



Data Article

Dataset on antioxidant system of non-model halophytes *Urochondra setulosa* and *Dichanthium annulatum* in saline environment

Anita Mann^{a,*}, Naresh Kumar^{a,b}, Charu Lata^{a,c}, Ashwani Kumar^a, Arvind Kumar^a, B.L. Meena^a

^a ICAR-Central Soil Salinity Research Institute, Karnal, Haryana, India

^b Eternal University, Baru Sahib, Himachal Pradesh, India

^c ICAR- Indian Institute of Wheat and Barley Research, RRS, Flowerdale, Shimla, Himachal Pradesh, India

ARTICLE INFO

Article history:

Received 13 February 2023

Revised 5 May 2023

Accepted 19 May 2023

Available online 25 May 2023

Dataset link: [Antioxidant metabolism dataset of Halophytes \(Original data\)](#)

Keywords:

Antioxidative enzymes

Halophytes

Salinity, *Urochondra*

Dichanthium

ABSTRACT

The antioxidant potential of halophytes, *Dichanthium annulatum* and *Urochondra setulosa*, was examined under the influence of high salinity. These halophytes were grown in lysimeters filled with saline soil and further irrigated with saline water to maintain different salt levels of ECe 30, 40 and 50 dS m⁻¹ along with the one set in normal field soil without saline irrigation serving as control. The leaf samples were collected after saline irrigation and analyzed for the antioxidative enzymes *i.e.*, Catalase (CAT), Peroxidase (POX), Superoxide dismutase (SOD), Ascorbate peroxidase (APX), Monodehydroascorbate reductase (MDHAR), Dehydroascorbate reductase (DHAR) and Glutathione reductase (GR), including the ROS metabolites such as H₂O₂ content, malondialdehyde content (MDA), ascorbic acid content and total glutathione content. The mechanism of scavenging the reactive oxygen species in both the halophytes was characterized.

© 2023 The Author(s). Published by Elsevier Inc.

This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>)

* Corresponding author.

E-mail address: Anita.Mann@icar.gov.in (A. Mann).

Specifications Table

Subject	Agricultural and biological science
Specific subject area	Molecular Biology
Type of data	Tables and Figure
How the data were acquired	Spectrophotometer (SPECORD-210 PLUS, Analytic Zena, Germany)
Data format	Raw data
Description of data collection	Leaves of <i>Urochondra setulosa</i> and <i>Dichanthium annulatum</i> were collected in ice bucket and used immediately for the extraction of enzymes and other metabolites in three replications for each treatment per plant species. Enzyme activities were measured at different wavelengths on Spectrophotometer and relative activity for each was calculated as Units mg^{-1} protein FW.
Data source location	Division of Crop Improvement ICAR-Central Soil Salinity Research Institute Karnal-132,001 Haryana India
Data accessibility	Mann, Anita; Kumar, Naresh; Lata, Charu; Kumar, Arvind; Meena, BL; Kumar, Ashwani (2023), "Antioxidant metabolism dataset of Halophytes", Mendeley Data, V1, doi: 10.17632/xd698v9w9s.1 Raw data for both the plants <i>D. annulatum</i> and <i>U. setulosa</i> has been deposited in the repository at Mendeley data and is available at https://data.mendeley.com/datasets/xd698v9w9s

Value of the Data

- The dataset shows the antioxidant potential of two halophytic grasses - *Dichanthium annulatum* and *Urochondra setulosa*, where *Dichanthium annulatum* is moderately salt tolerant with tolerance upto salinity of EC 30 dS m^{-1} (~ 300 mM NaCl), while *Urochondra setulosa* is highly salt tolerant, surviving up to EC 50 dS m^{-1} (~ 500 mM NaCl).
- The survival of these plants under high salt levels make them suitable for exploring their tolerance mechanism and hence, the dataset generated will be useful for plant researchers working on abiotic stresses in related plant species.
- Potential molecular markers/SNPs or m-QTLs can be identified for ROS system for inclusion in genome-editing programs to develop better salt-adaptive crop plants.
- These halophytes, especially *U. setulosa*, can survive with sea water salinity; the potential for saline water irrigation may be investigated in the face of expected freshwater supply limitations, providing a new path for the expansion of biosaline agriculture into unreachable areas.

1. Objective

The objective of this work was to analyze the quantitative role of antioxidative system, the first line of defense, in un-explored halophytes such as *Dichanthium annulatum* (moderately salt tolerant) and *Urochondra setulosa* (extremely salt tolerant) for salinity tolerance mechanism at different salinity levels to pinpoint the exact pathway of detoxification of reactive oxygen species under saline conditions.

