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NaCl Induced Oxidative Stress on Two Different Cultivars of Sunflower (*Helianthus annuus* L.)

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A B S T R A C T

Sunflower (*Helianthus annuus* L.) is an oil seed crop, grown all over the world while the yield potential is strongly affected by salinity stress. The study aims at the response of two cultivars NSSH-1084 and SWATI to salt stress. The extent of influence of NaCl on various biochemical parameters and anti-oxidative enzymes, catalase (CAT), Guaiacol peroxidase (GPX) and superoxide dismutase (SOD) were investigated at vegetative, flowering and post flowering stages. The chlorophyll, protein and soluble sugar contents were enhanced upto 100mM and 50mM in NSSH-1084 and SWATI var. respectively. Comparatively proline and MDA (Malondialdehyde) content were more than control in all concentrations of salt stress. The activity of CAT and SOD were increased with depletion in GPX with all the concentration of NaCl than control in NSSH-1084.However in SWATI the activity of CAT and GPX increased upto 50mM with a gradual enhancement of SOD from control to 200mM. The result indicates the tolerance potential of NSSH-1084 compared to SWATI cultivar.

Introduction

The physiology behind plant growth and development is a complex phenomenon. Primarily environmental stresses have a great impact on the growth and development of plants. Salinity is a major environmental stress affecting plant productivity and constitutes a problem concerning many areas, with an emphasis on regions of hot and dry climates. It is a serious threat to crop plants that reduces ground level yield.

Agricultural production is significantly affected by salt stress. High salt stress disrupts the homeostatic balance of water potential and ion distribution within a plant. It delays the germination events, resulting in reduced plant growth and final crop yield[1,2]. Overall it inhibits seed germination, root length, shoot length, flowering and fructification of plant[3].It also affects photosynthesis, protein synthesis, lipid metabolism, leaf chlorosis and senescence. Plants develop to adapt biochemical and molecular strategies to resist the problem of

salinity that include alleviation of osmotic stress, compartmentalization of ionic toxicity, an effective ROS scavenging mechanism and expression of salt tolerant genes[4].

Osmotic stress is developed as first line of action to mitigate the effect of salt stress. In response to alleviate osmotic stress caused by salinity, plants produce many osmolytes that maintain the metabolic potential of plant cell [5,6,7]. This results in the accumulation of inorganic and organic solutes [8,9]. Proline is synthesized as the first line of defence under salt stress. It is a low molecular weight, water soluble, non toxic osmolyte that raises the osmotic potential of plant cell[10].Proline content can be used as a physiological index for tolerance to salt stress[11]. Enhancement of osmotic potential can also be observed by accumulation of small soluble glycans[12].Therefore, an increase in the content of proline and soluble sugar can be used as a physiological indicator for salt tolerance. Some plants also develop another mechanism of compartmentalization of Na^+ into vacuoles through a Na^+/H^+ antiporter and maintains a high K^+/Na^+ ratio in the cell[13].Reactive oxygen species (ROS) like superoxide radicals, hydrogen peroxide(H2O2), hydroxyl radicals are produced in oxidative stress. However a balance was maintained between the production and scavenging of ROS under physiological steady state [14, 15]. This homeostasis is disturbed by salt induced stress. Plants develop defence mechanism to scavenge ROS by many enzymes like superoxide dismutase (SOD), peroxidase(GPX,APX) and Catalsae (CAT). A balance of these enzyme activities is crucial for suppressing toxic ROS level within cells and thus provides tolerance against salt stress[16].

