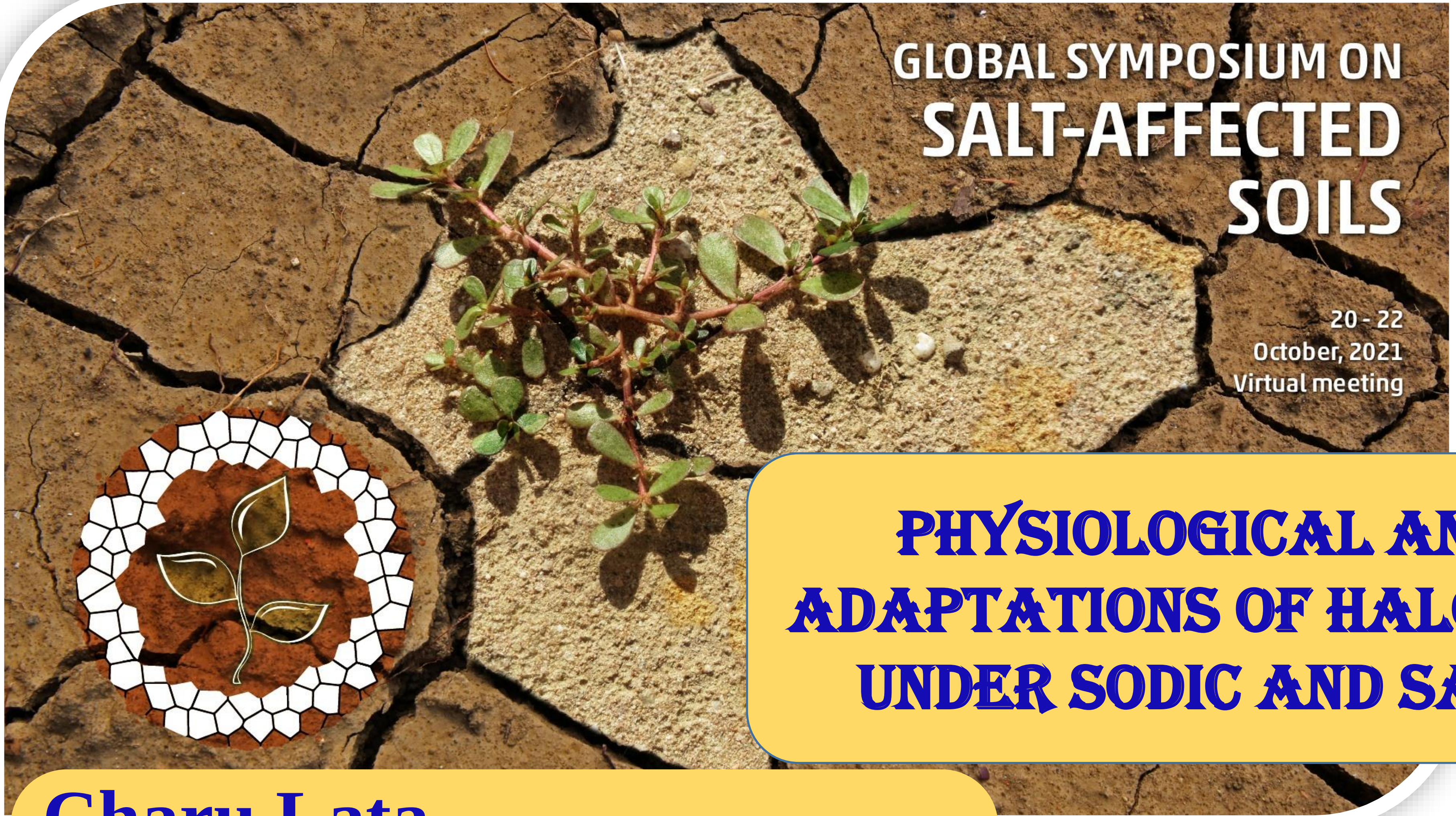

PHYSIOLOGICAL AND MOLECULAR ADAPTATIONS OF HALOPHYTIC GRASSES UNDER SODIC AND SALINE STRESSES

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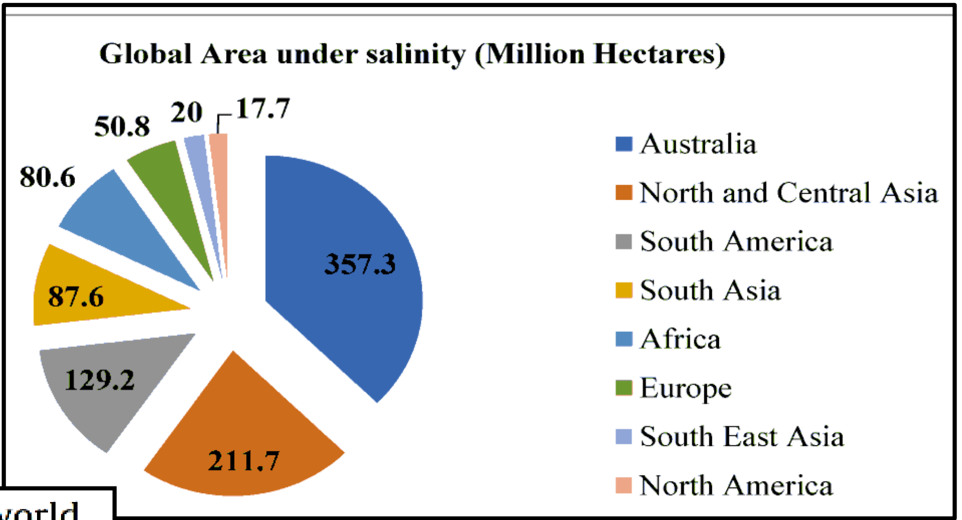
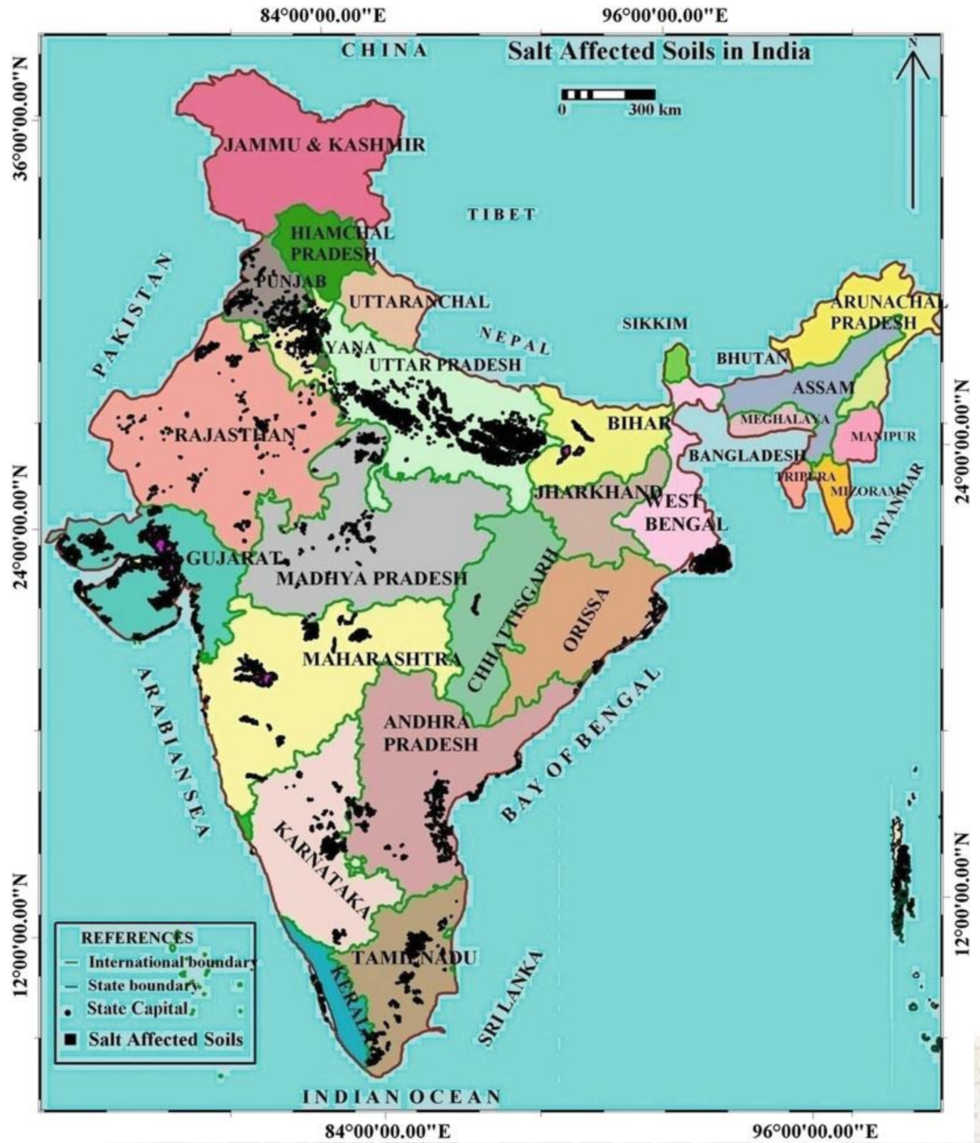
EXTENT & DISTRIBUTION OF SAS : GLOBAL SCENARIO & INDIAN PERSPECTIVE



Salt-affected soils in all over the world.

Name of country	Salt-affected area
Australia	30% (total area)
Thailand	30% (total area)
Egypt	9.1% (total area)
Hungry	10% (total area)
Iran	28% (irrigated land)
Kenya	14.4% (total area)
Nigeria	20% (irrigated land)
Russia	21% (irrigated land)
Syria	40% (irrigated land)
Tunisia	30% (total area)
USA	25–30% (irrigated land)
India	4.2% (total arable land)
China	4.88% (total available land)

Sl. No.	State	Saline Soils (ha)	Sodic Soils (ha)	Total (ha)
1	Andhra Pradesh	77598	196609	274207
2	Andaman & Nicobar	77000	0	77000
3	Bihar	47301	105852	153153
4	Gujarat	1680570	541430	2222000
5	Haryana	49157	183399	232556
6	Karnataka	1893	148136	150029
7	Kerala	20000	0	20000
8	Maharashtra	184089	422670	606759
9	Madhya Pradesh	0	139720	139720
10	Orissa	147138	0	147138
11	Punjab	0	151717	151717
12	Rajasthan	195571	179371	374942
13	Tamil Nadu	13231	354784	368015
14	Uttar Pradesh	21989	1346971	1368960
15	West Bengal	441272	0	441272
	Total	2956809	3770659	6727468



