

Physiological and biochemical responses of BARI sunflower-2 (*Helianthus annuus* L.) to sodium chloride-induced salinity stress

F.M. Tonmoy Chowdhury, Farjana Rahman Lopa, M.A. Halim and Nahid Akhtar*

Plant Physiology and Biochemistry Laboratory, Department of Botany,
Jahangirnagar University, Savar, Dhaka-1342, Bangladesh

Abstract

An experiment was conducted to evaluate the physiological and biochemical responses of BARI Sunflower-2 to NaCl-induced salinity stress (0–300 mM). Increasing salinity significantly reduced chlorophyll content, carotenoids, and relative water content, while water saturation deficit and water uptake capacity increased. However, compared to the control, the protein, proline and catalase content increased to 342.14%, 374.55%, and 272.30%, respectively, at 300 mM NaCl treatment. Proline accumulation and enhanced antioxidant enzyme activity indicate adaptive biochemical responses under salt stress. There was also an increase in WSD (67.64%) and WUC (450.47%) at 300 mM NaCl than that of control plants due to increasing NaCl levels. The findings suggest that BARI Sunflower-2 exhibits moderate salinity tolerance through osmotic adjustment and antioxidant defense mechanisms.

Keywords: Salinity, Chlorophyll, Carotenoid, Sunflower, Proline, Catalase.

INTRODUCTION

Salinity stress is one of the serious threats which affected the plant growth and production all around the world (Khanam *et al.*, 2025). Total area of salt affected soil in the world is nearly 7% (Hussain *et al.*, 2013). Sodium (Na^+) is a basic ion which plays a central role in toxicity because it is rapidly absorbed and taken up by root cells of plants (Hasegawa, 2013). Physiological and biochemical changes as well as the process of growth, photosynthesis, respiration, translocation, ion uptake, nutrient metabolism and growth attributes are also affected by salt stress in plants (Schroeder *et al.*, 2013). Salinity can disturb the physiological and biochemical functions of the plant cell, leading to cell death (Afrin *et al.*, 2021a). In addition, salt stress also leads to oxidative stress through an increase in the production of reactive oxygen species. One of the best mechanisms for salinity tolerance is the accumulation of proline into the plant cells. Proline plays a very important role in cell osmotic potential, stability of membrane and detoxification of toxic ions in plants under saline conditions (Akhtar *et al.*, 2021). Sunflower (*Helianthus annuus* L.) has a great importance all over the world because of oil but its production is decreasing in different areas where saline toxicity is rapidly increasing (Caterina *et al.*, 2007). The germination percentage and the yield of sunflower seedlings were significantly reduced by the elevated saline concentration (Chowdhury *et al.*, 2018, 2023). Despite previous reports on sunflower salinity responses, limited information is available regarding the physiological and biochemical adaptation mechanisms of BARI Sunflower-2 under

* Corresponding author. Email: nahid_akhtar98@yahoo.com

variable NaCl stress. Therefore, this study aimed to evaluate water relations, pigment composition, osmolyte accumulation, and antioxidant enzyme activity to understand the cultivar's tolerance mechanism.

