respectively. [Srivastava et al. \(2015\)](#) also observed an increase in SOD activity in 45-day-old *B. juncea* and *S. portulacastrum* plants, when exposed to NaCl treatments.

Catalase (CAT: EC.1.11.1.6) is a heme-containing enzyme that breaks hydrogen peroxide into hydrogen monoxide and oxygen in peroxisomes. In the present study, CAT activity increased by 59% in response to maximum stress concentrations of NaCl (200 mM). Similar to our results, [Abbasi et al. \(2016\)](#) observed increase in CAT activity in leaves of salt sensitive and salt tolerant maize hybrids under NaCl stress. [Esfandiari et al. \(2007\)](#) also observed significant increase in CAT activity in two cultivars of *T. aestivum* L. (Alvand and Sardari) at low levels of NaCl, however at higher NaCl concentrations (200mM), catalase activity significantly decreased. [Sairam et al. \(2002\)](#); [Heidari \(2009\)](#) and [Barakat et al. \(2011\)](#) and also observed similar results of increase in CAT activity.

Ascorbate peroxidase (APX: EC.1.1.1.1) is a member of Ascorbate-Glutathione (ASC-GSH) cycle that dismutates hydrogen peroxide into hydrogen monoxide and Dehydroascorbate (DHA) in the cytoplasm and chloroplast. In the present study, APX activity also increased by 80% in response to maximum stress concentrations of NaCl (200 mM). Similar to our results, APX activity significantly increased under salt stress in other studies also. [Haddadi et al. \(2016\)](#) observed continuous and significant increase in APX activity in *Mentha aquatica* L. seedlings in response to NaCl stress at 50, 100, 150 mM concentrations. [\(Abbasi et al., 2016\)](#) also observed higher APX activity in leaves of two maize hybrids (26204 and 8441) at 100 mL⁻¹ NaCl concentration compared to control. In another study conducted by [Daneshmand et al. \(2010\)](#), % increase in APX activity at maximum NaCl concentration (120mM) in three Solanum species; *S. stoloniferum*, *S. bulbosum* and *S. acaule* was 330%, 162% and 169% respectively.

Guaiacol peroxidase (GPX: EC.1.11.1.7) is a heme-containing antioxidant enzyme that catalyzes the removal of extra hydrogen peroxide under normal and salinity stress conditions. In the present study, GPX activity increased by 80% at maximum concentrations of NaCl (200 mM) as compared to control. Similarly, GPX activity has also been found to be increased under NaCl stress in *Carthamus tinctorius* L. cultivars, TSF1 and SM ([Vijayalakshmi et al., 2016](#)) and rice varieties, Nonabokra and Pokkali ([Ghosh et al., 2011](#)).

Glutathione Reductase (GR: EC.1.6.4.2) is a flavoprotein oxidoreductase that reduces oxidized glutathione (GSSG) into reduced glutathione (GSH) using NADPH. In the present study, GR activity increased by 320% in *S. amara* seedlings exposed to maximum concentrations of NaCl (200 mM). The increase in GR activity clearly indicating its role in mitigating ROS effects in *S. amara* seedlings. Our results are in corroboration with other studies in which GR activity significantly increased in response to salt stress. [Abbasi et al. \(2016\)](#) observed significant increase in GR activity in leaves of two maize hybrids (26204 and 8441) in response to NaCl, Cd and NaCl+Cd stresses.

5. Conclusions

Present study revealed severe lipid peroxidation, reduction in leaf relative water content and photosynthetic pigments due to toxicity of salt stresses at higher concentrations in *S. amara* seedlings. At higher salt concentrations (150 and 200 mM NaCl), *S. amara* seedlings also showed symptoms of leaf chlorosis, wilting, and drooping. However, in response to salt stress, activities of all antioxidant enzymes (SOD, CAT, APX, GPX and GR) significantly increased. Similarly, increase in concentrations of both non-enzymatic oxidants (proline and cysteine) was observed. A significant increase in the activities of both enzymatic and non-enzymatic antioxidants reveals that *S. amara* has better defensive mechanism against salt stress, but up to 100 mM concentration. At 150 and 200 mM NaCl, salt toxicity hampered growth of the plants. Present study could be very helpful in making strategies of *S. amara* large scale cultivation on wastelands particularly high saline lands.