2. Data Description

The activities of antioxidative enzymes *i.e.*, Catalase (CAT), Peroxidase (POX), Superoxide dismutase (SOD), Ascorbate peroxidase (APX), Monodehydroascorbate reductase (MDHAR), Dehydroascorbate reductase (DHAR) and Glutathione reductase (GR) of both halophytes at various salt treatments (Control, EC_e 30 dS m^{-1} , 40 dS m^{-1} and 50 dS m^{-1}) are depicted in [Table 1](#).

Table 1
Antioxidative enzyme activities in *Urochondra setulosa* and *Dichanthium annulatum* at different salinity levels.

Enzyme activity (Units mg ⁻¹ protein FW)							
<i>Urochondra setulosa</i>							
Treatments	Catalase (CAT)	Peroxidase (POX)	Superoxide dismutase (SOD)	Ascorbate peroxidase (APX)	Monodehydro-ascorbate reductase (MDHAR)	Dehydroascorbate reductase (DHAR)	Glutathione reductase (GR)
Control (EC _e 0.69 dS m ⁻¹)	32.47 ± 0.52 ^D	44.19 ± 1.03 ^D	78.45 ± 3.21 ^D	207.30 ± 8.17 ^C	87.44 ± 3.78 ^C	59.14 ± 2.53 ^D	38.59 ± 1.44 ^C
EC _e 30 dS m ⁻¹	48.35 ± 1.18 ^C	60.66 ± 2.30 ^C	112.53 ± 4.77 ^C	350.48 ± 14.22 ^B	98.94 ± 4.01 ^B	65.11 ± 2.41 ^C	58.68 ± 2.33 ^B
EC _e 40 dS m ⁻¹	59.12 ± 2.56 ^B	64.85 ± 2.69 ^B	136.21 ± 5.53 ^B	363.04 ± 13.74 ^B	111.02 ± 4.80 ^A	78.94 ± 0.36 ^B	62.14 ± 2.13 ^A
EC _e 50 dS m ⁻¹	71.85 ± 1.62 ^A	67.23 ± 2.18 ^A	168.74 ± 3.95 ^A	449.89 ± 16.63 ^A	105.87 ± 4.58 ^{AB}	84.97 ± 2.07 ^A	56.11 ± 0.30 ^B
<i>Dichanthium annulatum</i>							
Control (EC _e 0.69 dS m ⁻¹)	56.00 ± 2.29 ^B	29.75 ± 1.31 ^B	67.11 ± 2.84 ^B	111.96 ± 4.64 ^B	62.49 ± 2.78 ^A	34.11 ± 1.29 ^B	24.56 ± 1.01 ^B
EC _e 30 dS m ⁻¹	144.57 ± 6.26 ^A	33.83 ± 1.34 ^A	87.76 ± 3.72 ^A	156.07 ± 6.89 ^A	68.22 ± 2.71 ^A	39.61 ± 1.61 ^A	28.96 ± 1.25 ^A

*Values in the column represents the mean ± SE from three independent experiments; Alphabets in superscript denote significant difference at $p \leq 0.05$.

Table 2

Hydrogen peroxide (H₂O₂), malondialdehyde (MDA), ascorbic acid and total glutathione content in *Urochondra setulosa* and *Dichanthium annulatum* at different salinity levels.

Urochondra setulosa				
Treatments	mmoles g ⁻¹ FW		µg g ⁻¹ FW	
	H ₂ O ₂	MDA	Ascorbic acid	Total glutathione
Control (EC _e 0.69 dS m ⁻¹)	4.37 ± 0.19 ^C	1.52 ± 0.12 ^C	112.36 ± 1.55 ^D	51.14 ± 1.83 ^D
EC _e 30 dS m ⁻¹	5.91 ± 0.26 ^B	1.89 ± 0.13 ^B	148.14 ± 3.61 ^C	70.51 ± 0.95 ^C
EC _e 40 dS m ⁻¹	6.03 ± 0.24 ^B	2.06 ± 0.11 ^B	164.78 ± 4.46 ^B	86.27 ± 3.42 ^B
EC _e 50 dS m ⁻¹	7.76 ± 0.33 ^A	2.98 ± 0.21 ^A	181.44 ± 2.13 ^A	103.11 ± 1.67 ^A
Dichanthium annulatum				
Control (EC _e 0.69 dS m ⁻¹)	2.86 ± 0.10 ^B	4.33 ± 0.17 ^B	69.14 ± 2.24 ^B	25.61 ± 0.23 ^B
EC _e 30 dS m ⁻¹	4.19 ± 0.18 ^A	5.41 ± 0.22 ^A	76.41 ± 2.41 ^A	39.47 ± 1.00 ^A

*Values in the column represent the mean ± SE from three independent experiments; Alphabets in superscript denote significant difference at $p \leq 0.05$.

