RESEARCH ARTICLE Indian Journal of Agricultural Research, Volume 59 Issue 12: 1809-1818 (December 2025)

Antioxidant and Agronomical Screening of *Oryza sativa* L. in Response to Abiotic Stress from Local Landraces

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10.18805/IJARe.A-6468

ABSTRACT

Background: Climate change is predicted to aggravate abiotic stresses such as salinity, drought and temperature, harshly affecting crop plants, especially rice plants and reducing their productivity. Soil quality is affected by stress conditions, which makes it a big challenge to secure food resources in rice-growing regions. Abiotic stress-tolerant rice is necessary to grow in extreme environmental conditions for food safety.

Methods: The present investigation was carried out by randomly selecting ten local rice varieties and screening them naturally for salt and drought tolerance. In this study, the salinity-sensitive rice variety (IR64) was taken as a control. We analysed biochemical parameters such as H_2O_2, chlorophyll, proline content, relative water content (RWC), lipid peroxidation (MDA) content, CAT (catalase), GPX (guaiacol peroxidase), GR (glutathione reductase), leaf disc assay, electrolytic leakage and hormone assay. The photosynthetic parameters were also determined to identify the salinity and drought-tolerant rice lines.

Result: Our results showed that stress-tolerant rice varieties, such as T2 (Jagannath) and T5 (Swarnamayee), T3 (Parijata) and T9 (Prativa) have higher chlorophyll, endogenous ion and soluble sugar contents than other varieties. The present study can help to develop stress-tolerant rice plants worldwide and increase food availability in salinity and drought environments.

Key words: Abiotic stress, Antioxidant, Drought, Local landraces, Rice, Salinity, Yield.

INTRODUCTION

Plants are exposed to abiotic stresses such as temperature extremes, drought, salinity and heavy metals, severely limiting plant distribution, disrupting growth and development and lowering agricultural output (Yanyan *et al*., 2018). Climate change has significantly increased the frequency and intensity of these abiotic stresses, adversely affected plants and reduced their productivity (Sachdev *et al*., 2021). Salinity and drought are important abiotic stressors that directly impact crop yield (Palei *et al*., 2024). Both stresses can affect germination, plant growth and yield production by inducing various morphological, physiological, biochemical and metabolic changes in crops (Tripathy, 2024). Salinity is the second most detrimental abiotic threat to crop productivity after drought (Chen *et al*., 2020). Excessive irrigation water consumption with inadequate drainage, poor irrigation water quality containing excessive salt levels and flooding from seawater are the main causes of salinity in arable land (Angon *et al.,* 2022; Liu *et al.,* 2021).

Rice (*Oryza sativa* L.) plays a vital role in global agriculture as half of the world's population relies on it as a staple food. In terms of dietary energy supply, rice leads the way by contributing 20%, surpassing both wheat and maize, which contribute 19% and 5% respectively (Kabange *et al.,* 2023). When soil salinity rises above 6 dS/m, the production of rice can be significantly decreased by more than 50% (Atta *et al.,* 2023 and Liu *et al*., 2021). Rice has been described as a salt-sensitive crop; nevertheless, its salt tolerance varies with different developmental stages (Sonsungsan *et al.,* 2021). At the seedling stage, rice is salt-sensitive; at the vegetative stage, it is moderately salt-tolerant; and at the reproductive stage, it is highly salt-sensitive (Shen *et al.,* 2020). The tolerant to salinity during the seedling stage is critical for the successful establishment of crops, particularly in coastal areas (Jamal *et al.,* 2023). Saline soil affects the photosynthetic machinery and reactive oxygen species (ROS), restricting availability of water/ nutrient and also interfering with sodium (Na^+)/potassium (K^+) ion in plants (Khan, 2022).

Unlike salinity stress, drought also limits rice production worldwide, resulting in huge economic losses. Drought is highly responsible for reducing turgor pressure, the water content of leaves and stomatal activity and it also limits cell growth and proliferation (Bhandari *et al.,* 2023). A recent study reported that drought is highly responsible for oxidative damage in the rice plant by over-accumulating H_2O_2 content (Sandeep and Godi, 2023). The present study aims to screen naturally available salinity and drought-stress-tolerant rice varieties so that they can be helpful for farmers in cultivating

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How to cite this article: Mohapatra, M.D. and Sahoo, R.K. (2025). Antioxidant and Agronomical Screening of *Oryza sativa* L. in Response to Abiotic Stress from Local Landraces. *Indian Journal of Agricultural Research*. **59(12):** 1809-1818. doi: 10.18805/IJARe.A-6468.

Submitted: 25-09-2025 **Accepted:** 24-11-2025 **Online:** 22-12-2025

rice crops in adverse environmental conditions. For this purpose, we randomly collected ten rice varieties and screened them through different salinity and drought stress parameters. This study will help to identify suitable rice varieties to study the mechanism of tolerance at the molecular level to meet the global food demand and protect the livelihood and sustainability of farmers.

