



Effect of different polyamines on some physiological traits, growth, and development of basil (*Ocimum basilicum* L.) in salt stress under hydroponic culture conditions

Khatereh Nejadasgari Chokami¹, Vahid Abdossi^{1*}, Saeid Samavat², Alireza Ladan Moghadam³, Pezhman Moradi⁴

¹Department of Horticulture Science, Science and Research Branch, Islamic Azad University, Tehran, Iran

²Soil and Water Research Institute, Tehran, Iran

³Department of Horticultural Science, Garmsar Branch, Islamic Azad University, Garmsar, Iran

⁴Department of Horticultural Sciences, Saveh Branch, Islamic Azad University, Saveh, Iran

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ABSTRACT

As basil occurs in the arid and semi-arid regions, the drought and salinity decrease the vegetative growth and leaf area. This research was carried out in Pakdasht private greenhouse to evaluate the effect of putrescine, spermine, and spermidine on quality and quantity of basil under conditions of salt stress. This research was done as a factorial experiment in a completely randomized block design with three replications. The treatments included application of putrescine, spermine, and spermidine at four levels (0, 50, 100, and 150 mg/l), salinity stress at four levels (0, 50, 100, and 150 mM), and control treatment. The results showed that the interaction effects between polyamines, salinity, and concentration on plant height, fresh/dry shoot weight, fresh/dry weight of root, fresh/dry leaf weight, leaf chlorophyll content, catalase, peroxidase and guaiacol peroxidase antioxidant enzymes, the ratio of K/Na ratio, ion leakage, and proline were statistically significant at 1% level. Interaction and simultaneous exposure of 150 mg/l spermidine and low salinity had a positive effect on all the studied plant traits. In addition, the results showed that the concentration of 150 mM sodium chloride solution reduced the mentioned traits. However, spermidine improved this condition, and symptoms of stress and damages were less observed in spermidine-treated plants. Therefore, it can be used to withstand the oxidative stress of plants.

1. INTRODUCTION

Basil (*Ocimum basilicum* L.) is one of the most important medicinal herbs belonging to the plant family Lamiaceae. This plant species is very sensitive to drought and salinity [1]. Therefore, optimal soil moisture for growth, yield, and essential oil production is necessary during the growing season. As this plant occurs in the arid and semi-arid regions, drought and salinity decrease the vegetative growth and leaf area. Due to the indigenous nature of this plant in Iran and its widespread uses, various aspects of this plant have to be understood in detail.

Polyamines are a new group of plant growth regulators. The presence of polyamines in all plant organs reflects their key role in plant growth regulation and maintaining the quality of horticultural products. Polyamines are widely used in physiological processes such as the growth and development, cell division, plants DNA, proteins, break down the embryogenesis, root formation, flowering, development of reproductive organs, growth and emergence of fruits, aging and reacting to living and non-living environmental stresses. Polyamines are divided into three groups, such as putrescine, spermidine, and spermine [1].

Hydroponic culture allows farmers to grow more crops in a shorter time with less effort. It is well proven that plant does not need soil to grow if the necessary mineral elements are supplied. Generally, hydroponic agro products are better in terms of nutrition and hygiene than crop products originating from soil cultivation. Moreover, this is due to the control of the elements and substances

*Corresponding Author

Vahid Abdossi, Department of Horticulture, Science and Research Branch, Islamic Azad University, Tehran, Iran. E-mail: abdusivahid@gmail.com

that are acquired by the plant. Studies have shown that salinity, polyamines, and their interactions can have a significant effect on the plant traits, and the use of polyamines tends to have a positive effect on plants like bean [2].

In another study [3] involving two types of polyamines under saline conditions in hydroponic system, putrescine increased and spermidine reduced the growth of tobacco. Therefore, the aim of this study was to evaluate the effect of spermidine, spermine, and putrescine on salinity stress conditions in hydroponic culture on basil.

