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Salinity driven oxidative stress in *Gerbera jamesonii* cv. Bolus

Javeria Uzma, Sai Krishna Talla#, Ebenezer Madam and Praveen Mamidala*

*Department of Biotechnology, Telangana University, Dichpally, Nizamabad, Telangana, 503322, India. [2]Department of Biotechnology, Telangana University, Dichpally, Nizamabad, Telangana, 503322, India. [3]Department of Biotechnology, Telangana University, Dichpally, Nizamabad, Telangana, 503322, India. *E-mail: pmamidala@gmail.com; pmamidala@telanganauniversity.ac.in. # shares the equal authorship with the first author.*

Abstract

Salinity adversely affects various plant's metabolic processes, negatively influencing their productivity and crop yield. *Gerbera jamesonii cv.* Bolus is a commercially important ornamental plant cultivated globally annually for its cut flower production in polyhouses. During cultivation in a polyhouse, repeated fertigation may cause salinity in *Gerbera,* affecting its flower quality and yield, indicating functional alterations in the basal level of cellular antioxidative defense systems. In the current study, *Gerbera's* salt sensitivity level was verified with varying NaCl concentrations (0-200 mM) using *in vitro* leaf disc approach and various antioxidative enzymatic/non-enzymatic defense systems besides MDA and chlorophyll content was measured. Treatment with higher salt concentrations (above 100 mM NaCl) exhibited severe bleaching in leaf discs, followed by elevated levels of H_2O_2, lipid peroxidation and proline. Besides, our study also revealed a decrease in the total chlorophyll content; activities of superoxide dismutase, catalase, glutathione reductase, and ascorbate peroxidase. The observed results showed 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 salinity stress and antioxidative defense systems may help develop salt-tolerant varieties in *Gerbera* and several other ornamental plants of Asteraceae.

Key words*: Gerbera;* reactive oxygen species (ROS); antioxidative defense; salinity; oxidative stress; fertigation

Introduction

Plants regularly confront abiotic and biotic stress or both in their habitats. Abiotic stress factors like temperature extremes, salinity, drought, cold, *etc.,* lead to plants' morphological, biochemical and molecular-driven physiological adaptations (Huang *et al.*, 2012). Despite several metabolic adjustments, abiotic stress has become a crucial factor affecting crop yield. 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 (Nasibi and Kalantari 2009).

The excessive production of reactive oxygen species (ROS), which results in cellular oxidative damage, is one of the critical elements in plants that emerge during salinity stress. Plants employ several antioxidative defense mechanisms to counteract this toxicity (Gill and Tuteja 2010). 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), and glutathione reductase (GR), *etc.* (Zhao *et al.*, 2021). Monitoring altered levels of these components in plants may help better understand plants' physiological changes under salt stress (Abdelgawad *et al.*, 2016).

In the present scenario, there is a growing demand for sustainable agriculture, especially protected cultivation of ornamental and vegetable crops. However, over time, repeated fertigation may drastically hamper plant's productivity and threaten its existence (Liang *et al.*, 2014). *Gerbera* (*Gerbera jamesonii*) of the family Compositae is an important ornamental plant commercially grown under protected cultivation due to its extreme sensitivity towards various stresses (Chakrabarty and Datta 2008). Due to its floral demand, Gerbera is cultivated all the year round and ranks fifth in global cut flower sales after roses, carnations, chrysanthemums and tulips (Bhatia *et al.*, 2009).

Often polyhouse cultivated and regularly fertigated, *Gerbera* is prone to salinity due to the accumulation of fertilizers and inorganic chemicals on the soil surface (Bres *et al.*, 2016). Given Gerbera's economic importance, the development of salt-tolerant varieties may help boost its productivity and yield. To date, limited studies have attempted to assess the effect of salinity on *Gerbera*. Though several factors induce salinity in general, we have focussed on NaCl as it is one of the major components of fertigation. In salt-stressed plants, monitoring altered levels of antioxidative defense systems may help better understand their physiological changes. However, little to no studies have been attempted on salinity-induced oxidative damage in *Gerbera* to date.

