Journal of Applied Biology & Biotechnology Vol. 11(4), pp. 141-147, Jul-Aug, 2023
Available online at http://www.jabonline.in
DOI: 10.7324/JABB.2023.120550

Insights into the impact of spermidine in reducing salinity stress in *Gerbera jamesonii*

Javeria Uzma, Sai Krishna Talla, Praveen Mamidala*

Department of Biotechnology, Telangana University, Nizamabad, Telangana, India.

ARTICLE INFO

Article history:
Received on: January 12, 2023
Accepted on: March 18, 2023
Available online: June 04, 2023

Key words:
Abiotic stress,
Antioxidant defenses,
Gerbera,
Reactive oxygen species,
Salinity,
Spermidine.

ABSTRACT

The present study was carried out to investigate the role of spermidine (Spd) in alleviating salinity stress in an ornamental plant, *Gerbera jamesonii* cv Bolus ex Hook. f. From our laboratory studies, we could establish that *G. jamesonii* plants grown in a polyhouse are hugely exposed to salts due to repeated fertigation and differ in their sensitivity toward salt stress. In the present study, we have assessed changes in the salinity tolerance levels across *G. jamesonii* genotypes when treated with exogenous supplementation of Spd. The sodium chloride (NaCl) sensitive (white), moderately sensitive (pink), and tolerant (yellow) *G. jamesonii* varieties were subjected to 200 mM NaCl treatment followed by an exogenous foliar spray of Spd (1.0 mM). Our findings revealed that Spd treatment increased leaf growth, soluble protein, proline, chlorophyll content and lowered lipid peroxidation, and hydrogen peroxide accumulation. Enhanced enzyme activities of superoxide dismutase, catalase, ascorbate peroxidase, and glutathione reductase following treatment with Spd suggest its role in conferring salt tolerance in *G. jamesonii* genotypes. Supplementation of 1.0 mM Spd has drastically improved efficiency of photosynthesis by minimizing salt stress initiated cellular injury by scavenging highly reactive oxygen radicals.

1. INTRODUCTION

Polyamines (PAs) are considered a novel class of growth regulators in plants besides acting as secondary messengers with a key role in cell signaling. They are organic/nitrogenous low molecular weight cationic amines, associated with cell differentiation and act as signaling molecules during plant's exposure to environmental stresses [1]. Although the accurate role of PAs in a plant when in stress is speculative, the huge research information from recent years on polyamine metabolism help in understanding their active role in plant growth regulation [2]. In brief, PAs are known to minimize oxidative damage, reduce free radicals, and alleviate lipid peroxidation during stress in plants. The PAs are also crucial and modulate growth in plants during salinity stress through increasing the rate of photosynthesis, accumulation of carbohydrates, proline, and other osmolytes [3]. Besides this, PAs in plants regulate activities of antioxidative defense-related enzymes and proteins and improve their ability to counteract oxidative stress [4].

Research on abiotic stress biology particularly negating the detrimental results of salinity in plants by deployment of PAs such as putrescine, spermidine (Spd), and spermine has a great scope at the current scenario. Several reports also demonstrated that endogenous levels of PAs get increased under stressful conditions [5]. Despite enormous research being done on the applications of exogenous supplementation of Spd in mitigating salt stress, to our knowledge, no studies were attempted on net house grown *Gerbera jamesonii* cv Bolus ex Hook. f. which face huge salt stress conditions. *G. jamesonii,* a decorative plant belonging to *Asteraceae*, is popular worldwide for its attractive cut flowers and is also used as a decorative potted plant [6]. Increased demand for *G. jamesonii* across the globe has resulted in potential market value for floriculture both in domestic and international markets. The turnover of floriculture industry is estimated to be more than 8.2 billion USD (US dollars) in 2022 which is thought to be exponentially increasing [7].

Although *G. jamesonii* is grown in green/polyhouses under controlled conditions pertaining to its susceptibility to inconsistent environmental conditions, it gets prone to salinity stress over a period of time [8]. Greenhouse grown *G. jamesonii* are subjected to regular fertigation with inorganic chemicals and fertilizers which accumulate on the upper layers of soil bed forcing plants to absorb more salts through their roots resulting in salinity [9]. Salinity, a major abiotic stress, restricts a plant from attaining its maximum productivity. More than optimal levels of salt concentrations in plants disturb their ionic balance and induce osmotic stress which ultimately leads to oxidative damage of its cellular components [10]. Salinity stress in *G. jamesonii* causes wilting, thickening, and curling of leaves in addition to functional impairment of photosynthetic process due to reduced synthesis

*Corresponding Author:
Praveen Mamidala,
Department of Biotechnology, Telangana University,
Nizamabad, Telangana, India.
E-mail: pmamidala @ gmail.com

of chlorophylls and carotenoids [11]. During this phase, plants go through a plethora of phenotypical, molecular, and physicochemical changes resulting in altered metabolic processes. Some of these include adjustments in physiological parameters such as increase in malondialdehyde (MDA), hydrogen peroxide (H_2O_2) and proline content, and modifications in enzymatic/non-enzymatic antioxidant activities. These physiologically induced biochemical changes act as key stress indicators and maintain cellular redox balance depending on the resistance/sensitivity of different cultivars of *G. jamesonii* toward salinity stress [12].