Acknowledgement

The authors are thankful to the Department of Life Sciences, School of Basic Sciences and Research, Sharda University, Greater Noida, India. Thanks are also conveyed

to the Directors/Professors of University of Agricultural Sciences (U.A.S.), Bangalore, Karnataka, India for permitting us to collect *S. amara* seeds.

Author details

Amit K Das
Department of Life Sciences, School of Basic Sciences and Research (SBSR), Sharda University, Greater Noida - 201310, Uttar Pradesh, India.
Amit Kumar Rai
Institute of Bioresources and Sustainable Development, Mizoram Node, Aizawl, India.
Kaushal Bhargava
Department of Botany, Zakir Husain Delhi College, University of Delhi, Delhi, India.
Jatin Kumar
Department of Life Sciences, School of Basic Sciences and Research (SBSR), Sharda University, Greater Noida - 201310, Uttar Pradesh, India.

Citation information

Cite this article as: Das, A. K., Rai, A. K., Bhargava, K., & Kumar, J. (2022). Biochemical and physiological tolerance evaluation of the oil-yielding paradise tree (*Simarouba amara* Aubl.) under salt stress. *Journal of Innovation in Applied Research*, 05, Article 05. doi: [10.51323/JIAR.5.1.2022.39-51](https://doi.org/10.51323/JIAR.5.1.2022.39-51)

