



Research Article

DETERMINATION OF SALINITY TOLERANT POTENTIAL OF INDIGENOUS LANDRACE AND WHITE RICE VARIETY

Rajalakshmi S, Parameshwari R and Indra Arulselvi P*

Plant and Microbial Biotechnology Laboratory, Department of Biotechnology, Periyar University,
Salem, Tamil Nadu, 636011, India

Article History: Received 19th October 2022; Accepted 27th November 2022; Published 09th December 2022

ABSTRACT

The purpose of this study was to evaluate how salt stress affected the activity of antioxidant enzymes in rice plants. The improved cultivar of White Ponni and the conventional cultivar of Kalundai were the two genotypes used in the experiment, which had a factorial design, complete randomization, three replicates, and five salt levels (0, 50, 100, 150, and 200 mM NaCl). Catalase (CAT) and superoxide dismutase (SOD), two antioxidant enzymes, as well as the total soluble protein content, physiological characteristics, and chlorophyll content were all measured. While the White Ponni cultivar's CAT and SOD activities showed no discernible trend, the activity of all examined enzymes in the Kalundai cultivar rose with increasing salt stress treatments. Regression analysis results for antioxidant enzymes in the Kalundai cultivar revealed a strong relationship between salt stress levels and antioxidant enzymes. In contrast, the correlation between CAT and SOD activity was modest in the white ponni cultivar. In summary, the current study's findings showed that the newly produced cultivar of white ponni was less resistant to salt stress than the Kalundai cultivar.

Keywords: Klundai, SOD, CAT, Chlorophyll, Oxidative stress.

INTRODUCTION

Rice (*Oryza sativa*, Linn) is one of the major food crops deeply affected by salt stress in about 20% of arable land in the world (Dramalis *et al.*, 2021). India retains remarkable procurement in rice production yet faces a nourishing 1.8 billion by 2050. The intention to feed the population under the elevated conditions of abiotic factors like soil salinities exerted dynamic pressure on rice production in 23 million ha of affected land (Mythili and Goedecke, 2016). Tamil Nadu is in sixth place among the Indian states under soil salinization restoring 13.231 saline and 354.784 sodic soil in million ha of finite land resources (Mandal *et al.*, 2018). Indigenous rice exists as prime in Tamil Nadu which is predominantly confined in the local food security. Based on indigenous people's knowledge-rich pool of landraces have the capability that grows under the increasing conditions of a saline environment (Rathna Priya *et al.*, 2019). However, recent scientific investigations have identified few indigenous rice accountable for salinity tolerance are far from being wealthy reservoirs.

The salinity level of ($3dSm^{-1}$) affects all stages in rice genotypes due to the elevation level of osmotic pressure. The osmosis of Na^{+} and Cl^{-} ions generates toxicity that hinders normal cellular and molecular processes inward and reduces biomass, root and shoots length at the early seedling stage. The salinity likewise influences crop productivity at the reproductive stage (tiller, spikelet number, panicle length, spikelet fertility) (Krishnamurthy *et al.*, 2016). The potential for water is reduced by high NaCl accumulation at the root zone, which limits water extraction and causes osmotic stress. The negative CO_2/O_2 ratio in the chloroplasts is caused by the low conductivity of the stomata due to the high salt concentration. High NaCl concentrations just at root zone lower the amount of water that could be extracted and cause osmotic stress. The negative CO_2/O_2 ratio in the chloroplasts is caused by the high concentration of salt's decreased stomatal conductivity, which limits the uptake of leaf CO_2 (Remorini *et al.*, 2009). The high rise of salt amount impairs the function of thylakoids in chloroplast gradually declining the activity in photosynthetic systems (I and II)

*Corresponding Author: Indra Arulselvi P, Plant and Microbial Biotechnology Laboratory, Department of Biotechnology, Periyar University, Salem, Tamil Nadu, 636011 India Email: iarulselvibiotech@gmail.com .