Keeping in view of the above context, we have chosen three different *G. jamesonii* genotypes (white, pink, and yellow) exposed to sodium chloride (NaCl) and further assessed the protective role of Spd in them. In this study, our approach is to apply and elucidate the effect of exogenous Spd (1.0 mM) as a foliar spray on whole plant and examine the morphology, physiology, and biochemical activities associated with NaCl (salt) tolerance/susceptibility. More specifically, the regulatory role played by Spd in salt sensitive (White – Latara), moderately sensitive (Pink – Basic), and tolerant genotypes (Yellow – Faith) of *G. jamesonii* was studied. We hypothesized that treatment with Spd would contribute to salt tolerance in *G. jamesonii* genotypes yielding positive results. Physiological parameters such as contents of proline, chlorophyll, levels of antioxidant enzymatic defense systems, namely, superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), and glutathione reductase (GR), H_2O_2 content, and the degree of lipid peroxidation were measured. This study mainly emphasized understanding the antioxidant defense responses in *G. jamesonii* against NaCl stress and the precise role of PAs (Spd) in alleviating it.

2. MATERIALS AND METHODS

2.1. Plant Growth Conditions and Treatments

2.1.1. Establishing healthy plants

One-month-old plantlets of three *G. jamesonii (*white' – white flower, G. jamesonii (Pink, and yellow flower varieties) were purchased from SLN Biologicals, Nizamabad, India. These plantlets were sowed in potting mixture comprising sand: cocopeat:red soil mixture in a ratio of 1:1:1 in a green/net house and were grown in controlled conditions (day/night temperatures of 27–29°C/23–25°C and 45–51% relative humidity, respectively).

2.1.2. Treatment with NaCl followed by exogenous supplementation of Spd

The experiments in the present study were designed as a randomized complete block containing three treatment groups with three replicates per treatment: (a) Control (0 mM NaCl), (b) salt alone (200 mM NaCl) as per Uzma *et al*. [7], and (c) salt + Spd (200 mM NaCl + 1.0 mM Spd). Before the design of the treatment groups, Spd concentration of 1.0 mM was standardized by treating *G. jamesonii* plants using a range of concentrations of Spd (0.25 mM, 0.50 mM, 0.75 mM, and 1.0 mM) and the resulting concentration of 1.0 mM was optimized (data not shown). The Spd stock solution used as a foliar spray was dissolved in water at pH 9.2. For salt treatments, *G. jamesonii* plants in Group B and Group C were treated with 200 mM NaCl daily up to 5 days. Following NaCl treatment, to Group C, a foliar spray of Spd (1.0 mM) further for 5 days was given from the 6th day to 10th day. Aftercompletion of the period of treatment, the leaves from treated plants were excised and crushed using liquid nitrogen immediately and froze under −20°C to be used for biochemical experiments.

2.2. Measurement of Size of the Leaves

In control and treated plants, the length of leaves was scaled from one end to the other along the midrib, that is, from tip of the lamina to the point where lamina joins the petiole. Leaf width was scaled along the axis perpendicular to the midrib between the most comprehensive lobes of the lamina. Leaf length (L) in cm and leaf width (W) in cm were calculated as per Cho *et al*. [13].

2.3. Detection of H_2O_2

Detection of H_2O_2 was done by 3,3-diaminobenzidine (DAB) staining method as per Daudi and O'Brien [14], with slight modifications. The leaves excised from control and treated whole plants were fed with 1 mg/mL DAB solution (pH 3.8) for 1 h in low light (50 µE/m^2/S). Post-DAB treatment, the resulting leaves were fixed with a mixture of ethanol: lactic acid: glycerol (3:1:1, v: v:v; which acts as bleaching agent) for 15 min. Following this, the samples are washed with 75% methanol and the treated leaf samples were viewed for the detection of dark brown color using LED light source.

2.4. Determination of H_2O_2 Content

Leaf extract approximately 0.1 g was subjected to homogenization in 100 mM potassium phosphate buffer (pH 7.8) and was centrifuged at 19,000× *g* for 20 min. The collected supernatant was allowed to react with potassium iodide (KI) as per Alexieva *et al*. [15] for the measurement of H_2O_2 content with slight modifications. The reaction mixture contains 0.5 mL of 0.1% trichloroacetic acid (TCA), 50 µg protein, 100 mM sodium phosphate buffer (pH 7.8), and 2 mL of 1 M KI reagent. The blank consists of 0.1% TCA without a protein sample. The reaction mixture was incubated in dark for 1 h and the absorbance was recorded at 390 nm and the quantity of H_2O_2 was measured using a standard curve. The standard curve equation for H_2O_2 was given as $y = 0.024 \times 0.006$ with an R^2 value of 0.975.

2.5. Determination of MDA Content

Lipid peroxidation of samples was calculated by measuring the content of MDA using thiobarbituric acid (TBA) reaction as per the protocol of Heath and Packer [16] following slight changes. Leaf extract (1 g) was homogenized in 0.5% TCA (1 mL) and subjected to centrifugation (19,000× *g*, 20 min). To the supernatant, equal volume of TBA reagent (15% TCA (w/v) and 0.375% TBA (w/v) in 0.25 M HCl) was added and subjected to heat treatment (95°C, 15 min). The reaction is cooled immediately on ice and again centrifuged (15,000× *g*, 15 min). The supernatant was used to measure absorbance at a wavelength of 532 nm and turbidity was subtracted at 600 nm. The amount of MDA produced was measured using the extinction coefficient (155 mM^{-1}/cm) and the data were expressed as µmol per g fresh weight (FW).