2. METHODS AND MATERIALS

Seeds of basil were purchased from Sepahan Rooyesh company and sterilized for 5–7 minutes with 5% commercial sodium hypochlorite and then rinsed with distilled water. The seeds were then placed on wet perlite in the dark for germination. After 7 days, the seedlings were transferred to light for 24 hours; after the leaf emergence, the seedlings were transferred to the hydroponic medium. Then, 10-day-old seedlings were adapted to Hoagland solution for 1 week. Solution was sterilized before use. Diameter of each plate through hole was 1–2 cm.

Four-weeks-old hydroponic seedlings were transferred to nutrient solution containing different concentrations of NaCl (50, 100, and 150 mM). After 1 week, putrescine, spermidine, and spermine at concentrations of 50, 100, and 150 mg/l mM were added to the nutrient solution. Therefore, salinity treatment (NaCl) was applied for 2 weeks and polyamide for 1 week. The plants were kept in a growth room at 23°C–23°C, 70%–80% RH, and 7/17 hours in light/dark period. The nutrition solution was adjusted every 6 weeks. Six weeks after the initiation of the treatments (2 weeks after salinity treatment), the plants were harvested and transferred to the laboratory for measurements. The total experimental period was about 8 weeks.

Fresh weight of shoot and root was weighted by digital balance with accuracy of 0.1 g. In addition, dry weight was determined with digital scale. To determine the amount of chlorophyll in leaves of basil, Arnon *et al.* [4] method was used. The longest root and shoot length was measured with a ruler. The peroxidase activity, catalase, malondialdehyde, and proline were measured by nitro-blue tetrazolium test (NBT) method, as explained by Nakano and Asada [5]. Ion leakage was estimated according to Lutz *et al.* [6].

This study was conducted as completely randomized design with three replicates. The data were subjected to analysis of variance

(ANOVA) and Duncan's test was used for mean comparison at 1%. All statistical analyses were calculated using Minitab 16 Statistical software and the graphs were drawn in Microsoft Office Excel 2013.

3. RESULTS AND DISCUSSION

3.1. Plant Height

Polyamine application and salinity significantly influenced plant height (Table 1). Plants treated with 150 mg/l spermine were the tallest and plants in 150 mM NaCl (negative control) were the shortest (Fig. 1). An increase in plant height was evident with increasing polyamine concentration. The results showed that increasing salt concentration decreased plant height (Table 1). The increase of salt concentrations in plant growth medium may decrease the water content of the plant through impaired xylem function on laboratory tubes, which is followed by a decrease in shoot elongation. These results are consistent with Lasof *et al.* [7]. The decrease in plant height under salinity stress is probably due to the effect of NaCl on both processes of cell division and cell elongation. Osmotic stress caused by salinity reduces both of these cell processes. Under salinity stress, the production and transfer of cytokinin and gibberellins hormones, which play an important role in the proliferation of cells, decrease. If the abscisic acid increases, the stomatal pores tend to close and eventually photosynthesis will decrease, such changes result in lower plant growth and in turn plant height than control [8].

4. FRESH AND DRY WEIGHT OF LEAVES

The effect of salinity, concentrations, and application of polyamines on fresh and dry weights of leaf was significant (Table 1). The application of 150 mg/l spermine + and 50 mM NaCl had maximum fresh weight, whereas lowest was in spermine control treatment with 150 mM NaCl (negative control) (Figs. 2 and 3). The highest dry weight was obtained in the same treatment. The reduction in leaf weight, like other plant organs, results from the negative effects of salinity stress, which produces lightweight leaves and stems by seedling. Ionic toxicity, nutritional imbalance, and osmotic regulation are the reported effects of salinity stress in wheat plants [9].

Some studies have shown that polyamines increased plant height, leaf number, and fresh and dry leaf weights in different plant species like *Matthiola*, *Gladiolus*, and *Dahlia*. The results of the present study are consistent with the findings of those studies [10–12].