(Formentin *et al.*, 2018). Na⁺ toxicity generates the production of ROS (e.g., OH, H₂O₂ and O²⁻) that deteriorate biomolecules (lipids, proteins, and DNA) in molecular processes leading to plant death. Salt-tolerant rice plants have defense systems such as catalase (CAT), superoxide dismutase (SOD), and Ascorbate peroxidase (APX) against ROS (Sharma *et al.*, 2012). The upregulation of transcriptional levels during stress in salt-tolerant plants counteract ROS with non-enzymatic such as ascorbate, carotenoids, flavonoids, etc. The stress deteriorates the peroxidation of unsaturated fatty acids in membranes (lipids) will accumulate malondialdehyde (MDA) as a final product imprint of ROS.

In Tamil Nadu, the insights of rice landraces featured from ancient scripts have been incredibly tolerant to stress and are renowned as the “Rice Granary of South India” (Sathya, 2014). In recent decades, studies are focusing more on the investigation of the antioxidants' defense mechanism in crop plants under salt stress. Therefore these studies highlighted the significance of salt-tolerant mechanisms in traditional rice landraces. In this research, we did a preliminary study of Kalundai, traditional rice for its salt-tolerant ability under salt stress conditions. In addition, we have compared Kalundai with improved variety white ponni, a commercial rice variety

MATERIALS AND METHODS

Plant material and NaCl treatment

Kalundai and white ponni were obtained from Tamil Nadu Agricultural University (TNAU), Coimbatore, Tamil Nadu, and India. Rice seeds were surface-sterilized with 2.5% sodium hypochlorite solution and washed with distilled water. The seeds were then allowed to germinate in sterile filter papers soaked in distilled water for 72 h in the dark. Constantly selected germinated seeds were grown in 0.5% Murashige and Skoog (MS) medium maintained under 30°C /25°C day and night temperature, 70 - 80% of relative humidity, and 700 - 800µmol m⁻² s⁻¹ of light intensity over a 12 h photoperiod (semi-controlled plant tissue culture room). The nutrient solution was air circulated continuously and replaced with a fresh medium once in two days. Three weeks old rice seedlings were treated with different concentrations of NaCl (0, 50, 100, 150, 200mM). Plants were harvested a week after the treatment for further analysis.

Morphological parameters

Indigenous rice Kalundai and white ponni were harvested after salt treatment. Root and shoot length and biomass were recorded.

Physiological measurements

Chlorophyll and Carotenoids

Chlorophyll *a* and *b* were estimated following the method adopted by Roy Choudhury *et al.* (2007). Nearly, 100 mg

of fresh leaf tissue was collected for chlorophyll measurement. Ice-cold homogenized in pre-chilled mortar & pestle with ice-cold 80% of (v/v) acetone and centrifuged at 10,000 rpm for 10 min. This was re-extracted till clear supernatant appears and collected supernatant in a separate tube. The extract was filtered and analysed with a spectrophotometer at 660 nm & were 5 nm for chlorophyll *a* & *b* respectively. Carotenoids were also measured at 470 nm. The results were expressed in mg/ g of fresh weight and calculated by the equations of Lichtenthaler.

Protein content

Protein extracted from leaves as well as the treated plants were estimated according to Bradford's method. The estimation is based on the capability of proteins bound to Coomassie brilliant blue G-250 dye with an expected extinction coefficient greater than the free dye. Various concentrations of protein standards were prepared from the stock solution (0.1, 0.2 to 1 ml) into test tubes and the volume was made up to 1ml. The 100µg of rice roots and leaves were ground using 2 ml of the extraction buffer, and the mixture was filtered. Nearly, 100µl of the filtered mixture was taken in a test tube and 1 ml of distilled water and 2.5ml of Bradford reagent were added. The mixture was mixed with a vortex mixer and incubated at room temperature for 10-30 mins. The protein was estimated using the absorbance at 595 nm.

Enzymatic Assays

Enzyme extraction

Root and leaf samples weighing 500µg were homogenized in ice cold 0.1 M phosphate buffer (pH=7.5) containing 0.5 mM EDTA and 2% (w/v) polyvinylpolypyrrolidone (PVPP) with pre-chilled pestle and mortar. Each homogenate was transferred to cold centrifuge tubes and was centrifuged at 4°C in Beckman refrigerated centrifuge for 15 min at 15000×g. The supernatant was used for enzyme activity assay (Esfandiari *et al.*, 2007).