2.6. Measurement of Content of Free Proline

The content of free proline was measured spectrophotometrically as given by Ninhydrin method and absorbance was read at 540 nm as described by Bates *et al*. [17] and data were expressed as µmol per g FW. In brief, 100 mg of leaf sample was homogenized in 3% sulfosalicylic acid and centrifuged at 15,000× *g* for 5 min at room temperature. To the supernatant, a reaction mixture comprising 100 µL 3% sulfosalicylic acid, 200 µL glacial acetic acid, and 200 µL acidic Ninhydrin is added and mixed well. The samples were then incubated at 96°C for 60 min. The reaction tubes were punctured on the lid surface to avoid accidental opening and sample burst out. Following incubation, the samples were extracted using toluene and absorbance

was read at 520 nm to the chromophore obtained. The concentration of proline in the samples was determined using a standard proline concentration curve. The standard curve equation for proline was given as y = 0.004 × 0.008 with an R^2 value of 0.995.

2.7. Estimation of Content of Total Chlorophyll

The content of chlorophyll was determined spectrophotometrically by measuring absorbance of samples at 646.6 nm and subtracting the turbidity at 663.6 nm as per Lichtenthaler [18]. In short, samples were extracted with acetone (80% v/v) to calculate chlorophyll a, chlorophyll b, and total chlorophyll contents and data were expressed as mg per g FW.

The formulae used to calculate chlorophyll a, chlorophyll b, and total chlorophyll contents were:

$$\text{Chl a} = 12.25*A^{663.6} - 2.55*A^{646.6}$$

$$\text{Chl b} = 20.31*A^{646.6} - 4.91*A^{663.6}$$

$$\text{Chl a + Chl b} = 17.76*A^{646.6} + 7.34*A^{663.6}$$

2.8. Determination of Antioxidant Enzyme Activities

To determine enzymatic activities, 100 mg of leaf extract was homogenized in 1 mL ice-cold extraction buffer containing 100 mM potassium phosphate (pH 7.0) and 1 mM ethylenediaminetetraacetic acid (EDTA). The obtained homogenate was sieved using a muslin cloth and subjected to centrifugation (5000× *g*, 15 min). The supernatant obtained was analyzed to determine the activities of SOD, CAT, APX, and GR.

For all the enzyme activities, the total soluble protein quantity was estimated using bovine serum albumin, BSA (Sigma Aldrich Co., USA) as standard at 640 nm according to the method of Lowry [19]. The obtained values were expressed as mg per g FW. The standard curve equation for BSA was given as y = 0.01× 0.001 with an R^2 value of 0.997.

The SOD activity (EC 1.15.1.1) was monitored as per the protocol of Beyer and Fridovich [20]. To determine the activity of SOD, the reaction in the above obtained supernatant was initiated with the photochemical reduction of nitroblue tetrazolium (NBT). In brief, enzymes from leaf samples were extracted using extraction buffer supplemented with PVP (1% (w/v) polyvinyl-pyrrolidone) and 0.5% (v/v) Triton X and centrifuged (10,000 × g for 20 min). A reaction mixture comprising leaf extract containing 50 µg protein, 1.5 M Na_2CO_3 (sodium carbonate), 200 mM methionine, 3 mM EDTA, and 2.25 mM NBT was made up to 3 mL with 100 mM potassium phosphate (pH 7.0) buffer. Reaction was initiated by adding 60 µM riboflavin and placing the tubes below a light source of two 15 W fluorescent lamps for 15 min. Following this, the reaction was stopped by removing the light source. Activity of one unit of SOD was expressed as the amount of enzyme consumed resulting in 50% inhibition of NBT at 560 nm.

CAT activity, CAT (1.11.1.6), was determined following the protocol of Aebi [21], wherein, decrease in concentration of H_2O_2 was monitored at a wavelength of 240 nm and the absorbance was quantified using the molar extinction coefficient of H_2O_2 (36 M^{-1}/cm). Enzyme activity was expressed as micro moles of H_2O_2 decreased min^{-1} mg^{-1} protein. In brief, to the leaf extract containing 50 µg protein which acts as an enzyme source, 10 mM H_2O_2 is added and the reaction mixture is made up to 1 mL by adding 100 mM potassium phosphate (pH 7.0) buffer and the rate of reaction was determined.

The activity of APX (1.11.1.1) was calculated as per Nakano and Asada [22]. The activity of enzyme was measured at an absorbance of 290 nm and quantified by the molar extinction coefficient of ascorbate (2.8 mM^{-1}/cm). In brief, the reaction mixture comprising leaf extract, 0.1 mM ascorbate, 0.3 mM H_2O_2, and 100 mM potassium phosphate buffer were used to record the enzyme activity up to 3 min. The calculated values are expressed as micromoles of ascorbate decreased min^{-1} mg^{-1} protein.

The activity of GR (1.6.4.2) was determined as per the protocol of Foyer and Halliwell [23] at an absorbance of 340 nm and was calculated using the extinction coefficient of nicotinamide adenine dinucleotide phosphate (NADPH) (6.2 mM^{-1}/cm). In short, a reaction was initiated with a reaction mixture containing leaf extract, 1 mM GSSG (oxidized glutathione), 0.1 mM NADPH, 0.08 mM DTNB (5, 5^1-dithio bis-2- nitrobenzoic acid), and 50 mM potassium phosphate buffer to record the enzymatic activity of GR wherein a unit of GR was defined as 1 m mol/mL GSSG reduced min^{-1}.