Table 1: ANOVA of traits.

SOV	DF	Shoot length	Shoot fresh weight	Shoot dry weight	Root fresh weight	Root dry weight	Catalase	Peroxidase	Malondialdehyde
Time of spraying	2	0.34 ^{ns}	0.07 ^{ns}	0.28 ^{ns}	0.34 ^{ns}	0.07 ^{ns}	0.28 ^{ns}	0.24 ^{ns}	0.21 ^{ns}
treatment	6	19.28**	0.85**	11.20**	19.28**	0.85**	11.20**	11.30*	0.15*
Time of spraying × treatment	12	0.40*	0.04*	0.20*	0.40*	0.04*	0.10*	0.20*	0.20*
error	32	0.09	0.02	0.07	0.09	0.02	0.03	0.07	0.09
CV	20	12.14	9.45	12.14	12.14	9.45	12.14	11.34	12

*and **: Significant at the 5% and 1% probability levels, respectively; "ns" is not significant.

5. FRESH AND DRY WEIGHT OF SHOOT AND ROOT

The effect of interaction between salinity, concentrations, and application of polyamines on fresh and dry weight of shoot and root was significant (Table 1). Fresh and dry weight of shoot and root in 150 mg/l spermine + and 50 mM NaCl was more than others and treatment with 150 mM NaCl was the lowest (Figs. 4–7).

Previous studies do indicate that increasing salt concentrations decrease fresh and dry weights of shoots [10,11]. Therefore, reduction in shoot and root growth is the first physiological response to stress resulting in reduced biomass, which was significant in the presence of salt. The results of this study showed that decreasing salinity along with polyamine application increased the fresh and dry weights of shoots and roots in comparison with control (negative control).

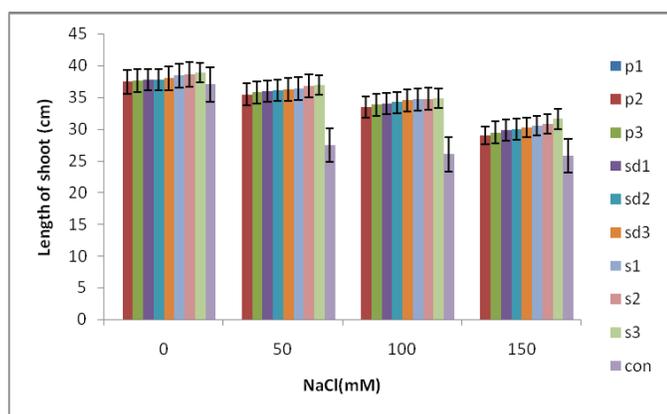


Figure 1: Changes in plant height in response to polyamine application under salinity stress. p1: putrescine (50 mg), p2: putrescine (100 mg), p3: putrescine (150 mg), sd1: spermidine (50 mg), sd2: spermidine (100 mg), sd3: spermidine (150 mg), s1: spermine (50 mg), s2: spermine (100 mg), s3: spermine (150 mg); error bar indicates standard deviation.

6. PEROXIDASE ACTIVITY

The results showed that increasing salt concentration reduced the peroxidase enzyme activity. The enzyme activity increased as compared to control (negative control) with decreasing salinity levels in the presence of polyamines. Therefore, the highest peroxidase activity was observed in plants treated with 150 mg/l spermine +50 mM NaCl, and the lowest level at a concentration of 150 mM NaCl (Fig. 8).

These results accord with the observations of an increased antioxidant enzyme activity under salinity stress conditions in barley plants [10]. The application of treatments showed activity of peroxidase enzyme; the cell wall is the main source of accumulation of peroxidase isoenzymes. On the other hand, some of the peroxidase isoenzymes have also been linked to the cell surface, which can easily be introduced into the apoplastic solution under different conditions of stress [13].