MATERIALS AND METHODS

Seeds of BARI Sunflower-2 were collected from Bangladesh Agricultural Research Institute (BARI) and were used as plant material. The seeds were surface sterilized by soaking the seeds in 0.1% sodium hypochlorite for three minutes followed by washing 3 times with tap water and distilled water. Five seeds of uniform size were directly sown into sandy loam soil in the 15 inches earthen pots. Tap water was applied to all pots until seedling emergence. After seedling establishment, tap water was applied to the control pots and 12.5 mM NaCl solution was applied to salt treatments. When the first leaf appeared i.e., ten days after emergence (DAE), actual amount of NaCl solution was applied. Seedlings in the control group were irrigated with tap water. The experiment was carried out in a randomized block design with three replications. On the basis of previous research information (Carbonell-Barrachina and Burlo-Carbonell, 1997), six concentrations of sodium chloride (NaCl) solution, $T_1=50$ mM, $T_2=100$ mM, $T_3=150$ mM, $T_4=200$ mM, $T_5=250$ mM, $T_6=300$ mM and T_0 = distilled water as control were used as treatments. Manures and fertilizers were applied as per the recommendation of Fertilizer Recommendation Guide (2005). The physiological and biochemical analyses were carried out in the Plant Physiology and Plant Biochemistry laboratory of Department of Botany, Jahangirnagar University, Savar, Dhaka. The amounts of Chlorophyll-a, Chlorophyll-b and carotenoids were estimated after 30 DAT (days after treatment) in terms of mg/g fresh weight of the leaf. The fresh, turgid and dry weights of the leaves were used to determine RWC (Relative water content), WRC (Water retention capacity), WSD (Water saturation deficit) and WUC (Water uptake capacity) (Sangakkara *et al.*, 1996). Protein content of leaves was estimated by the method of Lowry *et al.* (1951). Proline content of leaf was extracted and measured by the method of Bates *et al.*, (1973). The proline concentration was determined from a standard curve using D-proline and calculated on a fresh weight basis ($\mu\text{mol/g}$ fresh leaf weight proline). The activity of Catalase (CAT) was determined by the method of Aebi (1983). The activity of CAT was calculated using the extinction coefficient, $0.04 \text{ mM}^{-1} \text{ cm}^{-1}$. The CAT activity was expressed as μkat of $\text{H}_2\text{O}_2 \text{ m}^{-2}$. The data recorded on different parameters were statistically analyzed with the help of Microsoft Excel 2013 and Duncan multiple range test (DMRT) was performed at the 0.05 level of significance using SPSS Program 16.00. The least significant difference (LSD) was also found out after performing ANOVA (Gomez & Gomez, 1984).

RESULTS AND DISCUSSION

Physiological Parameters

Chlorophyll and Carotenoid content: Chlorophyll content of the leaves decreased with the increasing NaCl levels (Table 1). High level of salt stress cause chlorophyll a and chlorophyll b to decrease in rate (Khanam *et al.*, 2025). Reddy and Vora (1986) reported that a decrease in chlorophyll concentration in salinized plants, could be attributed to

increase activity of the chlorophyll degrading enzyme chlorophyllase. The carotenoid contents decreased under NaCl stress as compared to control, which was reported by El-Tayyab (2005). In the present investigation, the mean value of chlorophyll-a decreased from 2.41 mg/g fresh leaf in the control to 0.22 mg/g fresh leaf at 300 mM NaCl level. The mean value of chlorophyll-b decreased from 1.97 mg/g fresh leaf in the control to 0.12 mg/g fresh leaf at 300 mM NaCl level. The mean value for total chlorophyll decreased from 4.38 mg/g fresh leaf in the control to 0.34 mg/g fresh leaf at 300 mM NaCl level, which indicates the ionic toxicity. In case of carotenoid, the contents decreased from 10.49 mg/g fresh leaf in the control to 1.16 mg/g fresh leaf at 300 mM NaCl level.

Table 1. Effect of different levels of salinity on leaf pigments of BARI Sunflower -2

Treatments (NaCl)	Chlorophyll-a (mg/g fresh leaf)	Chlorophyll-b (mg/g fresh leaf)	Total Chlorophyll (mg/g fresh leaf)	Carotenoid (mg/g fresh leaf)
0 mM	2.41a	1.97a	4.38a	10.49a
50 mM	2.01b (83.41%)	1.46b (74.12%)	3.47b (79.22%)	8.04b (76.64%)
100 mM	0.67c (27.8%)	0.37c (18.78%)	1.04c (23.74%)	3.41c (32.50%)
150 mM	0.53d (21.00%)	0.29d (14.72%)	0.82d (18.72%)	2.56d (24.40%)
200 mM	0.46d (19.08%)	0.27d (13.71%)	0.73d (16.67%)	2.37d (22.59%)
250 mM	0.36d (14.93%)	0.22d (11.17%)	0.58e (13.24%)	1.91e (18.20%)
300 mM	0.22e (9.13%)	0.12e (6.09%)	0.34e (7.77%)	1.16e (11.05%)
Cultivar Mean	0.95	0.67	1.63	4.28
LSD (5%)	2.33	2.42	2.85	3.16
CV%	13.52	20.78	14.84	3.5

* Means in a column followed by same letters do not differ significantly at 5% level by DMRT.