Therefore, we aimed to investigate the effect of NaCl on H_2O_2, chlorophyll, malondialdehyde (lipid peroxidation), proline levels and activities of antioxidant enzymes in *Gerbera* using a leaf disc culture system. This study may give insight into intricate defense mechanisms involved in encountering salt stress in *Gerbera*.

Methods and materials

Fourth (4^{th}) youngest leaf from the same age group plants of *Gerbera jamesonii* cv Bolus L. (Terraregina Latara - white colored flower variety) was excised. The leaves were surface

sterilized (Talla *et al.*, 2019) and placed in distilled water. Leaf discs of approximately 11 mm diameter were punched by immersing in a water tray to minimize the mechanical stress and designed into six treatment groups (25 mM, 50, 75, 100, 150 and 200 mM NaCl) and a control (without NaCl) as per Talla *et al.* (2011). Approximately 200mg fresh weight (FW) leaf discs were incubated in Petri plates containing 20 mM MES buffer with two mM $CaCl_2.2H_2O$ (pH.5.6) (Talla *et al.*, 2016) in combination with various levels of NaCl. The leaf discs were incubated for 5 days under controlled conditions (photoperiod of 16/8 hrs at 25˚C and RH 60%-80%). Measurement of H_2O_2 content was done spectrophotometrically as per Alexieva *et al.* (2001) with slight modifications. Lipid peroxidation was determined spectrophotometrically using the malondialdehyde (MDA) method as per Heath and Packer (1968). Proline content by the ninhydrin method was measured spectrophotometrically at an absorbance of 520 nm, according to Bates *et al.* (1973). Chlorophyll content was determined spectrophotometrically at an absorbance of 646nm and 663nm, (Arnon, 1969).

For enzyme assays, control and treated leaf discs were ground in liquid nitrogen and then transferred to 1 mL, 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 (1951). Superoxide dismutase activity (EC 1.15.1.1) was monitored by the method of Beyer and Fridovich (1987), and 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 (1981) at an absorbance of 290 nm. Glutathione Reductase, GR (1.6.4.2) activity was measured at 340 nm absorbance according to Jiang and Zhang (2001). The data presented are the average values (±SE) of results from three replicates. The same letters on the bars indicate they are insignificant (P<0.01) as per the statistical analysis of Duncan's Multiple Range Test performed using Sigma Plot version 12.

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 (Katsuhara *et al.*, 2005). In the present study, it was evident that the content of H_2O_2 levels increased proportionately along with MDA upon increasing levels of NaCl (Fig.1A & B). This increase in MDA was significantly more pronounced with about six folds increments, particularly in 200 mM NaCl treatment compared to control indicating the sensitivity of *Gerbera*. Similar observations on salt sensitivity were reported in alfalfa (Wang *et al.*, 2007), maize (Abdelgawad *et al.*, 2016) and cucumber seedlings (Shu *et al.*, 2013). A recent study in the