MATERIALS AND METHODS

Collection and raising of rice seeds

Rice (*Oryza sativa* L.) varieties available in Odisha, India were collected from different locations. Out of 20 samples, the ten most consumed varieties (Tulsi, Jagannath, Parijata, Kalajeera, Swarnamayee, Khandagiri, Parbati, Pradip, Prativa, Navin) were selected for salinity and drought tolerance screening. Seeds of selected rice varieties were germinated inside the net house of Centurion University of Technology and Management, Bhubaneswar, Odisha, India. Approximately 21 days old seedlings were taken for salinity as well as drought stress tolerance assay. For control, we used a salinity-susceptible variety, *i.e*., IR 64, for easy comparison. We abbreviations for easy presentation of data as follows (T1: Tulsi, T2: Jagannath, T3: Parijata, T4: Kalajeera, T5: Swarnamayee, T6: Khandagiri, T7: Parbati, T8: Pradip, T9: Prativa, T10: Navin and C: IR64).

Analysis of rice lines during abiotic stresses (salinity and drought)

Selected rice varieties (T1-T10) and control rice plants were raised in a pot filled with soil. 25-day-old rice seedlings were subjected to different abiotic stress treatments. The stressors included 200 mM NaCl for salinity and 25% PEG for drought. Leaf samples were collected at 1 h, 2 h, 6 h, 12 h and 24 h intervals for further analysis.

Leaf disc senescence assay and chlorophyll content for salinity tolerance

This experiment was conducted in the year 2022-2023, by taking healthy rice leaves with a size of 1 cm × 1 cm from 10 experimental rice varieties as well as those of the control plant. The leaf discs were for 72 hrs floated in 100 mM and 200 mM solutions of NaCl. All the procedures were carried out as described earlier with three replicates (Tuteja *et al.,* 2015).

Assay of antioxidant gene expression for salinity stress

We selected 21-days-old seedlings of experimental rice plants for this experiment. Plants were raised in 200 mM NaCl (24 hrs) and then the leaf samples were taken from treated plants for the biochemical assays. These assays include MDA content, H_2O_2, ion leakage and RWC (relative water content) were determined by previous method (Tuteja *et al.,* 2015). According to the protocol of Garg *et al.* (2012), proline contents were determined. Antioxidants like APX (activities of ascorbate peroxidase), CAT (catalase), GPX (guaiacol peroxidase) and GR (glutathione reductase) were analysed under 200 mM NaCl.

Agronomic analysis of rice plants

All 10 experimental rice varieties were allowed to grow under 200 mM NaCl up to maturity for 30 days and their agronomic parameters were recorded. Rice plants were subjected to a treatment of 200 mM NaCl and a control of 0 mM NaCl over a period of 30 days to evaluate their physiological responses to varying salinity levels. Various agronomic parameters, such as the measurement of leaf area was conducted with precision using an advanced leaf area meter from Systronics, based in Hyderabad, India. Fresh leaves samples were dried at 80°C for 96 hours (4 days) in a hot air oven and incubated in a desiccator to achieving a precise and consistent dry weight. A meter scale is used to measure the shoot and root length of rice plants (Memmert, Model 500, Germany).

Endogenous ion and hormone (GA, Zeatin and IAA) assay

For the estimation of endogenous ions like phosphorus, sodium, potassium and nitrogen content, we have used experimental rice plant leaves after 8 weeks of planting. These plants were previously exposed for 24 hrs to 200 mM NaCl or water (control). The total nitrogen and phosphorus content was analysed in a spectrophotometer (Sarker and Oba, 2020). Potassium content was determined with the help of a flame ionization photometer and the sodium content was calculated by the process of Munns (2011). Plant hormones such as GA, Zeatin and IAA also determined in this experiment.

Measurement of soluble sugar (glucose and fructose) for drought stress

The final estimation was the determination of glucose, fructose. This experiment was done by taking the roots and shoot samples of stress-treated experimental and control rice plants. Stress treatments were inflicted by 12 days of exposure to 200 mM NaCl for salinity.

Drought tolerance assay of rice plants

The ten local experimental rice varieties and IR64 (control) were evaluated under both drought stress and non-stress conditions. These rice plants were grown in a pot in a greenhouse environment and the drought stress was induced by stopping the watering for fifteen days. Both the experimental and control plants were re-watered for the next ten days.

Measurement of photosynthetic characters, endogenous ion and sugar content

For drought tolerance assay, we have analyzed sugar content (glucose and fructose) and photosynthetic parameters such as Intracellular CO_2 concentration, transpiration rate, stomatal conductance, proline content, RWC, CAT was performed by infrared gas analyzer (IRGA;

LI-COR, LI-6400XT System) which was previously described by (Tuteja *et al.,* 2015).