Catalase (CAT) activity

CAT (EC.1.11.1.6) was measured following the method adopted by Aebi (1984). CAT was estimated by the residual H₂O₂ in the reaction mixture. The activity was measured by monitoring the decrease in the absorbance from 0-3 min at 240 nm using a UV visible spectrophotometer. CAT was calculated using the extinction coefficient of 39.4mM⁻¹. One unit of CAT activity was defined as the amount of enzyme required to scavenge 1µmol of H₂O₂µg fresh weight under assay conditions.

Superoxide dismutase activity (SOD)

The SOD (EC.1.15.1.1) activity was measured by the method described by Dhindsa *et al.* (1981). The reaction was started by adding 60µm riboflavin to the sample extracts exposed to light. A complete reaction mixture without enzyme served as control and a complete reaction mixture (unexposed to light) served as blank. The reaction

was stopped by unexposed to light. The absorbance was measured at 560 nm against the blank using UV- a visible spectrophotometer (Sytronics, India). One unit of the SOD enzyme activity was defined as the quantity of SOD required to produce a 50% reduction of NBT (expand) under assay conditions. The enzyme activity was taken as units per gram for the fresh weight of the plant tissue.

Statistical Analysis

All treatments were carried out in three repetitions to ensure statistical validity when measuring plant growth, biomass, photosynthetic pigment concentrations, antioxidative enzyme activity, and protein content. Using SPSS 20 was used to do the ANOVA and the Duncan's multiple-range test (DMRT). The Student-Newman-Keuls test, with a significance level of $P < 0.05$, was used to assess the mean differences.

RESULTS AND DISCUSSION

When seedlings were subjected to salt stress, the growth characteristics, including shoot height, root length, fresh weight, and dry weight, demonstrated a significant shift. Shoot heights were lowered as a result of salinity. In comparison to the control of both genotypes, seedling

shoots length dropped by around 39.41% and 26.40% on white ponni and kalundai respectively. On the other hand the salinity stressed seedling roots also dropped by around 57.24% in white ponni and 38.57% in kalundai. The growth of the rice varieties was significantly impacted by the growing medium's various salt concentrations. The effects of various doses of NaCl treatments on seedling biomass were depicted in (Table 1). The seedling biomass was gradually decreased with increasing the salinity dose level in the growth medium. In NaCl treated seedlings of both varieties, the greatest biomass reduction observed was 35.50% and 52.96% when compared to the control. The effects of stress exposure were found to be detrimental to both seedling development and total biomass. At higher salt stress concentrations, the inhibition was more pronounced in root tissues than shoot tissues. Roots' inability to absorb nutrients restricted the growth of the entire plant, ultimately resulting in a reduction in plant biomass. A significant slowdown in growth, as was previously found in rice seedlings under salinity stress (Pattanagul and Thitisaksakul, 2008). Salinity also had a significant impact on the fresh and dry weights of treated rice seedlings of both varieties. Additionally, Sobahan *et al.* (2012) demonstrated that the biomass decreased under salinity stress.

Table 1. Effect of salt stress treatment on the development of two distinct rice varieties White ponni and kalundai.