2.9. Statistical Analysis

The data recorded in this study are presented as mean values (±SE) of experiments performed in three replicates at different time points which were statistically analyzed by one-way analysis of variance (ANOVA) using the method of Holm-Sidak in a software program SigmaPlot (Version 12.0).

3. RESULTS AND DISCUSSION

3.1. Effect of Exogenous Spd on Morphological Characteristics in Three Varieties of *G. jamesonii*

In the present morphological observation, it became evident that NaCl stress reduced plant growth in *G. jamesonii* which was found to increase by the application of Spd. In brief, the leaf samples of three genotypes of *G. jamesonii* (yellow, pink, and white) treated with NaCl showed conspicuous morphological changes with consistent bleaching of leaves. However on application of Spd, in all the three genotypes, the bleached leaves regained green color [Figure 1]. This observation was more prominent in white compared to pink and yellow genotypes. These results are in line with recent research outcomes which state the protective role of PAs in improving plant growth during salinity conditions [24].

3.2. Effect of Spd on Leaf Size in Three Different Genotypes of NaCl-Treated *G. jamesonii*

A noticeable difference in leaf length and width was noticed across three genotypes of *G. jamesonii* [Table 1]. On NaCl treatment, we noticed decrease in the leaf length and leaf width of all three genotypes which was particularly high in white. Exposure of these treated plants to Spd showed a marginal improvement in leaf size in comparison to control.

3.3. Histochemical Reactive Oxygen Species (ROS) Detection and H_2O_2 Content in Three Different Genotypes of *G. jamesonii* on Exposure to NaCl and Spd Treatment

The morphological results of NaCl stress in plants can be noticed by subjecting the leaves to DAB staining. During salinity stress, several ROS burst out in the aerial parts of plants like leaves and damage their pigment complexes. Thus due to staining by DAB, dark brown color indicating high increases in H_2O_2 content can be seen in salt-

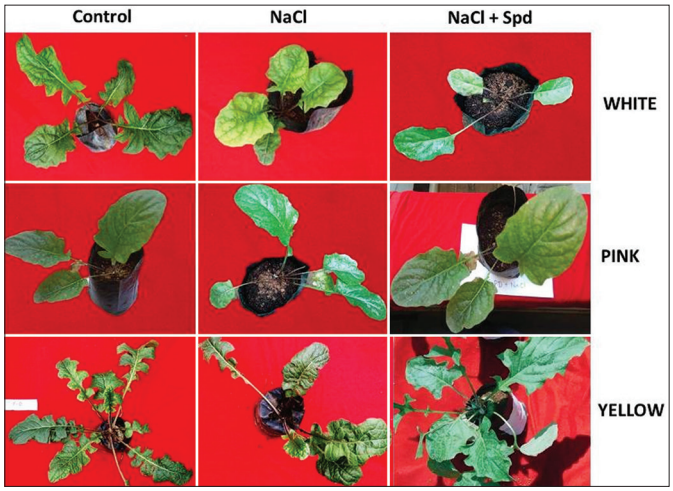

Figure 1: Effect of Spd treatment on morphological observations of salt-treated *Gerbera* varieties (white, pink, and yellow). Spd treatment indicates exogenous foliar spray of 1.0 mM concentration of spermidine and salt stress indicates treatment with 200 mM NaCl. The untreated leaves act as control.

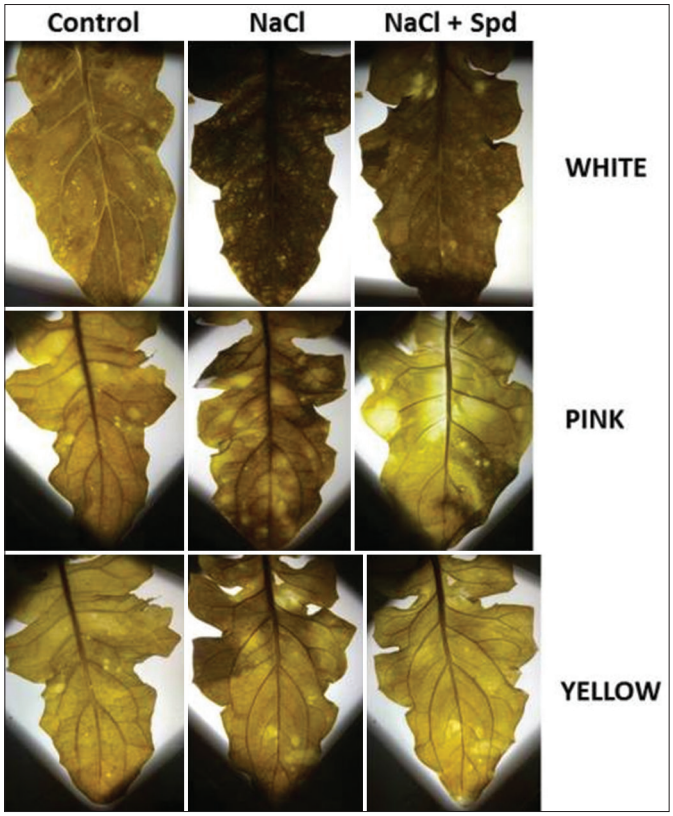

Figure 2: Histochemical ROS detection through method of DAB staining in Spd-treated leaves of three different genotypes of salt-treated *Gerbera* (white, pink, and yellow) to detect the H_2O_2 content. Spd treatment indicates exogenous foliar spray of 1.0 mM concentration of spermidine and salt stress indicates treatment with 200 mM NaCl. The untreated leaves act as control.

affected seedlings. This over accumulation of ROS (H_2O_2 in particular) indicates increased lipid peroxidation thereby reducing the integrity of cell membranes leading to ion leakage. Exogenous PAs like Spd mitigate the negative effects of ROS accumulation in the leaves. H_2O_2 staining is seen in leaves of all the three genotypes when treated with

Table 1: Effect of Spd on leaf size and leaf width in three different genotypes of salt-treated *Gerbera* (white, pink, and yellow).