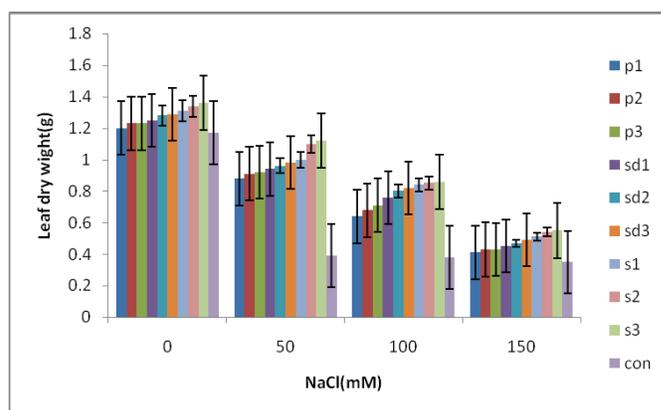


Figure 3: Changes in leaf dry weight to polyamine application under salinity stress. p1: putrescine (50 mg), p2: putrescine (100 mg), p3: putrescine (150 mg), sd1: spermidine (50 mg), sd2: spermidine (100 mg), sd3: spermidine (150 mg), s1: spermine (50 mg), s2: spermine (100 mg), s3: spermine (150 mg); error bar indicate standard deviation.

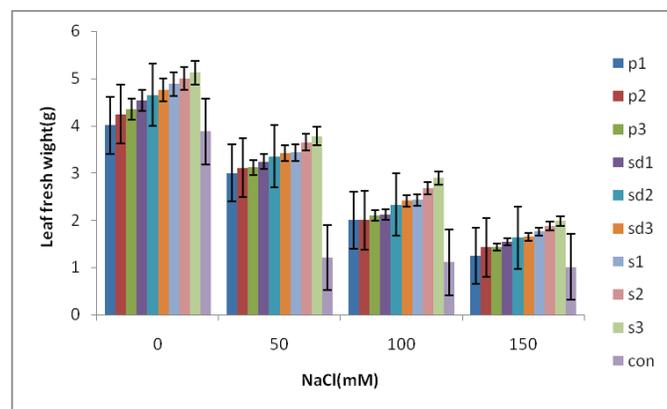


Figure 2: Changes in leaf fresh weight to polyamine application under salinity stress. p1: putrescine (50 mg), p2: putrescine (100 mg), p3: putrescine (150 mg), sd1: spermidine (50 mg), sd2: spermidine (100 mg), sd3: spermidine (150 mg), s1: spermine (50 mg), s2: spermine (100 mg), s3: spermine (150 mg); error bar indicate standard deviation.

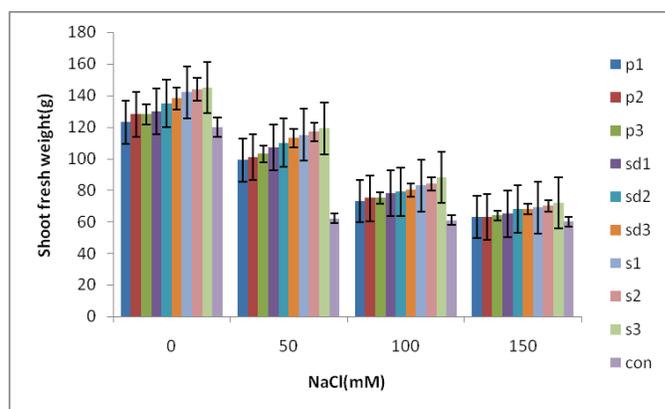


Figure 4: Changes in shoot fresh weight to polyamine application under salinity stress. p1: putrescine (50 mg), p2: putrescine (100 mg), p3: putrescine (150 mg), sd1: spermidine (50 mg), sd2: spermidine (100 mg), sd3: spermidine (150 mg), s1: spermine (50 mg), s2: spermine (100 mg), s3: spermine (150 mg); error bar indicate standard deviation.