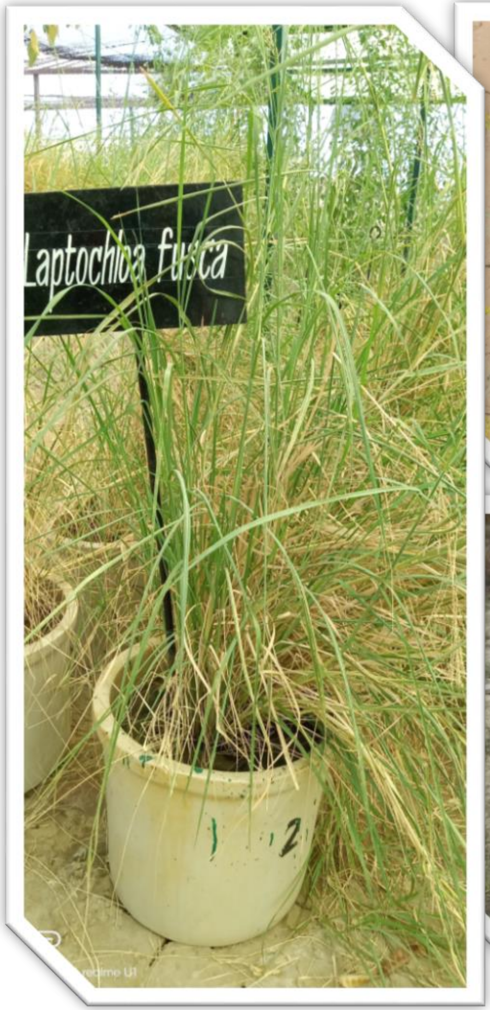
RATIONAL / BACKROUND

- Soil salinity and sodicity are prolific abiotic soil-related problems afflicting nearly 20% of total arable lands, affecting the crop yields and quality.
- Feasible solutions to harness the potential of SAS»»» use of halophytes
- 'Halophytes' occupy ~1% of the entire flora present on the earth's surface.
- Potentiality of halophytes to outlive such harsh conditions depends on various morphological, physiological, biochemical and molecular traits that helps them to adapt, grow and flourish.
- Improved knowledge of halophytes at physiological and molecular level is important in understanding our natural world and to enable the use of some of these fascinating plants in land re-vegetation, as forages for livestock, and to develop salt-tolerant crops.
- Evaluated three halophytes: *Urochondra setulosa*, *Leptochloa fusca* and *Sporobolus marginatus* under saline and sodic stress at physiological and molecular levels.


Halophytes

OBJECTIVES

Urochondra setulosa



Leptochloa fusca

Sporobolus marginatus

- ✓ **Evaluating phyto-remediation potential of grass halophytes.**
- ✓ **Studying physiological and enzymatic perspectives of salinity tolerance in grass halophytes.**
- ✓ **Expression profiling of candidate salt tolerance genes in halophytic grasses at different sodicity and salinity levels.**

METHODOLOGY

- The present investigation was carried out on 3 halophytic grassess- *Urochondra setulosa, Leptochloa fusca and Sporobolus marginatus* in screen house at ICAR-CSSRI, Karnal (29°43`N, 76°58`E, and 245 m above the mean sea level).
- The root cuttings and seed material was initially collected from RRS, ICAR-CAZRI, Bhuj and RRS, ICAR-CSSRI, Lucknow. These grasses were raised in screening blocks filled with sandy soil under controlled conditions. After establishment, these grasses were transferred to micro-plots (2.5m × 1.5m × 0.5m).
- The screen house was covered with HDPE polythene sheets to avoid the entry of rain water and maintain the desired salinity stress as per treatments.

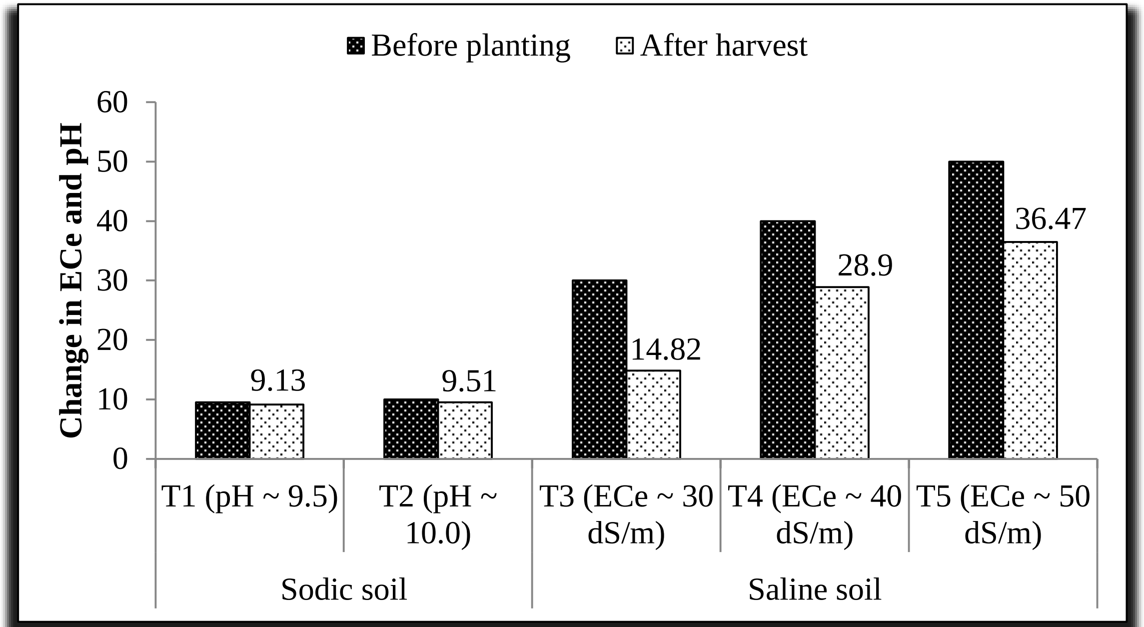
Treatments: Six

- Control
- Sodic stress — Soil pH: 9.5 and 10.0
- Salinity stress — Soil EC_e: 30, 40 and 50 dSm^{-1}