Plant phenotype	Control	200 mM NaCl	200 mM NaCl+1 mM Spd
Leaf width (in cm)			
Yellow	18.900±0.208^{a}	16.333±0.240^{b}	17.067±0.769^{ab}
Pink	16.267±0.203^{a}	13.553±0.158^{b}	14.900±0.666^{ab}
White	7.500±0.115^{a}	6.633±0.176^{b}	6.933±0.0882^{bc}
Leaf length (in cm)			
Yellow	18.333±0.260^{a}	13.333±0.667^{b}	17.333±0.809^{ac}
Pink	17.267±0.176^{a}	16.133±0.120^{b}	16.467±0.260^{ab}
White	14.533±0.203^{a}	12.467±0.328^{b}	14.333±0.0882^{ac}

*Spd treatment indicates exogenous foliar spray of 1.0 mM concentration of spermidine and salt stress indicates treatment with 200 mM NaCl. The untreated leaves act as control. Bars represent mean±standard error of three replicates per treatment executed randomly at different time points. Different letters on bars indicate that the values are statistically significant as determined by one-way ANOVA (Holm-Sidak method).

NaCl as brown patches which was more prominent in white followed by pink and yellow [Figure 2]. With the exogenous supplementation of Spd, accumulation of ROS in leaves was decreased across all three genotypes as indicated by reduced browning.

The H_2O_2 levels which were on a rise when treated with 200 mM NaCl decreased considerably on treatment with Spd in all three genotypes [Figure 3b]. The amount of H_2O_2 particularly got more accumulated in salt stressed white genotype, which got reduced after treatment with Spd. Similar observations were recorded in *Arabidopsis* wherein endogenous Spd resulted in decrease in H_2O_2 levels, thereby increasing multiple abiotic stress tolerance in it [25].

3.4. Content of MDA in Three Varieties of *G. jamesonii* on NaCl and Spd Treatment

The cellular environment in plants, when exposed to saline environments, trigger over production of proactive/ROS such as superoxide (O^{2-}), H_2O_2, singlet oxygen, and hydroxyl radical (OH^{-}). These highly unstable species of oxygen react with biomolecules such as carbohydrates, proteins, lipids, and nucleic acids and accelerate programmed cell death or senescence. For example, lipids play a major role in maintaining the integrity of cell membranes. During stress, ROS particularly peroxides react with lipids associated with cell membranes to form a highly unstable compound, MDA resulting in lipid peroxidation reaction [4]. Damage to these lipid molecules may loosen the membrane structure and leads to cell leakage. In this study, we noticed a gradual rise in MDA contents when treated with NaCl which decreased considerably on treatment with Spd in all three genotypes [Figure 3a]. The MDA accumulation was more pronounced particularly in NaCl stressed white genotype. On par with this, the revival was also high in Spd-treated white genotype. In the present study by applying salt stress, we noticed increased MDA content levels in *G. jamesonii* leaves as observed in pot marigold [26] and *Chrysanthemum* [27]. The Spd treatment (1.0 mM) effectively reversed this response in all three cultivars of *G. jamesonii* reducing the MDA contents of white, pink, and yellow. This observation is in similarity to stress studies in rice [28], citrus [4], Zoysia grass [29], and bluegrass [24]. This might be due to the action of PAs which help maintain membrane integrity and prevent cellular leakage by decreasing the peroxidation of lipids by ROS, thereby decreasing the levels of accumulated MDA.