Stress concentrations (mMNaCl)	Shoot length (cm)	Root length (cm)	Biomass FW(mg)	Biomass W(mg)
White ponni control	27.15 ± 0.05 ^a	13.80 ± 0.60 ^a	1.69 ± 0.05 ^a	0.68 ± 0.02 ^a
50	25.80 ± 0.10 ^a	13.00 ± 0.65 ^a	1.70 ± 0.00 ^a	0.51 ± 0.05 ^b
100	22.55 ± 0.55 ^b	10.05 ± 0.80 ^b	1.40 ± 0.02 ^b	0.46 ± 0.08 ^c
150	19.00 ± 0.20 ^c	7.8 ± 0.55 ^c	1.23 ± 0.02 ^c	0.30 ± 0.01 ^d
200	16.45 ± 0.45 ^d	5.9 ± 0.45 ^c	1.09 ± 0.03 ^d	0.27 ± 0.09 ^e
Kalundai Control	31.05 ± 0.70 ^a	16.85 ± 0.35 ^a	2.53 ± 0.001 ^a	1.61 ± 0.10 ^a
50	30.95 ± 0.80 ^a	16.30 ± 0.45 ^a	2.26 ± 0.023 ^b	0.95 ± 0.02 ^b
100	28.06 ± 0.75 ^b	15.5 ± 0.90 ^b	1.83 ± 0.015 ^c	0.71 ± 0.016 ^c
150	25.20 ± 0.50 ^c	13.85 ± 0.65 ^c	1.59 ± 0.032 ^d	0.66 ± 0.012 ^d
200	22.85 ± 0.70 ^d	10.35 ± 0.80 ^d	1.19 ± 0.011 ^e	0.57 ± 0.17 ^e

Note. Data are presented as mean ± standard error (n ¼ 3). Mean values followed by different superscript letters (a–f) within the column are statistically significant at $p \leq 0.05$ level.

Table 2. Effect of salt stress treatment on photosynthetic pigment of two distinct rice varieties White ponni and kalundai.

Stress concentrations (mMNaCl)	Photosynthetic pigment contents level (mg g ⁻¹ FW)		
	Chlorophyll a	Chlorophyll b	Carotenoids
White ponni Control	27.59 ± 0.05 ^a	9.96 ± 0.34 ^a	6.30 ± 0.01 ^a
50	27.04 ± 0.10 ^a	9.13 ± 0.53 ^{ab}	5.65 ± 0.20 ^b
100	25.56 ± 0.14 ^b	7.81 ± 0.00 ^b	4.64 ± 0.14 ^c
150	23.42 ± 0.48 ^c	5.03 ± 0.44 ^c	3.87 ± 0.07 ^d
200	21.98 ± 0.30 ^d	3.14 ± 0.24 ^d	2.63 ± 0.29 ^e
Kalundai Control	33.15 ± 0.28 ^a	14.13 ± 0.55 ^a	9.36 ± 0.39 ^a
50	32.22 ± 0.33 ^b	13.65 ± 0.23 ^{ab}	8.20 ± 0.06 ^b
100	29.82 ± 0.15 ^c	12.29 ± 0.18 ^{bc}	6.22 ± 0.18 ^c
150	26.09 ± 0.02 ^d	11.42 ± 0.19 ^c	5.22 ± 0.07 ^{cd}
200	25.68 ± 0.64 ^e	9.05 ± 1.08 ^d	5.01 ± 0.27 ^d

Note. Data are presented as mean ± standard error (n ¼ 3). Mean values followed by different superscript letters (a–f) within the column are statistically significant at $p \leq 0.05$ level.

The amount of chlorophyll in the leaves as stated in table 2 was impacted by salt treatment. After NaCl treatments, there was a drop in the amount of carotenoid and chlorophyll a in the leaves. Chlorophyll b content in both varieties decreased during salt stress by (68.47%) in white ponni (35.95%) in kallundai than chlorophyll a (20.33%) and (22.53%) on white ponni and kallundai were less influenced than chlorophyll b. In white ponni and kallundai with large amounts of NaCl at 200 mM, the carotenoid content fell by almost 58.25% and 46.47%, respectively. The salt-induced suppression of carotenoid and chlorophyll production, which may be brought on by nutrient deficiencies such as Mn, Cu, Fe, and P, may be responsible for the lower amount of photosynthetic pigments (Sobrinho-Plata *et al.*, 2009). The deficiency cause is that the growing medium's salinity were initially contacted by plant roots. Under salt stress, similar outcomes were also noted in *Medicago sativa* (Zhou *et al.*, 2007).