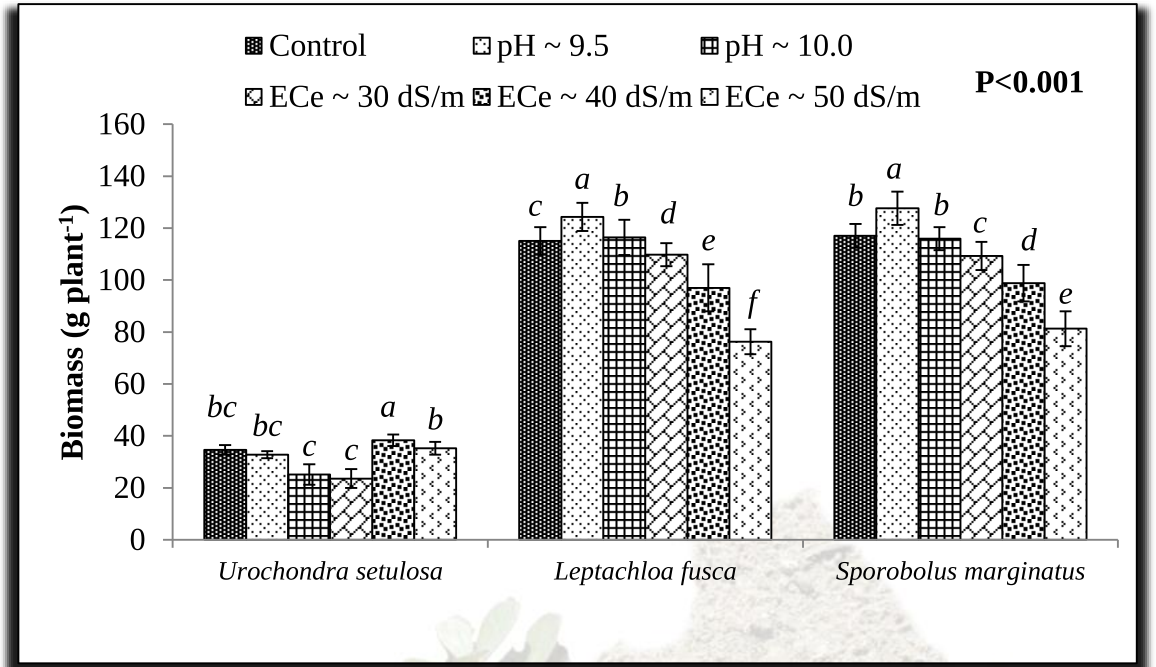
Replication: Three   Design: RBD

OBJECTIVES	ACTIVITIES	OBSERVATIONS RECORDED
▪ Evaluation of phyto-remediation potential	▪ Soil ECe and pH were measured ▪ Biomass recording	▪ Soil ECe (electrical conductivity of saturated soil extract) and pH were measured before planting and after harvesting of halophytes ▪ Biomass (fresh weight) was recorded after every 3 months.
▪ Studying physiological and enzymatic perspectives of salinity tolerance in grass halophytes	▪ Photosynthetic attributes ▪ Reactive oxygen species (ROS)/ antioxidant system imparting salt tolerance. ▪ Osmoprotectants analysis ▪ Ionic observations	▪ Gas exchange attributes; using IRGA (LI-6400, LICOR Inc., Lincoln, NE, USA) and Chlorophyll fluorescence (Fv/Fm) was measured by portable pulse modulated fluorescence measurer (Junior PAM Chlorophyll Fluorometer, Germany). ▪ H_2O_2 content, ROS content, Antioxidant enzymes (Superoxide dismutase, Glutathione reductase, Catalase, Peroxidase and Ascorbate peroxidase) ▪ Osmoprotectants (proline, glycine betaine and K^+) and biochemicals (Total soluble proteins, Epicuticular wax load) ▪ Na^+, K^+ and Ca^{2+} with di-acid [HNO_3:$HClO_4$ (3:1)] digestion using AAS, Z eenit 700P, Analytical Zena, Germany.
▪ Expression profiling of candidate salt tolerance genes at different sodic and salinity levels	▪ RNA isolation ▪ Expression profiling of salt responsive genes	▪ Using Trizol reagent and cDNA was synthesised using R2D 1st strand cDNA synthesis kit (GCC Biotech, Kolkata, India) ▪ q-PCR was performed using SSO Fast Eva Green Supermix" (Bio-Rad) on CFX96 Real-Time PCR system (Bio-Rad) using gene specific primers and The gene expression analysis was carried out using $2^{-\Delta\Delta CT}$ method

RESULTS



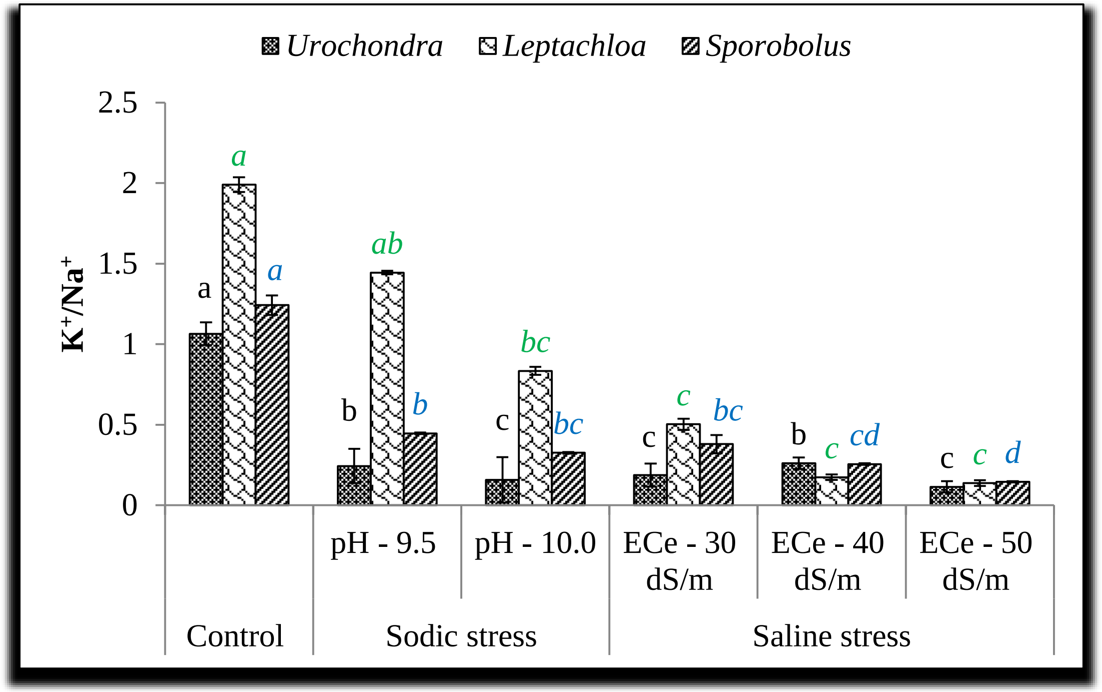
Changes in soil salinity (ECe) and sodicity (pH) before and after halophyte planting