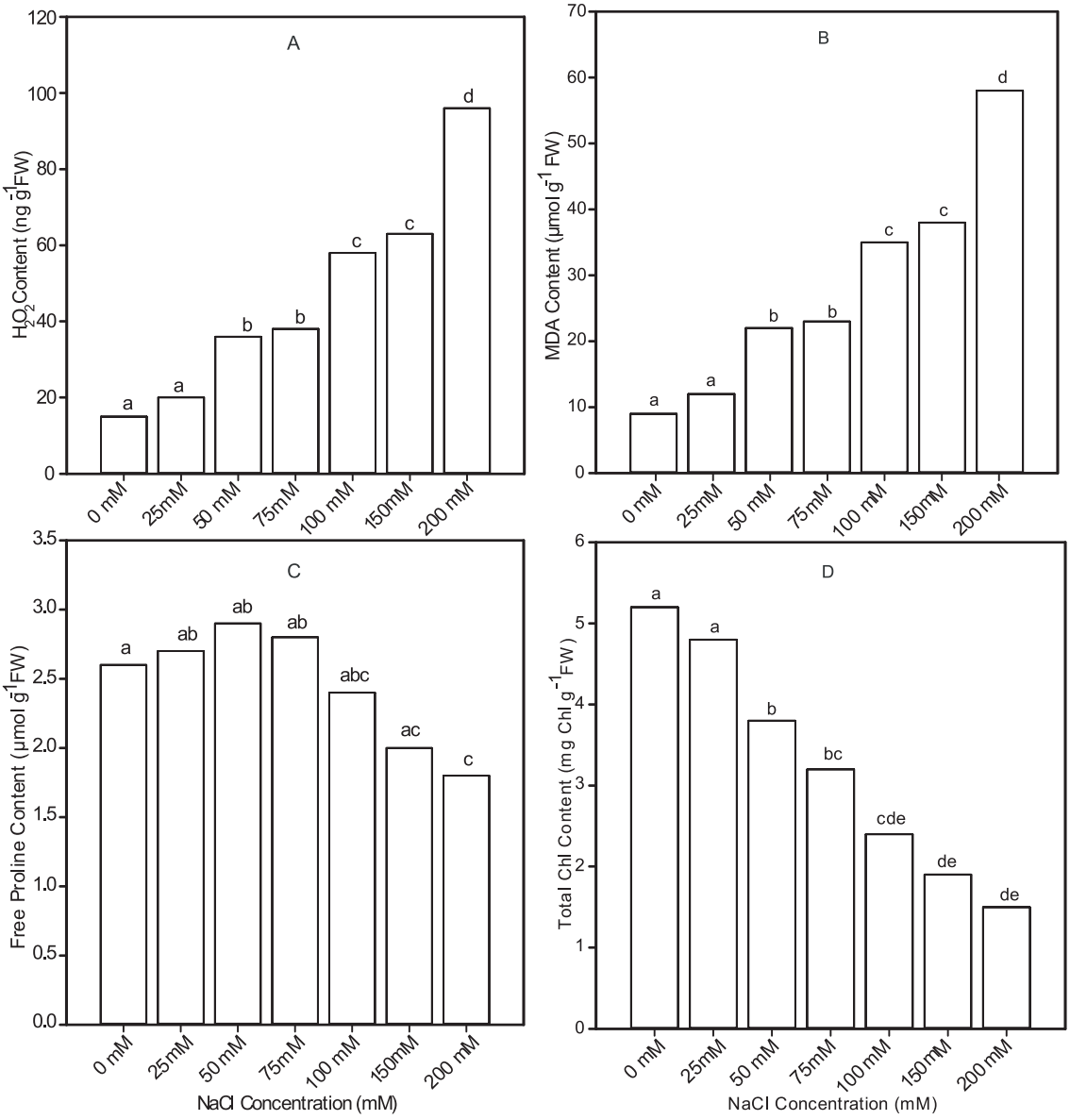

Fig. 1. H_2O_2 content (A), MDA content (B), free proline content (C) and Total chlorophyll content (D) in *Gerbera* leaf discs treated with different NaCl concentrations: Each bar is represented as mean average ± standard deviation of three replicates per treatment performed randomly at different time periods.