Salinity is a major challenge in India on account of its vast coastal belts and inland sporadic precipitation.32% of agricultural land is affected by salinity. Crop productivity is much hampered in absence of selective remedial measures. So the purpose of studying plant tolerance is to cultivate tolerant varieties in such saline agricultural land that allow optimum crop yield. Cash crops have derived more attention in this regard because of crop rotation, short duration, less demand for irrigation. Sunflower is an important oil seed cash crop in the country. It is mostly cultivated in agricultural land as a replacement to Rabi crops. Odisha is a potent state for sunflower production in India. Likely the productivity is subjected to low yield under salt stress. In view of the importance of sunflower as a prominent oil seed cash crop in the country the present scenario aims at investigating the tolerance potential of two varieties of sunflower (NSSH-1084 and SWATI) against salt induced stress. They are subjected to different intensities of salt concentration and were evaluated for their salt tolerance potentials. The main objective of this study is to estimate the differential effect of salinity stress on several biochemical parameters in both the varieties.

Materials and Methods

Plant material

Seeds of sunflower (*Helianthus annuus* L.) of two varieties, NSSH-1084 and SWATI, were surface sterilised with 0.1 % HgCl2 for 2-3 min. Approximately 3-4 seeds were planted onto the cemented pot filled with 8 kg of soil in the ratio of soil: vermi-compost: sand(2:1:1/2). One healthy seedling out of 3 was allowed to grow after 10 days of germination. The salt solution of NaCl prepared in different concentrations of 50mM, 100mM, 150mM and 200mMof NaCl was supplemented once to 10 days old plantlets. The biochemical and antioxidative enzyme activities were quantitatively analysed from two varieties at three phases of growth of the

plant i.e. vegetative, flowering and post flowering.

Estimation of chlorophyll and carotenoid

The second leaf of the healthy plant of Helianthus annuus from the top was sampled for the experimental purpose. 0.1g of leaf sample (finely cut leaf tissue) was grinded to fine pulp with addition of chilled 80% acetone. Absorbance was taken at 645nm and 663nm for chlorophyll estimation and 470nm of carotenoid content. Total chlorophyll content in the leaves was estimated[17].

Chlorophyll a = [(12.7×OD 663-2.69×OD 645) ×V/FW×1000]

Chlorophyll b = [(22.9×OD 645-4.68×OD 663) ×V/FW×1000]

Total Chlorophyll = [(20.2×OD 645-8.02×OD 663) ×V/FW×1000]

Carotenoid =1000×A470-3.27×chl a-104×chl b/229×0.1

Soluble Protein estimation

Soluble protein from healthy leaf was estimated using Bovine Serum Albumin (BSA) as standard[18]. The absorbance of each sample was recorded at 750nm after 30 min incubation. The concentration of protein content was determined with reference to standard curve made by using standard BSA (Bovine Serum Albumin). Finally the absorbance of protein extract and BSA was recorded at 750 nm.

Soluble sugar estimation

Carbohydrate of leaf sample was estimated and the content of the sample was quantified by using a standard curve of glucose with OD at 620nm[19].

Lipid peroxidation estimation

Lipid peroxidation was carried out as per the standard procedure by measuring the amount of Malonodialdehyde (MDA) generated due to thiobarbituric acid reaction[20]. Leaves were grounded with a pestle and mortar in 1% TCA and centrifuged at 10,000 rpm for 5 min. To 1.0 ml of supernatant in a separate test tube, 4.0 ml of 0.55 TBA was followed by heating at 95^{o}C for 30 min and cooling in ice-cold water with further centrifugation at 5,000 rpm for 5 min. Absorbance was measured at 532nm and corrected for unspecific turbidity by subtracting the value at 600nm. The blank contained 1 % TBA in 20% TCA. MDA content was calculated using an extinction coefficient of 155mM^{-1}cm^{-1} and the results expressed as µmol MDAg^{-1}F.W.

Proline estimation

Proline content of leaf estimated and further modified based on proline's reaction with ninhydrin[21, 22]. For proline colorimetric determinations, a 1:1:1 solution of proline, ninhydrin and glacial acetic acid was incubated at 100ºC for 1 hour. The reaction was arrested in an iced bath and the cromophore was extracted with 4 ml toluene and its absorbance was visualized 520 nm.