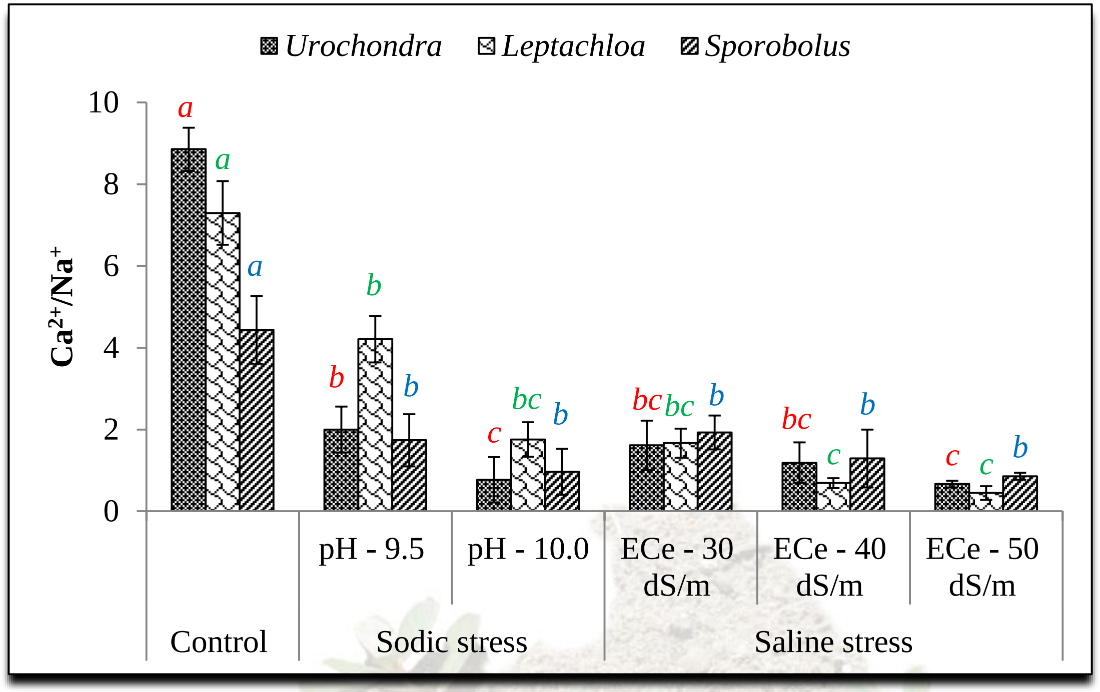
Effect of salinity and sodicity stresses on biomass (g $plant^{-1}$) production in halophytic plants

Effect of salinity and sodicity stresses on gas exchange attributes of three halophytic plants differing in their tolerance

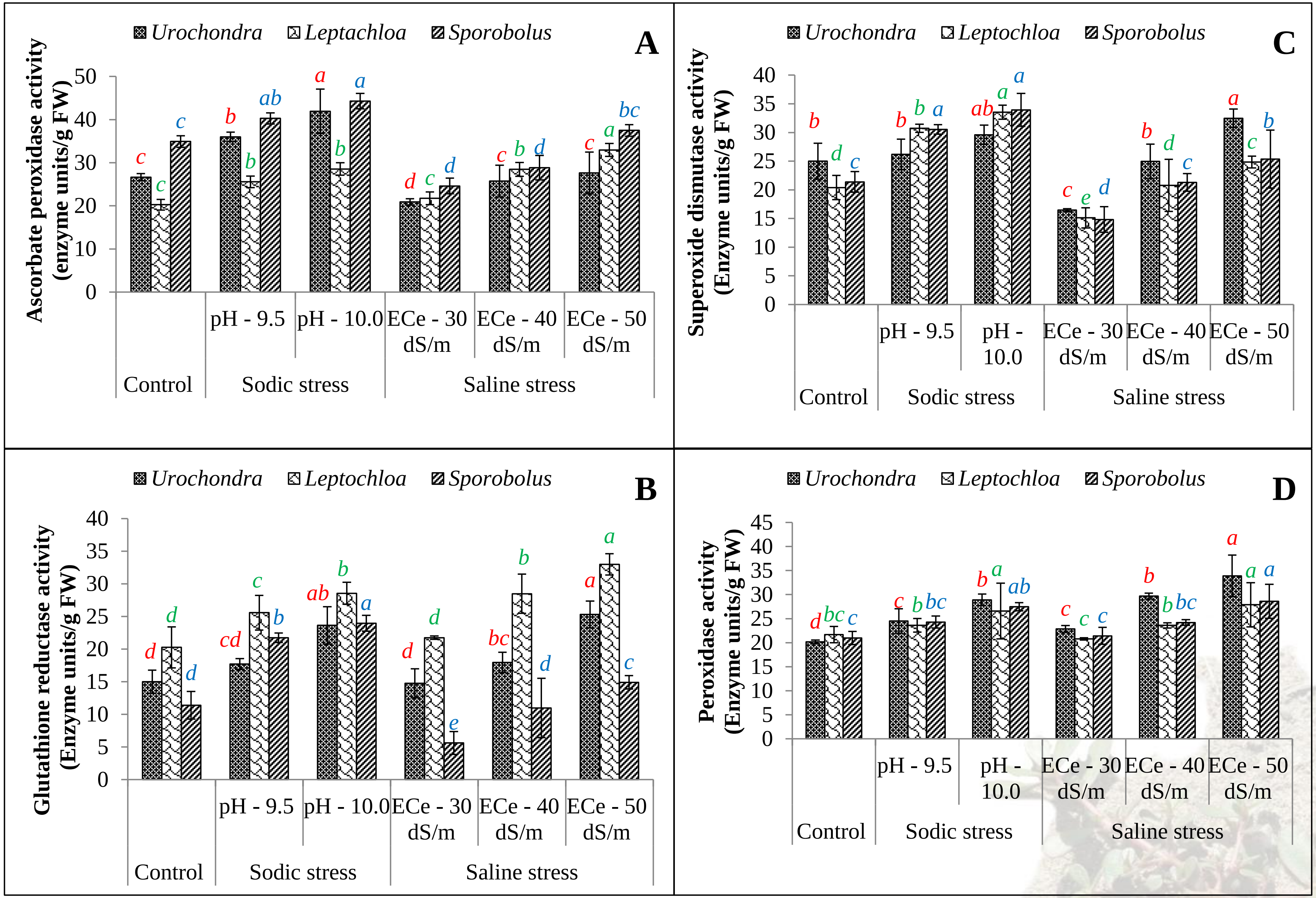
Treatment/Traits		*Urochondra setulosa*				*Leptachloa fusca*				*Sporobolus marginatus*			
		pN	gS	E	Fv/Fm	pN	gS	E	Fv/Fm	pN	gS	E	Fv/Fm
Control		34.80^{A}	0.65^{A}	16.69	0.80^{A}	28.13^{A}	0.53^{A}	13.04^{A}	0.72^{A}	31.54^{A}	0.58^{A}	14.02	0.75^{A}
Sodic Stress	pH ~ 9.5	31.81^{BC}	0.61^{C}	15.73	0.79^{A}	27.44^{A}	0.50^{A}	12.13^{AB}	0.71^{AB}	30.53^{AB}	0.53^{BC}	13.04	0.74^{B}
	pH ~ 10.0	27.65^{D}	0.54^{E}	13.08	0.75^{C}	24.10^{B}	0.45^{B}	10.07^{CD}	0.69^{BC}	27.21^{D}	0.51^{CD}	10.13	0.71^{C}
Saline stress	ECe ~ 30 dSm^{-1}	32.71^{B}	0.63^{B}	16.23	0.80^{A}	27.32^{A}	0.52^{A}	11.29^{BC}	0.71^{AB}	29.46^{BC}	0.55^{B}	10.89	0.74^{B}
	ECe ~ 40 dSm^{-1}	30.72^{C}	0.61^{C}	15.59	0.79^{AB}	23.15^{B}	0.51^{A}	9.63^{D}	0.69^{BC}	28.44^{CD}	0.53^{BC}	12.38	0.70^{C}
	ECe ~ 50 dSm^{-1}	28.20^{D}	0.57^{D}	11.43	0.77^{BC}	20.10^{C}	0.42^{B}	8.12^{E}	0.68^{C}	25.12^{E}	0.49^{D}	9.02	0.68^{D}
General Mean		30.98	0.60	14.79	0.78	25.04	0.49	10.71	0.70	28.72	0.53	11.58	0.72
CV (%)		2.42	0.93	10.68	0.96	3.31	2.66	5.44	1.37	2.43	1.70	11.89	0.50
SE(d)		0.749	0.006	1.579	0.008	0.828	0.013	0.583	0.010	0.696	0.009	1.377	0.004
LSD at 5%		1.9244	0.0144	NS	0.0193	2.128	0.0336	1.4985	0.0245	1.7904	0.0231	NS	0.0092



