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Assessment of Salinity Tolerance Deploying Antioxidant Defense Systems in *Gerbera Jamesonii*

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Inconsistency in the environment exposes plants to various abiotic stresses. This results in damage of a plant's cellular components due to excessive accumulation of unstable reactive oxygen. Besides, it also disrupts enzymatic/non-enzymatic detoxification mechanisms in plants making them more sensitive. Salinity is one such abiotic stress which disrupts regular physiological mechanisms in plants. In this study, we examined the effects of salinity using NaCl in four different genotypes of Gerbera jamesonii cv Bolus, an important ornamental plant of family Compositae. We hypothesized that, upon treatment with NaCl (50, 100, 150 200mM concentration), alterations in the morphological features along with elevated levels of H2O2, MDA, proline, and degradation of chlorophyll will be observed. The enzymatic antioxidant defenses were also hypothesized to differ among genotypes based on their level of tolerance. These parameters were monitored on the 5th and 20th day of NaCl treatment and results were recorded. The observations suggest that 1. the Lattara genotype of Gerbera is sensitive to NaCl and 2. Faith is tolerant, while 3. Alcatras and Basic are moderately tolerant. These findings accompanied by further research on the physiological parameters responsible for attaining salinity tolerance may help in developing salt-tolerant varieties in Gerbera. Future studies on decoding molecular networks associated with the antioxidative defense system in Gerbera can help improve breeding and create novel germplasm in various ornamentally important plants besides Gerbera.

Keywords: Antioxidant Defense Systems; Gerbera; Ornamental Plant; ROS; Salinity.

Various kinds of abiotic stress like salinity, drought, temperature extremes, UV radiation, nutrient deficiency, air pollution, etc. deter the physiological functioning of plants[20]. The extreme levels of these stress factors lead to protein denaturation, imbalance in production/scavenging of unstable oxygen species, and oxidative damage in plants [26]. To counteract these, plants accumulate a variety of compatible solutes, induce defensive proteins and accelerate reactive oxygen species (ROS) defending systems[59]. The most common ROS defending system in plants is the antioxidative defense system which includes compounds like phenolics, ascorbic acid, tocopherols, glutathione, carotenoids, etc., [17] and enzymes like superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), and glutathione reductase (GR). Apart from this, overproduction of compatible solutes like proline, trehalose, glycine betaine, sucrose, etc.,) also help in mitigating and repairing the oxidative damage caused [17, 50].

Salinity is becoming a serious concern in crop and ornamental plants which mainly rely on continuous fertigation. The ability of roots

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to absorb water gets badly affected by salinity, resulting in reduced plant growth, causing a suite of physiological adaptations in plants [43, 14, 15]. Under salinity stress, increased stomatal closure via transpiration results in an imbalance in the carbon dioxide to oxygen content leading to ROS generation[21]. The generated ROS content triggers cellular reactions such as lipid peroxidation, DNA denaturation and protein deprivation causing reduced plant growth, yield loss and eventual death[24, 42, 58]. Minimizing soil salinization and/or increasing salinity stress tolerance of crops have become serious issues which are needed to be resolved immediately [43].

Daisies or Gerbera (*Gerbera jamesonii*) are important ornamental plants of Compositae/ Asteraceae family widely known for their attractive flowers and long shelf/vase life. *Gerbera* being sensitive to abiotic/biotic stresses are commercially grown under protected and controlled cultivation (net/poly house) [11]. Gerbera is cultivated throughout the year and ranks fifth after Rose, Carnation, Chrysanthemum, and Tulip in cut flower trade[09, 45]. Several *Gerbera* cultivars were being grown throughout the world promoting employability and sustainability in the agribusiness sector.

Despite being polyhouse cultivated, *Gerbera* has a high risk of salinity-induced oxidative stress due to excessive fertigation on the soil surface in a polyhouse. The accumulated chemicals present in fertilizers may cause a decline in the plant's productivity causing a threat to its existence[6]. Therefore the development of salt tolerance in Gerbera varieties may protect its commercial significance with regard to its aesthetic value and productivity. To date, little to no studies was attempted on the effect of salinity in several commercially important cultivars of *Gerbera*. Therefore, in this study, we examined the effect of salinity using NaCl and its influence on potential stress indicators to understand the stress-induced changes in four commercially important genotypes of *Gerbera*.