Antioxidant enzymes appear to have a protective *function* against oxygen species. Therefore, it is concluded that the spraying solution would break down the hydrogen peroxide (H_2O_2) in the cells. This prevents the reactive oxygen species (ROS) production and, therefore, increasing in the enzyme activity, lessening ROS damage in plant since peroxidase (POX) and catalase (CAT) enzymes are essentially known as the most important H_2O_2 -scavenging enzymes. Therefore, spraying with the mentioned compounds, it is expected to improve the tolerance of the plant with the activity of more enzymes and higher levels of antioxidants. Therefore, it is likely that high concentrations of polyamine have been able to induce the activity of these enzymes [14].

7. CATALASE ACTIVITY

Regarding the comparison of the mean, the highest content of CAT was observed at 150 mg/l spermine + 50 mg/l NaCl content and

its lowest was at 150 mM NaCl (Table 1). The results showed that increasing the content of polyamines increased the amount of this enzyme (Fig. 9).

The activity of antioxidant enzymes in response to salt stress raised in plant treated with polyamine. Tolerance to salt stress and drought stress in plants is associated with changes in the activity of antioxidant enzymes. Catalase, glutathione peroxidase (GPX), and POX are important scavengers of H_2O_2 . The activity of enzymes has been shown by treatment with polyamines under salt stress conditions. The interaction of polyamines, concentration, and salinity stress on the activity of all enzymes was significant. Therefore, regarding the enzymes studied, their activity levels were generally influenced by the type and concentration of polyamines and the salinity stress. In fact, the above-mentioned treatments show higher levels of plant tolerance by increasing the activity of this enzyme.

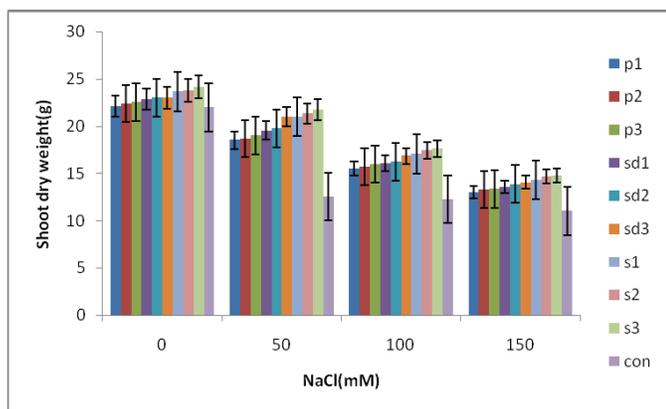


Figure 5: Changes in shoot dry weight to polyamine applications under salinity stress. p1: putrescine (50 mg), p2: putrescine (100 mg), p3: putrescine (150 mg), sd1: spermidine (50 mg), sd2: spermidine (100 mg), sd3: spermidine (150 mg), s1: spermine (50 mg), s2: spermine (100 mg), s3: spermine (150 mg); error bar indicate standard deviation.

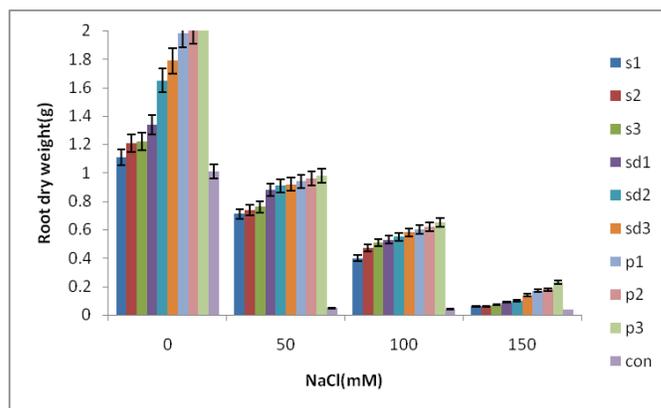


Figure 7: Changes in root dry weight to polyamine application under salinity stress. p1: putrescine (50 mg), p2: putrescine (100 mg), p3: putrescine (150 mg), sd1: spermidine (50 mg), sd2: spermidine (100 mg), sd3: spermidine (150 mg), s1: spermine (50 mg), s2: spermine (100 mg), s3: spermine (150 mg); error bar indicate standard deviation.