Measurement of soluble sugar (glucose and fructose) content and Chlorophyll a fluorescence assay for drought stress

Experimental and control rice plants were grown with photosynthetically active radiation (750 µmol m^{-2} s^{-1}) for 16 hrs at 25°C. A chlorophyll a (Chl a) fluorescence assay was determined from the leaf samples of a 25-day-old control rice plant and the samples of rice seedlings were measured with a PAM-2100 fluorometer (Walz, Germany). Before the measurement, all the samples were placed in a dark place for 20 min.

Statistical analyses

In this study, data were obtained from three distinct experiments and the mean values along with their standard errors are presented here. ANOVA (one-way analysis of variance) was performed by using SPSS (12.0 Inc., USA) for the significant data. This technique was used to determine the least significant difference (LSD). We analysed and identified differences in the average values among the various treatments and we observed that the means of the treatments were different. Here, the results were analysed using Duncan's multiple range tests (DMRT).

RESULTS AND DISCUSSION

Relative expression of salinity-responsive rice lines during abiotic stresses

We grew 10 rice samples (T1-T10) and control rice plants for the salinity tolerance assay (Fig 1 a-b).

After 72 hrs of salinity stress (100 mM and 200 mM NaCl), we observed that T2 and T5 rice varieties were naturally tolerant to the stress (Fig 1b) while T3 and T9 rice varieties performed well under drought conditions (Fig 2).

Photosynthesis assay during saline stress

Both T2 and T5 rice lines were exposed to the photosynthetically active radiation, the chlorophyll fluorescence increased from 'O' level or 'F_o' (low minimum level) to 'P' level or F_m (higher maximum level). The maximum primary photochemical efficiency, estimated from Fv/Fm, was the most identical sample as a comparison to the control plant. Therefore, the chlorophyll content was higher in T2 and T5 rice plants under salt stress (Fig 3).

Antioxidant assay of selected rice line

The results of this experiment indicated significant reductions in MDA content, H_2O_2 levels and ion leakage. Additionally, the relative water content (RWC) was noticeably higher in the T2 and T5 rice varieties (Fig 4).

Similarly, we have found that proline content, antioxidant (CAT, APX, GPX, GR) content was increased in the same

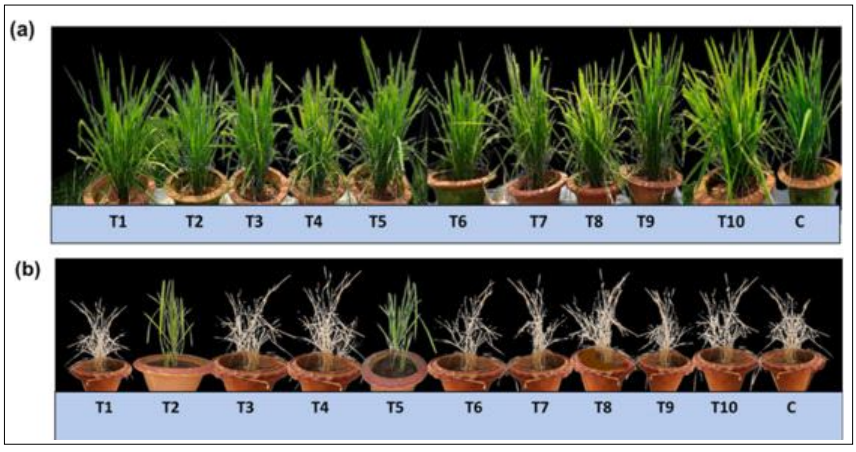


Fig 1: Tolerance activity of T2 and T5 rice varieties to the salinity stress in 200 mM NaCl concentrations.

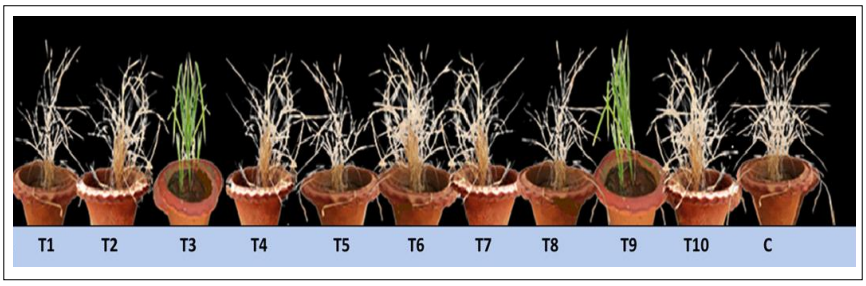


Fig 2: Tolerance activity of T3 and T9 rice varieties under drought stress.

rice varieties (T2 and T5) as compared to control plants (C) and other rice varieties under salinity stress of 200 mM Sodium Chloride (Fig 5 and Fig 6a).

Photosynthetic characteristics and agronomic assay of rice plants

The photosynthetic characteristics of all experimental plant samples were observed in 0 mM NaCl and 200 mM NaCl of salt treatments. Under 200 mM NaCl stress, we observed that the experimental rice plants' growth significantly increased as compared to the control rice plants. Here, the increased plant growth was found to be in the traits such as plant height, root length and dry weight, leaf area, number of tillers and panicles or plants, number of chaffy grains or panicles, straw weight (dry), 100 number of grain weight and number of filled grains or panicles. We identified that under the stress environment, both T2 and T5 plant samples increased their photosynthetic characteristics more than the control plant (IR64).