K^+/Na^+ ratio under saline and sodic stress in halophytes



Ca^{2+}/Na^+ ratio under saline and sodic stress in halophytes

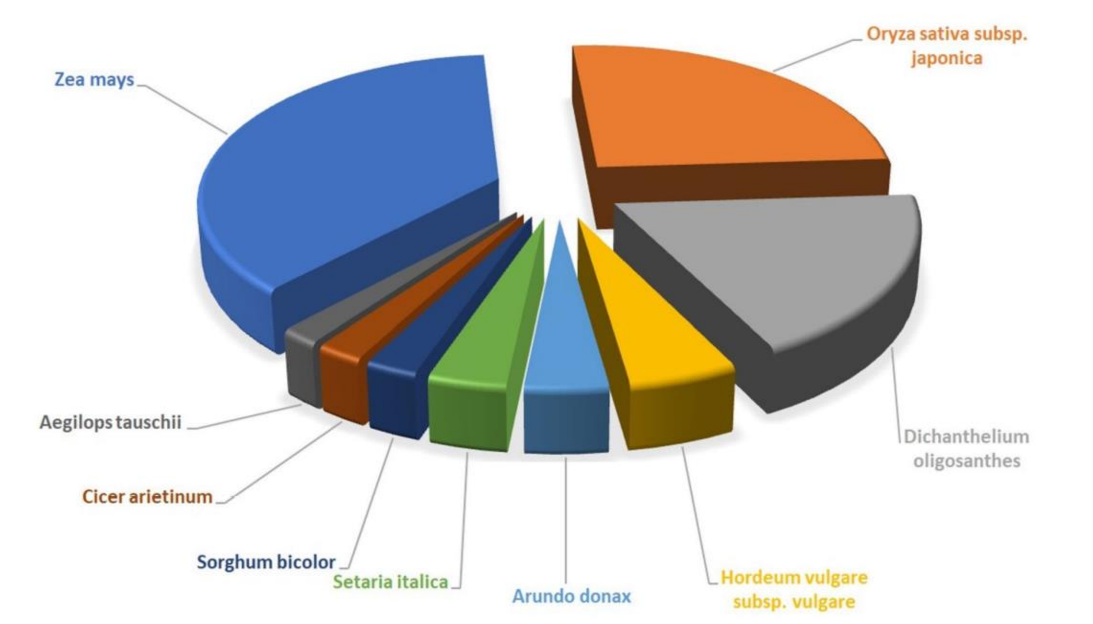


Changes in antioxidant enzymes {APX (A), GR (B), SOD (C) and POX (D)} of halophytes at saline and sodic stresses