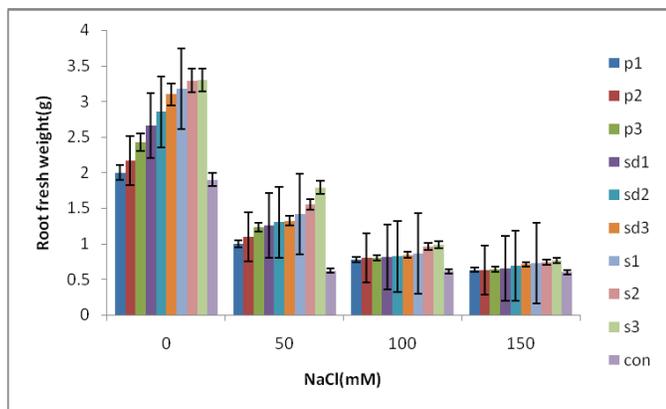


Figure 6: Changes in root fresh weight to polyamine application under salinity stress. p1: putrescine (50 mg), p2: putrescine (100 mg), p3: putrescine (150 mg), sd1: spermidine (50 mg), sd2: spermidine (100 mg), sd3: spermidine (150 mg), s1: spermine (50 mg), s2: spermine (100 mg), s3: spermine (150 mg); error bar indicate standard deviation.

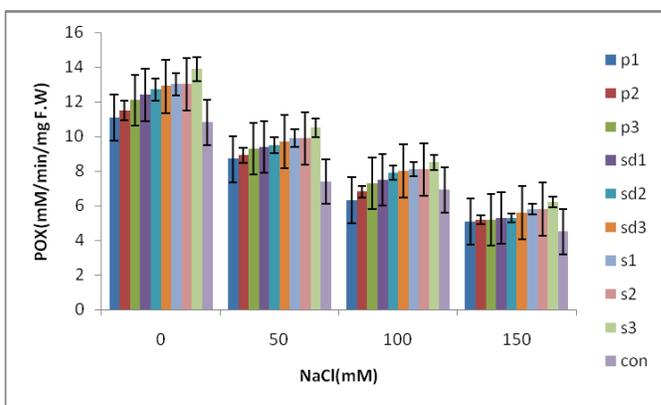


Figure 8: Changes in POX activity to polyamine application under salinity stress. p1: putrescine (50 mg), p2: putrescine (100 mg), p3: putrescine (150 mg), sd1: spermidine (50 mg), sd2: spermidine (100 mg), sd3: spermidine (150 mg), s1: spermine (50 mg), s2: spermine (100 mg), s3: spermine (150 mg); error bar indicate standard deviation.

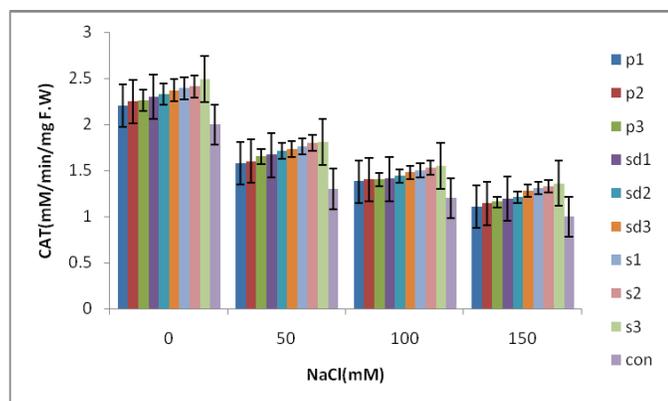


Figure 9: Changes in CAT activity to polyamine application under salinity stress. p1: putrescine (50 mg), p2: putrescine (100 mg), p3: putrescine (150 mg), sd1: spermidine (50 mg), sd2: spermidine (100 mg), sd3: spermidine (150 mg), s1: spermine (50 mg), s2: spermine (100 mg), s3: spermine (150 mg); error bar indicate standard deviation.