Antioxidant Enzyme extraction and Assay

Fresh leaves (0.5g) of helianthus annuus L. were homogenised with a mortar and pestle under chilled conditions with phosphate buffer (0.1M, pH 7.5) and EDTA (0.5mm).The homogenate was centrifuged at 14,000 rpm for 10 min at 4^{o}C. The resulting supernatant was used for assay of different enzymes.

Catalase (CAT)

Catalase activity of control and stressed plants of Helianthus annuus was estimated[23]. About

3 ml reaction mixture containing 1.5 ml of 100 mM potassium phosphate buffer (pH=7), 0.5 ml of 75 mM H2O2, 0.05 ml enzyme extraction and distilled water to make up the volume to 3 ml. Reaction started by adding H2O2 and decrease in absorbance recorded at 240 nm for 1 min. Enzyme activity was computed by calculating the amount of H2O2 decomposed.

Guaiacol Peroxidase (GPX)

GPX was assayed and the reaction mixture comprises of phosphate buffer (pH= 6.0, 50 mM), H2O2 (10 mM), guaiacol (2.25 mM) and 50 µl of enzyme extract[24]. The subsequent increase in absorbance of oxiguaiacol was measured at 470 nm and was defined as µmol of H2O2 per min.

Superoxide Dismutase (SOD)

The assay of superoxide dismutase was done[25] and this method comprises, 1.4ml aliquots of the reaction mixture (comprising 1.11 ml of 50 mM phosphate buffer of pH 7.4, 0.075 ml of 20 mM L-Methionine, 0.04ml of 1% (v/v) Triton X 100, 0.075 ml of 10 mM Hydroxylamine hydrochloride and 0.1ml of 50 mM EDTA) was added to 100 ul of the sample extract and incubated at 30ºC for 5 minutes. 80 ul of 50 mM riboflavin was then added and the tubes were exposed for 10 min to 200 W-Philips fluorescent lamps. After the exposure time, 1ml of Greiss reagent (mixture of equal volume of 1% sulphanilamide in 5% phosphoric acid) was added and the absorbance was measured at 543 nm. One unit of enzyme activity was measured as the amount of SOD capable of inhibiting 50% of nitrite formation under assay conditions.

Statistical analysis

All results are presented as the mean values ± standard errors. The statistical significances of differences between mean values were assessed by analysis of variance and Duncan's multiple range tests. $P < 0.05$ was considered significant.

Results and Discussion

Chlorophyll and carotenoid

Statistical analysis of Chlorophyll and carotenoid (Table 1 and 2) of Helianthus annuus revealed that the interaction between salinity and two cultivars had a significant effect on chlorophyll a, b, total chlorophyll and carotenoid content at different growth stages i.e. vegetative, flowering and post-flowering. Chlorophyll a, b, and total chl evidence maximum enhancement upto 100mM followed by 50mM in NSSH-1084 and SWATI respectively with a gradual declination in the vegetative stage. Additionally there were no significant differences of pigments in plants grown in presence of 150mM when compared to control plants in NSSH-1084. Whereas during flowering stage all the pigment increased upto 50mM in both the variety but a constancy in all pigment at 100mM NaCl was observed as compared to control in NSSH-1084. Above all a decrease in pigment was noticed in both the varieties during post flowering stage. However the quantity of different pigments along with total chlorophyll goes on decreasing during different stages of growth in the individual variety concerned. In a comparison NSSH-1084 synthesized maximum pigment with respect to chl a, chl b, and total chl than SWATI at all the different stages of growth.

Similarly carotenoid content of NSSH-1084 of Helianthus annuus increased with the increased NaCl concentration, upto 200mM in flowering stage with a decline at post flowering stage. While SWATI cultivar synthesized higher amount of carotenoid

during vegetative stage up to 50mM. The rest two growth stages showed a decrease in carotenoid content with the increase salt concentration as compared to control.