In *U. setulosa*, the enzyme activities of peroxidase, superoxide dismutase, ascorbate peroxidase were higher at all the three saline treatments including the higher activities of Asc-Glu-pathway enzymes as well, i.e., MDHAR, DHAR and GR. On the other hand, higher catalase activity was observed in *D. annulatum* with lower activities of all other enzymes at salinity level of EC 30 dS m⁻¹ although the specific enzyme activities of ROS system increased with increasing levels of salinity in both the halophytes. In terms of the products of ROS system, MDA content was higher in *D. annulatum*, although an increase with salinity was observed in both the plants (Table 2). Similarly increased accumulation of H₂O₂ content was observed with increasing salt concentrations in both the species, although higher rate of accumulation in *D. annulatum* with 46.5% than 35.2% in *U. setulosa* at EC 30 dS m⁻¹. The range of increase in ascorbic acid content was 32–62% in *U. setulosa* with only 10% increase in *D. annulatum*. At lower salt intensity (30 dS m⁻¹), total glutathione accumulation was observed towards higher side in *D. annulatum* (54%) than in *U. setulosa* (37.8%), although it was approximately double at higher salinity of EC (50 dS m⁻¹) in *U. setulosa*. The replicated raw data of both the halophytic plants has been submitted in Mendeley data repository [1]. Briefly, experimental findings indicated that higher accumulation of H₂O₂ in *D. annulatum* are being detoxified by increased activities of SOD, APX and CAT, while in case of *U. setulosa*, POX dominantly removes H₂O₂ along with DHAR and MDHAR differentiate detoxification of reactive oxygen species and survival mechanism of both the halophytes at variable salinity levels. In nutshell, the experimental findings indicated that higher production of H₂O₂ under saline stress is detoxified through the hyper activity of SOD and through AsA-GSH pathway. In the plant species *D. annulatum*, (which adaptive upto salinity of EC 30 dS m⁻¹) the higher accumulation of H₂O₂ is detoxified through combined activities of SOD, APX and CAT. Alternately in *U. setulosa*, hyper activity of POX takes over CAT to detoxify the higher production of H₂O₂ along with DHAR and MDHAR. The differential salinity stress levels modulate enzymatic and non-enzymatic components of the antioxidant system differentially in both these plants. Consequently a joint action of ROS-detoxifying compounds (H₂O₂, MDA, Ascorbate and Glutathione contents) and enzymatic antioxidant system, have a fundamental role in the cellular protection of *U. setulosa* and *D. annulatum* halophytes under higher salinity conditions.

3. Experimental Design, Materials and Methods

The schematic overview of experimental design has been shown in Fig. 1. The study was conducted at ICAR – Central Soil Salinity Research Institute (CSSRI), Karnal, Haryana, India (29°43' N latitude, 76°58' N longitude). The site comes under semi-arid region having very hot summer (45.4 °C) and extremely cold winter (1.6°C). During the establishment of experiment (year 2017), a total rainfall of 793.4 mm was received in 44 rainy days at the experimental site in

