Salinity Driven Oxidative Stress in *Gerbera jamesonii* cv Bolus-an Ornamental Plant with High Aesthetic Value

**Abstract**

**Introduction:** Salinity adversely affects a variety of plant’s metabolic processes influencing its productivity and crop yield. *Gerbera jamesonii* Bolus is a commercially important ornamental plant cultivated globally throughout the year for its cut flower production in polyhouses. During cultivation in polyhouse, repeated fertigation may cause salinity in *Gerbera* negatively affecting its flower yield which will be indicated by functional alterations in basal level of antioxidative defense systems in it. Though salinity is induced by several factors, we have focussed on NaCl as it is one of the major components of fertigation. Monitoring altered levels of antioxidative defense systems in plants may help to better understand the physiological changes of plants under salt stress. However, little to no studies were attempted on salinity induced oxidative damage in *Gerbera* till date.

**Methods:** We verified the salt sensitivity level of *Gerbera* with varying concentrations of NaCl (0mM-200mM) using *in vitro* leaf disc approach and measured various antioxidative enzymatic/non enzymatic defense systems besides MDA and chlorophyll determination.

**Results:** Treatment with higher salt concentrations (above 100 mM) exhibited severe bleaching in leaf discs followed by elevated $\text{H}_2\text{O}_2$, lipid peroxidation and proline levels. Besides our studies also revealed a decrease in total chlorophyll contents, activities of superoxide dismutase, catalase, glutathione reductase and ascorbate peroxidase.

**Conclusion:** The observed results left a clue that *Gerbera* may not tolerate higher levels of NaCl as it could be detrimental to its cellular activities. Future studies on decoding molecular networks associated with antioxidative defense system in *Gerbera* may help in developing salt-tolerant varieties in *Gerbera*.

**Keywords:** Gerbera; Reactive oxygen species (ROS); Antioxidative defense; Salinity; Oxidative stress; Fertigation

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**Introduction**

Plants are regularly confronted to abiotic and/or biotic stress or a combination of both in their habitats. Abiotic stress represents environmental factors like temperature extremes, salinity, drought, cold, etc. leading to morphological, biochemical and molecular driven physiological adaptations in plants [1]. Despite several metabolic adjustments, abiotic stress has become a crucial factor affecting crop yield [2-4]. In response to abiotic stress, plants develop newer/alternative metabolic pathways (accumulating low molecular weight metabolites and proteins), detoxification mechanisms and altered phytohormone levels developing tolerance [5].

One of the crucial factors in plants that arise during salinity stress is excess production of its Reactive Oxygen Species (ROS) levels causing oxidative damage to cells. To overcome this toxicity, several antioxidative defense systems play a protective role [6]. The antioxidative defense system comprises non-enzymatic antioxidants/compatible solutes viz., ascorbic acid, glutathione, osmolytes like proline, etc. and antioxidant enzymes such as Superoxide Dismutase (SOD), Catalase (CAT), Ascorbate Peroxidase (APX), Guaiacol Peroxidase (GPX), Glutathione Reductase (GR), etc. [7]. Monitoring altered levels of these components in plants may help to better understand the physiological changes of plants under salt stress [8].

In the present scenario, there is a growing demand for sustainable agriculture, especially protected cultivation of ornamental and vegetable crops. However, over a period of time repeated fertigation may drastically hamper plant’s productivity and threaten its existence (Haynes et al. 1987). Gerbera (Gerbera jamesonii) of family Compositae, is an important ornamental plant commercially grown under protected cultivation (tunnel), proline and activities of antioxidant enzymes in plants under salt stress [8].

In the current study, excess accumulation of ROS was quantified as an equivalent to the level of MDA, a decomposition product of polyunsaturated fatty acids routinely used as a biomarker for lipid peroxidation [19-21]. In the present study, it was evident that the content of H$_2$O$_2$ levels increased proportionately along with MDA upon increasing levels of NaCl (Figure 1b). This increase in MDA was significantly more pronounced with about ≥ 6 folds increment particularly in 200mM NaCl treatment compared to control indicating the sensitivity of Gerbera. Similar observations on salt sensitivity were reported in Alfalfa [22], Cotton [7], Maize [8], Wheat [23], Mulberry [24] and Cucumber seedlings [25]. Recent study in ornamental plant, Amsonia orientalis revealed similar pattern which corroborate our findings in Gerbera [26]. The H$_2$O$_2$ content in our study was found to be similar with the results obtained in Wheat [27] and Pismat sativum [28].