Figure 1 depicts how salt stress affected the overall amount of soluble protein. In comparison to control, white ponni and kallundai shoots treated with 100 and 150 mM NaCl had a higher total soluble protein content. For both genotypes' shoot tissues, the highest levels of total soluble protein were recorded at concentrations of 100 and 150

mM, or 126% and 128%, respectively. In contrast, the total protein content in root tissues rises to 116% at 50 mM for white ponni and 194% at 200 mM for kallundai. In both the shoot and root tissue of white ponni and the shoot tissue of kallundai, decreases in total protein content beyond the ideal salt concentration are directly correlated with the NaCl treatment. The total protein content level rose to the maximum salt concentration in the kallundai root tissue (200mM). At greater salt concentrations, the protein level marginally reduced. This rise may be caused by additional metal sequestration systems, which are involved in the detoxification of salt dosages, becoming more active. Both leaves and roots of plants under salt stress and during recovery circumstances had significantly lower levels of total protein than controls. Durchan *et al.* (2012) also noted that greater stress concentrations in algae were accompanied by protein level declines. Similar to pearl millet, green pea, and other plants, stress at higher doses greatly increased the leaf total soluble protein levels (Tarafdar *et al.*, 2014; Mukherjee *et al.*, 2016). Our results are consistent with Zhao *et al.* (2012)'s work, which found that CeONP treatment of corn increased the expression of stress proteins. According to a study, proteins shield cells from possible oxidative damage brought on by the deposition of nanoparticles in plants (Zhao *et al.*, 2012).

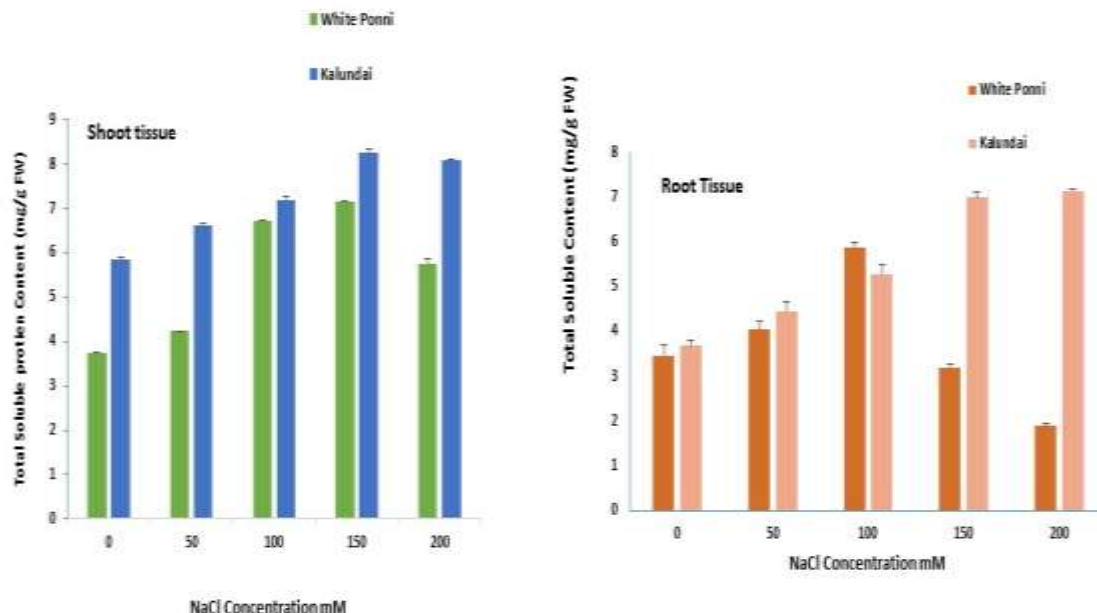


Figure 1. Analysis of total soluble protein content levels in both shoot and root tissues of rice plants grown under NaCl treatment.