Hormone (GA, Zeatin and IAA), Endogenous ion and sugar assay

For salinity stress, we analysed hormones like GA, Zeatin and IAA were higher similarities in rice varieties T2 and T5 than the other samples and the control plant (Fig 6b-d). Similarly, sugar (glucose and fructose) content was also observed and found higher in T2 and T5 varieties (Fig 7a, b).

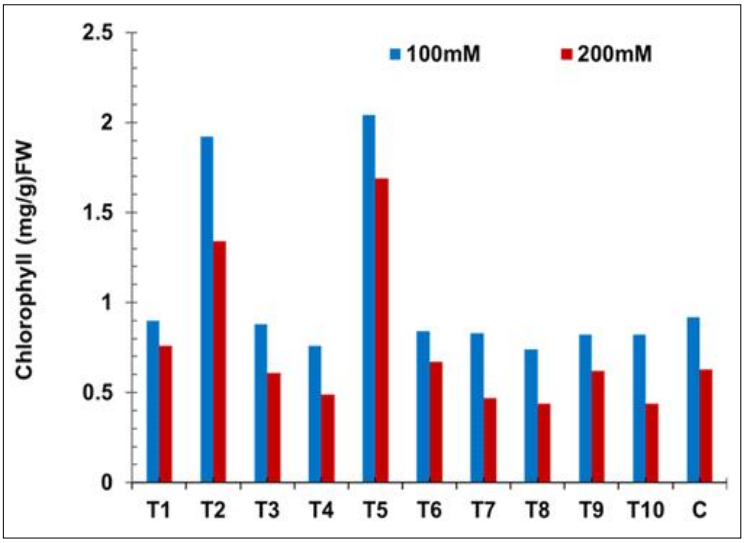


Fig 3: Chlorophyll assay of rice line under salinity stress.

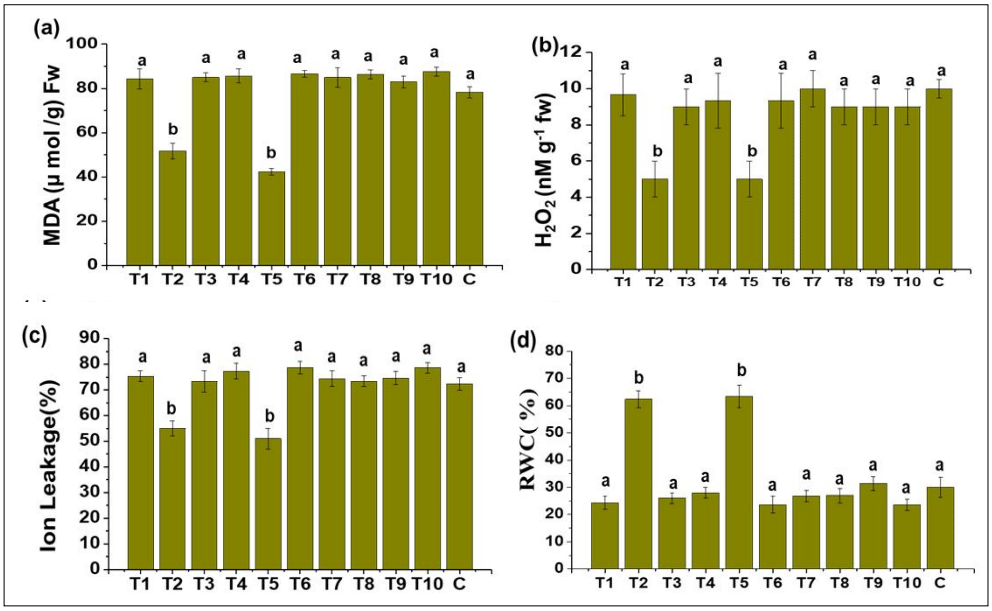


Fig 4: MDA, H_2O_2, ion leakage, RWC assay of rice line under salinity stress.