* Values within parentheses indicate percentage change over control.

Effect of NaCl on water relations of BARI Sunflower-2

Relative Water Content (RWC): Salinity decreases water potential of salt solution and plants cannot uptake water freely, and consequently RWC decreased (Orcutt and Nilsen, 2000). Results presented in Table 2 revealed that salinity decreased relative water content. In the present study, at the control, the relative water content was 67.45 and the RWC decreased to 45.42 at the highest salinity level (300 mM). RWC was found to be 96.39, 89.16, 76.93, 73.66, 71.43 and 67.33% at 50, 100, 150, 200, 250 and 300 mM NaCl salinity, respectively. However, at the highest salinity level, relative water content decreased to 32.67% than that of the control treatment.

Water Saturation Deficit (WSD): Water saturation deficit indicates the degree of water deficit in plants. Salinity increased the WSD in all the treatments (Table 2). This finding reveal that BARI Sunflower-2 suffered more from water deficit especially at high level of

salinity (Orcutt & Nilsen, 2000). In the present study, at the control, the WSD was 32.55 and at the highest salinity level (300 mM) the WSD increased to 54.57, representing an increase of 67.64%. At 200 mM salinity level, 54.53% WSD was found, whereas only 7.46% WSD was exhibited in 50 mM NaCl salinity level.

Water Retention Capacity (WRC): Water retention capacity (WRC) illustrates the capacity of plant cell to retain water at a particular stage, which is determined by cell structures. Salinity decreased the WRC in all the treatments (Table 2). The higher reduction in WRC (% to the control) indicated a great damage in cell structure due to salinity (Sangakkara *et al.*, 1996). Salinity decreased the WRC from 58.32 at the control to 12.66 at the highest level of salinity (300mM). 78.30% WRC was decreased at 300 mM NaCl salinity. In the present study, WRC was found 45.66, 27.19, 22.62, 19.55 and 14.62 at 50, 100, 150, 200, 250 and 300 mM NaCl salinity, respectively.

Water Uptake Capacity (WUC): The water uptake capacity (WUC) quantifies the capacity of plant cells to absorb water per unit dry weight at a particular stage. A higher WUC under saline condition means a plant is subjected to water stress at a greater degree, because the plant would absorb more water to reach turgidity than the plant under control condition. More water uptake capacity helps crops to uptake relatively more amount of water to reach turgidity (Sangakkara *et al.*, 1996). Results presented in Table 2 showed that, salinity increased the WUC in all the treatments. In the present study, salinity resulted in an increasing WUC from 12.58 in control to 56.67 at the highest level of salinity (300 mM). WUC increased drastically in the highest treatment level of NaCl salinity (300 mM) level which was more than 350.47% than that of control treatment.

Biochemical parameters

Protein content in leaves: Protein contents of leaves gradually increased with the increasing NaCl salinity (Table 3). Uzun *et al.*, (2013) and Kapoor and Srivastava (2010) also reported that increasing salinity level tended to enhance the crude protein synthesis on *Vigna mungo* L. and bitter vetch. In the present study, at the highest salinity level (300 mM), the protein content in leaves increased to 13.52 mg/g fresh tissue. Protein content increased by 30.71, 61.67, 88.83, 135.28, 181.47 and 243.14% in 50, 100, 150, 200, 250 and 300 mM NaCl salinity level, respectively.