References

- Abbasi, et al. (2016). Profiling of anti-oxidative enzymes and lipid peroxidation in leaves of salt tolerant and salt sensitive maize hybrids under NaCl and Cd stress. *Sains Malay*, 45, 177-184.
- Acosta-Motos, et al. (2017). Plant responses to salt stress: adaptive mechanisms. *Agronomy*, 7, 18-18.
- Aebi, et al. (1984). In: Catalase in Vitro. *Methods in Enzymology. Colowick SP*, 105, 114-121.
- Akcin, & Yalcin. (2016). Effect of salinity stress on chlorophyll, carotenoid content, and proline in *Salicornia prostrata* Pall. and *Suaeda prostrata* Pall. subsp. *prostrata* (Amaranthaceae). *Brazilian Journal of Botany*, 39, 101-106.
- Al-Huraby, et al. (2022). The effect of salinity stress on the *Phaseolus vulgaris* L. plant. *African Journal of Biological Sciences*, 4(1), 94-107.
- Amirjani, M. R. (2015). Effect of salinity stress on seed germination and antioxidative defense system of *Catharanthus roseus*. *ARPN Journal of Agricultural and Biological Science*, 10, 163-171.
- Arnon, et al. (1949). Copper enzymes isolated chloroplasts, polyphenoloxidase in *Beta vulgaris*. *Plant Physiology*, 24, 1-15.
- Ashraf, & Harris. (2013). Photosynthesis under stressful environments: An overview. *Photosynthetica*, 51, 163-190.
- Ashraf, Y., et al. (2020). Economic utilization of salt-affected wasteland for plant production: A case study from Pakistan. *Handbook of Halophytes: From Molecules to. Ecosystems towards Biosaline Agriculture*, 1-24.
- Awate, Gaikwad, et al. (2014). Influence of growth regulators on secondary metabolites of medicinally important oil yielding plant *Simarouba glauca* DC. under water stress conditions. *Journal of Stress Physiology and Biochemistry*, 10, 222-229.
- Barakat, et al. (2011). Oxidative stress markers and antioxidant potential of wheat treated with phytohormones under salinity stress. *Journal of Stress Physiology & Biochemistry*, 250-267.
- Bates, et al. (1973). Rapid determination of free proline for water stress studies. *Plant and Soil*, 39, 205-207.
- Beyer, & Fridovich. (1987). Assaying for superoxide dismutase activity: some large consequences of minor changes in conditions. *Analytical Biochemistry*, 161, 559-566.
- Bradford, et al. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, 72, 248-254.
- Bres, et al. (2016). Responses of pelargonium (*Pelargonium x hortorum* L.H. Bailey) to long-term salinity stress induced by treatment with different NaCl doses. *Acta Physiologiae Plantarum*, 38, 1-11.
- Breś, W., Kleiber, T., Markiewicz, B., Mieloszyk, E., & Mieloch, M. (2022). The effect of NaCl stress on the response of Lettuce. *Lactuca sativa* L.). *Agronomy*, 12(2), 244-244.
- Cuartero, et al. (2006). Increasing salt tolerance in the tomato. *Journal of Experimental Botany*, 57, 1045-1058.
- Daneshmand, et al. (2010). Physiological responses to NaCl stress in three wild species of potato in vitro. *Acta Physiologiae Plantarum*, 32, 91-101.
- Esfandiari, et al. (2007). The effect of salt stress on antioxidant enzymes' activity and lipid peroxidation on the wheat seedling. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, 35, 48-56.
- Eshghizaden, et al. (2012). The mechanisms of salinity tolerance in the xero-halophyte Blue Panicgrass (*Panicum antidotale* Retz). *Notulae Scientia Biologicae*, 4, 59-64.
- Evelin, et al. (2019). Mitigation of salinity stress in plants by arbuscular mycorrhizal symbiosis: current understanding and new challenges. *Frontiers in Plant Science*, 10, 470-470.
- Farhoudi, et al. (2015). Effect of salt stress on physiological and morphological parameters of rapeseed cultivars. *Journal of Scientific Research and Development*, 2, 111-117.
- Fediuca, et al. (2005). O-acetylserine (thiol) lyase activity in *Phragmites* and *Typha* plants under cadmium and NaCl stress conditions and the involvement of ABA in the stress response. *Journal of Plant Physiology*, 162, 865-872.
- Ghosh, et al. (2011). Differential responses of two rice varieties to salt stress. *Plant Biotechnology Reports*, 5, 89-103.
- Haddadi, et al. (2016). Effect of salinity and waterlogging on growth, anatomical and antioxidative responses in *Mentha aquatica* L. *Acta Physiologiae Plantarum*, 38, 1-11.
- Hassan, A., et al. (2015). Effects of salt and water stress on plant growth and on accumulation of osmolytes and antioxidant compounds in cherry tomato. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, 43, 1-11.
- Heath, Packer, et al. (1968). Photoperoxidation in isolated chloroplasts. I. Kinetics and stoichiometry of fatty acid peroxidation. *Archives of Biochemistry and Biophysics*, 125, 189-198.
- Heidari. (2009). Antioxidant activity and osmolyte concentration of sorghum (*Sorghum bicolor*) and wheat (*Triticum aestivum*) genotypes under salinity stress. *Asian Journal of Plant Science*, 8, 240-244.
- Joshi, Joshi, et al. (2008).
- Kaydan, & Yagmur. (2008). Germination, seedling growth and relative water content of shoot in different seed sizes of triticale under osmotic stress of water and NaCl. *African Journal of Biotechnology*, 7, 2862-2868.
- Kumar, Agrawal, et al. (2015). Study of some economically important under-utilized crops for cultivation on wastelands and biotechnology approaches for propagation and gene cloning. *International Journal of Research in BioSciences*, 4, 9-24.
- Lichtenthaler, Wellburn, et al. (1983). Determination of total carotenoids and chlorophyll a and b of leaf extract in different solvents. *Biochemical Society Transactions*, 11, 591-592.
- Mehrzi, et al. (2012). Copper effects on growth, lipid peroxidation, and total phenolic content of Rosemary leaves under salinity stress. *Journal of Agriculture*, 14, 205-212.
- method, G., et al. (1967). A spectrophotometric method for the direct determination of cysteine in the presence of other naturally occurring amino acids. *Biochemical Journal*, 104, 627-633.

