Physiological and Biochemical Effect of Sodium Chloride (NaCl) Induced Salinity Stress on Three Cultivars of Radish

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***Abstract*— Salinity, is a major environmental factor that adversely affects plant growth and development and limits agricultural production. A very effective and simple screening technique for the identification of salt-tolerant and salt-sensitive radish genotypes was observed. In the present study, the effect of increasing NaCl concentrations was investigated on three varieties (*Pusa chetki, Mino early long,* and *Red round radish*) of *Raphanus sativus.L*. Seedlings were grown and irrigated with distilled water for 20 days and thereafter treated with different NaCl concentrations (0, 25, 75, 125 and 175mM) for 20 days. The relative tolerance of selected varieties was studied by analyzing differences in various parameters like Germination percentage, Seedling vigor index, Electrolyte leakage, Relative water content, Total chlorophyll content, Proline, Protein, Lipid Peroxidation, and Antioxidant enzymatic assays (Catalase Activity, Peroxidases Activity, and Superoxide Dismutases Activity). Physiological and biochemical properties were reduced significantly by the induction of salinity stress in all cultivars. Germination percentage, Leaf relative water content, Malondialdehyde concentration, Total chlorophyll content, Proline concentration, Protein content, and Enzymatic activity of *Pusa chetki* were determined to be higher than *Red round radish* and *Mino early long* Cultivars. Electrolyte leakage value was higher in the *Mino Early Long* cultivar than that in *Red Round Radish* and *Pusa chetki*. Our results showed that among all the three genotypes, *Pusa Chetki* and *Red Round Ra Red round radish* are salt-tolerant and *Mino early long* is a salt-sensitive genotype.**

***Index Terms*— Antioxidant, Catalases, Dismutases, Electrolyte leakage, Lipid Peroxidation, Peroxidases, Proline, Superoxide.**

I. INTRODUCTION

The stabilized gene pool of living being controls and governs almost all life leading mechanisms, reactions, pathways, and the entire schedule of the life cycle. The physiological reactions and manifestations are under genetic control. The synthesis of enzymes/ catalysts is also a genetically governed process. Thus, it is said that the genome of life forms plays a vital role in all life leading manifestations of living beings. The aspect of stress study, therefore, has been undertaken and illustrated in the present work to understand the response of plants to salt tolerance. Stress is usually defined as an external factor that exerts an unpropitious influence on the plant. In most cases, stress is measured concerning Plant endurance, Crop yield, Growth (biomass accumulation), or the Primary assimilation process (carbon dioxide and mineral uptake), which are related to overall growth. Because stress is defined exclusively in terms of plant responses, it is sometimes called Strain in conformance with engineering terminology. The concept of stress is informally associated with stress resilience, which is the plant's fitness to cope with an unfavorable environment. Soil salinity is one of the most important and desolating abiotic stress in agriculture [31]. Salinity, which is generally deleterious to plant growth adversely affects the metabolism of plants and causes important modifications in gene expression in plants.

Numerous genes seem to perform in concern to increase salinity tolerance, and certain proteins involved in salinity stress stability have also been recognized. Therefore, the development of methods and strategies to mitigate the detrimental effects of salt stress on plants have received ample attention. Such adaptations lead to multiplication or depletion of certain metabolites resulting in an imbalance in the levels of a proportionately small set of cellular proteins, which could increase, decrease, appear or disappear after salt treatment [21].

To survive the strain, multitudinous morphological, physiological, and biochemical changes occur in various plants species. Salinity can be mitigated

through either soil redemption or growing tolerant crops. The alteration of protein synthesis and degradation is one of the fundamental, metabolic processes that may influence salt stress tolerance [31].

Radish (*Raphanus sativus L.*) is native to Europe and Asia. It is cultivated almost everywhere in India, especially near the town markets. Commonly radish is a cool-season crop and it is cultivated during the winter season at any time between September and January. Asiatic varieties can resist more heat than European varieties. The enlarged edible roots are fusiform and of various sizes and colors from white to red. A large number of indigenous and introduced varieties are cultivated like *Pusa chetki*, *Mino early long* and *Red round Radish*.

Investigation of salt effects on biochemical and physiological traits *of Raphanus sativus* cultivars is necessary to understand salt stress effects on plant growth and yield and identify important tolerance mechanisms used in plants. For this reason, Germination percentage, Seedling vigor index, Electrolyte leakage, Leaf relative water content, Photosynthetic pigment content and Photosynthesis, Proline, Protein, Lipid peroxidation, and Antioxidant enzymatic assays (Catalase activity, Peroxidases activity, and Superoxide dismutases activity have been evaluated in three Radish cultivars.

II. MATERIAL AND METHOD

Raphanus sativus was selected as an experimental system for the present study. The seeds of three Radish genotypes *Pusa chetki*, *Mino early long*, and *Red round radish* was collected from the Mumbai local market.