Protein

Total protein quantity of NaCl treated plants of Helianthus annuus was estimated by Lowry method (1951) and was given in Figure 1. In NSSH-1084, the protein content increased with NaCl upto 100mM both at vegetative and flowering stage. Whereas SWATI cultivar had enhanced protein content upto 50mM NaCl with a gradual decline in vegetative and flowering stage. Towards post flowering stage both NSSH-1084 and SWATI cultivar exhibited insignificant protein content in all the NaCl concentrations. In comparison NSSH-1084 was more potent than SWATI cultivar at all the different growth stages.

Soluble sugar

In the present investigation the total carbohydrates in the leaves of sunflower Helianthus annuus was depicted in Figure 2. NSSH-1084 variety exhibited an increment in carbohydrate with increasing salinity level in all the growth stages. The total carbohydrate decreased in a sequence of vegetative to flowering and ultimately to post flowering. Carbohydrate content was found to be insignificant for SWATI during vegetative stage. However significant increase was noticed up to 100mM during flowering with a declination during post flowering stage in SWATI cultivar of Helianthus annuus. Comparatively carbohydrate content were found to be higher in NSSH-1084 than SWATI cultivar during different stages of growth.

Lipid peroxidation

Under increased NaCl concentrations membrane lipids get damaged by ROS because of lipid peroxidation. Lipid peroxidation increased with increasing salinity which was estimated by the synthesis and quantification of MDA. The rate of lipid peroxidation in both the varieties increased when the plants were exposed to high salinity level as compared to control (Figure 3). The rate of increment was seen to be higher in SWATI cultivar than NSSH-1084 of Helianthus annuus. Both the cultivar showed an increase of MDA content with increasing NaCl concentration for all the growth stages.

Proline

Proline content at vegetative, flowering and post flowering stage of both the cultivar of Helianthus annuus revealed that there are significant difference in individual cultivar (Figure-4). Both NSSH-1084 and SWATI executed an increased accumulation of proline from 50mM to 200mM of NaCl as compared to control for all the growth stages. Proline synthesis was enhanced from vegetative to post flowering through flowering stage for both the cultivars. A significant accumulation of proline was observed in NSSH than SWATI for all treatments and growth stages.

Antioxidant enzymes

The activity of antioxidant enzymes plays a major role for evaluation of tolerance in plants. The CAT, GPX and SOD activities recorded for both cultivars of Helianthus annuus during the salinity experiments were depicted in Figures 5-7.There were striking differences in antioxidant enzyme activity between the two sunflower cultivars with increasing NaCl concentration.NSSH-1084 exhibited a sharp increase in CAT activity from control to 200mM of salt stress with respect to all the growth stages. A sharp increase of CAT activity was seen at 200mM NaCl during vegetative stage of growth. However the CAT activity differs from vegetative to post flowering showing a

decrease in trend from the former to the later at their respective treatments. The activity of catalase during post flowering stage even fall much below the value of flowering stage at their respective treatments. Whereas SWATI exhibited enhanced CAT activity up to 50mM NaCl both during vegetative and flowering stage, with a declination in post flowering stage as compared to control.

Table.1(A) Effect of saline stress at different concentrations of NaCl on chlorophyll a, chlorophyll b and total chlorophyll content (mgg-1 FW) of leaf during vegetative stage (± SE)

NSSH 1084 variety	**Chlorophyll a**	**Chlorophyll b**	**Total chlorophyll**
Control	1.02±0.03	0.38±0.07	1.45±0.05
50mM	1.08±0.07	0.39±0.01	1.58±0.09
100mM	1.41±0.01	0.48±0.02	1.90±0.01
150mM	1.07±0.08	0.30±0.02	1.47±0.01
200mM	0.52±0.01	0.20±0.08	0.82±0.04
SWATI variety	Chlorophyll a	Chlorophyll b	Total chlorophyll
control	0.92±0.02	0.37±0.06	1.32±0.09
50mM	1.03±0.01	0.40±0.05	1.43±0.06
100mM	0.98±0.03	0.38±0.01	1.36±0.04
150mM	0.49±0.01	0.31±0.06	0.80±0.03
200mM	0.32±0.07	0.27±0.01	0.59±0.01