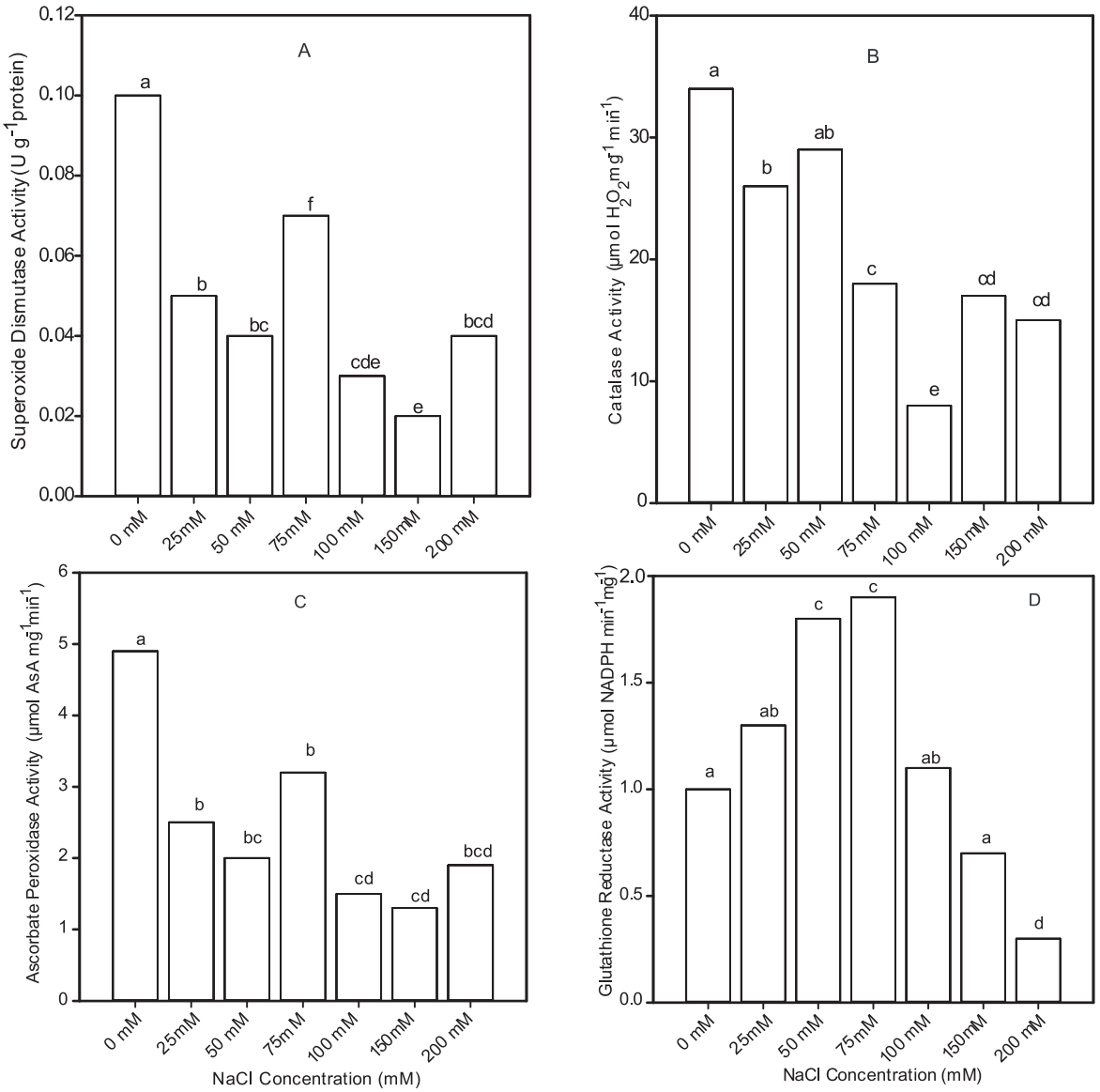

Fig. 2. Effect of NaCl concentrations (0-200 mM) on Superoxide Dismutase (A), Catalase (B), Ascorbate peroxidase (C) and Glutathione reductase (D) enzyme levels in leaf discs of *Gerbera*: Each bar is represented as mean average ± standard deviation of three replicates per treatment performed randomly at different time periods.

ornamental plant, *Amsonia orientalis* revealed a similar pattern, corroborating the findings in *Gerbera* (Acemi *et al.*, 2017). The H_2O_2 content in our study was similar to the results obtained in wheat (Mandhania *et al.,* 2006) and *Pisum sativum* (Noreen *et al.*, 2009).