Randomly selected seeds were surface sterilized with 0.01% Mercuric chloride solution for three minutes and rinsed in tap water. The experiment to study the effect of salinity on these radish cultivars was conducted in two sets. In the first set, a laboratory experiment was conducted, wherein, the seeds of each genotype were placed in sterilized Petri dishes containing Whatman filter paper number 2, moistened with distilled water (controlled), and different treatment solutions (25 mM, 75 mM, 125 mM, and 175 mM of sodium chloride) using the forceps. A five-milliliter solution of each concentration was applied to each Petri plate as per treatment, and the same volume of deionized water was added to the control Petri plate using disposable syringes. All the Petri plates containing treated and control seeds were covered with lids and incubated at room temperature. With the respective treatment concentrations, all the seeds in the Petri plates were moistened evenly. This was endured throughout the experiment. The whole procedure was reciprocated for 15 days. The seeds were used for the evaluation of morphological parameters.

In the second set, a pot experiment was conducted in which the seeds of each genotype were sown in plastic pots (300 mm diameter) filled with 3 kg of a 1:1:1 soil mixture containing red soil, sand, and farmyard manure (FYM). Four seeds were sown per pot and all the pots were watered with tap water up to 30 days after sowing them. On day 15 the pots were irrigated with tap water as control or with different treatment solutions of 25 mM, 75 mM, 125 mM, and 175 mM of Sodium Chloride (NaCl) respectively. The seedlings were used for the assessment of the following biochemical and physiological parameters-

A. Germination / Emergence Percentage -

Sprouted seeds were counted from the first day itself. The emergence count was made throughout the experimental period until all the seeds were either germinated or perished.

$$Germination\ \% = \frac{no.of\ germinated\ seeds}{no.of\ total\ seeds} \times 100$$

B. Seedling Vigor Index-

The seedling Vigor Index was calculated by dividing germination percentage by seedling dry weight.

$$\text{Seedling vigor index} = \frac{\text{germination percentage}}{\text{seedling's dry weight}}$$

C. Measurement of electrolyte leakage/ leaf membrane stability index (MSI In %)-

Leaf Membrane Stability Index (MSI) was measured as ion leakage from the leaves. 0.1 gram washed leaves were cut into 1 cm pieces and placed in a glass beaker containing 10 ml of double-distilled water in two sets. One set was kept at 40 °C for 30 minutes and the conductivity (C1) of the solution was measured by a conductivity meter. Another set was boiled at 100 °C for 15 minutes and then its conductivity (C2) was measured after cooling the solution to room temperature [13].

$$\text{Leaf membrane stability index} = 1 - \frac{C1}{C2} \times 100$$

D. Plant Water Status/ leaf Relative Water Content (LRWC)-

Five fresh leaves of the same size and same age of five plants from each treatment were collected and weighed (FW). Leaf segments were kept immersed in distilled water for 24 hours at room temperature in the dark. The turgid weight (TW) of leaves was measured and then they were oven-dried at 80 °C for 72 hours until a constant weight was obtained (DW). Values of FW, TW, and DW were used to calculate LRWC [29].

$$\text{Leaf relative water content (\%)} = \frac{FW - DW}{TW - DW} \times 100$$

E. Total Chlorophyll Content Estimation-

Accurately weighed 0.5g of fresh plant leaf sample was taken and homogenized in mortar and pestle with the addition of 10 ml of 80% acetone solvent and a pinch of $MgCO_3$. The homogenized sample mixture was centrifuged at 10,000 rpm for 15 minutes at 40 °C. The supernatant was separated and again extracted repeatedly with 80% Acetone until the solution became clear. The solution mixture was analyzed for Chlorophyll-a, Chlorophyll-b, and total chlorophyll content Spectrophotometrically [4].

$$\text{Chlorophyll a (mg/ml)} = 12.7\,(A_{663}) - 2.69\,(A_{645})$$
$$\text{Chlorophyll b (mg/ml)} = 22.9\,(A_{645}) - 4.68\,(A_{663})$$

Where,

A645 = absorbance at a wavelength of 645 nm; A663 = absorbance at a wavelength of 663 nm.

Total Chlorophyll (mg/ml) = Chlorophyll a + Chlorophyll b

Total Chlorophyll (mg) in original tissue sample = Chlorophyll (mg /mL) x final volume (mL)

Total Chlorophyll a (mg) in original tissue sample = Chlorophyll a (mg /mL) x final volume (mL)

Total Chlorophyll b (mg) in original tissue sample = Chlorophyll b (mg /mL) x final volume (mL)