**Methods**

In the given study, we have excised the fourth (4th) youngest leaf from same age group plants of Gerbera jamesonii cv Bolus L., (Terraregina Lata - white colored flower variety). The leaves were surface sterilized [12] and were placed in distilled water. Leaf discs of approximately 11mm diameter were punched by immersing in water tray to minimise the mechanical stress and designed into six treatment groups (25mM, 50mM, 75mM, 100mM, 150mM and 200mM NaCl) and a control (without NaCl) as per Talla et al. [13]. Approximately 200mg Fresh Weight (FW) leafdiscs were incubated in petriplates containing 20mM MES buffer with 2mM CaCl$_2$.2H$_2$O (pH5.6) (as per Talla et al. [14]) in combination with various levels of NaCl. The leaf discs were incubated for 5 days under controlled conditions (photon period of 16/8 hrs at 25°C and RH 60-80 %). Measurement of H$_2$O$_2$ content was done spectrophotometrically as per Alexieva et al. (2001) with slight modifications. Lipid peroxidation was determined spectrophotometrically using Malondialdehyde (MDA) method as per Heath and Packer [15]. Proline content by ninhydrin method was measured spectrophotometrically at an absorbance of 520 nm according to Bates et al. 1973. Chlo- rophyll content was determined spectrophotometrically at an absorbance of 646nm and 663nm as per Arnon [16].

For enzyme assays, control and treated leaf discs were ground in liquid nitrogen and then transferred to 1ml ice cold extraction buffer (100 mM potassium phosphate buffer pH 7.0, 1mM EDTA). The homogenate was filtered and centrifuged at 5,000 rpm for 15 min, and the supernatant was used for enzyme assays. In all assays, soluble protein concentration was determined using bovine serum albumin, BSA as a standard at 750nm according to Lowry’s method [17]. Superoxide dismutase activity (EC 1.15.1.1) was monitored as per the method of Beyer and Fridovich (1987), Catalase activity, CAT (1.11.1.6) was monitored at 240nm as per Aebi et al. 1974. Ascorbate Peroxidase, APX (1.11.1.1) activity was determined according to Nakano and Asada [18] at an absorbance of 290 nm. Glutathione Reductase, GR (1.6.4.2) activity was measured at A340 nm absorbance according to Jiang and Zhang (2001). Data presented are the average values (±SE) of results from three replicates and statistical analysis was done using one way ANOVA (Holm-Sidak method) through SigmaPlot Version 13.0.

**Results and discussion**

In the current study, excess accumulation of ROS was quantified as an equivalent to the level of MDA, a decomposition product of polyunsaturated fatty acids routinely used as a biomarker for lipid peroxidation [19-21]. In the present study, it was evident that, the content of H$_2$O$_2$ levels increased proportionately along with MDA upon increasing levels of NaCl (Figure 1b). This increase in MDA was significantly more pronounced with about ≥ 6 folds increment particularly in 200mM NaCl treatment compared to control indicating the sensitivity of Gerbera. Similar observations on salt sensitivity were reported in Alfalfa [22], Cotton [7], Maize [8], Wheat [23], Mulberry [24] and Cucumber seedlings [25]. Recent study in ornamental plant, Amsonia orientalis revealed similar pattern which corroborate our findings in Gerbera [26]. The H$_2$O$_2$ content in our study was found to be similar with the results obtained in Wheat [27] and Pismat sativum [28].