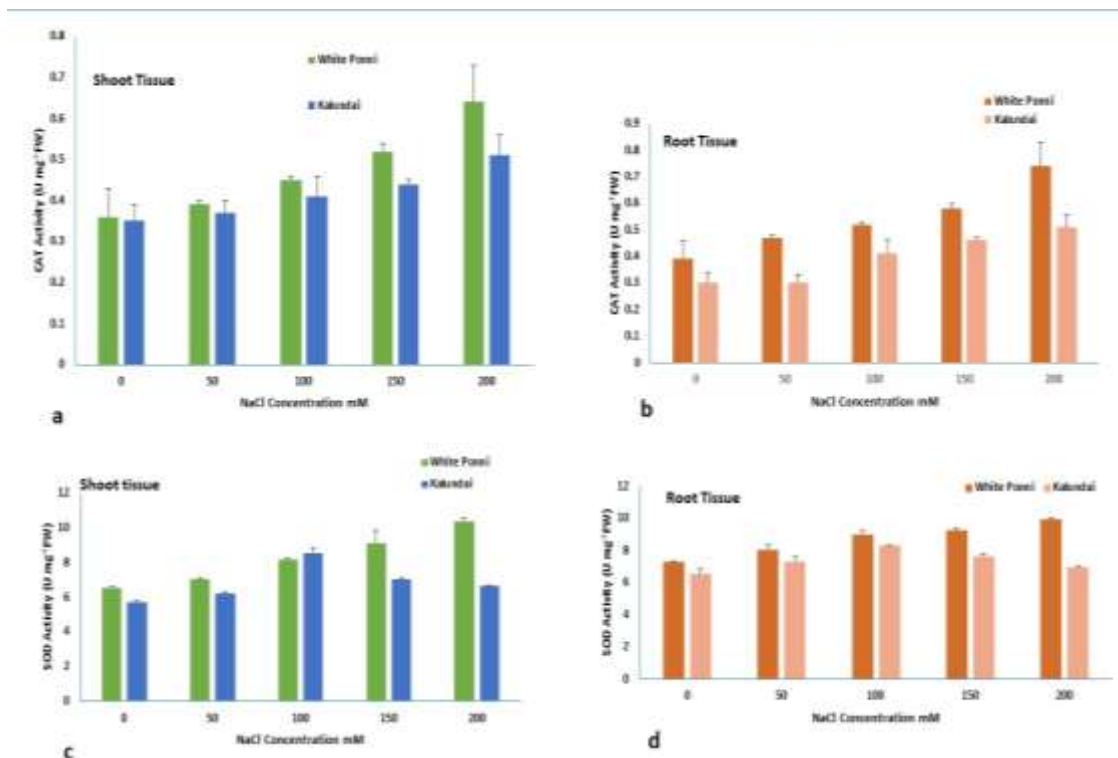


Figure 2. Estimation of (a and b) CAT and (c and d) SOD antioxidative enzyme activities in both shoot and root tissues of two genotypes of rice cultivar (white ponni and kalundai) plants grown under NaCl treatments.

One of the most powerful antioxidant enzymes is CAT, which is essential for maintaining the redox equilibrium inside the cell. (Shaw, 1995). The detoxification of H₂O₂ is carried out by the antioxidant defense enzymes CAT and POX, which turn free radicals into water (H₂O) and oxygen (O₂) (Ma *et al.*, 2015). CAT activity in salt-stressed plants' shoots and roots changed in both genotypes over the course of the experiment Figure.2a. It's noteworthy to note that when salt stress levels in the medium grew, the level of CAT activity also considerably increased, reaching maximum levels of 177.7% and 145% on doses of white ponni and Kalundai, respectively, at 200 mM. White Ponni and Kalundai both have somewhat higher CAT activity in the root tissue, at 189.7% and 170%, respectively. The CAT assay results clearly show that the cultivated variety (white ponni) has a higher level of CAT Activity than the wild variety (kalundai). In the NaCl stress treatment, the intensity of the CAT isoforms dropped, but it remained constant in the control. Similar reports were seen with tobacco as well (Badawi *et al.*, 2004).

In the current investigation, increasing the concentration of NaCl treatment up to 200 mM generally raised the SOD activity in the shoot and root of white ponni. The white ponni has a maximum elevated level of SOD activity of 158.5% in the shoot and 136% in the root tissue. On the other hand, elevations in Kalundai's SOD activity ceased and began to decline once the tissues had received 100 mM of the optimal NaCl treatment. The SOD results of plants under salt stress show unequivocally that wild variety is appropriate for salinity area. With increasing

salinity and time, there was a change in CAT and SOD activity. As the duration of the stress increased, the differences in CAT and SOD activity demonstrated that SOD was more important in oxidative damage than CAT. The enhanced SOD and CAT activity in rice can be viewed as circumstantial proof that wild kalundai have more developed salt tolerance mechanisms than white ponni.