MATERIALS AND METHODS

Plant Material

Plantlets of *Gerbera jamesonii* cv Bolus L., (Terraregina Latara - white colored flower variety, Basic - pink colored flower variety, Alcatras – red colored flower variety and Faith - yellow colored flower variety) aged one month were procured from Virat Agri Biotech company. The plants were potted in sand: cocopeat: soil (1:1:1) mixture and raised in a greenhouse by maintaining controlled conditions (27-29°C day and 23-25°C night temperatures and relative humidity of 45-51%, respectively for the entire growth period).

Optimization of NaCl concentrations based on the morphology of leaves

Preliminary morphological examinations were done for a period of 30 days and the concentration of NaCl to be applied for salinity treatments were assessed using 100–300mM of NaCl concentration. The response to salt treatment was initiated on the 5th day which was aggravated on the 20th day of treatment (data not shown).

Salinity Treatments

For initiating salinity treatments, 45 days old plants with at least four true leaves were selected. The experiment was planned as a complete randomized block which included four treatments and a control (1mM NaCl) each comprising of three replicates. The desired NaCl concentrations of 1mM, 50mM, 100mM, 150mM, and 200mM NaCl were supplemented every third day by dissolving in water in their respective concentrations to the whole plant for twenty days. Sampling was done to these treated plants on the 5th and 20th day of treatment. Secondary leaves were harvested and stored at -20°C after freezing in liquid nitrogen until experiments were carried out. The samples were used for further biochemical studies and Spectrophotometrical assays using UV 3000+ spectrophotometer (Shimadzu).

Investigations of salt affected parameters

Determination of lipid peroxidation by measuring MDA levels

Using the standard protocol of Heath and Packer (1968)[22] with slight modifications, the content of malondialdehyde (MDA) was determined which gives us the extent of peroxidation of lipids in cellular membranes. In brief, 1 gram of frozen leaf material was ground to a fine paste in a mortar and pestle by adding 1 mL of 0.5% TCA and the collected sample was centrifuged at 19000 g for 20min. The supernatant is used as a crude extract. TBA (thiobarbutiric acid) reagent was prepared by adding 15% trichloroacetic acid (w/v) and 0.375% TBA(w/v) in 0.25 M HCl. Equal volumes (0.4 mL)

of crude extract and TBA reagent in a microfuge tube were heated for 15 min at 95°C, immediately shifted to an ice bath. Following this, the mixture is centrifuged for 15 min at 15000 g. The supernatant obtained is read for absorbance at 532 nm by subtracting turbidity at 600 nm. The quantity of MDA was calculated from the extinction coefficient of 155 $mM^{-1}cm^{-1}$. Data were expressed as ìmol per g FW.

Determination of Content of Free Proline

Using the method of Ninhydrin, the content of free proline was determined as per Bates et al. (1973)[07]. In a spectrophotometer, absorbance was measured at 540nm. Data were expressed as ìmol per g FW.

Determination of Chlorophyll Content

The content of chlorophyll was determined as per the standard protocol of Lichtenthaler and Wellburn (1983)[33]. Absorbance was read at 646nm and turbidity obtained at 663 nm was subtracted from it. In brief, leaf samples were extracted using 80% (v/v) acetone and the quantities of chlorophyll a, b, and total chlorophyll were calculated. Data were expressed as mg per g FW.

Determination of Antioxidant Enzyme Assays

For enzyme assays, 100mg of sample was thoroughly ground to a paste in a mortar and pestle by adding 1mL of ice cold extraction buffer. The extraction buffer consists of 100mM potassium phosphate buffer (pH 7.0) with 1mM EDTA. The obtained homogenate was centrifuged at 5,000 rpm for 15 min (Eppendorf Centrifuge 5430 R, Hamburg, Germany) and the supernatant was used to analyze enzyme activities.

The quantity of total soluble protein was determined as per the Lowry's method (1951)[36] using bovine serum albumin (BSA) as a standard at 640nm. In brief, leaf samples of 100 mg were ground in 10 mM ice cold potassium phosphate buffer of pH 6.8 and centrifuged for 20 min at 15 000 g. The obtained supernatant was used to determine soluble protein content. Values were expressed as mg per g fresh weight (FW).