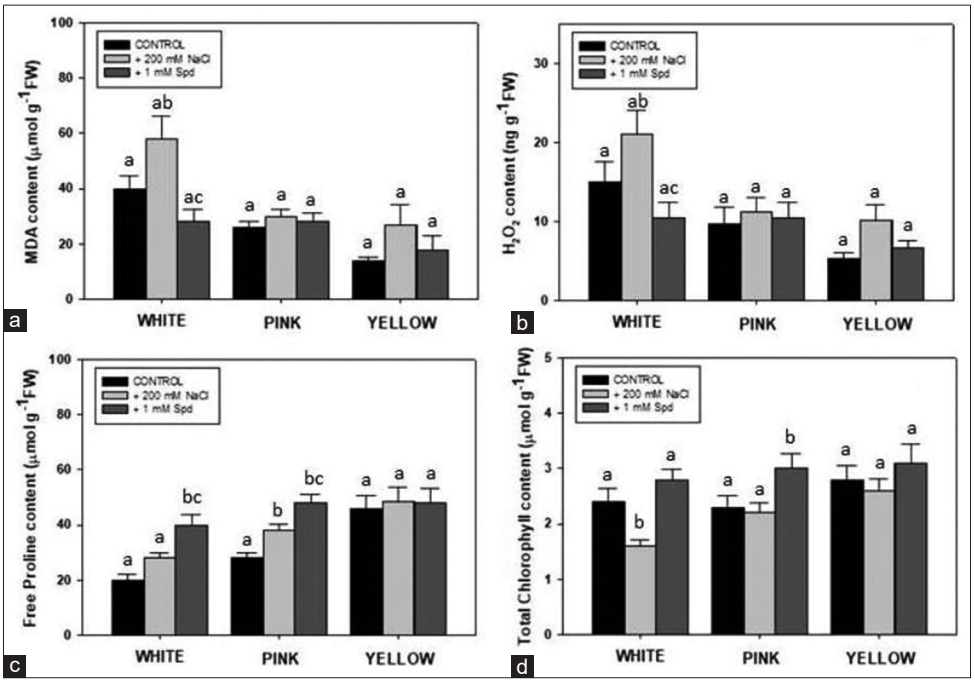

Figure 3: Effect of Spd on (a) malondialdehyde content (lipid peroxidation), (b) H_2O_2 content, (c) free proline content, and (d) content of total chlorophyll in three varieties of salt-treated *Gerbera* (white, pink, and yellow). Spd treatment indicates exogenous foliar spray of 1.0 mM concentration of spermidine and salt stress indicates treatment with 200 mM NaCl. The untreated leaves act as control. Bars represent mean ± standard error of three replicates per treatment executed randomly at different time points. Different letters on bars indicate that the values are statistically significant as determined by one-way ANOVA (Holm-Sidak method).

3.5. Free Proline Content in Three Varieties of *G. jamesonii* on Salinity Stress Followed by Treatment with Spd

We noticed an elevated concentration in the content of free proline when treated with NaCl and this increase was further elevated upon treatment with Spd, particularly in white and pink without much alteration in yellow when compared to control [Figure 3c]. Plants in response to salinity stress generally over synthesize osmolytes such as proline and glycine betaine, reduce the differential osmotic potential between a cell and its cellular environment, and improve the water uptake capacity in them. One of the most ubiquitous of these osmolytes is proline. PAs regulate the negative effects of stress by inducing proline synthesis and accumulation in plants [30]. In the present study, salinity led to an increased proline content in *G. jamesonii* which further increased by the application of Spd. This kind of observation is similar in abiotic stress studies in pot marigold [26], citrus [4], rice cultivars [28], and Kentucky bluegrass [31]. Further, our study also noted that NaCl sensitive species have more tendencies to aggregate proline when compared to salt-tolerant species. These findings were similar to other studies recorded in Guerrier *et al*. [32].

3.6. Total Chlorophyll Content in Three Varieties of NaCl Treated *G. jamesonii* on Spd Application

On NaCl treatment, the total chlorophyll content was gradually decreased which revived back to its normal state on Spd treatment in all three genotypes. This pattern was more prevalent particularly in white genotype [Figure 3d]. Exogenous application of PAs in plants subjected to various environmental stresses results in increased photosynthesis as indicated by improved levels of chlorophyll and improved plant growth. The findings in our study are in agreement with Li *et al*. [33] (tomato subjected to salt stress) and Khoshbakht *et al*. [4] (salt stressed citrus). A decrement in the content of pigments related to photosynthesis in salt stressed plants may be attributed to disorganization of chloroplasts and photo-oxidation or peroxidation of chlorophyll pigments [28].

3.7. SOD, CAT, APX, and GR Activity in Three Varieties of Salt Stressed *G. jamesonii* on Spd Treatment

Resistant varieties of plants usually synthesize compatible solutes, osmoprotectants, and osmolytes in their cellular compartments to neutralize the toxic effects of salinity. To further stabilize their cellular defense, production of antioxidative defense enzymes such as CAT, peroxidase (POD), SOD, APX, and GR is stimulated in the cellular environment particularly in cell organelles such as chloroplast and mitochondria to scavenge the over produced ROS [34]. On treatment with 1.0 mM Spd post-NaCl exposure, the activity of CAT was further increased in all the three genotypes. Whereas the activities of SOD, APX, and GR were enhanced only in white, there was little to no increase in the other two genotypes after subjecting the NaCl-treated plants to 1.0 mM Spd [Figure 4]. Levels of SOD, CAT, GR, and APX were assessed to know about the ROS scavenging ability in *G. jamesonii* genotypes. In these results, it can be noted that the antioxidative enzyme activities are significantly more in Spd-treated plants particularly in salt sensitive variety which might be the possible reason for reduction of ROS and improved tolerance in it. In the current research work, antioxidant enzymes exhibited varied responses during salinity stress and in response to Spd. This gives us an implication that the three different varieties of *G. jamesonii* exhibit different levels of salinity tolerance as indicated by their distinct antioxidant enzymatic defense levels which were similar to results drawn in pot marigold [26], Zoysia grass [29], and rice [27,35].