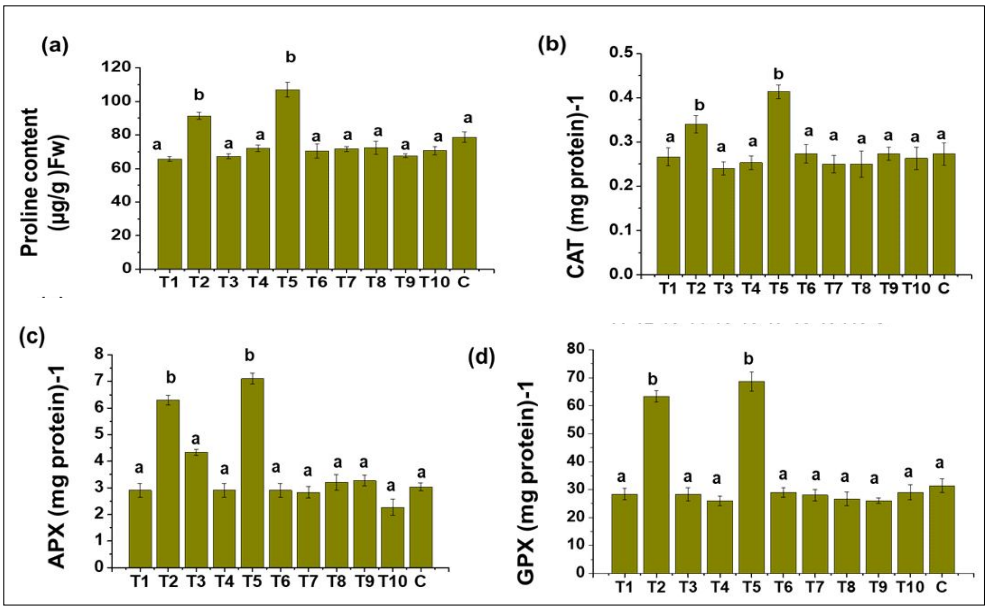


Fig 5: Proline, CAT, APX, GPX analysis of roots and shoots of rice line under salinity stress.

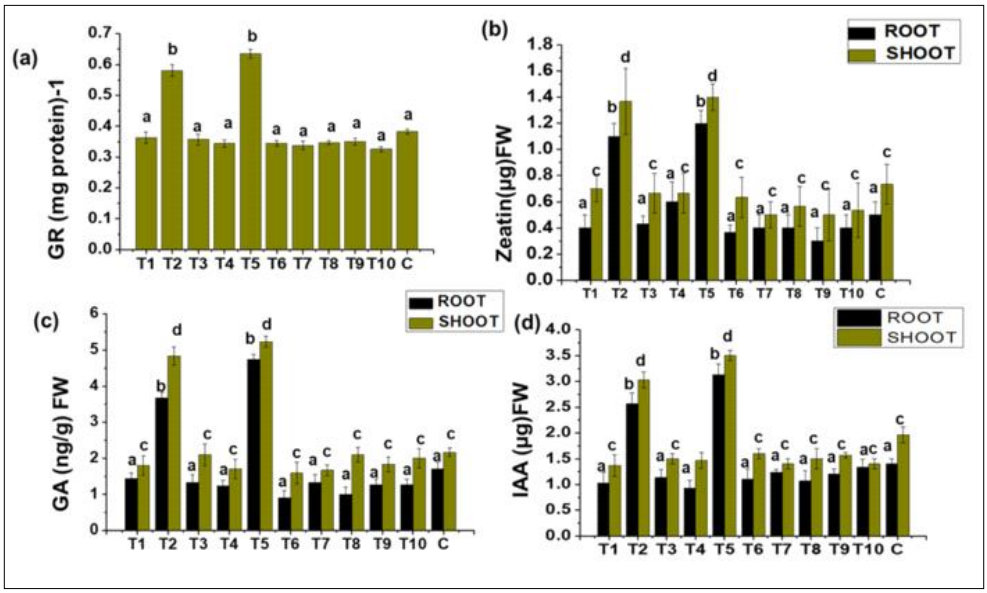


Fig 6: GR, Zeatin, Ga, IAA analysis of roots and shoots of rice line under salinity stress.

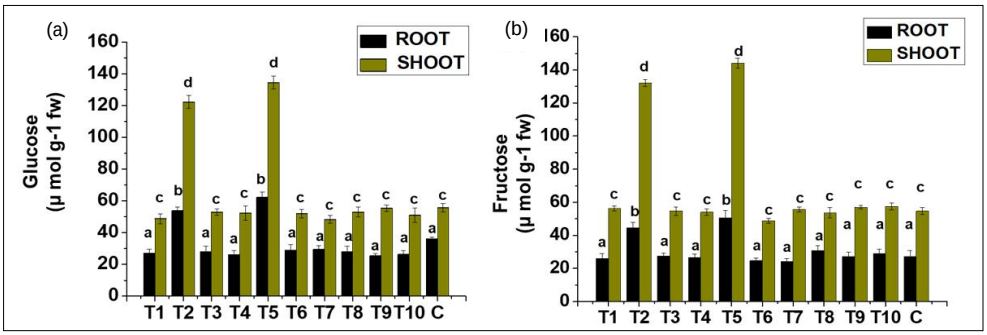


Fig 7: Sugar assay (Glucose and fructose) of plant roots and shoots for salinity stress.

The potassium phosphorus and nitrogen content in T2 and T5 plants were higher, whereas sodium content was lower as compared to C plants (Table 1).

Analysis of drought stress tolerance rice plants

In drought stress screening, we have selected two rice lines such are T3 and T9 from 10 rice varieties (T1-T10). we have analysed both T3 and T9 rice lines that increased their photosynthetic characteristics than the control plant and other rice lines (Table 2).