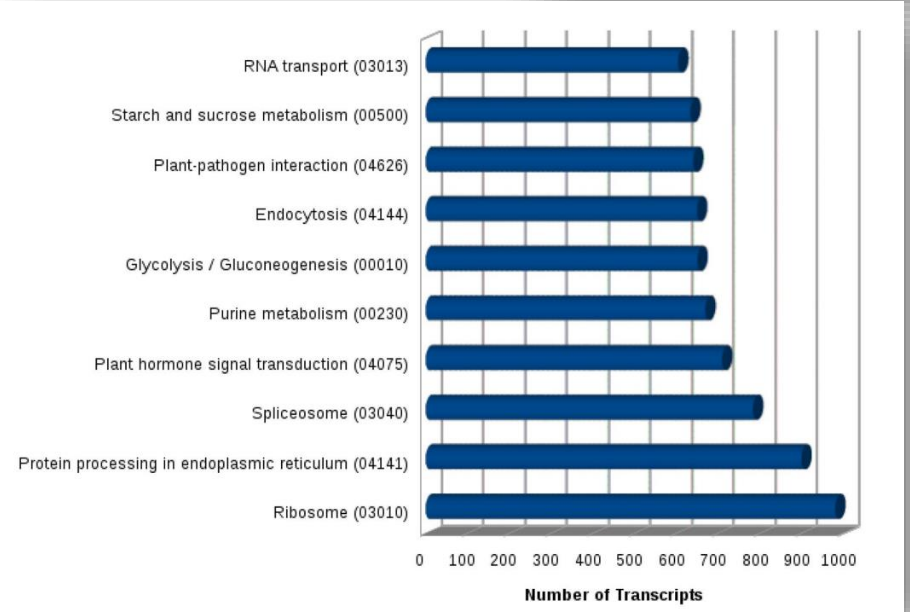
Effect of sodicity and salinity stresses on expression of *MnSOD*, *NHX1* and *FuSOS1* genes in three halophytic grasses

Treatments		Urochondra setulosa			Leptachloa fusca			Sporobolus marginatus		
		MnSOD	NHX1	FuSOS	MnSOD	NHX1	FuSOS	MnSOD	NHX1	FuSOS
Sodic Stress	pH ~ 9.5	1.37 ± 0.1^{e}	1.24 ± 0.08^{d}	2.25 ± 1.5^{c}	4.33 ± 0.3^{c}	6.3 ± 1.9^{c}	2.27 ± 0.6^{d}	1.21 ± 0.03^{bc}	1.38 ± 0.2^{c}	2.21 ± 0.69^{d}
	pH ~ 10.0	8.06 ± 0.2^{b}	10.0 ± 0.6^{b}	3.07 ± 0.2^{b}	19.7 ± 0.85^{a}	24.86 ± 1.0^{a}	10.99 ± 0.9^{a}	1.33 ± 0.14^{b}	1.94 ± 0.3^{b}	3.72 ± 0.8^{c}
Saline stress	ECe ~ 30 dSm^{-1}	4.45 ± 0.1^{d}	2.94 ± 0.5^{c}	1.97 ± 1.2^{d}	1.37 ± 0.09^{d}	1.18 ± 0.1^{e}	3.01 ± 0.5^{c}	1.12 ± 0.01^{c}	1.41 ± 0.17^{c}	2.3 ± 0.4^{d}
	ECe ~ 40 dSm^{-1}	5.32 ± 0.4^{c}	3.07 ± 0.2^{c}	2.02 ± 0.8^{d}	1.38 ± 0.2^{d}	4.93 ± 0.78^{d}	3.09 ± 0.6^{c}	1.13 ± 0.07^{c}	1.82 ± 0.19^{bc}	4.59 ± 0.4^{b}
	ECe ~ 50 dSm^{-1}	14.47 ± 0.9^{a}	16.5 ± 3.4^{a}	4.33 ± 0.3^{a}	8.02 ± 0.23^{b}	9.24 ± 1.1^{b}	4.33 ± 0.3^{b}	7.49 ± 0.9^{a}	59.6 ± 1.95^{a}	6.07 ± 0.8^{a}
General Mean		6.73	6.75	2.73	6.96	9.30	4.74	2.46	13.23	3.78
CV (%)		1.69	2.15	2.81	4.08	1.18	3.26	2.93	2.00	2.11
SE(d)		0.093	0.118	0.062	0.232	0.09	0.126	0.059	0.216	0.065
LSD at 5%		0.2066	0.2636	0.1392	0.5169	0.1996	0.2814	0.1308	0.4823	0.1453

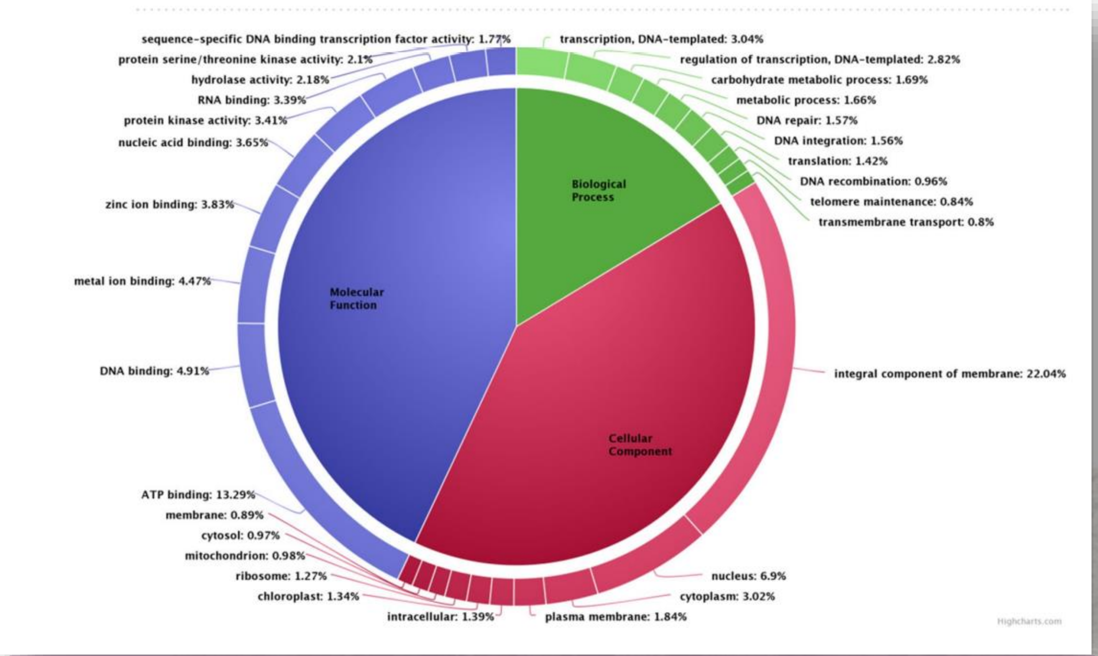
Mann et al, 2021



Sequence similarity index of *Urochondra* transcripts with other species



Top 10 most highly represented pathways in *Urochondra setulosa*



Frequency of top 10 abundant GO terms under biological process, molecular function and cellular component categories in *Urochondra setulosa*

