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**Research Article**

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

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## 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 restoring13.231 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 CO2/O2 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)

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(Formentin *et al*., 2018). Na+ toxicity generates the production of ROS (e.g., OH, $H_2O_2$ and $O^{2-}$) 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 30ºC /25ºC 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 &were5 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 (Systronics, 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 verities White ponni and kalundai.

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

**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 verities White ponni and kalundai.

| Stress concentrations (mMNaCl) | Photosynthetic pigment contents level (mg $g^{-1}$ FW) | | |
|---|---|---|---|
| | Chlorophyll a | Chlorophyll b | Carotenoids |
| White ponni Control | $27.59 \pm 0.05^{a}$ | $9.96 \pm 0.34^{a}$ | $6.30 \pm 0.01^{a}$ |
| 50 | $27.04 \pm 0.10^{a}$ | $9.13 \pm 0.53^{ab}$ | $5.65 \pm 0.20^{b}$ |
| 100 | $25.56 \pm 0.14^{b}$ | $7.81 \pm 0.00^{b}$ | $4.64 \pm 0.14^{c}$ |
| 150 | $23.42 \pm 0.48^{c}$ | $5.03 \pm 0.44^{c}$ | $3.87 \pm 0.07^{d}$ |
| 200 | $21.98 \pm 0.30^{d}$ | $3.14 \pm 0.24^{d}$ | $2.63 \pm 0.29^{e}$ |
| Kalundai Control | $33.15 \pm 0.28^{a}$ | $14.13 \pm 0.55^{a}$ | $9.36 \pm 0.39^{a}$ |
| 50 | $32.22 \pm 0.33^{b}$ | $13.65 \pm 0.^{23ab}$ | $8.20 \pm 0.06^{b}$ |
| 100 | $29.82 \pm 0.15^{c}$ | $12.29 \pm 0.18^{bc}$ | $6.22 \pm 0.18^{c}$ |
| 150 | $26.09 \pm 0.02^{d}$ | $11.42 \pm 0.19^{c}$ | $5.22 \pm 0.07^{cd}$ |
| 200 | $25.68 \pm 0.64^{e}$ | $9.05 \pm 1.08^{d}$ | $5.01 \pm 0.27^{d}$ |

**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 verities 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 (Sobrino-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 kalundai 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 kalundai. In both the shoot and root tissue of white ponni and the shoot tissue of kalundai, 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 kalundai 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).