Superoxide dismutase activity, SOD of (EC 1.15.1.1) was done by the method of Beyer and Fridovich (1987)[08]. Leaves were extracted using extraction buffer supplemented with PVP (1% (w/v) polyvinyl-pyrrolidone) and 0.5% (v/v) Triton X. The photochemical reduction of nitro blue tetrazolium (NBT) was recorded at 560 nm. The amount of SOD used up in the reaction to inhibit 50% of NBT gives us one unit of SOD activity.

Catalase activity, CAT (1.11.1.6) was measured as per Aebi et al. 1974[3]. Briefly, the decrease in the substrate H_2O_2 incubated with Leaf Extract was monitored at 240 nm and quantified by its molar extinction coefficient of 36 $M^{-1}cm^{-1}$. The activity of catalase was expressed as ìmoles of H_2O_2 decreased min^{-1} mg^{-1} protein.

Ascorbate Peroxidase, APX (1.11.1.1) activity was done by the method of Nakano and Asada (1981)[44]. The absorbance of ascorbate at 290 nm was quantified using molar extinction coefficient (2.8 $mM^{-1}cm^{-1}$) and activity of APX expressed as ìmoles of ascorbate decreased min^{-1} mg^{-1} protein.

Glutathione Reductase, GR (1.6.4.2) activity was measured according to Foyer and Halliwell (1976)[16]. The absorbance at 340 nm was calibrated using a molar extinction coefficient of NADPH (6.2 $mM^{-1}cm^{-1}$). One unit of GR was defined as 1 mmol ml^{-1} GSSG (glutathione) reduced min^{-1}.

Statistical Analysis

The data presented are the average values (±SE) of results from three experiments conducted on different time points including four *Gerbera* species and five salinity levels. The data were subjected to further statistical analysis by one-way ANOVA (Holm-Sidak method) using SigmaPlot Version 14.0.

RESULTS

Effect of NaCl treatment on phenotypic characteristics in four different genotypes of Gerbera

In this study, we treated all the four genotypes of *Gerbera* (Red, Yellow, Pink, and White) with a concentration of 200 mM NaCl for 20 days (Figure 1). Upon morphological observation, bleaching was observed which was more pronounced in White followed by Red and Pink genotypes combined with a decrease in leaf size and shape. NaCl stress also inhibited the emergence of young leaves showing stunted growth. In contrast the leaves of the Yellow genotype exhibited little to no effect.

Levels of H_2O_2 in four different genotypes of Gerbera on exposure to salinity stress

In the given study, the four different genotypes of *Gerbera* were treated with a 200 mM concentration of NaCl and the H_2O_2 levels were monitored at two different time points (5th and 20th DOT). In the initial days of treatment, i.e., on the 5th day we observed a rise in H_2O_2 levels upon treatment with 200 mM NaCl particularly in Pink and White *Gerbera* genotypes (Fig.2A), while there was a considerable decrease in the levels of H_2O_2 in Yellow and Red *Gerbera* genotypes compared to control (Fig.2A). At 20th DOT, the levels of H_2O_2 were in significant increment in the Pink and White genotype of *Gerbera* when compared to control (Fig.2A) whereas no such increase was observed in other genotypes (Fig. 2A).

Levels of MDA in four different genotypes of Gerbera on exposure to salinity stress

In the current study, after treating the four different genotypes of *Gerbera* with 200 mM NaCl, the MDA levels were monitored at two different time points (5th and 20th DOT). Initial days of treatment, i.e., 5th day we observed a significant rise in MDA levels upon treatment with NaCl particularly in Red, Pink, and White *Gerbera* genotypes (Fig.2B), while there was no considerable change in MDA levels in Yellow genotype (Fig.2B). Similar to the 5th day even on 20th day of DOT, we observed a significant increment in MDA levels in all genotypes of *Gerbera* when compared to control, except Yellow which also exhibited an increase but was not significant (Fig.2B).