Proline content in leaves: Proline contents of leaves gradually increased with the increasing NaCl salinity (Table 3). Ramteke & Karibasappa (2005) observed that significant increase in proline accumulation both in leaves and grains under salt stress relates to osmotic adjustment and it seems that proline performs as osmoticum and therefore, it interferes in salt tolerance. The amount of free proline in the stressed plants increased dramatically as the salinity escalated (Afrin *et al.*, 2021b). In the present study, at the highest salinity level (300 mM), the proline content in leaves was 105.88 mg/g fresh leaf. Proline content increased dramatically at the highest level of NaCl salinity (300 mM), reaching 374.55% higher than that of the control plants. Among seven treatments, proline contents were gradually increased from control treatment to 300 mM NaCl salinity treatments for maintaining the osmotic adjustment. Among these moderate salinity level (150 mM NaCl), proline content was increased 26.47% than that of control plants.

Table 2. Effect of different levels of salinity on relative water content (RWC), water saturation deficit (WSD), water retention capacity (WRC) and water uptake capacity (WUC) of BARI Sunflower-2.

Treatments (NaCl)	RWC	WSD	WRC	WUC
0 mM	67.45a	32.55c	58.32a	12.58d
50 mM	65.02ab (96.39%)	34.98c (107.46%)	45.66b (78.29%)	17.73c (140.93%)
100 mM	60.14ab (89.16%)	39.86bc (122.45%)	27.19c (46.62%)	21.23c (168.75%)
150 mM	51.89ab (76.93%)	48.11bc (147.80%)	22.62c (38.78%)	28.94bc (230.04%)
200 mM	49.69bc (73.66%)	50.30ab (154.53%)	19.55d (33.52%)	35.67ab (283.54%)
250 mM	48.18c (71.43%)	51.81ab (159.17%)	14.62e (25.06%)	40.12ab (318.91%)
300 mM	45.42c (67.33%)	54.57a (167.64%)	12.66e (21.70%)	56.67a (450.47%)
Cultivar Mean	55.4	44.58	28.66	30.42
LSD (5%)	3.64	4.76	2.32	3.67
CV%	19.91	24.73	13.22	22.12

* Means in a column followed by same letters do not differ significantly at 5% level by DMRT.

* Values within parentheses indicate percent change over control.

Table 3. Effect of different levels of salinity on Proline (mg/g) and protein (mg/g) contents and CAT (μkatg^{-1}) enzyme activity of BARI Sunflower-2

Treatments (NaCl)	Protein content (mg/g fresh leaf)	Proline content (mg/g fresh leaf)	CAT activity (μkatg^{-1})
0 mM	3.94d	27.20c	12.82d
50 mM	5.15c (130.71%)	27.60c (101.47%)	14.96d (116.69%)
100 mM	6.37c (161.67%)	32.40bc (119.11%)	17.15c (133.77%)
150 mM	7.44bc (188.83%)	34.40bc (126.47%)	19.47c (151.87%)
200 mM	9.27bc (235.28%)	35.20bc (129.41%)	25.85bc (201.62%)
250 mM	11.09ab (281.47%)	61.20ab (225.00%)	30.66ab (239.16%)
300 mM	13.52a (342.14%)	101.88a (374.55%)	34.91a (272.30%)
Cultivar Mean	8.11	45.84	22.26
LSD (5%)	3.12	5.35	3.02
CV%	22.31	16.63	8.42

* Means in a column followed by same letters do not differ significantly at 5% level by DMRT.

* Values within parentheses indicate percent change over control.