F. Estimation of Proline-

The acid Ninhydrin method of Bates was used for the estimation of Proline content [7]. The extract was made by homogenizing 0.5 g of plant material in 10 ml of 3% aqueous sulpho salicylic acid. The homogenate was filtered through Whatman number 2 filter paper. 2 ml of filtrate was taken in a test tube and 2 ml of glacial acetic acid and 2 ml of acid Ninhydrin were added in a sequence. The mixture was heated in the boiling water bath for 1 Hour. The reaction was stopped by placing the tube in an ice bath. 4 ml toluene was added to the reaction mixture and was stirred for 20-30 seconds. Separation of the Toluene layer was done and it was warmed to room temperature. Later, the red color intensity was measured at 520 nm. A series of standards with pure proline in a similar way was made and a standard curve was obtained. Thus the amount of proline in the test sample was determined from the standard curve.

$$\mu moles\ Proline\ /gram\ tissue = \frac{(\mu g\ Proline/ml) \times toluene\ (ml)}{115.5 \mu g/\mu\ mole} \times \frac{5}{gram\ sample}$$

G. Estimation of Protein -

Protein estimation was done by Lowry's method [23]. Extraction was done by homogenizing 0.5 gm of plant material with 5-10 ml of phosphate buffer (pH 7.0). The homogenate was centrifuged at 10,000 rpm for15min at 40C. The separated supernatant was used for protein estimation. The working standards (0.2, 0.4, 0.6, 0.8, and 1.0 ml) were pipetted out into a series of test tubes. 0.5ml and 1ml of the sample extract were taken into another test tube. The final volume was made to 1ml with distilled water in all test tubes. Test tubes with 1ml distilled water served as blank. 5ml of Alkaline copper tartrate solution (reagent C) was added to each test tube including the blank, mixed well, and allowed to stand for 10 minutes. Follins - ciocalteau reagent (reagent D) was added to these test tubes, mixed well, and allowed to incubate at room temperature in dark for 30 minutes. The blue color was developed. The readings were taken at 660 nm. By plotting a standard graph the amount of protein in the sample was calculated.

Total Protein in a tissue sample (µg) = protein (µg/ml) × Volume of original extract (ml)

H. Lipid peroxidation –

Lipid peroxidation rate was determined by measuring the Malondialdehyde equivalents following Hodges [18]. 0.5 gm of plant material was homogenized in a mortar and pestle using 80% ethanol and the final volume was made to 10 ml. Then the homogenate was centrifuged at 15,000 RPM at 4°C and the pellet was extracted twice with the same solvent. The supernatant was cooled and 1ml of this sample was added to two test tubes, one with an equal volume of + TBA solution and another with an equal volume of -TBA solution. The +TBA solution contained 20% trichloroacetic acid (TCA), 0.01% butylated hydroxyl toluene (BHT) and 0.65% thiobarbituric acid (TBA) while the – TBA solution contained only 20% trichloroacetic acid (TCA),

0.01% butylated hydroxyl toluene (BHT). Samples were heated at 100°C for 15 minutes in a water bath and cooled at room temperature. Absorbance was read at 440 nm, 532 nm, and 600 nm and was used to calculate MDA equivalents expressed as $cm^2$of tissue.

$$[((A_{532}) + TBA) - ((A_{600} + TBA)] - [((A_{532}) - TBA) - ((A_{600} - TBA)] = A$$

$$[((A_{400}) + TBA) - ((A_{600} + TBA)] \times 0.0571 = B$$

$$\text{MDA equivalents (n moles/ml)} = \left(\frac{A-B}{157000}\right) \times 10^6$$

I. Superoxide Dismutase (SOD) Activity –

The 0.5 gm of plant material was homogenized under N_2 Atmosphere (0-4 °C) in a pre-chilled mortar and pestle using 100mM sodium phosphate buffer (pH 7.0). Then the homogenate was centrifuged at 15,000 RPM at 4°C. The supernatant was separated and taken into a different test tube. 1ml of this sample extract was added with an equal volume of 100mM sodium phosphate buffer, 0.05% nitro-blue tetrazolium chloride. 0-minute absorbance (A) was recorded at 580 nm before adding 1ml sodium azide solution and after adding 1ml sodium azide solution increase in absorbance (A_1) was recorded at 580nm [8].

$$SOD\ activity\ (\mu moles/mg/min) = \frac{(A_1 - A_0)\ x100}{time} \text{ x } \frac{\text{total volume of exract}}{\text{volume used}} \text{x} \frac{1}{\text{fresh weight of tissue sample}}$$

J. Peroxidases (H_2O_2) activity –

The plant material (0.5 gm) was homogenized with 10ml of a solution containing 0.1M phosphate buffer (pH 7.5), 0.5M Ethylene diamine tetraacetic acid (EDTA), and chilled with 0.5% trichloroacetic acid (TCA) using pre-chilled mortar and pestle and the homogenate was centrifuged at 15,000 RPM at 4°C. The supernatant (0.5ml) was taken into another test tube containing 0.5 ml 100 mM potassium phosphate buffer and 1ml freshly prepared 1M potassium iodide (KI) and 1ml distilled water. The reaction was allowed to develop in dark for 1 Hour and absorbance was read at 360 [2].