Fig. 1. Effect of NaCl on phenotypic characteristics of four different genotypes of Gerbera (White, Pink, Red and Yellow) of A) Control, B) 5th DOT and C) 20th DOT. NaCl treatment indicates leaves treated with 200 mM of NaCl. The untreated leaves (0 mM NaCl) served as experimental control

Content of total chlorophyll in four genotypes of Gerbera on exposure to salinity stress

In the given study, we determined the content of total chlorophyll in four *Gerbera* genotypes after giving a treatment of 200 mM NaCl for twenty days and measured the content of chlorophyll at two time points (5th and 20th DOT). At 5th day, we observed no considerable change in the chlorophyll content in Yellow/ Pink genotypes of *Gerbera* whereas in White and Red genotypes a non-significant decrease in the chlorophyll content was seen (Fig.2C). Similarly, at 20th DOT we observed a drop in total chlorophyll content in NaCl treated plants particularly in Pink, Red and White genotypes of *Gerbera* (while in Yellow we did not noticed any change in chlorophyll content when compared to control (Fig.2C).

Content of free proline in four Gerbera genotypes upon exposure to salinity stress

Further, we determined the content of free proline in four *Gerbera* genotypes treated with 200 mM concentration of NaCl at two time points (5th and 20th DOT). At 5th day, we observed increased accumulation of free proline in Red and White genotypes of *Gerbera* in NaCl treated plants. While there was little to no enhancement in proline accumulation in Yellow and Pink genotypes of *Gerbera* compared to control (Fig.2D).Whereas, at 20th DOT we observed significant shoot up in proline levels in White, Pink and Yellow genotypes of *Gerbera* (in Yellow this increase was not significant). In contrast, the Red genotype of *Gerbera* did not exhibit much alteration upon treatment with 200 mM of NaCl concentration (Fig. 2D).

Enzymatic activity in four Gerbera genotypes on exposure to salinity stress

In the present study, we monitored the effect of salinity on SOD in four different genotypes of *Gerbera* after treating with 200 mM NaCl at two time-points (5th and 20th DOT). On the 5th day, except in the White genotype, a significant increase in the SOD activity was noticed in NaCl treated plants of all genotypes of *Gerbera* compared