We have found the intracellular CO_2 concentration, transpiration rate, Photosynthetic yield and stomatal conductance of T3 and T9 rice lines were significantly higher than the other model plant samples and the control plant (Table 3).

Similarly, we have found higher proline, RWC, CAT and sugar content whereas ion leakage was lower than the other model samples and the control plant (Fig 8-9).

In this study, we identified the two best stress-tolerant rice varieties from 10 local rice varieties along with empty control (C) under salt stress and drought stress. Among the 10 rice varieties we have identified two varieties (T2: Jagannath and T5: Swarnamayee) were tolerance towards salinity stress and T3: Parijata and T9: Prativa rice varieties were tolerant towards drought stress. High salinity results in a higher influx of Ca^{2+} through the cyclic nucleotide-gated channel (CNGC). Elevated Ca^{2+} activates the SOS signalling pathway, which results in the efflux of Na+ from the cell. Na+ and Cl^- are compartmentalized inside the vacuoles of plant cells by OsNHXs and OsCLC transporters, respectively, reducing Na+ toxicity. High salinity stress also activates ABA signaling, which activates many transcription factors to provide salinity tolerance. However, ethylene negatively regulates salinity stress. Furthermore, during high salt concentrations, the gated outwardly rectifying K^+ (GORK) channel regulates K^+ efflux by utilizing ATP. Higher ROS concentrations inside the cell is generated by NADPH oxidase, OsRBOHA/F, which may result in cellular damage. Salt stress-induced ROS signal transduction processes include MAP kinase cascades to activate stress-responsive transcription factors. However, the plant cell neutralizes ROS by turning on the stress-responsive gene *OsCPK1*, which then activates OsAPX to decrease ROS. Genes, including *OsP5CS1*, *OsP5CR* and *OsTPS*, increase the amounts of osmolyte, proline and trehalose-6-phosphate, respectively, helping in osmotic adjustments. *Oryza sativa* late embryogenesis abundant (LEAs) are also upregulated to provide salinity tolerance (Fig 10).

T2 and T5 rice varieties were found to have high chlorophyll content in the presence of excess NaCl (200 mM). In leaf disks assay these varieties leaves were maintained green whereas the control (C) leaves became yellow. These results indicate that two varieties were stable and no chlorophyll deficiency in high saline environment (Chen *et al.,* 2021). Different growth parameters also showed tolerance to the salinity and drought stress. In (T2 and T5) (T3 and T9) varieties accumulation of H_2O_2, MDA

Table 1: Endogenous ion assay of rice lines under salinity and drought tolerance.

Attributes	Salinity stress						Drought stress			
	Control plants		T2		T5		T3		T9	
	0	200	0	200	0	200	0	200	0	200
Phosphorus (%)	0.243±0.011^{b}	0.126±0.004^{c}	0.269±0.017^{a}	0.133±0.013^{a}	0.272±0.012^{a}	0.129±0.018^{a}	0.2400.015^{a}	0.243±0.002^{a}	0.244±0.015^{a}	0.126±0.011^{a}
Sodium (%)	0.043±0.002^{a}	0.055±0.002^{a}	0.047±0.002^{a}	0.047±0.002^{a}	0.041±0.002^{a}	0.041±0.002^{a}	0.043±0.002^{a}	0.043±0.002^{a}	0.042±0.002^{a}	0.042±0.002^{a}
Potassium (%)	0.133±0.003^{b}	0.082±0.001^{c}	0.164±0.003^{a}	0.119±0.002^{a}	0.157±0.003^{a}	0.124±0.0.005^{a}	0.132±0.003^{a}	0.080±0.002^{a}	0.131±0.004^{a}	0.079±0.002^{a}
Nitrogen (%)	0.284±0.015^{b}	0.109±0.003^{c}	0.312±0.016^{a}	0.284±0.011^{a}	0.321±0.013^{a}	0.274±0.016^{a}	0.284±0.011^{a}	0.107±0.009^{a}	0.281±0.011^{a}	0.110±0.012^{a}

Table 2: Agronomic assay of salinity and drought tolerance of rice line.

Attributes	Rice plants		Abiotic stress-grown rice plants							
			Salinity stress				Drought stress			
	Control plants		Line T2		Line T5		Line T3		Line T9	
	0	200	0	200	0	200	0	After drought stress	0	After drought stress
Plant height (cm)	72±5.2^{a}	33.6±1.6^{b}	76±3.9^{a}	74±4.5^{a}	77±6.1^{a}	75±3.6^{a}	75±5.1^{a}	73±2.6^{a}	78±3.1^{a}	76±3.6^{a}
Root length (cm)	25±0.5^{a}	12±0.03^{b}	32±0.5^{a}	28±1.0^{a}	29±0.7^{a}	25±1.0^{a}	33±0.5^{a}	28±0.6^{a}	32±0.5^{a}	30±1.4^{a}
Root dry weight (g)	2.7±0.22^{a}	1.7±0.05^{c}	2.6±0.14^{a}	2.4±0.9^{a}	3.2±0.12^{a}	2.7±0.5^{a}	2.9±0.12^{a}	2.2±1.3^{a}	2.7±0.06^{a}	2.4±0.2^{a}
Leaf area (cm^2/plant)	92±2.4^{ab}	47.54±2.3^{c}	95±1.7^{a}	83±1.2^{ab}	94±1.2^{a}	85±0.9^{ab}	98±2.7^{a}	84±1.5^{ab}	94±1.7^{a}	81±1.0^{ab}