$$H_2O_2\ activity\ (\text{unit/mg/min}) = \frac{A_{390}x100}{time} \text{ x } \frac{\text{total volume of exract}}{\text{volume used}} \text{x} \frac{1}{\text{fresh weight of tissue sample}}$$

K. Catalase activity –

The plant material (0.5gm) was homogenized with 10ml of a solution containing 0.1M phosphate buffer (pH 7.5), 0.5M Ethylene diamine tetraacetic acid (EDTA). Homogenate was centrifuged and the supernatant was used for the determination of catalase activity. 0.5 ml of this supernatant was taken into another test tube containing 1.5 ml of 100mM potassium phosphate buffer (pH 7.0) and 2.5 ml of distilled water. 0 min absorbance (A) was read at 240 nm then 0.5 ml of 75 mM hydrogen peroxide (H_2O_2) was added into the test tubes and after a 1-minute decrease in absorbance (A_1) was read at 240 nm [1].

$$CAT\ activity\ (\text{unit/mg/min}) = \frac{(A_1 - A_0)\ x100}{time} \text{ x } \frac{\text{total volume of exract}}{\text{volume used}} \text{x} \frac{1}{\text{fresh weight of tissue sample}}$$

III. RESULT AND DISCUSSION

A. Germination Percentage-

Germination percentage is a significant indicator of salt tolerance. While observing the effect of different salinity stresses imposed using sodium chloride at 25 mM, 50 mM, 125 mM, and 175 mM levels on germination percentage, the results revealed that the growth of the radish plant was drastically reduced in all three varieties (*Pusa chetki*, *Mino early long* and *Red round radish*), due to NaCl at all the studied concentrations (Fig. I). Elevated NaCl concentration caused a decline in germination percentage. The germination percentage was obtained as 80%, 40%, and 78% from the *Pusa chetki*, *Mino early long*, and *Red round radish*, respectively, which was the highest at controlled treatment (0 mM). However, there were no significant differences in germination percentage between cultivars. These results were concurrent with Kaymak et al. (2009) who found that the lowest concentration of NaCl does not affect radish seed germination crucially. In general, germination percentage decreased significantly with increasing NaCl concentration treatment. For 75 mM, 125 mM, and 175 mM of NaCl concentration, the germination percentage was recorded as 76%, 58%, and 57.9% for the *Pusa chetki* cultivar (Fig. I). The second highest germination percentage was obtained as 62%, 62%, and 20% for *Red Round Radish* respectively.

The lowest germination percentage was found as 20%, 20%, and 0% for *Mino early long* cultivars in 75 mM, 125 mM, and 175 mM NaCl concentrations (Fig. I). Jamil et al. (2005) reported a decrease in the germination of *Brassica species* with an increase in the salinity concentration. The germination percentage of *Pusa chetki* was comparatively higher than *Red round radish* and *Mino early long* cultivars. It suggests that seeds of *Pusa chetki* could tolerate and germinate well at the highest

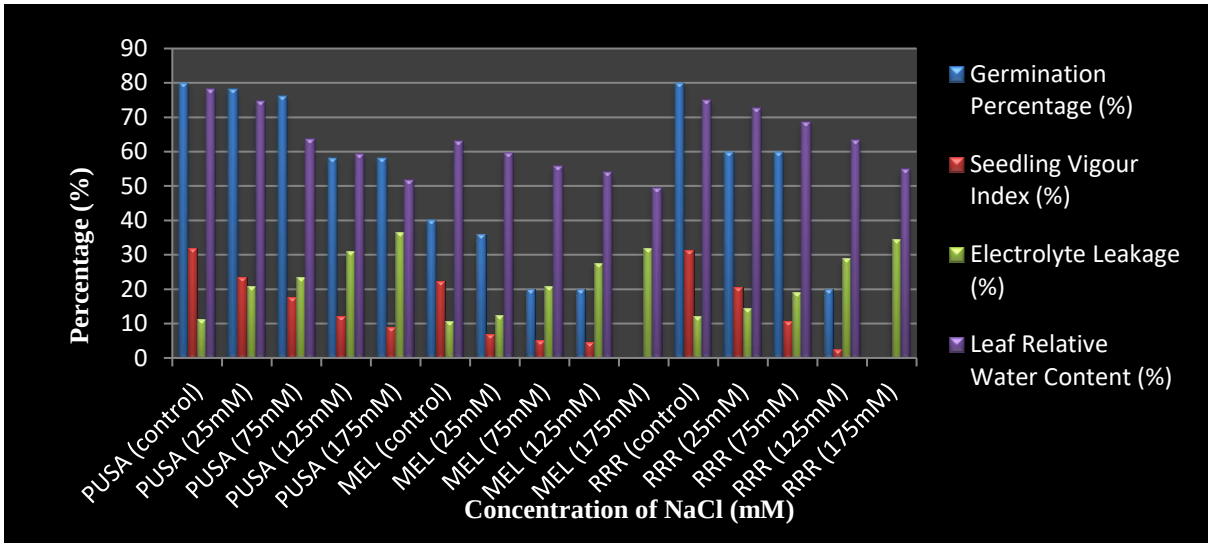

Fig I- Evaluation of some growth parameters on salinity induction mediated by NaCl treatment in Radish varieties

concentration of NaCl (175 mM). Jamil et al. (2006) suggested that seeds absorb sufficient water during germination and thus the tissue water potential plays a critical role. The activity of the enzyme α-amylase and higher concentration of salt reduces the water potential in the medium which hinders water absorption by germinating seeds and thus reduces germination. Thus it appears that a decrease in germination is related to salinity-induced disturbance of the metabolic process leading to an increase in phenolic compounds.