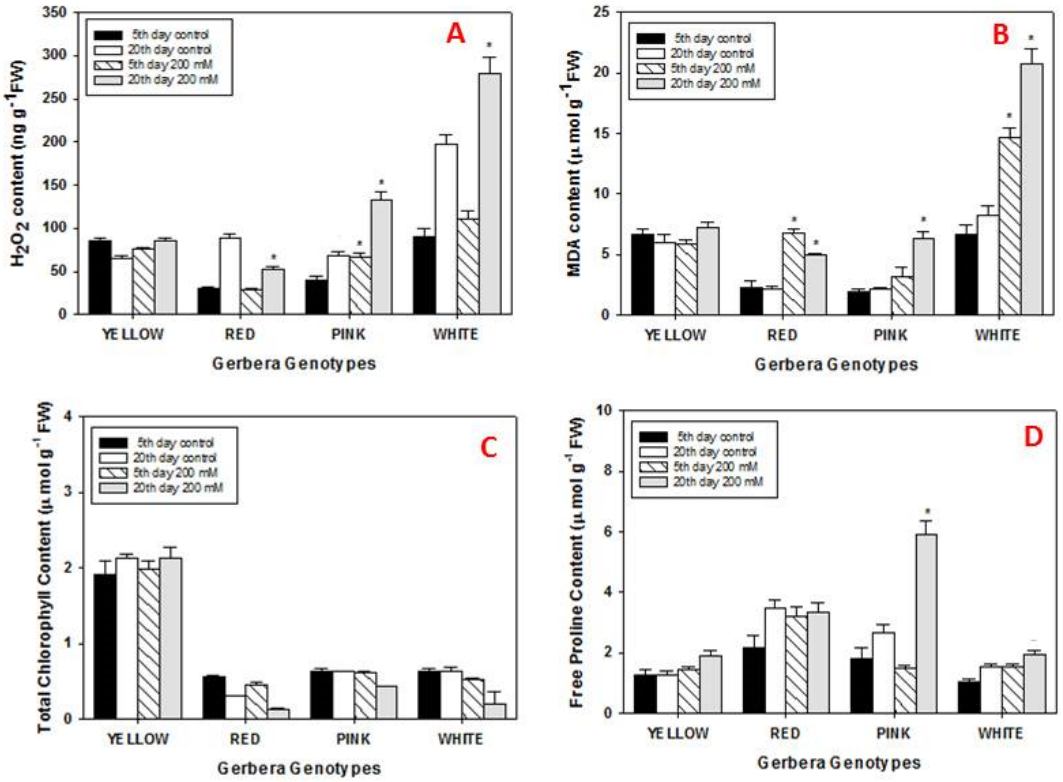

Fig. 2. Effect of NaCl on A) H2O2 content, B) MDA content (lipid peroxidation), C) total chlorophyll content, D) free proline content in Yellow, Red, Pink and White genotypes of Gerbera respectively at 5th DOT and 20th DOT

to control (Fig. 3A). Similarly at 20th DOT we observed the same pattern of results when studying the activity of SOD in *Gerbera* (Fig.3A).

Thereafter, we monitored the activity of CAT in four different genotypes of *Gerbera* treated with 200 mM NaCl at two time-points (5th and 20th DOT). At 5th day, a noticeable increase in the CAT activity on treatment with 200 mM NaCl concentration was seen in Yellow and Pink and a slight decreased activity was seen in White and Red genotypes of *Gerbera* (Fig.3B). Whereas, at 20th DOT we observed a noteworthy increase in the activity of CAT in Yellow, Red, Pink genotypes, in contrast CAT activity in White genotype showed little to no change in NaCl treated plants when compared to control.

We also examined the activity of APX in all four genotypes of *Gerbera* upon treatment with 200 mM NaCl at two time points (5th and 20th DOT). On the 5th day, we observed a gradual increase in APX activity compared to control upon treatment with 200 mM NaCl concentration in all genotypes of *Gerbera* (particularly significant in Yellow and Pink)(Fig.3C). Whereas, at 20th DOT we observed an increase in APX activity with increasing concentrations of NaCl particularly in Yellow, Pink and Red genotypes of *Gerbera* which was significant, in contrast, the APX activity in the white genotype showed a slight decrease at 200mM NaCl compared to control (Fig. 4(G)).

Finally, we studied the activity of GR in all four genotypes of *Gerbera* after subjecting it to salt stress by treating it with 200 mM NaCl for a period of twenty days. The observations were recorded at two different time points (5th and 20th DOT). On the 5th day, we observed a significant shoot in the activity of GR compared to control in Yellow and Red genotypes of *Gerbera*, while in both Pink and White genotypes we observed a decline in GR activity (Fig.3D). At 20th DOT a noticeable decrease in the activity of GR in all genotypes except Yellow was observed (Fig.3D).