Effect of NaCl on Catalase (CAT) enzyme activity: Salinity increased the Catalase enzyme activity in all the treatments (Table 3). The catalase activity was increased due to salinity stress as reported by Esfandiari *et al.* (2007). Increased SOD, POD and CAT activities are closely related to salt tolerance of many plants as reported in various researches (Rahnama and Ebrahimzadeh, 2005; Azevedo Neto *et al.*, 2006). In the present study, catalase enzyme activity was $34.91 \mu\text{katg}^{-1}$. Catalase enzyme activity was increased dramatically in the highest level of NaCl salinity (300 mM) level which was 172.30% more than that of control plants. At the moderate salinity level (150 mM NaCl), catalase enzyme activity increased 51.87% than that of control plants. This increase in catalase activity indicates that the plant activates its antioxidant defense mechanism to detoxify the excess H_2O_2 produced during salt stress. This response is considered a protective mechanism and an indicator of salinity tolerance in BARI Sunflower-2.

In conclusion, the present study demonstrates that increasing NaCl-induced salinity significantly affected the physiological and biochemical attributes of BARI Sunflower-2. Therefore, despite the adverse effects on growth-related physiological traits, BARI Sunflower-2 exhibits considerable biochemical adaptability under salinity stress, indicating its potential tolerance to high NaCl levels. The increased proline accumulation and catalase activity suggest activation of osmotic and antioxidant defense pathways. However, severe pigment degradation at 300 mM NaCl indicates that tolerance is partial rather than complete. These findings highlight the cultivar's suitability for cultivation in moderately saline environments and provide valuable insights for future salinity management and crop improvement strategies.

REFERENCES

- Aebi, H.E. 1983. Catalase. In: Bergmeyer, H.U., Ed., Methods of enzymatic analysis, Verlag Chemie, Weinheim. 273- 286.
- Afrin, S., Akhtar, N., Khanam, T. and Hossain, F. 2021a. Application of zinc mitigates the salt-induced effects on growth of soybean (*Glycine max* L.). *Int. J. Ecotoxicol. Ecobiol.* **6**(3): 65-70.
- Afrin, S., Akhtar, N., Khanam, T. and Hossain, F. 2021b. Alleviative effects of zinc on biomass yield and antioxidative enzymes activity in leaves of soybean (*Glycine max* L.) under salt stress. *Am. J. Agric. For.* **9**(3):147-155.
- Akhtar, N., Karim, A., Afrin, S. and Hossain, F. 2021. Effects of gibberellic acid and kinetin on germination and ion accumulation in a Bangladesh wheat variety under salt stress conditions. *Eur. J. Biophys.* **9**(2): 86-91.
- Ashraf, M. and Foolad, M.R. 2007. Roles of glycine-betaine and proline in improving plant abiotic stress resistance. *Environ. Exp. Bot.* **59**(2): 206-216.
- Azevedo Neto, A.D. Prico, J.T. Eneas-Filho, J. Bragade Abreu, C.E. and Gomes-Filho, E. 2006. Effect of salt stress on antioxidative enzymes and lipid peroxidation in leaves and roots of salt-tolerant and salt-sensitive maize genotypes. *Environ. Exp. Bot.* **56**(1): 235-241.
- Bangladesh Agricultural Research Council (BARC). 2005. Fertilizer Recommended Guide- 2005. Bangladesh Agricultural Research Council, Farmgate, Dhaka, Bangladesh.
- Bates, L.S., Waldren, R.P. and Teare, L.D. 1973. Rapid determination of free proline for water stress studies. *Plant Soil.* **39**: 205-207.