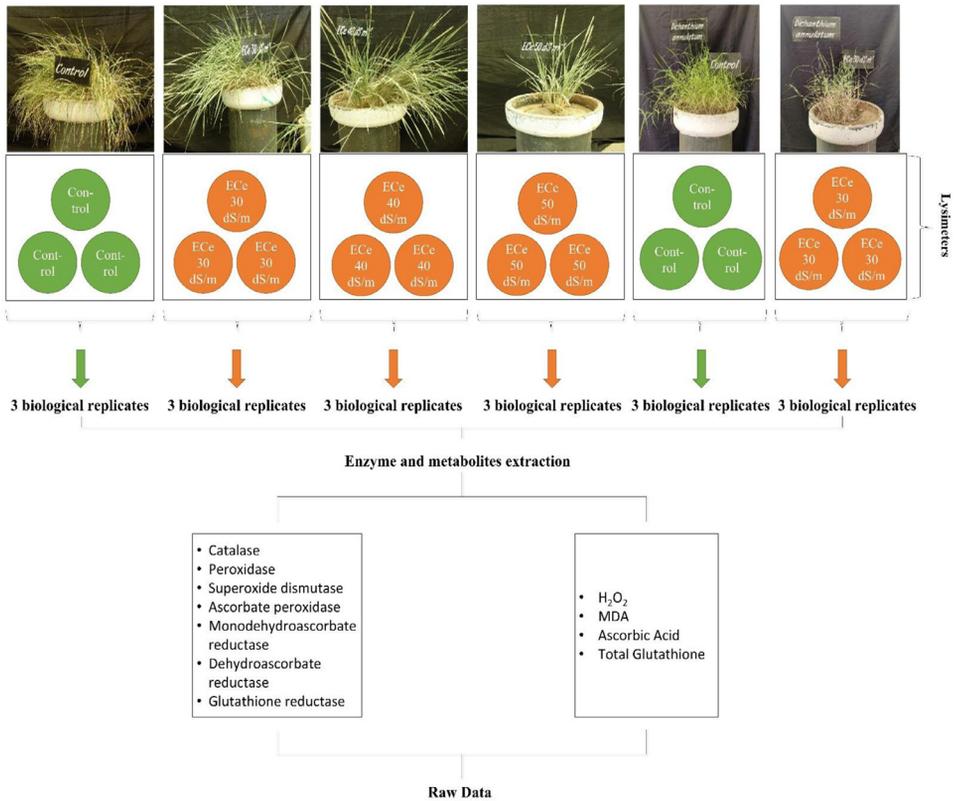


Fig. 1. A schematic overview of experimental design in *U. setulosa* and *D. annulatum*. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

comparison to the mean annual rainfall of 737.4 mm. The relative humidity was lowest (9%) on 10th April and 14th April while the highest (100%) was recorded on several occasions during the year. The total open pan evaporation during the year was 1324.0 mm, which was more than 1.67% than the annual rainfall. The halophytes, *Dichanthium annulatum* (moderately salt tolerant) and *Urochondra setulosa* (extremely salt tolerant) were initially collected from their native environment in saline Kutch plains near Bhuj, Gujarat, India. The soil pH of the collection sites varied from 7.7 to 9.1. However in the top 0 to 15 cm layer, the EC_e varied from 0.15 to 63.1 dS m⁻¹. The soil samples reflected very low organic carbon status i.e. 0.02 to 0.26% in the top layer (0–15 cm). Water soluble anions (Na⁺, K⁺, Ca²⁺, Mg²⁺) and cations (Cl⁻ and SO₄²⁻) varied significantly according to the spatial and depth of soil samples. Na⁺ content varied from 3.1 to 138.9 ppm. The root cutting of these halophytes were planted in lysimeters (40 × 70 cm) filled with sandy soil having saline soil of EC_e ~16 dS m⁻¹ with three replications in a completely randomized design (CRD) in covered net house. Initially, soil with sandy loam in nature having EC_e 0.69 dS m⁻¹, pH₂ 7.8, organic carbon 0.33% with 16% clay was filled in control lysimeters. However natural saline soils (collected from CSSRI, Nain Farm Panipat) of sandy loam in texture having the EC_e 16.2 ± ± 1.4 dS m⁻¹ and pH₂ 8.2 ± ± 0.2 was filled in saline treatment lysimeters. The organic carbon (%) was 0.46 while the available N, P and K were recorded in the tune of 142.0, 60.1 and 222.3 kg ha⁻¹, respectively. After the plants established fully, chloride-dominated [3(Cl):1(SO₄)] saline water irrigations were given to maintain levels of ECe 30, 40 and 50 dS/m. One set with good quality irrigation water served as control. Leaves were sampled for antioxidant enzymes and other ROS components.

Catalase (CAT; EC 1.11.1.6) activity was determined using the method of Aebi [2]. One unit of catalase was defined as the amount of enzyme required to catalyze the breakdown of 1 μmol H_2O_2 per minute at 240 nm, which was estimated using the extinction coefficient ($0.036 \text{ cm}^2 \text{ mol}^{-1}$) of absorbance for H_2O_2 at 240 nm. Peroxidase (POX; EC 1.11.1.7) activity was measured through the rate of guaiacol oxidation in the presence of H_2O_2 at 470 nm [3] and the amount of enzyme required to oxidize one nmol of guaiacol $\text{min}^{-1} \text{ ml}^{-1}$ is defined as one unit of peroxidase activity. Superoxide dismutase (SOD; EC 1.15.1.1) activity was measured using the procedure of Beauchamp and Fridovich [4] and expressed as Units mg^{-1} protein FW where one unit is defined as the amount of enzyme required to inhibit the photo-reduction of one μmol of NBT. Ascorbate peroxidase (APX; EC 1.11.1.11) activity was calculated using extinction coefficient of $2.8 \text{ mM}^{-1} \text{ cm}^{-1}$ as per method given by Nakano and Asada [5] where one enzyme unit is expressed as the amount of enzyme required to oxidize one nmol of ascorbate min^{-1} . The monodehydroascorbate reductase (MDHAR; EC 1.6. 5.4) activity was based on NADH oxidation with a decrease in A_{340} [6]. Dehydroascorbate reductase (DHAR; EC 1.8.5.1) activity was assayed using modified method of Hossain and Asada [7]. Glutathione reductase (GR) was quantified as per unit enzyme for oxidation of one nmol of NADPH min^{-1} [8] where one enzyme unit is defined as amount of enzyme required to oxidize one nmol of NADPH min^{-1} . The activities of all antioxidative enzymes were quantified on spectrophotometer (SPECORD-210 PLUS, Analytic Zena). The H_2O_2 content was calculated as per the method given by Junglee [9] using standard curve of H_2O_2 . Malondialdehyde (MDA) accumulation was measured as an indicator of lipid peroxidation as given by Heath and Packer [10]. Ascorbic acid content was estimated by the method of Desai and Desai [11]. Total glutathione was estimated using 2-nitrobenzoic acid as per method of Salbitani [12].