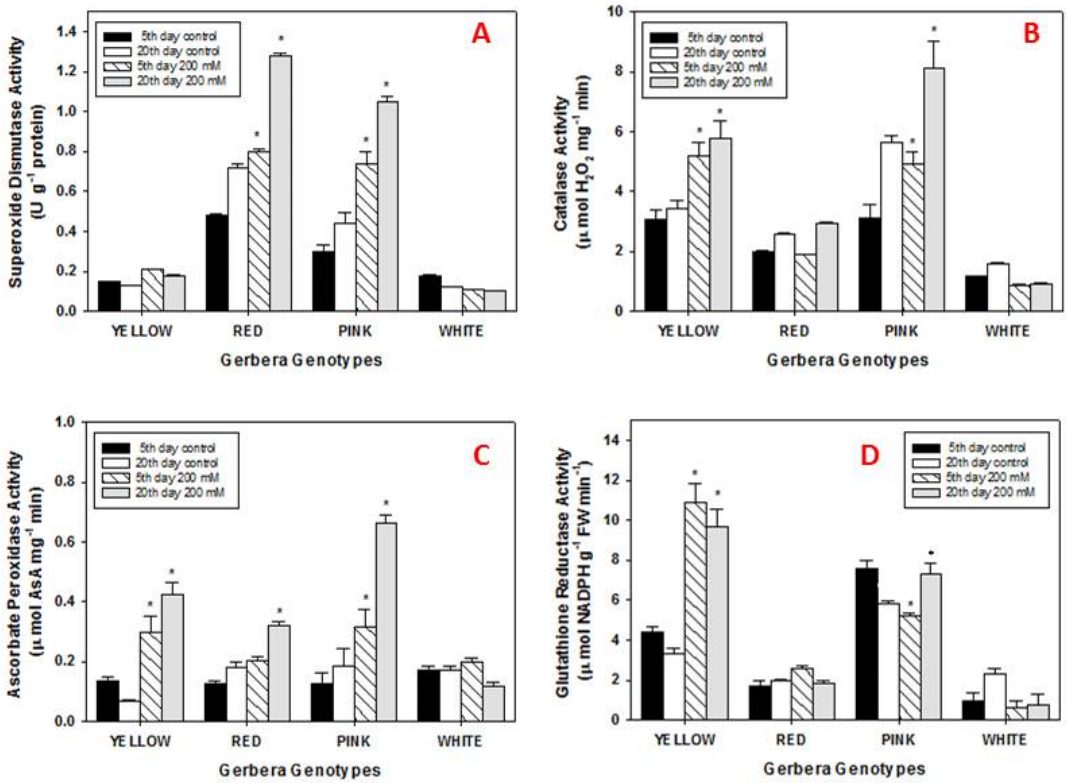

Fig. 3. Effect of NaCl on A) Superoxide Dismutase, B) Catalase, C) Ascorbate Peroxidase and D) Glutathione Reductase in Yellow, Red, Pink and White genotypes of Gerbera respectively at 5th DOT and 20th DOT

DISCUSSION

Salinity is a major concern in crop and ornamental plant species negatively affecting growth and productivity[19]. Although much research has been conducted in crop plants, salinity studies in ornamentals are still at a budding stage urging stress biologists to focus their scientific research and studies on salt tolerance[18]. In the given study we chose *Gerbera* as a research model in ornamental plants to study salt tolerance across four different genotypes. As a part of our study, we investigated the intensity of oxidative damage caused by salinity stress in *Gerbera* and the mechanisms to mitigate it. Earlier, we carried out some preliminary studies on the white genotype of *Gerbera* to assess its sensitivity towards NaCl using leaf disc culture system and found the White genotype to be sensitive (data not shown). This provoked us to move to a further level in determining the ability of *Gerbera* to confer salt tolerance across its other genotypes which have huge market demand in the floriculture sector (red, pink and yellow *Gerbera* varieties).

In this regard, we initially performed preliminary phenotypic experiments in four genotypes of *Gerbera* by treating them with increasing NaCl concentrations (50 mM, 100 mM, 150 mM, and 200 mM) for a period of 30 days. Based on phenotypic/morphological observations, the toxicity of NaCl was evident from5th day onwards which aggravated in 20 days of salt treatment (symptoms like wilting, disfiguration of leaves were seen after 20th day) (data not shown) which made us plan further biochemical studies at 5th day and 20th day of NaCl treatment.

Salinity effect on plant growth and morphology in four different genotypes of Gerbera

In this study, we characterized salt-tolerant ability in four different genotypes of *Gerbera* based on their morphological observations upon NaCl treatment. We observed varied responses in plant height, leaf size, and color with increasing concentrations of NaCl, particularly in white genotype with conspicuous yellowing/bleaching of leaves signifying the intensity of oxidative damage. These findings are in line with the previous reports [4, 38, 43] which show a similar response. The results obtained in our study reports a detrimental effect of salinity on plant growth, causing a gradual reduction of leaf area.

Altered levels of H_2O_2 content in four different genotypes of Gerbera upon salt stress

In plants, the accumulation of H_2O_2 signifies an intercellular physiological breakdown and signals the plant defense system to activate genes involved in stress responses, such as catalase, peroxidase, etc., [31]. We attempted to look into it by estimating the accumulation of H_2O_2 levels in NaCl treated leaves of four *Gerbera* genotypes. We observed that on 5th and 20th DOT, when compared with its respective controls, in Pink and White genotypes NaCl treatment showed a considerable rise in H_2O_2 levels. Whereas, H_2O_2 levels in the Red genotype remained unaltered. In contrast, the yellow genotype exhibited a lower accumulation of H_2O_2 content. These findings give us a hint that a rise in H_2O_2 levels in Pink and White Gerbera was also may be due to the induction of SOD in plants to defend itself against oxidative stress as a part of H_2O_2 defense signaling[28]. Our findings are similar to the previous reports in *Dianthus superbus* [37] and Rice [29] which suggested that there is an increased content of H_2O_2 under salt stress.