B. *Seedling Vigor Index*-

The effect of salt stress on the vigor index of seedlings of all the varieties was assessed at different salinity levels (Fig. I). The seedling vigor index was significantly altered at all three levels of salinity. Salinity at 125 mM concentration had a significant reduction in the mean vigor index over control. The maximum vigor index of 12.06% was recorded by the *Pusa chetki* cultivar. The vigor index of *Mino early long* was significantly reduced when compared to control. Significant varietal variations were observed in the vigor index as a result of salinity stress. Djanaguiraman et al. (2003) reported that the rice varieties are more sensitive to NaCl in relevance to the seedling vigor index.

C. *Electrolyte Leakage*-

Substantial electrolyte leakage (EL) was noticed due to the salt stress. Electrolyte leakage was elevated with increasing salinity levels as compared to the control radish plants in all three cultivars. Electrolyte leakage was markedly greater in salt-stressed plants than those in the non-stressed plants (Fig. I). The increase in electrolyte leakage recorded under salt stress might be partly because of the decreased chlorophyll content according to Kaya et al. (2001). Heightened electrolyte leakage was reported to be correlated with degradation in leaf senescence-induced chlorophyll concentration following reports of Dhindsa et al. (1981), and Chen et al. (1991). The Electrolyte leakage values of salinized plants were higher in the cultivar *Mino early long* (36.72%) than that in Red round radish (34.44%) and *Pusa chetki* cultivar (32.03%). A similar phenomenon was already observed in cucumber, rice, and sugar beet by Munns and Tester (2008). This states that the *Mino early long* is a salt-sensitive species and *Pusa chetki* and *Red round radish* are salt-tolerant cultivars.

D. Leaf Relative Water Content (RWC)-

Cultivars *Pusa chetki* and *Red round radish* maintained higher RWC at 0 mM conditions, whereas *Mino early long* had the lowest RWC values (Fig. I). The RWC of the leaf was decreased from 78.09%, 62.93%, and 74.99% in control to 51.63%, 49.4%, and 54.77%, at 175mM NaCl treatment in *Pusa chetki*, *Mino early long*, and *Red round radish* cultivars respectively (Fig. I). In this connection at the end of the experimental period, the leaves of NaCl treated plant showed an observable symptom of chlorosis and necrosis. Techawongstin et al. (1993) have also reported this phenomenon in water-stressed *Piper nigrum.*

E. Lipid Peroxidation-

Lipid peroxidation levels in leaves were assessed as the content of Malondialdehyde (MDA) which is a

more reliable indicator of salt stress tolerance. The content of MDA, a decomposition product of polyunsaturated fatty acids produced during peroxidation of membrane lipids escalates significantly with increasing salt stress. MDA content and membrane permeability are used as biomarkers for lipid peroxidation. In our study, an increase in MDA content under salt treatment was observed. The salt stress-induced MDA increase was 4.4, 4.490, and 5.960 µmoles at control (0 mM) salt treatment to 9.250, 5.882, and 5.534 µmoles at 175 mM of NaCl levels in *Pusa chetki*, *Mino early long* and *Red round radish* respectively (Fig. II). A similar effect was observed in cultivars of radish (*Raphanus sativus L.*) in an experiment conducted by Noreen and Ashraf (2009). It has been suggested that a decrease in membrane stability reflects the extent of lipid peroxidation caused by reactive oxygen species which generated oxidative stress in plants. The MDA reactive product (lipid peroxidation) showed a noteworthy effect of treatments. Thus highest MDA concentration was observed in *Pusa chetki* (9.250 µM) then in *Red round radish* (5.882 µM) and the lowest MDA concentration has been observed In *Mino early long* (5.534 µM) at the highest NaCl concentration of 175mM.