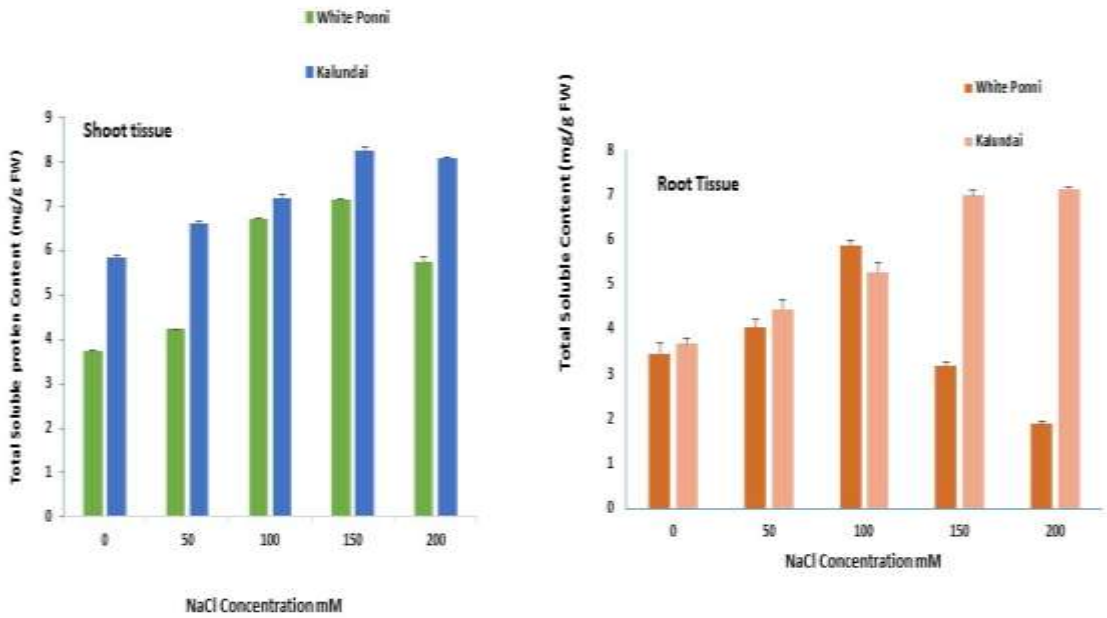


**Figure 1.** Analysis of total soluble protein content levels in both shoot and root tissues of rice plants grown under NaCl treatment.

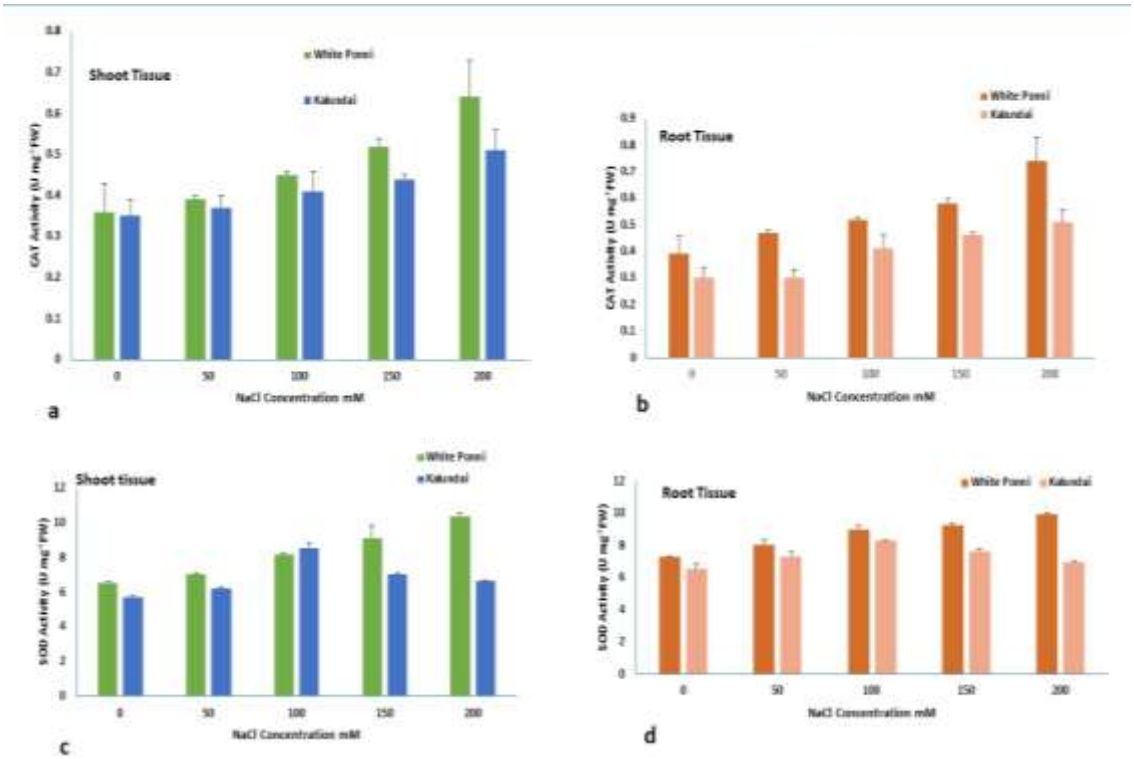


**Figure 2.**Estimation of (a and b) CAT and (c and d) SOC antioxidative enzyme activities in both shoot and root tissues of two genotypes of rice cultivar (white ponni and kalundai) plants grown under NaCl treatments.

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 verity (white ponni) has a higher level of CAT Activity than the wild verity (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.

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