Altered levels of MDA content in four different genotypes of Gerbera upon salt stress

Accumulation of malondialdehyde, produced due to peroxidation of lipids in cellular membranes signals oxidative damage in plants subjected to stress conditions. We attempted to look into it by estimating the accumulation of MDA levels in NaCl treated leaves of four *Gerbera* genotypes. We observed that on 5th DOT, when compared with its respective control, Red and White genotypes exhibited high levels of MDA, whereas the MDA levels in the Pink genotype remained unaltered. In contrast, the Yellow genotype exhibited a lower accumulation of MDA content. These findings signify that the White genotype is the first and foremost one to show the stress response. In addition to this, the MDA content on the 20th day was also significantly higher in White followed by Pink, Red and Yellow indicating the hyper sensitivity of White genotype when compared to others. However, in all genotypes, MDA content was found to be higher compared to their respective control in which Yellow genotype exhibited negligible increase (1

fold) suggesting the ability of Yellow genotype to withstand stress.

Our findings are similar to the previous reports in Sunflower *(Helianthus anuus L),* which suggested that there is an increase in lipid peroxidation under salt stress[13]. Apart from this, the salt sensitive cultivar Wuxi of Jerusalem Artichoke *(Helianthus tuberosus)* has also exhibited significant enhancement in MDA levels upon NaCl stress[58].

Modulation of Free Proline content in four different genotypes of Gerbera upon salt stress

Further, we investigated free proline content as it is a major organic osmolyte that is known to accumulate in plants under stressful conditions[40]. Particularly, in salt-induced oxidative stress proline protects cellular membrane by enhancing activities of various antioxidants[47]. In our study also, at 5th DOT we noticed a high accumulation of proline in all genotypes except Yellow with respect to their control. Even at 20th DOT, proline accumulation was highly significant in pink genotype followed by Yellow and White genotypes suggesting the role of proline in minimizing ROS production. While in the Red genotype, we observed a slight increase in proline content which was not significant. Among all genotypes, both at 5th and 20th DOT the Yellow genotype of *Gerbera* accumulated significantly higher proline content under saline conditions.

The findings of this present study are concurrent with earlier published data which clearly demonstrated that salt stresses markedly enhanced free proline content upon treatment with NaCl in eight sunflower cultivars[51] and in roots of Chrysanthemum[25].

Degradation of total chlorophyll content in different genotypes of Gerbera on treatment with NaCl

It is a well-known fact that any oxidative stress would definitely cause either a decrease in chlorophyll synthesis or degradation of its complexes[39,50]. This is evident by previous reports which suggest that salt sensitive cultivar Wuxi of Jerusalem artichoke; an ornamental plant exhibited a dramatic decrease in chlorophyll content with increasing salt concentrations[58]. *Similarly, in Helianthus anuus* L. plants also salt treatment exhibited a decreased production of chlorophyll[51]. In corroboration with these reports, in our study particularly at 20th DOT, the total chlorophyll content was decreased to a larger extent in all genotypes except yellow.

Modulation of antioxidant enzyme activities by NaCl treatment in four different genotypes of Gerbera