Plant cells accumulate compatible osmolytes like proline to scavenge free radicals and protect metabolic enzymes (Hayat *et al.*, 2012). The levels of proline in this study continued to increase upon increasing concentrations of NaCl up to 75 mM. After that a slight downfall was observed (significant at 150 mM and 200 mM NaCl) (Fig. 1C). This reflects *Gerbera*'s inability to accumulate proline, making it susceptible, which may be due to little synthesis or higher degradation of proline under high salinity stress (Kibria *et al.*, 2017). The results were similar to the salt stress reports on other ornamental species, *Amsonia orientalis* (Acemi *et al.*, 2017), *Pelargonium* (Bres *et al.*, 2015) and *Catharanthus roseus* (Jaleel *et al.*, 2007). These studies show that at a certain level of NaCl exposure (≥75 mM NaCl), *Gerbera* restricts its synthesis of proline, one of the crucial osmolytes produced during stress.

In the current study, we noticed a gradual decrease in total chlorophyll content upon increasing NaCl concentrations (Fig.1D). This significant decrease in chlorophyll content, particularly above 100 mM NaCl, serves as preliminary evidence that *Gerbera* is sensitive towards salinity stress (Ambede *et al.*, 2012). Recent studies on salt tolerance in ornamental plants like *Dianthus superbus* (Ma *et al.*, 2017), *Brassica oleraceae* (Salachna *et al.*, 2017) and *Pelargonium* (Bres *et al.*, 2015) also depicted an apparent decrease in chlorophyll contents with increased salinity levels.

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 NaCl treatment and decreased after that (Fig. 2A). This suggests the role of SOD in combating salinity stress up to a certain concentration of NaCl as recorded in *Nicotiana tabacum* cv. Xanthi (Lee *et al.*, 2013). The CAT activity was found to be higher in the control compared to NaCl-treated samples. With increasing salinity, we observed a decrement in CAT activity up to 50 mM NaCl, which was insignificant (Fig 2B). With further

increase of NaCl concentrations (75 to 200 mM), a significant decrease in CAT activity, particularly at 100 mM NaCl (Fig. 2B) was observed, which was consistent with salt tolerance studies in *Amsonia orientalis* (Acemi *et al.*, 2016).

In the present study, we observed a significant decrease in the activity of APX upon increasing concentrations of NaCl (Fig. 2C), which align with reports on the salt-sensitive cultivar of cucumber seedlings (Shu *et al.*, 2013). It suggests that the ascorbate-glutathione cycle is important in maintaining the redox poise in plant cells against abiotic stress (Saxena *et al.*, 2011). Contrary to CAT and APX, GR activity increased gradually up to 75 mM NaCl and decreased significantly at 200 mM NaCl concentration (Fig. 2D). This is in corroboration with salinity studies in rice (Wu *et al.*, 2015).

We reported Gerbera's salt-sensitive response by performing differential antioxidant profiles and responses under salinity conditions. Salinity significantly reduced chlorophyll, CAT, APX and GR activities, while proline and MDA contents increased. This sensitivity of *Gerbera* towards salinity indicates the efficiency of the plant defense system to combat ROS accumulation, disturbing the redox homeostasis and integrity of cellular components. However, we must also focus on understanding salinity stress due to several other factors besides NaCl including antioxidant activities which might be useful in future studies as biochemical markers for improving salt tolerance in *Gerbera* and other ornamental plants of Compositae. To our knowledge, this is the first report on antioxidative damage studies in *Gerbera* upon exposure to salt stress which opens the door to manipulating antioxidative defense systems at the molecular level for developing salt-tolerant varieties in *Gerbera*.

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