- Carbonell-Barrachina, A.A. and Burlo-Carbonell, F. 1997. Effect of sodium arsenite and sodium chloride on bean plant nutrition (macronutrients). *J. Plant Nutr.* **20**(11): 1617-1633.
- Caterina, R.D., Giulian, M.M. Rotunno, T.A. Caro, D.A.D. and Flagella, Z. 2007. Influence of salt stress on seed yield and oil quality of two sunflower hybrids. *Ann. Appl. Biol.* **151**(2): 145-154.
- Chowdhury, F.T., Halim, M., Hossain, F. and Akhtar, N. 2018. Effects of sodium chloride on germination and seedling growth of Sunflower (*Helianthus annuus L.*). *Jahangirnagar Univ. J. Biol. Sci.* **7**(1):35-44.
- Chowdhury, F.T., Halim, M., Hossain, F. and Akhtar, N. 2023. Effects of sodium chloride salinity on growth and yield of BARI Sunflower-2 (*Helianthus annuus L.*). *Jahangirnagar Univ. J. Biol. Sci.* **11**(1&2):13-20.
- El-Tayeb, M.A. 2005. Response of barley grains to the interactive effect of salinity and salicylic acid. *Plant Growth Regul.* **45**(3): 215-224.
- Esfandiari, E., Shakiba, M. R, Mahboob, S. Alyari, H. and Toorchi, M. 2007. Water stress, antioxidant enzyme activity and lipid peroxidation in wheat seedling. *J. Food Agric. Environ.* **5**(1): 149-153.
- Gomez, K.A. and Gomez, A.A. 1984. Statistical Procedure for Agricultural Research. 2nd edin, John Wiley, Newyork.
- Hasegawa, P.M. 2013. Sodium (Na⁺) homeostasis and salt tolerance of plants. *Environ. Exp. Bot.* **150**: 19-31.
- Hussain, S., Khaliq, A. Matloob, A. Ashfaq, M. and Afzal, A. 2013. Germination and growth response of three wheat cultivars to NaCl salinity. *Soil Environ.* **32**(1): 36-43.
- Kapoor, K. and Srivastava, A. 2010. Assessment of salinity tolerance of *Vigna mungo* var. Pu-19 Using Ex Vitro and In Vitro Methods. *Asian J. Biotech.* **2**(2): 73-85.
- Khanam, T., Kim, K., Karim, Md. A., Afrin, S., Lopa, F.R., Hoque, M.M., Higuchi, H., Walitang, D.I., Roy, S.K., Sa, T., Akhtar, N. 2025. Salinity tolerance of two rice cultivars is related to enhanced activities of enzymatic antioxidants and higher proline content. *Appl. Biol. Chem.* **68**(1):58.
- Lowry, O.H., Rosenbrough, N.J. Farr, A.L. and Randall, R.J. 1951. Protein measurement with the Folin Phenol Reagent. *J. Biol. Chem.* **193**(1): 265-275.
- Orcutt, D.M. and Nilsen, E.T. 2000. The physiology of plants under stress. John wily and sons, Inc., 605 Third Avenue, New York, NY. 10158-0012, USA. pp. 177-235.
- Rahnama, H. and Ebrahimzadeh, H. 2005. The effect of NaCl on antioxidant enzyme activities in potato seedlings. *Biol. Plant.* **49**(1): 93-97.
- Ramteke, S.D. and Karibasappa, G.S. 2005. Screening of grape (*Vitis vinifera*) genotypes for drought tolerance. *Indian J. Agric. Sci.* **75**(6): 355-357.
- Reddy, M.P. and Vora, A.B. 1986. Changes in pigment composition, hill reaction activity and saccharides metabolism in bajra. *Photosynthetica.* **20**(1):50-55.
- Sangakkara, U.R., Hartwig, U.A and Nosberger, J. 1996. Response of root branching and shoot water potentials of Rench Bean (*Phaseolus vulgaris L.*) to soil moisture and fertilizer potassium. *J. Agron. Crop Sci.* **177**(3): 165-173.
- Schroeder, J.I., Delhaize, E. and Frommer, W.B. 2013. Using membrane transporters to improve crops for sustainable food production. *Nature.* **497**(7447): 0-66.
- Uzun, S., Uzun, O. Kaplan, M. and Ilbas, A.I. 2013. Response of bitter vetch lines to salt stress. *Bulgarian J. Agric. Sci.* **19**(5): 1061-1067.
- Xiong, L. and Zhu, J.K. 2002. Molecular and genetic aspects of plant responses to osmotic stress. *Plant Cell Environ.* **25**(2): 131-139.