F. *Total Chlorophyll Contents-*

Photosynthesis is an essential process in plants that are altered by salt stress [12]. The photosynthetic machinery such as pigments, stomatal functioning, gaseous exchange, structure and function of the thylakoid membrane, electron transport, and enzymes were reported to be damaged at numerous levels by the oxidative effect of salt stress [33]. Chlorophylls have a key role in photosynthesis, therefore, any changes in their level can affect plant growth. Total leaf chlorophyll contents significantly decreased with increasing NaCl levels. The decrease in total chlorophyll contents was 79.6%, 24.93%, and 48.52% at 175mM of salt stress in *Pusa chetki*, *Mino early long*, and *Red round radish* respectively as compared to non-treated plants (Fig. II). Yasar et al. (2008), Kusvuran (2010), and Nazarbeygi et al. (2011) reported the decline in the content of chlorophyll under salinity stress. In the salt-resistant genotypes (*Pusa chetki* and *Red round radish*), the chlorophyll content was conserved possibly because of the high antioxidant enzyme activities that prevented degeneration of leaf chlorophyll. Chlorophyll a, b, and total chlorophylls in radish were reduced due to Salinity stress (Fig. II). Similar results have been recorded in *Thellungiella halophile* [32], *Brassica juncea* [33]. Rao and Rao (1981) correlated the decrease in the chlorophyll content with the instability of protein complexes and enhancement of Chlorophyllase activity. In *Lycospercum esculentum* total chlorophyll content was suppressed considerably due to drought stress induced by polyethylene glycol [24].

G. *Proline Content-*

Proline content of the leaves of radish cultivars was significantly enhanced with NaCl at all tested concentrations (Fig.III). The increase of proline concentration in the leaves was observed to be by 47%, 22.7%, and 28.54% in 175 mM of NaCl for

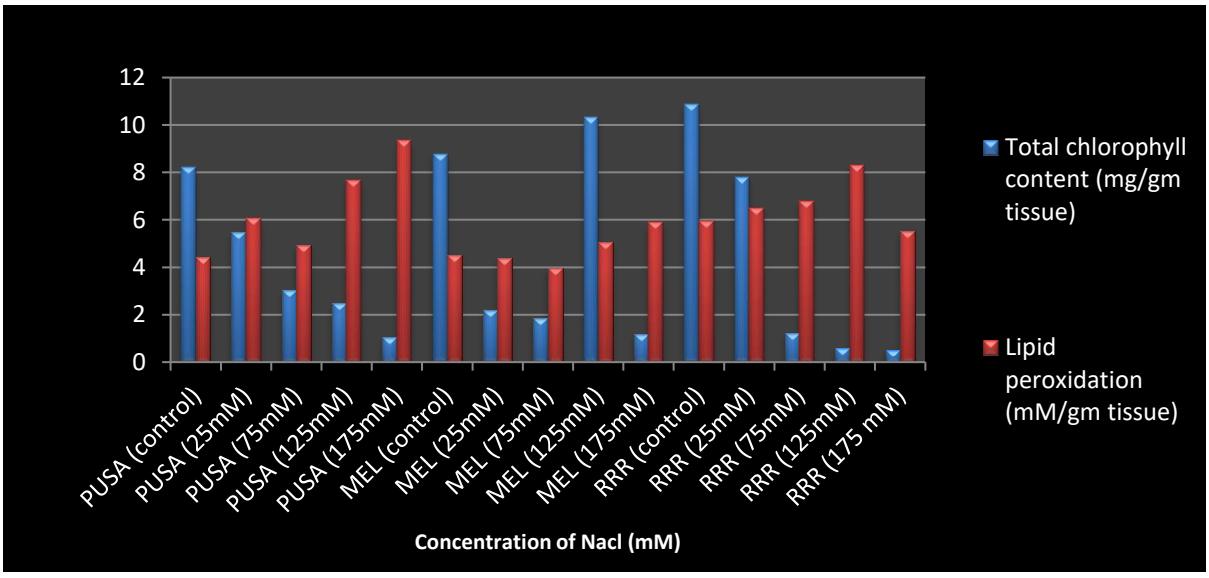

Fig II- Assessment of Chlorophyll and lipid content in radish varieties treated with varying concentration of NaCl