[Plant height (cm), root length (cm), root dry weight (g) and leaf area (cm^2) are the growth parameters. Each value indicates the mean of three replicates±SE and is compared by ANOVA. The same letters of data (a, b and c) in a row are minimal significance different at P>0.05 as determined by the LSD test].

Table 3: Photosynthetic parameters such as intracellular CO_2, transpiration rate, photosynthetic yield (Fv/Fm) and stomatal conductance assay of salinity and drought stress tolerance rice lines.

Attributes	Salinity stress						Drought stress			
	Control plants		T2		T5		T3		T9	
	0	200	0	200	0	200	0	200	0	200
Intracellular CO_2 (Ci, µ mol mo^{-1})	223±11.2^{a}	102.1±4.3^{b}	234±10.3^{a}	212±10.6^{a}	238±11.1^{a}	221±10.4^{a}	213±10.8^{a}	207±10.42^{a}	231±11.5^{a}	209±10.71^{a}
Transpiration rate (mol m^{-2} s^{-1})	1.52	0.81	1.96	1.07	1.83	1.12	1.78	1.04	1.87	1.14
Photosynthetic yield (Fv/Fm) (PN, µ mol CO_2 m^{-2} s^{-1})	9.25±0.5^{b}	6.03±0.23^{c}	11.19±0.4^{a}	8.26±0.25^{a}	12.04±0.7^{a}	9.31±0.22^{a}	10.51±0.7^{a}	9.32±0.34^{a}	10.38±0.47^{a}	9.17±0.242^{a}
Stomatal conductance (gs, mmol m^{-2} s^{-1})	243±12.2^{a}	112.3±5.6^{b}	242±12.4^{a}	146±11.9^{a}	252±12.4^{a}	144±11.9^{a}	252±12.6^{a}	123±6.2^{a}	256±13.1^{a}	138±6.7^{a}

[Photosynthesis parameters [Intracellular CO_2 concentration; transpiration rate (µ mol CO_2 m^{-2} s^{-1}), Photosynthetic yield (Fv/Fm) (PN, µ mol CO_2 m^{-2} s^{-1}) and stomatal conductance (mmol m^{-2} s^{-1})]. Each value indicates the mean of three replicates ±SE and is compared by ANOVA. The letters of a and b in the data row have minimal significance difference at P>0.05 as determined by the LSD test].

and ion leakage were significantly decreased under both salinity and drought stress. This report was consistent previously with stress-tolerance plants such as rice and sunflower, including watermelon (Khan *et al.,* 2024). antioxidant enzymes CAT, GR, GPX and RWC are significantly increased in two samples of rice plants (Sahoo *et al.,* 2022). Moreover, proline has been shown to help plants deal with various stresses, including salt. In our study, proline content accumulated and increased in (T2 and T5) rice samples under 200 mM salt stress. Glucose and fructose detoxify the reactive oxygen species (ROS) and protect the plant from salt stress (Sahoo *et al.*, 2022).

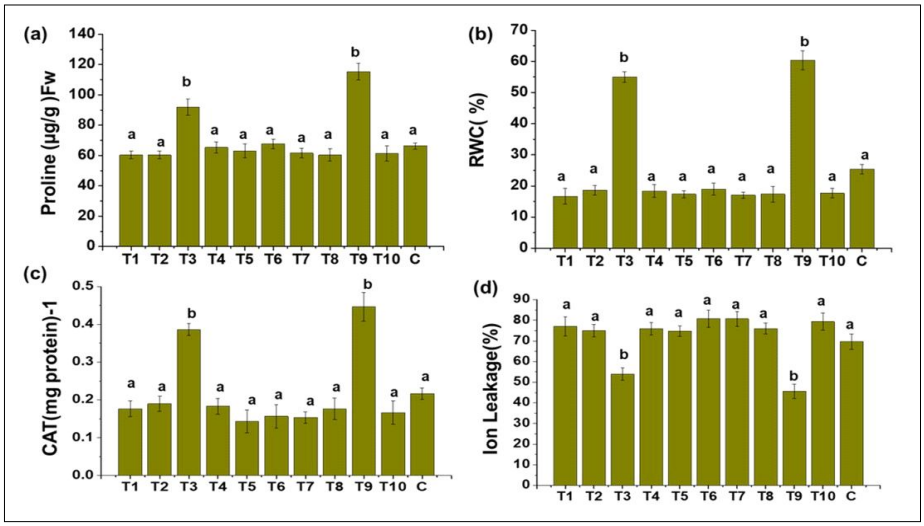


Fig 8: Proline, Ion leakage, RWC, CAT assay under drought stress.