Table.1(B) Effect of saline stress at different concentrations of NaCl on chlorophyll a, chlorophyll b and total chlorophyll content (mgg-1 FW)of leaf during flowering stage (±SE)

NSSH 1084 variety	**Chlorophyll a**	**Chlorophyll b**	**Total chlorophyll**
Control	0.93±0.07	0.35±0.01	1.33±0.04
50mM	1.08±0.06	0.42±0.02	1.68±0.09
100mM	0.91±0.03	0.33±0.03	1.35±0.08
150mM	0.69±0.04	0.25±0.02	0.95±0.08
200mM	0.42±0.10	0.12±0.01	0.64±0.01
Swati variety	**Chlorophyll a**	**Chlorophyll b**	**Total chlorophyll**
Control	0.70±0.88	0.36±0.05	1.06±0.03
50mM	0.85±0.05	0.47±0.03	1.32±0.88
100mM	0.53±0.21	0.29±0.57	0.82±0.15
150mM	0.39±0.15	0.18±0.21	0.57±0.24
200mM	0.27±0.08	0.13±0.18	0.40±0.11

$p < 0.05$, each mean represents 3 replicates

Table.1(C) Effect of saline stress at different concentrations of NaCl on chlorophyll a, chlorophyll b and total chlorophyll (mgg^{-1} FW) of leaf during post flowering stage (±SE)

NSSH 1084 variety	Chlorophyll a	Chlorophyll b	Total chlorophyll
Control	0.70±0.24	0.30±0.04	1.90±0.40
50mM	0.62±0.01	0.27±0.04	0.89±0.02
100mM	0.45±0.03	0.20±0.03	0.69±0.01
150mM	0.30±0.04	0.17±0.20	0.58±0.03
200mM	0.17±0.01	0.09±0.01	0.28±0.08

Figure 1: Effect of different concentrations of NaCl on soluble protein (µmg g $^{-1}$ FW)of Helianthus annuus L. on vegetative, flowering and post flowering stage

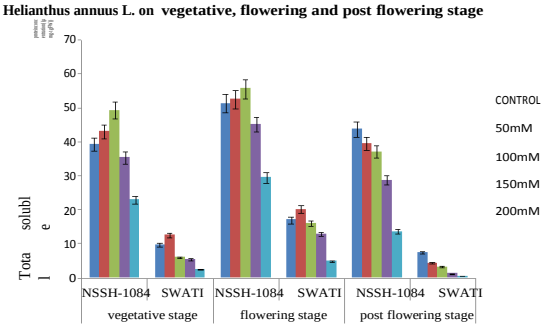
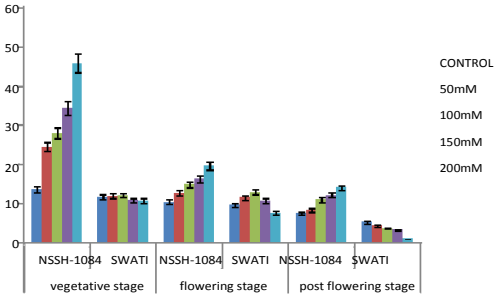
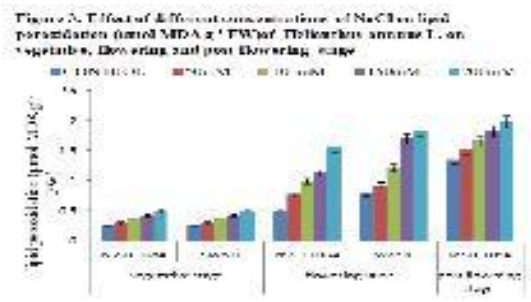