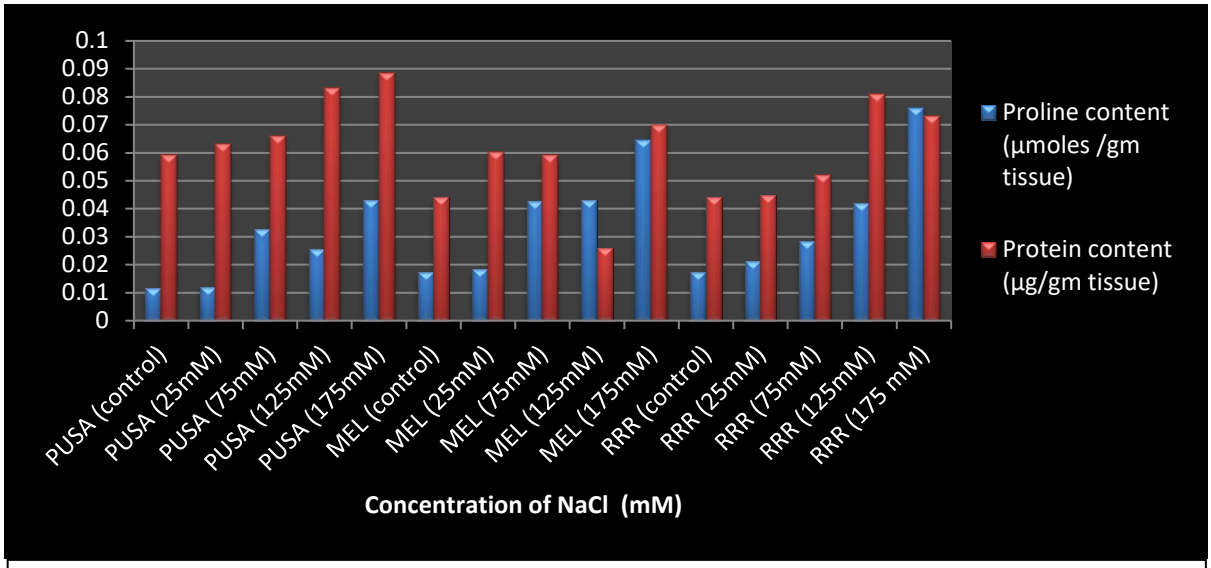

Fig III- Proline and Protein content in salinity induced radish cultivars

Pusa chetki, *Mino early long*, and *Red round radish* respectively, compared to the controls which were 12%, 13.2%, 14.5% respectively. Higher concentrations of proline might be the reason for higher salt tolerance or prevention of NaCl effects. Proline accumulation has been accepted as an indicator of environmental stress and is additionally considered to possess important protective roles. Environmental stress may lead to proline accumulation. In Arabidopsis, the gene of A'-pyrroline-5-carboxylate synthetase, a key enzyme in proline biosynthesis, is alleviated under the condition of salt stress [34]. Thus, high proline concentration in *Pusa chetki* followed by *Red round radish* indicates higher tolerance and prevention to stress.

H. *Protein content* -

A significant increase in leaf protein content was observed under salinity stress in all three radish cultivars. The magnitude of increase in protein contents under saline condition was high in *Pusa chetki* (0.08845µg/gm) and *Red round radish* (0.0816 µg/gm) as compared with that in *Mino Early Long* (0.07025 µg/gm) at 175mM (Fig.III). An increase in protein contents of both salt-tolerant and salt-sensitive radish cultivars has been reported during salinity stress by Karl and Läuchli (2000). In the present study, salt stress has caused an increase in leaf protein. However, this effect was more significant in *Pusa chetki*. The magnitude of increase in protein contents under saline condition was higher in *Pusa chetki* (0.08845µg/gm) and *Red round radish* (0.081608845µg/gm) as compared to *Mino early long* (0.07025) at 175mM. Hakimi and Hamada (2001) reported an increase in the leaf soluble protein on induction of salt stress in *Triticum vulgare*. Leaf soluble proteins contribute to the protection of membrane and membrane-bound enzymes.

I. *Antioxidant enzyme activities*-

To determine the response of radish to salt-induced oxidative stress, superoxide dismutase, catalase, and peroxidases, activities were evaluated in leaves of seedlings grown in control, 25 mM, 75 mM, 125 mM, and 175 mM NaCl.

Superoxide dismutase activities (SOD) of three radish genotypes under the effect of salt stress and control are depicted in (Fig. IV). The SOD activity was increased by salinity induction. The SOD activity was the highest in tolerant cultivars, *Pusa chetki* and *Red round radish*, 77.4 and 69.3 mmol/min/mg, respectively. These genotypes had increased SOD activity by 72.97%, 73.05%. Whereas, *Mino early long* exhibited a significant decrease in SOD activity (66.19%) with salt stress (Fig. IV).

Salt treatment increased Catalase activity (CAT) in all the genotypes when compared with their respective control groups. The increase in CAT activities in *Pusa chetki* and *Red round radish* was high compared to the salt-sensitive *Mino early long*. The CAT activity in *Pusa chetki* and *Red round radish* under salt stress was increased by 73.04 and 72.97 %, respectively, in comparison with that of the non-salinized control plants (Fig. IV). CAT activity in Salt sensitive genotypes (*Mino early long*) was only increased by 60.60%, respectively (Fig. IV).