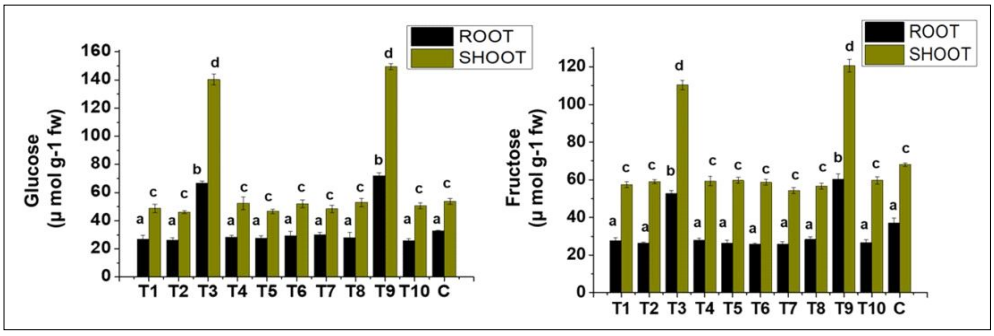


Fig 9: Sugar assay (Glucose and fructose) of plant roots and shoots for drought stress.

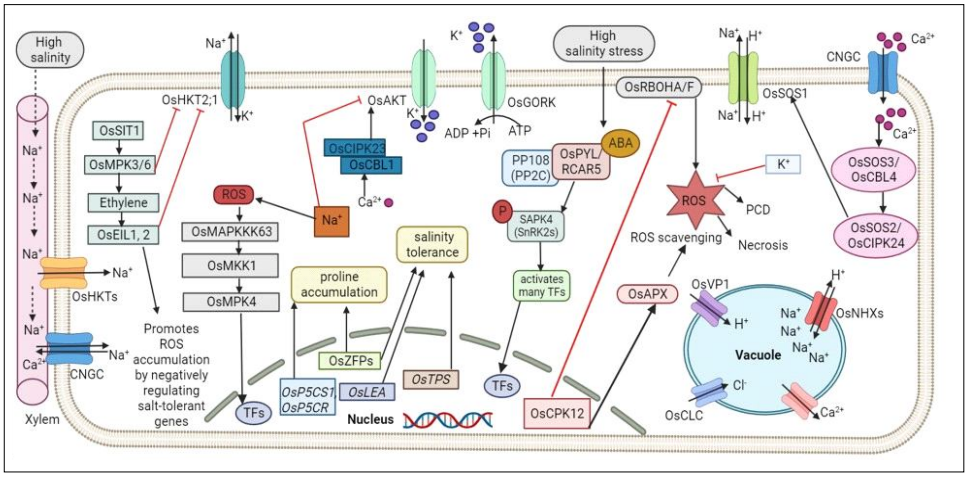


Fig 10: Tolerance mechanisms of salt stress in rice.

The sugar content in (T2 and T5), (T3 and T9) model plants was higher compared to C and other sample plants. Higher concentrations of potassium and nitrogen and lower concentrations of sodium were found in leaves of (T2 and T5), (T3 and T9) varieties as compared with C and model plants. The concentrations of potassium are higher and sodium concentrations are lower in T2 and T5. In T3 and T9 plantlets Na+/K+ ratio was lower and improved stress tolerance to salinity and drought compared to other plants including the control plant (C).

CONCLUSION

In this research work, we have identified the natural salinity and drought-tolerant rice varieties that were higher in antioxidant concentration and agronomic parameters. Out of ten rice varieties, we have identified four rice varieties, two varieties were suggested as salinity tolerant. In addition, antioxidant enzymes such as CAT, APX and GR show increased expression and enhanced activity during salinity/ drought stress. we have analysed higher concentrations of ascorbate peroxidase (APX), catalase (CAT), guaiacol peroxidase (GPX), glutathione reductase (GR) enzymes, hydrogen peroxide (H_2O_2) and proline content in stress-tolerance rice varieties (T2 and T5) and (T3 and T9) compared to other rice varieties. The findings of our study will be helpful and enhance agricultural products for both plant breeders and farmers in saline belts and drought areas.

ACKNOWLEDGEMENT

The author thanks Centurion University of Technology and Management, Bhubaneswar, India for providing the necessary support to complete this manuscript.

Author contributions

Monalisha Das Mohapatra, Ranjan Kumar Sahoo conceived the manuscript. Monalisha Das Mohapatra created figures and tables and wrote the manuscript with the help of Ranjan Kumar Sahoo.

Funding

No funding agency gave support for this work.

Conflict of Interest

The authors declare no conflicts of interest.

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