- Mohamed, et al. (2015). Role of hydrogen peroxide pretreatment on developing antioxidant capacity in the leaves of tomato plant (*Lycopersicon esculentum*) grown under saline stress. *International Journal of Advanced Research*, 3, 878-879.
- Nakano, & Asada. (1981). Hydrogen peroxide is scavenged by ascorbate specific peroxidase in spinach chloroplasts. *Plant Cell Physiology*, 22, 867-880.
- Omami, & Hammes. (2006). Interactive effects of salinity and water stress on growth, leaf water relations, and gas exchange in amaranth (*Amaranthus* spp.). *New Zealand Journal of Crop and Horticultural Science*, 34, 33-34.
- Qados, et al. (2011). Effect of salt stress on plant growth and metabolism of bean plant *Vicia faba* (L.). *Journal of the Saudi Society of Agricultural Sciences*, 10, 7-15.
- Rakhmankulova, et al. (2014). Effect of NaCl and isoosmotic polyethylene glycol stress on gas exchange in shoots of the C4 xerohalophyte *Haloxylon aphyllum* (Chenopodiaceae). *Photosynthetica*, 52, 437-443.
- Razavizadeh, & Ehsanpour. (2009). Effects of salt stress on proline content, expression of delta-1-pyrroline-5-carboxylate synthetase, and activities of catalase and ascorbate peroxidase in transgenic tobacco plants. *Biological Letters*, 46, 63-75.
- Romero, et al. (2001). Salt regulation of O-acetylserine (thiol) lyase in *Arabidopsis thaliana* and increased tolerance in yeast. *Plant Physiology and Biochemistry*, 39, 643-647.
- Sairam, et al. (2002). Differential response of wheat genotypes to long term salinity stress in relation to oxidative stress, antioxidant activity and osmolyte concentration. *Plant Science*, 163, 1037-1046.
- Sarwar, M., et al. (2022). Triacanthanol regulates morphological traits and enzymatic activities of salinity affected hot pepper plants. *Scientific Reports*, 12(1), 1-8.
- Schaedle, & Bassham. (1977). Chloroplast glutathione reductase. *Plant Physiology*, 53, 1011-1012.
- Shabani, et al. (2013). Growth and physiologic response of rapeseed (*Brassica napus* L.) to deficit irrigation, water salinity and planting method. *International Journal of Plant Production*, 7, 569-596.
- Shrivastava, Kumar, et al. (2015). Soil salinity: A serious environmental issue and plant growth promoting bacteria as one of the tools for its alleviation. *Saudi journal of biological sciences*, 22, 123-131.
- Srivastava, et al. (2015). (Vol. 3).
- Taher, et al. (2021). (Vol. 11).
- Tanveer, Yousaf, et al. (2020). Plant single-cell biology and abiotic stress tolerance. *Plant Life under Changing Environment*, 611-626.
- Thimmaiah, et al. (1999). *Standard methods of biochemical analysis*. New Delhi: Kalyani Publishers.
- Turner, Kramer, et al. (1980). *Adaptation of plants to water and high temperature stress* (N. Turner & P. Kramer, Eds.). New York: Wiley Interscience.
- Vijayalakshmi, et al. (2016). Salt stress induced modulations in growth, compatible solutes and antioxidant enzymes response in two cultivars of safflower (*Carthamus tinctorius* L. Cultivar TSF1 and Cultivar SM) differing in salt tolerance. *American Journal of Plant Sciences*, 7, 1802-1819.
- Vijendra, et al. (2016). Physiological and biochemical changes in Moth Bean (*Vigna aconitifolia* L.) under cadmium stress. *Journal of Botany*, 1-13.
- Vromman, et al. (2016). Salinity influences arsenic resistance in the xerohalophyte *Atriplex atacamensis* Phil. *Environmental and Experimental Botany*, 126, 32-43.
- Weimberg. (1987). Solute adjustments in leaves of two species of wheat at two different stages of growth in response to salinity. *Physiologia Plantarum*, 78, 381-388.
- Yan, et al. (2022). Effect of salt stress on the photosynthetic characteristics and endogenous hormones, and: A comprehensive evaluation of salt tolerance in *Reaumuria soongorica* seedlings. *Plant Signaling & Behavior*.
- Zhang, et al. (2016). Non-destructive determination of Malondialdehyde (MDA) distribution in oilseed rape leaves by laboratory scale NIR hyperspectral imaging. *Scientific Reports*, 6, 1-8.
- Zhang, et al. (2021). Salt tolerance mechanisms in trees: research progress. *Trees*, 35(3), 717-730.