Plant cells accumulate compatible osmolytes like proline to scavenge free radicals and protect metabolic enzymes [29]. The levels of proline in the given study continued to increase upon increasing concentrations of NaCl up to 75 mM and thereafter observed a slight downfall (significant at 150 mM and 200mM NaCl) (Figure 1c). The sudden downfall of proline levels from 150mM NaCl reflects Gerbera’s inability to accumulate proline making it susceptible which may be due to little synthesis or higher degradation under high salinity stress [30]. Our results were similar with the salt stress reports of other ornamental species, Amsonia orientalis [26], Pelargonium [31] and Catharanthus roseus [20]. From these studies, it is observed that Gerbera, at a certain level of NaCl exposure (≥ 75 mM NaCl) restricts its synthesis of proline, one of the crucial osmolyte produced during stress.
In the current study, we noticed a gradual decrease in total chlorophyll content upon increasing NaCl concentrations (Figure 1a). This significant decrease in chlorophyll content particularly above 100 mM NaCl serves as a preliminary evidence that *Gerbera* is sensitive towards salinity stress [32]. Recent studies on salt tolerance in ornamental plants like *Dianthus superbus* [33], *Brassica oleracea* [34] and *Pelargonium* [31] depicted apparent decrease in chlorophyll contents with increased salinity levels which is in agreement with our current findings.

The response of *Gerbera* towards salinity was also checked by monitoring the activities of key antioxidant enzymes (SOD, CAT, APX and GR). The activity of SOD increased gradually up to 75 mM of NaCl treatment and decreased thereafter suggesting its role in combating stress up to a certain point of salinity stress as recorded in Wheat [23]. The CAT activity was found to be higher in control compared to NaCl treated samples. With increasing salinity, we observed a decrement in CAT activity up to 50 mM NaCl which were not significant (Figure 2a). Further increase of NaCl concentrations (75 mM to 200 mM), a significant decrease in CAT activity particularly at 100 mM NaCl (Figure 2a) was observed which were in consistency with salt tolerance studies in *Amsonia orientalis* [26].

In the present study, we observed a significant decrease in the activity of APX upon increasing concentrations of NaCl (Figure 2b) which are in line with reports on salt sensitive cultivar of Foxtail millet (*Setaria italica* L.) [35] and *Cucumber* seedlings [25]. Evidences suggests that the ascorbate-glutathione cycle play an important role in maintaining the redox poise in plant cells against abiotic stress [36]. In contrary to CAT and APX, GR activity increased gradually up to 75 mM NaCl and decreased thereafter with a significant decrease at 200 mM NaCl concentration (Figure 2c) which is in corroboration with salinity studies in *Pisum sativum* cv. Challis [37-43]. To conclude with, we reported salt sensitive response of *Gerbera* by performing differential antioxidant profiles and responses under salinity conditions. Overall, salinity significantly reduced chlorophyll, CAT, APX and GR activities, while proline and MDA contents were increased. This sensitivity of *Gerbera* towards salinity indicates the inefficiency of the plant defense system to combat ROS accumulation, disturbing the redox homeostasis and integrity of cellular components. However, we also need to focus on understanding salinity stress due to several other factors besides NaCl [44-50]. These antioxidant activities might be useful in future studies as biochemical markers for improving salt tolerance in *Gerbera* and other ornamental plants of Compositae [51-65]. To our knowledge, this is the first report on antioxidative damage studies in *Gerbera* upon exposure to salt stress which opens the door to manipulate antioxidative defense systems at molecular level in *Gerbera* for developing salt tolerant varieties.

**Figure 1:** H$_2$O$_2$ content (A), MDA content (B), free proline content (C) and Total chlorophyll content (D) in *Gerbera* leaf discs treated with different NaCl concentrations: Each bar is represented as mean average ± standard deviation of three replicates per treatment performed randomly at different time periods. Asterisks indicate that the differences (p<0.05) between the control (untreated) and treated samples are statistically significant as determined by one way ANOVA (Holm-Sidak method).

**Figure 2:** Effect of NaCl concentrations (0-200 mM) on Superoxide Dismutase (A), Catalase (B), Ascorbate peroxidase (C) and Glutathione reductase (D) enzyme levels in leaf discs of *Gerbera*: Each bar is represented as mean average ± standard deviation of three replicates per treatment performed randomly at different time periods. Asterisks indicate that the differences (p<0.05) between the control (untreated) and treated samples are statistically significant as determined by one way ANOVA (Holm-Sidak method).

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**Compliance with ethical standards**

**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.
References