Figure 2: Effect of different concentrations of NaCl on Soluble sugar (mg g $^{-1}$ FW)of Helianthus annuus L. on vegetative, flowering and post flowering stage





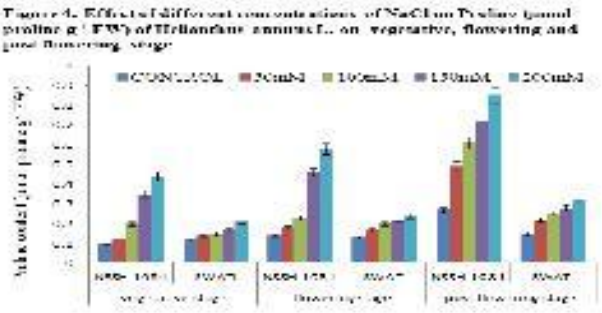


Figure 5: Effect of different concentrations of NaCl on Catalase activity(µmol min^{-1} mg^{-1}protein) of Helianthus annuus L. on vegetative, flowering and post flowering stage

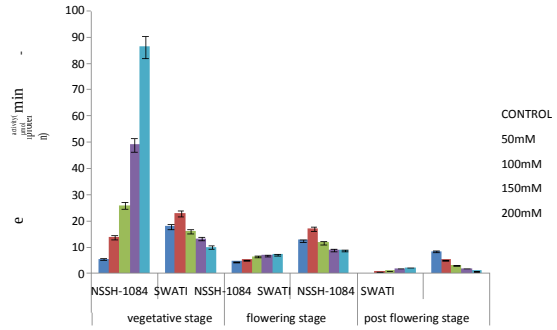


Figure 6: Effect of different concentrations of NaCl on Guaiacol Peroxidase activity (µmolmin $^{-1}$mg^{-1} protein) of Helianthus annuus L. on vegetative, flowering and post flowering stage

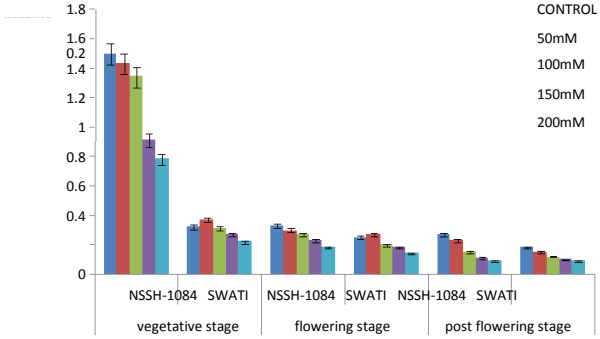
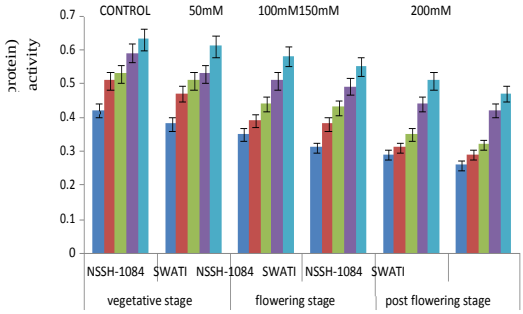


Figure 7: Effect of different concentrations of NaCl on SOD activity (µmol min $^{-1}$ $_{mg}$$^{-1}$protein)of Helianthus annuus L. on vegetative, flowering and post flowering stage



Similarly GPX activity for both the cultivar of Helianthus annuus under different salt concentration and growth stages was evaluated statistically. In NSSH-1084 a significant decrease in GPX activity was observed with 200mM of salt treatment than control at different stages of growth. The activity was seen to be highest during vegetative stage followed by flowering and then post flowering at their respective treatments. However in SWATI, a significant increase in GPX activity was observed from control to 50mM NaCl with subsequent decrease from 100mM to 200mM NaCl for the first two stages of growth. Nevertheless both the cultivars executed a decrease in CAT activity form control to 200mM for post flowering stage. NSSH-1084 had maximum GPX activity for all the treatments and stages of growth than SWATI.