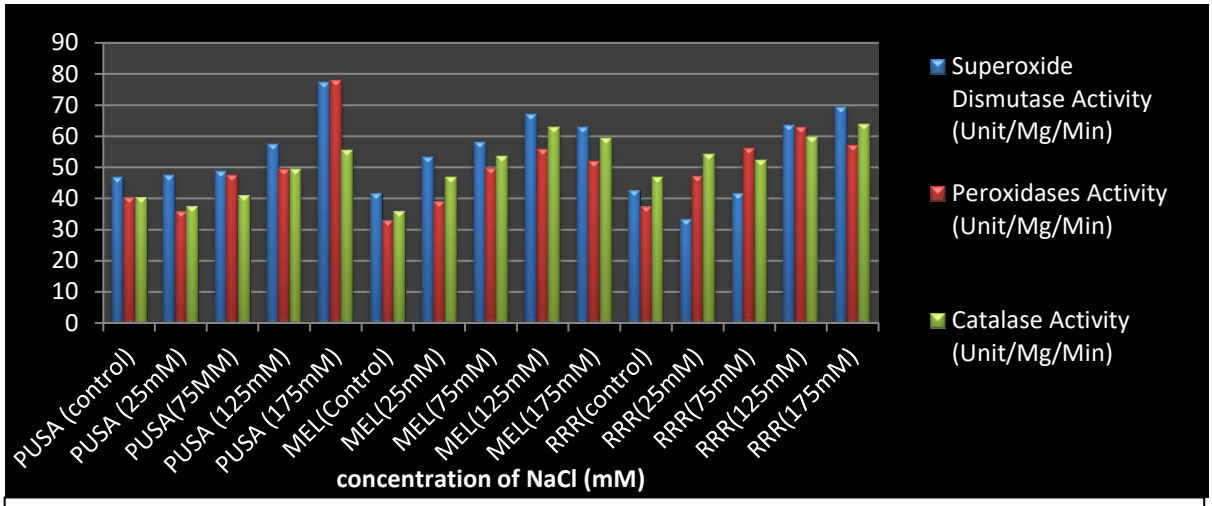

Fig IV- valuation of enzyme activities in three cultivars of radish subjected to NaCl mediated salinity stress cultivars

A significant increase in Peroxidases (H_2O_2) activity was observed in all three genotypes under salt stress conditions. However, *Mino early long* and *Red round radish* had the lowest Peroxidases activity under saline conditions. In contrast, *Pusa chetki* had the highest in Peroxidases activities of all cultivars under salt stress (Fig. IV). In response to the salt treatment, the Peroxidases activity was found to be enhanced in *Pusa chetki* and *Red round radish* genotype up to 78.0 and 57.0 mmol/min/mg under 175mM salt stress. Salinity stress resulted in the generation of excessive reactive oxygen species (ROS), which lead to cell toxicity, membrane deterioration, and cell death as reported by Chookhampaeng (2011). Among the active oxygen species superoxide is converted by Superoxide dismutase enzyme to H_2O_2, which is further scavenged by Catalases.

Our results showed that these enzyme activities in all genotypes were increased by induced salinity and were higher in salt-tolerant genotypes *Pusa chetki* and *Red round radish* than salt-sensitive genotypes (*Mino early long*). These enzymes were also reported to be important in salt-tolerant *Cucumis melo* by Yasar et al. (2006) and Kusvuran et al. (2007), in *Phaseolus vulgaris* by Yasar et al., 2008, and in *Glycine max by* Amirjani, (2010). Li (2009) and Wang et al. (2011) have also observed that salinity increased Peroxidases activity in salt-tolerant *Lycopersicum esculentum* and *Medicago sativa* genotypes.

IV. CONCLUSION

Stress is an extraneous factor that applies an adverse influence on the plant. Among the unfavorable environmental conditions, salinity stress is the most substantial factor that resentfully affects plant growth, development, and productivity.

The present study analyses changes in Germination percentage, Seedling vigor index, Total chlorophylls, Proline accumulation, Protein content, Lipid peroxidation, and Enzymatic assays (Superoxide dismutase, Catlases, and Peroxidases activities) in three cultivars of *Raphanus sativus* (*Pusa chetki*, *Mino early long* and *Red round radish).*

The radish cultivars were subjected to different salt concentrations of 25 mM, 75 mM, 125 mM, and 175mM. As the duration of salt stress increased, Germination percentage and Seedling vigor index values were considerably decreased between non-salty and low salt concentrations. Increasing salt concentrations after 75 mM caused a significant loss in the total chlorophyll content. A substantial increase in the Proline and Protein accumulation was noticed with the increase in the salt concentration. Different concentrations of NaCl resulted in variability in the lipid peroxidation activity. Anti-oxidative enzyme defense mechanisms played a protective role against salt stress and were effective in providing resistance to stress in Radish. In conclusion, tolerant and sensitive genotypes showed different responses under salinity.

The result of this study shows salt-tolerant genotypes *Pusa chetki* and *Red round radish* may have better protection against stress. Although there is lots of information on the salt tolerance of several vegetables, it is interesting to note how little significant research on salt tolerance has been done on the majority of the vegetable species. Because of the high cash value potential of many vegetable and herb species, and the multifarious germplasm accessible, there is a need for ample research in this

area. The plausible genetic multiformity among radishes to agglomerate a range of different ions and combinations of ions could add to the significance and potential of these species as bio accumulators. There is a growth in the use of poorer quality water for agriculture because of deficient high-quality water. Such waters include both saline groundwater, agricultural drainage, and municipal and industrial waste effluents. The confrontations for using such water effectively will depend on greater knowledge of salt tolerance.

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