Ethics Statements

All the authors hereby declare that all the experiments were conducted while maintaining all ethical rules and regulations. None of the studies included humans or animals.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

[Antioxidant metabolism dataset of Halophytes \(Original data\)](#) (Mendeley Data).

CRediT Author Statement

Anita Mann: Conceptualization, Project administration, Funding acquisition; **Naresh Kumar:** Data curation, Formal analysis, Writing – original draft; **Charu Lata:** Formal analysis, Validation; **Ashwani Kumar:** Data curation; **Arvind Kumar:** Visualization, Writing – review & editing; **B.L. Meena:** Methodology.

Acknowledgments

This work was funded by [Indian Council of Agricultural Research \(ICAR\)](#) - National Agricultural Science Fund (NASF), New Delhi, India (Award no [ABP-6027](#)). We thank the Director, ICAR-

Central Soil Salinity Research, Karnal, Haryana, India for providing necessary facilities to carry out the research work.

References

- [1] A. Mann, N. Kumar, C. Lata, A. Kumar, B.L. Meena, A. Kumar, Antioxidant metabolism dataset of Halophytes, *Mendley Data V1* (2023), doi:10.17632/xd698v9w9s.1.
- [2] H. Aebi, Catalase in vitro, in: *Methods in Enzymology*, Elsevier, 1984, pp. 121–126.
- [3] M.V. Rao, C.B. Watkins, S.K. Brown, N.F. Weeden, Active oxygen species metabolism in 'White Angel'x'Rome Beauty'apple selections resistant and susceptible to superficial scald, *J. Am. Soc. Horticult. Sci.* 123 (1998) 299–304.
- [4] C. Beauchamp, I. Fridovich, Superoxide dismutase: improved assays and an assay applicable to acrylamide gels, *Anal. Biochem.* 44 (1971) 276–287.
- [5] Y. Nakano, K. Asada, Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts, *Plant Cell Physiol.* 22 (1981) 867–880.
- [6] V. Mittova, M. Volokita, M. Guy, M. Tal, Activities of SOD and the ascorbate-glutathione cycle enzymes in subcellular compartments in leaves and roots of the cultivated tomato and its wild salt-tolerant relative *Lycopersicon pennellii*, *Physiol. Plant.* 110 (2000) 42–51.
- [7] M.A. Hossain, K. Asada, Purification of dehydroascorbate reductase from spinach and its characterization as a thiol enzyme, *Plant Cell Physiol.* 25 (1984) 85–92.
- [8] B. Halliwell, C.H. Foyer, Properties and physiological function of a glutathione reductase purified from spinach leaves by affinity chromatography, *Planta* 139 (1978) 9–17.
- [9] S. Junglee, L. Urban, H. Sallanon, F. Lopez-Lauri, Optimized assay for hydrogen peroxide determination in plant tissue using potassium iodide, *Am. J. Anal. Chem.* 5 (2014) 730.
- [10] R.L. Heath, L. Packer, Photoperoxidation in isolated chloroplasts: I. Kinetics and stoichiometry of fatty acid peroxidation, *Arch. Biochem. Biophys.* 125 (1968) 189–198.
- [11] A.P. Desai, S. Desai, UV spectroscopic method for determination of vitamin c (ascorbic acid) content in different fruits in south Gujarat region, *Int. J. Environ. Sci. Nat. Res.* 21 (2019).
- [12] C. Salbitani, C. Bottone, S. Carfagna, Determination of reduced and total glutathione content in extremophilic microalga *Galdieria phlegrea*, *Bio. Protoc.* 7 (2017) e2372–e2372.