CONCLUSION

In conclusion, increasing soil salinity is one of the major issues affecting agricultural productivity around the world, particularly for crop plants (Ben-saad *et al.*, 2012). Further study is required to understand how salinity stress affects various species, as the intensity and duration of stress have a significant impact on the composition and amounts of leaf photosynthetic pigments, physiological characteristics, and antioxidant enzymes (Shah *et al.*, 2017). Furthermore, a detailed evaluation of these characteristics would enable the presentation of plant health as well as a subtly reflected stress reaction. The results of this study indicate that the kalundai response to ROS build up and oxidative stress differs from that of white ponni in a few key ways.

ACKNOWLEDGMENT

The authors express sincere thanks to the Head of the Plant and Microbial Biotechnology Laboratory, Department of Biotechnology, Periyar University, Salem, Tamil Nadu, India for the facilities provided to carry out this research work.

REFERENCES

- Aebi, H. (1984). Catalase Invitro. *Methods in Enzymology*, 105, 121-126.
- Badawi, G.H., Yamauchi, Y., Shimada, E., Sasaki, R., Kawano, N., Tanaka, K., Tanaka, K. (2004). Enhanced tolerance to salt stress and water deficit by overexpressing superoxide dismutase in tobacco (*Nicotiana tabacum*) chloroplasts. *Plant Science*, 166, 919-928.
- Ben-Saad, R., Ben-Ramdhan, W., Zouari, N., Azaza, J., Mieulet, D., Guiderdoni, E., Ellouz, R., Hassairi, A. (2012). Marker-free transgenic durum wheat cv. Karim expressing the ALSAP gene exhibits a high level of tolerance to salinity and dehydration stresses. *Molecular Breeding*, 30, 521-533.
- Bradford, M.M. (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.
- Dhindsa, R.H., Plumb-Dhindsa, R., T.A. (1981). Thorpe: Leaf senescence correlated with increased level of membrane permeability, lipid peroxidation and decreased level of SOD and CAT. *Journal Experimental Botany*, 32, 93-101.
- Dramalis, C., Katsantonis, D., and Koutroubas, S. D. (2021). Rice growth, assimilate translocation, and grain quality in response to salinity under Mediterranean conditions. *AIMS Agriculture and Food*, 6, 255-272.
- Durchan, M., Tichy, J., Litvin, R., Slouf, V., Gardian, Z., Hřibek, P. (2012). Role of carotenoids in light-harvesting processes in an antenna protein from the chromophyte *Xanthonema debile*. *The Journal of Physical Chemistry B*, 116, 8880-8889.
- Formentin, E., Sudiro, C., Perin, G., Riccadonna, S., Barizza, E., Baldoni, E., Lavezzo, E., Stevanato, P., Sacchi, G.A., Fontana, P. (2018). Transcriptome and cell physiological analyses in different rice cultivars provide new insights into adaptive and salinity stress responses. *Frontiers in Plant Science*, 9, 204.
- Krishnamurthy, S.L., Gautam, R.K., Sharma, P.C., Sharma, D.K. (2016). Effect of different salt stresses on agromorphological traits and utilization of salt stress indices for reproductive stage salt tolerance in rice. *Field Crops Research*, 190, 26-33.
- Ma, C., White, J.C., Xing, B., Dhankher, O.P. (2015). Phytotoxicity and ecological safety of engineered nanomaterials. *Inter. Journal of Plant and Environment*, <http://dx.doi.org/10.18811/ijpen.v1i1.7110>.
- Mandal, S., Raju, R., Kumar, A., Kumar, P., and Sharma, P. C. (2018). Current status of research, technology response and policy needs of salt-affected soils in India – a review. *Journal of the Indian Society of Coastal Agriculture Research*, 36, 40-53.