Generally in plants exposed to stress, the first line of defense which are the earliest to respond are the antioxidant enzymes like SOD, CAT, APX, GR, POX, GPX etc[30,54]. There are several reports which demonstrated the role of antioxidant enzymes under salinity stress of different ornamental plants such as *Helianthus anuus* [51], *Helainthus tuberosus* [58], Artemesia [32], Catharanthus [27]. In the given study we mainly focused and examined SOD, CAT, APX and GR activities at 5th and 20th DOT by applying salt stress using 200 mM NaCl concentration in four different genotypes of *Gerbera*. The activity of SOD increased in all the NaCl treated genotypes except in the White genotype on both 5th and 20th DOT which are in compliance with the results obtained in Cotton[41], where the tolerant cultivar *(Pora)* showed higher activity of SOD activity upon exposure to NaCl stress when compared to sensitive cultivar *(Guazuncho)*. Similar results were also observed in *Kandelia candel* [56] and sensitive (cv *Challis*) and tolerant cultivar (cv *Granada*) of *Pisum sativum,* where the activity of SOD was found to be more intolerant cultivar upon treatment with NaCl when compared to sensitive cultivar [23]. These results leave us a clue that the White genotype may be sensitive to NaCl stress when compared to Red, Pink and Yellow genotypes. The activity of CAT at 5th and 20th DOT increased considerably in Pink and Yellow genotypes, whereas this increase in Red was not seen. In contrast the activity of CAT slightly decreased which was not significant upon exposure to 200 mM NaCl in White genotype. The white genotype exhibited contrasting CAT activity by showing decreasing pattern which may be due to extreme sensitivity of white genotype towards higher NaCl levels. Our findings are in line with previous reports which suggested that there was a reduction in the CAT activity in salt sensitive cultivars of various plant species such as *Echinea purpuria*[48], Jerusalem artichoke[58], *Amsonia orientalis*[2]. Besides, there are also reports which suggested increased CAT activity in salt tolerant cultivars of plant species [12, 1, 32].

In continuation with the previous studies on SOD and CAT, we have monitored APX activity at 5th and 20th DOT. At 5th DOT, we observed a considerable increase in APX activity in all four genotypes, wherein the activity of APX was more significant in Yellow and Pink genotypes. While in Red and White genotypes, the activity of APX slightly increased even after treatment with 200 mM NaCl. Whereas at 20th DOT, the activity of APX is significantly high in Pink, Yellow and Red genotype, in contrast the White genotype exhibited decreased APX activity in NaCl treated plants indicating its sensitivity towards NaCl stress. We noticed that our present observations correlate with previous reports which demonstrated that there was a reduction in the APX activity in *Helianthus annuus*[51]. There are also a few reports from which it is evident that enhanced APX activity in *Artemesia annua*[46], *Echinea purpuria*[48], *Calendula officianalis* [12], *Stevia roubidiana* [10].

Finally, we studied the activity of GR by applying NaCl stress at 5th and 20th DOT. At 5th DOT, we noticed a considerable increase in GR activity in Yellow genotype, in anomaly we noticed a significant decreasing pattern in White and Pink genotype with increasing concentrations of NaCl. Whereas at 20th DOT, the activity of GR is significantly high in Pink and Yellow genotypes, in contrast the Red and White genotypes exhibited decreased GR activities with increasing NaCl levels. Our results also support previous evidence in several plants species belonging to Asteraceae family like Chrysanthemum[25], *Artemesia annua*[46], *Calendula officianalis* [12] which exhibited enhanced GR activity upon salt stress. Contradictory to it, in certain plant species like *Helianthus annuus*[51], *Stevia roubidiana*[10] there was a decline in GR activity at higher saline conditions.

As per Abogadallah et al.[1] plants subjected to salinity show elevated activities of antioxidant enzymes in tolerant species than the sensitive ones. This relates with our study which has shown that SOD, APX, CAT and GR were highest in Yellow and all enzyme activities are lowest in White. These findings imply that Yellow genotype can tolerate the negative effects of salinity better than other studied genotypes.

CONCLUSIONS

To conclude, our study is the first of its kind in members of the Compositae family which deals with the antioxidative defense response of different genotypes of *Gerbera* to salinity stress. A clear disparity was observed in the defense action of four genotypes of *Gerbera* employing enzymatic and non-enzymatic components. This kind of study may pave a way for further understanding of varied defense responses and their associated key metabolic activities across different genotypes of *Gerbera*. Our findings may leave a clue to ornamental plant researchers in the development of salt-tolerant transgenic plants and incite them to do further research in the area of abiotic stress physiology in ornamentals. This may have a global impact on the floral market with improved and large-scale productivity of *Gerbera* species.

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Conflict of interest

No potential conflict of interest was reported by the authors.

Author contributions

JU and SKT performed experiments; EM gave technical support to JU and SKT in performing experiments; JU, SKT and PM designed the experiments, analyzed data, and wrote the paper.

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