SOD activity increased from control to 200mM for all the growth stages in both NSSH-1084 and SWATI. However the activity was highest in vegetative followed by flowering, post flowering in both the cultivars for their respective NaCl treatments. NSSH-1084 showed a slightly higher increased activity at different concentration and growth stages as compared to SWATI.

As a universal fact Salinity induces stress in plants, as a response of which the totipotent organism opens up several pathways to mitigate the effect of such stress. The mechanisms are very much complicated pathways, and it needs a balance between plant growth and development and the stress effectors mechanisms. Hence salt tolerant plants are the discussion of present days that needs to stabilize crop production even in saline soil. Salt tolerance capability can be summarized in four aspects; osmotic stress, ion toxicity, antioxidant enzymes, salt tolerant genes [4].The understanding of the above aspects is complex in itself. However it may provide a better understanding of salt tolerance in terms of osmolyte accumulation, ion selective absorption and compartmentalization, enhanced antioxidant enzymes activity. The plants are divided as glycophytes and halophytes in response to salinity. The present study undergoes a rigorous comparison between two varieties NSSH-1084 and SWATI behaving as halophytes and glycophytes with respect to one's ability to tolerate stress up to a wide range and the other's inability to do so.

It was observed that NSSH-1084 cultivar of Helianthus annuus tolerate salinity upto 100mM level as compared to SWATI, upto a level of 50mM. The level of chlorosis increased with increasing NaCl concentration in both the cultivars and all the three stages. Chlorosis is a common response to salinity, as

a result of which photosynthesis is inhibited. Chlorophyll content is considered as one of the parameters of salt tolerance in crop plants[26]. Thus pigment degradation is a rapid indicator of plant's response to salt stress. However the rate of degradation is more rapid in SWATI as compared to NSSH-1084. Reduction in photosynthetic capacity is also a consequence of inhibition of certain carbon metabolism processes by feedback from other salt-induced reactions[27].Under reduced water potential, stromal levels of the substrate fructose-1,6 - bisphosphate (FBP) accumulate and the FBPase reduced the substrate, so that FBPase becomes rate limiting to photosynthesis [28].

As an accessory pigment carotenoid is quite important that increases as a response to stress. It reduces the photo inhibitory and photo-oxidative damage. Enhancement in carotenoid synthesis has been evidenced in both the cultivars particularly during vegetative stage. Above all NSSH-1084 executed tolerance towards salt stress even at flowering stage registering an increase of carotenoid content from control to 200mM. The findings corroborates with the work [29] while working with salt tolerant lines of tobacco reported the increase in carotenoid content up to a level of 200mM.

As increase in protein content under stress has been reviewed extensively[30]. Generally protein accumulates in plants under saline condition. It may play a major role in osmotic adjustment. It has been concluded that a number of proteins induced by salinity are cytoplasmic that cause alterations in cytoplasmic viscosity of the cells [31]. A higher content of soluble protein in Helianthus annuus has been observed in NSSH-1084 as compare to SWATI during all the stages of growth. The higher protein content of NSSH-1084 cultivar of Helianthus annuus is indicative of its salt tolerance quality.