- Mittler, R. (2007). Oxidative stress, antioxidants and stress tolerance. *Trends in Plant Science*, 7, 405-410.
- Mukherjee, A., Sun, Y., Morelius, E., Tamez, C., Bandyopadhyay, S., Niu, G., White, J.C., Peralta-
- Videa, J.R., Gardea-Torresdey, J.L. (2016). Differential toxicity of bare and hybrid ZnO nanoparticles in green pea (*Pisum sativum* L.): a life cycle study. *Frontiers in Plant Science*, 6, 1242.
- Mythili, G., and Goedecke, J. (2016). "Economics of Land Degradation in India," (*Cham: Springer*), 431-469.
- Pattanagul, W., and Thitisaksakun, M. (2008). Effect of salinity stress on growth and carbohydrate metabolism in tree rice (*Oryza sativa* L.) cultivars differing in salinity tolerance. *Indian Journal of Experimental Biology*, 46, 736-742.
- RathnaPriya, T.S., Nelson, A.R.L.E., Ravichandran, K., and Antony, U. (2019). Nutritional and functional properties of coloured rice varieties of South India: a review. *Journal of Ethnic Food*, 6, 11.
- Remorini, D., Melgar, J. C., Guidi, L., Degl'Innocenti, E., Castelli, S., Traversi, M. L. (2009). Interaction effects of root-zone salinity and solar irradiance on the physiology and biochemistry of *Olea europaea*. *Environmental and Experimental Botany*, 65, 210-219.
- Roy Choudhury, A., Roy, C., Sengupta, D.N. (2007). Transgenic tobacco plants over expressing the heterologous lea gene Rab16A from rice during high salt and water deficit display enhanced tolerance to salinity stress. *Plant Cell Reports*, 26, 1839-1859.
- Shah, S.H., Houborg, R., McCabe, M.F. (2017). Response of chlorophyll, carotenoid and SPAD-502 measurement to salinity and nutrient stress in wheat (*Triticum aestivum* L.). *Agronomy*, 7, 61.
- Sharma, P., Jha, A.B., Dubey, R.S., Pessarakli, M. (2012). Reactive oxygen species, oxidative damage, and antioxidative defense mechanism in plants under stressful conditions. *Journal of Botany*, Article ID 217037 | <https://doi.org/10.1155/2012/217037>.
- Shaw, B. P. (1995). Effects of mercury and cadmium on the activities of antioxidative enzymes in the seedlings of *Phaseolus aureus*. *Biologia Plantarum*, 37, 4, 587-596.
- Sobahan, M.A., Akter, N., Ohno, M., Okuma, E., Hirai, Y., Mori, I.C., Nakamura, Y., Murata, Y. (2012). Effects of exogenous proline and glycine betaine on the salt tolerance of rice cultivars. *Bioscience Biotechnology and Biochemistry*, 76, 1568-1570.
- Sobrinho-Plata, J., Ortega-Villasante, C., Flores-Caceres, M.L., Escobar, C., Del Campo, F.F, and Hernandez, L.E. (2009). Differential alterations of antioxidant defenses as bioindicators of mercury and cadmium toxicity in Alfalfa. *Chemosphere*, 77, 946-954.

- Tarafdar, J.C., Raliya, R., Mahawar, H., Rathore, I. (2014). Development of zinc nanofertilizer to enhance crop production in pearl millet (*Pennisetum americanum*). *Agricultural Research*, 3, 257-262.
- Zhao, L., Peng, B., Hernandez-Viezcas, J.A., Rico, C., Sun, Y., Peralta-Videa, J.R., Tang, X., Niu, G., Jin, L., Varela-Ramirez, A. (2012). Stress response and tolerance of Zea mays to CeO₂ nanoparticles: cross talk among H₂O₂, heat shock protein, and lipid peroxidation. *ACS Nano*, 6, 9615-9622.
- Zhou, Z.S., Huang, S.Q., Guo, K., Mehta, S.K., Zhang, P.C., Yang, Z.M. (2007). Metabolic adaptations to mercury-induced oxidative stress in roots of *Medicago sativa* L. *Journal of Inorganic Biochemistry*, 101(1), 1-9.