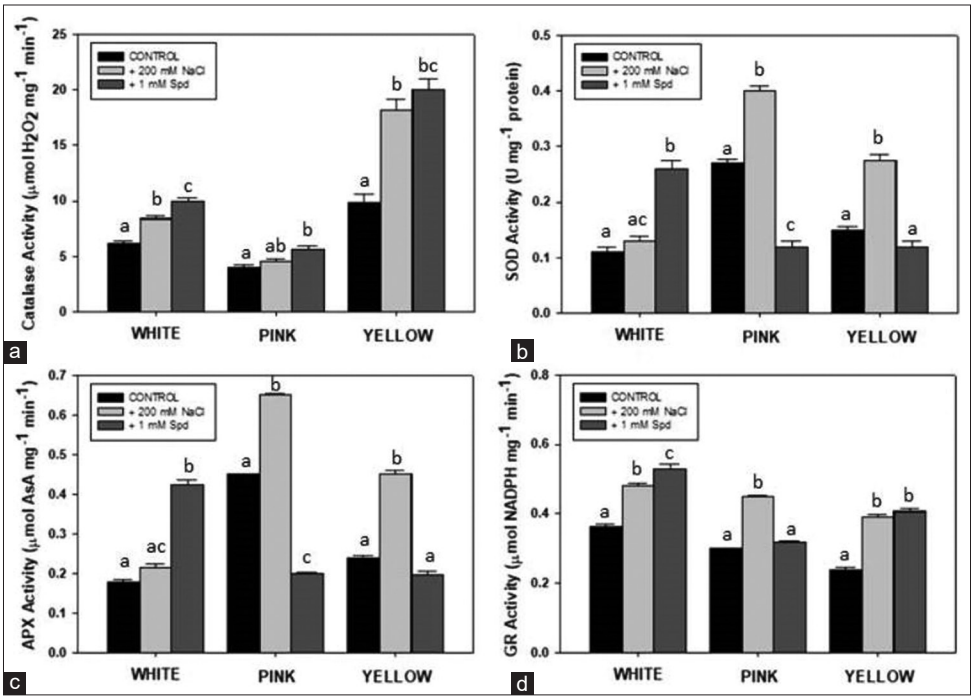

Figure 4: Effect of Spd on antioxidant enzyme activities of (a) catalase, (b) superoxide dismutase, (c) ascorbate peroxidase, and (d) glutathione reductase in three different genotypes of salt-treated *Gerbera* (white, pink, and yellow). Spd treatment indicates exogenous foliar spray of 1.0 mM concentration of spermidine and salt stress indicates treatment with 200 mM NaCl. The untreated leaves act as control. Bars represent mean ± standard error of three replicates per treatment executed randomly at different time points. Different letters on bars indicate that the values are statistically significant as determined by one-way ANOVA (Holm-Sidak method).

4. CONCLUSION

To conclude with, our study helps in understanding the significant role played by PAs in increasing salt stress tolerance as indicated by reduced cellular oxidative damage and enhanced antioxidant enzyme levels. In summary, this work confirms that foliar spray of Spd may ameliorate the detrimental results faced by *G. jamesonii* during exposure to salt stress as indicated by increase in leaf growth, rise in total chlorophyll content, lower lipid peroxidation, and decreased accumulation of H_2O_2. Future studies on identifying and characterizing key regulatory genes and proteins involved in PA-induced stress tolerance in *G. jamesonii* may shed light on generating transgenic *G. jamesonii* which can minimize the fertigation effects.

5. AUTHORS' CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agreed to be accountable for all aspects of the work. All the authors are eligible to be an author as per the International Committee of Medical Journal Editors (ICMJE) requirements/guidelines.

6. FUNDING

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

7. CONFLICTS OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

8. ETHICAL APPROVALS

This study does not involve experiments on animals or human subjects.

9. DATA AVAILABILITY

All the required data related to manuscript has been uploaded to editor and upon request, authors shall share available data.

10. PUBLISHER'S NOTE

This journal remains neutral with regard to jurisdictional claims in published institutional affiliation.

11. ACKNOWLEDGMENTS

We would like to express our sincere thanks to the Department of Biotechnology, Telangana University, Nizamabad, India, for providing laboratory equipment and instrument support.

REFERENCES

1. Chen D, Shao Q, Yin L, Younis A, Zheng B. Polyamine function in plants: Metabolism, regulation on development, and roles in abiotic stress responses. Front Plant Sci 2019;9:1945.
2. Masson PH, Takahashi T, Angelini R. Molecular mechanisms underlying polyamine functions in plants. Front Plant Sci 2017;8:14.
3. Peynevandi KM, Razavi SM, Zahri S. The ameliorating effects of polyamine supplement on physiological and biochemical parameters of *Stevia rebaudiana* Bertoni under cold stress. Crop Physiol 2018;21:123-31.
4. Khoshbakht D, Asghari MR, Haghighi M. Effects of foliar applications of nitric oxide and spermidine on chlorophyll fluorescence, photosynthesis and antioxidant enzyme activities of citrus seedlings