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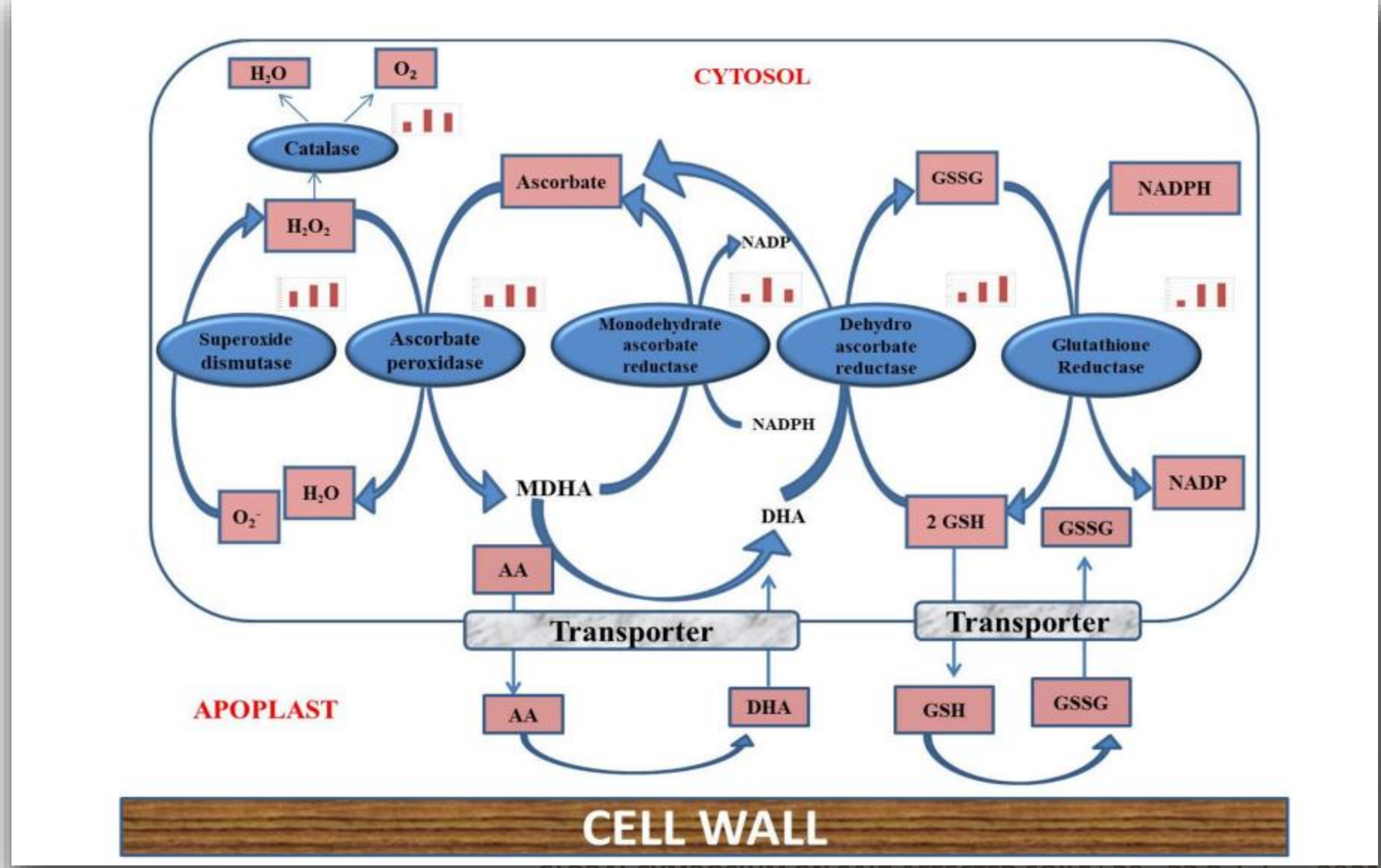
Differentially expressed genes of ROS pathway in response to saline stress



CONCLUSION

- The present study encapsulate understanding, how stress affects the metabolism of halophytic plants and in the similar way, how plants act in response to this interaction through their adaptive traits.
- Transcriptomic studies on *Urochondra* showed, how the regulation of transcription factors and signaling transcripts are influenced by salinity.
- The up-regulation of genes for photosynthetic enzymes, MAPK pathway, transcription factors, transporter proteins, antioxidative enzymes, cell membrane proteins and enzymes for synthesis of compatible solutes with increasing levels of salinity suggested the reasons for salt tolerance ability of halophyte *Urochondra*
- It is clear from the results that since *Urochondra setulosa* produces more biomass with extra salt load under salt stress, it may be grouped as highly salt tolerant.
- *Leptochloa fusca* produced higher biomass by maintaining higher K^+/Na^+ under sodic condition and could be categorized as sodicity tolerant grass.
- On the other hand, *Sporobolus marginatus* showed tolerance to both the stresses of salt or pH.
- Briefly, we can summarize that *U. setulosa* follows salt exclusion pathway, *L. fusca* showed ion homeostasis and *S. marginatus* survives through ion compartmentalization and hence, these grasses tolerate high levels of saline and alkaline stress conditions.

GLOBAL SYMPOSIUM ON SALT-AFFECTED SOILS

20 - 22
October, 2021
Virtual meeting