Sugar contributes upto 50% of the total osmotic potential in plants subjected to saline conditions. Soluble sugars stabilize membrane and protoplast [32].Moreover they protect soluble enzymes from high intracellular concentrations of inorganic ion. Under salinity, osmotic stress is caused by the increase of osmotic potential. Plants enhance their osmotic potential by accumulating small molecule-soluble glycans to resist this stress [33]. Therefore, the soluble sugar can be used as a physiological indicator of salt tolerance 12 evaluation. The accumulation of soluble carbohydrates in plants has been widely reported as a response to salinity or drought, despite a significant decrease in net CO2 assimilation rate[34]. Carbohydrates such as glucose, fructose, starch accumulate under salt stress, those play a leading role in osmo-protection, osmotic adjustment, carbon storage and radical scavenging. A greater soluble sugar in salt tolerant lines than the salt sensitive ones in five sunflower accessions [35]. In the present context NSSH-1084 cultivar of Helianthus annuus had a higher accumulation of soluble sugars than SWATI in their respective stages of growth. Lipid peroxidation is more pronounced during salt stress that can be measured through MDA. Hence MDA acts a parameter for evaluation of plant response to salinity stress [36].Present findings on Helianthus annuus had evidenced an increase in MDA content under different NaCl concentration from lower to higher. However the MDA content was seen to be higher in SWATI than NSSH-1084 during all stages of growth.

Quaternary amino acid derivates are the most common osmolytes which are produced in response to saline stress. Compatible solutes like proline are produced as a first line of defence to accommodate the ionic balance inside the cell [37, 38]. Proline accumulation and stress tolerance correlation have been reported in several studies. Also, a positive correlation

between magnitude of free proline accumulation and stress tolerance has been suggested as an index for determining stress tolerance potential of cultivars [39, 40, 41, 29]. In the present context with Helianthus annuus increased proline accumulation in all the growth stages supports the positive correlation towards salt stress tolerance.NSSH-1084 showed comparatively higher proline content as a line of defence over SWATI in all the three stages of growth for their respective treatments.

To safeguard normal cellular functioning and survival, cells have developed a number of defensive mechanisms, including the accumulation of antioxidant molecules containing thiol groups such as GSH and several antioxidant enzymes [42].Adaptation to high NaCl levels involves an increase in the antioxidant capacity of the cell to detoxify reactive oxygen species. It has been reported that salt stress produces an increase in superoxide anion[43, 44] which can be converted to H2O2 through both enzymatic and non-enzymatic reactions.

At present Helianthus annuus exhibited an increase in catalase activity with increase of salt stress as compared to control in NSSH-1084 cultivar which suggested the existence of an effective ROS-scavenging mechanism. Surprisingly the activity increases upto 200mM NaCl. This trend was shown to be similar for all the three stages of growth. But the activity was seen to be highest during vegetative followed by flowering and then

post flowering stage. Whereas SWATI exhibited decreased CAT activity with the varying salt concentrations that increased up to 50mM with a gradual decline in all the NaCl stressed during vegetative and flowering stages. But the activity declined from control to 200mM during the post flowering.

GPX activity was observed to decrease from control to 200mM for both the cultivars for all growth stages. However the GPX activity for SWATI in two of the growth stages (vegetative and flowering) was found to be insignificant. This finding goes in accordance which reported an increase in GPX activity reduced CAT activity in two oriental tobacco varieties [29].

In tune with the findings of several literatures the increased SOD activity with increased salt concentration in both the cultivars suggested an effective response towards scavenging the superoxide radical. However the activity was found to be higher in NSSH-1084 as compared to SWATI in all treatments and growth stages that indicated the tolerant potential of former over later. Similar responses have been observed in cotton[45], maize, [36] and cabbage[46] which were used as cash crops.

Therefore, the present scenario with enhanced CAT activity coordinated with the changes of SOD and GPX activities plays an important protective role in the ROS-scavenging process and the active involvement of these enzymes are related, at least in part, to salt-induced oxidative stress tolerance in both the sunflower cultivars.

It can be interpreted from present findings that changes in the levels of biochemical metabolites, i.e. soluble proteins, sugar, proline content, lipid peroxidation and antioxidative enzymes can be used to identify the sunflower genotypes having potential to tolerate salinity. The present case study indicates the tolerant potential of NSSH-1084 over SWATI variety of Helianthus annuus L. an oilseed cash crop.

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