under salinity stress. Photosynthetica 2018;56:1313-25.
5. Sanchez-Rodriguez E, Romero L, Ruiz JM. Accumulation of free polyamines enhances the antioxidant response in fruits of grafted tomato plants under water stress. J Plant Physiol 2016;190:72-8.
6. Faust JE. The global cut flower and foliage marketplace. In: Faust JE, Dole JM, editors. Cut Flowers and Foliages. Vol. 27. United Kingdom: CABI; 2021. p. 1-47.
7. Uzma J, Talla SK, Madam E, Mamidala P. Assessment of salinity tolerance deploying antioxidant defense systems in *Gerbera jamesonii*. Biosci Biotechnol Res Asia 2022a;19:243-54.
8. Maitra S, Shankar T, Sairam M, Pine S. Evaluation of Gerbera (*Gerbera jamesonii* L.) cultivars for growth, yield and flower quality under protected cultivation. Indian J Nat Sci 2020;10:60.
9. Uzma J, Talla SK, Madam E, Mamidala P. Salinity driven oxidative stress in *Gerbera jamesonii* cv Bolus. J Appl Hortic 2022b;24:2.
10. Isayenkov S, Maathuis FJ. Plant salinity stress: Many unanswered questions remain. Front Plant Sci 2019;10:80.
11. Chen W, Lin F, Lin KH. Growth promotion and salt-tolerance improvement of *Gerbera jamesonii* by root colonization of *Piriformospora indica*. J Plant Growth Regul 2022;41:1219-28.
12. Khorasgani OA, Emadi S, Mortazaienezhad F, Pessarakli M. Differential responses of three chamomile genotypes to salinity stress with respect to physiological, morphological, and phytochemical characteristics. J Plant Nutr 2017;40:2619-30.
13. Cho YY, Sungbong OH, MinOh M, EekSon J. Estimation of individual leaf area, fresh weight, and dry weight of hydroponically grown cucumbers (*Cucumis sativus* L.) using leaf length, width and SPAD value. Sci Hortic 2007;111:330-4.
14. Daudi A, O'Brien JA. Detection of hydrogen peroxide by DAB staining in *Arabidopsis* leaves. Bio Protoc 2012;2:e263.
15. Alexieva V, Sergiev I, Mapelli S, Karonov E. The effect of drought and ultraviolet radiation on growth and stress marker in pea and wheat. Plant Cell Environ 2001;24:1337-44.
16. Heath RL, Packer L. Photo peroxidation in isolated chloroplast, I. Kinetics and stoichiometry of fatty acid peroxidation. Arch Biochem Biophys 1969;125:189-98.
17. Bates LS, Waldren RP, Teare ID. Rapid determination of free proline for water-stress studies. Plant Soil 1973;39:205-7.
18. Lichtenthaler HK. Chlorophylls and carotenoids: Pigments of photosynthetic bio membranes. Methods Enzymol 1987;148:350-82.
19. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the folin phenol reagent. J Biol Chem 1951;193:265-75.
20. Beyer WF Jr., Fridovich I. Assaying for superoxide dismutase activity: Some large consequences of minor changes in conditions. Anal Biochem 1987;161:559-66.
21. Aebi H. Catalase *in vitro*. Methods Enzymol 1984;205:121-6.
22. Nakano Y, Asada K. Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in Spinach chloroplasts. Plant Cell Physiol 1981;22:867-80.
23. Foyer CH, Halliwell B. The presence of glutathione and glutathione reductase in chloroplast: A proposed role in ascorbic acid metabolism. Planta 1976;133:21-5.
24. Puyang X, An M, Han L, Zang X. Protective effect of spermidine on salt stress induce oxidative damage in two Kentucky bluegrass (*Poa pratensis* L.) cultivars. Ecotoxicol Environ Saf 2015;17:96-106.
25. Kasukabe Y, He L, Nada K, Misawa S, Ihara I, Tachibana S. Overexpression of spermidine synthase enhances tolerance to multiple environmental stresses and up-regulates the expression of various stress regulated genes in transgenic *Arabidopsis thaliana*. Plant Cell Physiol 2004;45:712-22.
26. Banaisadia F, Saffarib VR, Moudb AA. Physiological and growth responses of *Calendula officinalis* L. plants to the interaction effects of polyamines and salt stress. Sci Hortic 2018;234:312-7.
27. Zhang N, Shi X, Guan Z, Zhao S, Zhang F, Chen S, *et al*. Treatment with spermidine protects chrysanthemum seedlings against salinity stress damage. Plant Physiol Biochem 2016;105:260-70.
28. Paul S, Roychoudhury A. Effect of seed priming with spermine/spermidine on transcriptional regulation of stress-responsive genes in salt-stressed seedlings of an aromatic rice cultivar. Plant Gene 2017;11:133-47.
29. Li S, Jin H, Zhang Q. The effect of exogenous spermidine concentration on polyamine metabolism and salt tolerance in zoysia grass (*Zoysia japonica* steud) subjected to short-term salinity stress. Front Plant Sci 2016;7:1221.
30. Larher F, Aziz A, Deleu C, Lemesle P, Ghaffa A, Bouchard F, *et al*. Suppression of the osmoinduced proline response of rapeseed leaf discs by polyamines. Physiol Plant 1998;102:139-47.
31. Puyang X, An M, Xu L. Protective effect of exogenous spermidine onion and polyamine metabolism in Kentucky bluegrass under salinity stress. Hortic Environ Biotechnol 2016;57:11-9.
32. Guerrier G, Delumeau O, Renard M, Laval-Martin D. Proline metabolism and NAD kinase activity in Soybean Calli during short- and long-term exposures to light and NaCl. Biol Plant 1997;40:1-9.
33. Li J, Hu L, Li Z, Pan X, Hu X. Exogenous spermidine is enhancing tomato tolerance to salinity-alkalinity stress by regulating chloroplast antioxidant system and chlorophyll metabolism. BMC Plant Biol 2015;15:17.
34. Rasool S, Ahmad A, Siddiqui TO, Ahmad P. Changes in growth, lipid peroxidation and some key antioxidant enzymes in chickpea genotypes under salt stress. Acta Physiol Plant 2013;35:1039-50.
35. Roychoudhury A, Basu S, Sengupta DN. Amelioration of salinity stress by exogenously applied spermidine or spermine in three varieties of Indica rice differing in their level of salt tolerance. J Plant Physiol 2011;168:317-28.

How to cite this article:
Uzma J, Talla SK, Mamidala P. Insights into the impact of Spermidine in reducing Salinity Stress in *Gerbera jamesonii*. J App Biol Biotech. 2023;11(4):141-147. DOI: 10.7324/JABB.2023.120550