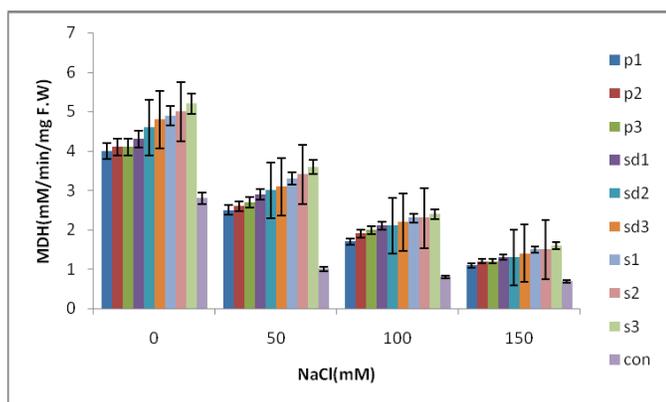


Figure 10: Changes in MDH activity to polyamine application under salinity stress. p1: putrescine (50 mg), p2: putrescine (100 mg), p3: putrescine (150 mg), sd1: spermidine (50 mg), sd2: spermidine (100 mg), sd3: spermidine (150 mg), s1: spermine (50 mg), s2: spermine (100 mg), s3: spermine (150 mg); error bar indicate standard deviation.

8. MALONDIALDEHYDE

Data analysis showed that the interaction of salinity stress and spraying of polyamine on the level of malondialdehyde (MDA) were significant. Mean comparison showed that the use of polyamine in all salinity levels increased MDA in comparison to control. The highest activity was in 150 mg/l spermine + 50 mg/l NaCl and its lowest level was observed in 150 mM NaCl (negative control) (Fig. 10).

When plants are exposed to salt stress, they usually change the structure of their lipids. The induced lipid peroxidation in membranes by salt stress indicates damage to the cell membrane, and the level of MDA produced during this process is considered as an indicator of oxidative damage [12,13]. Therefore, salinity stress may disrupt the electron transfer process in mitochondria and chloroplasts, and by producing reactive oxygen radicals, it causes oxidative damage to the membrane, resulting in an increased lipid peroxidation and induces MDA in treated plant. In this study, polyamines in high salinity stress significantly reduced the amount of MDA and reduced the impact of salinity stress in two groups, including polyamide treatment and salinity treatments. The role of polyamines in protecting plants against lipid peroxidation is a cellular mechanism to avoid undesirable oxidative damage [15].

9. PROLINE OF LEAVES

The results of ANOVA showed that the effect of salinity, polyamines, and their interactions for proline content was significant (Table 2). Comparison of means showed that the amount of proline increases with increasing concentrations of polyamines. Application of spermine, especially at 150 mg/l, at all salinity levels increased proline compared to the control treatment with 150 mM NaCl (negative control) (Fig. 11).

Increasing salinity levels in irrigation water raised the amount of proline in the basil leaf. Different kinds of polyamine have a significant effect on proline content of the leaf so that the highest amount of proline was obtained in spermine treatment.

Table 2: ANOVA of traits.

SOV	DF	Shoot length	Shoot fresh weight	Shoot dry weight	Root fresh weight	Root dry weight	Catalase	peroxidase	Malondialdehyde	Proline	Chlorophyll	Ion leakage
polyamine	3	18.29 ^{ns}	0.002 ^{ns}	0.002 ^{ns}	0.07 ^{ns}	0.28 ^{ns}	0.34 ^{ns}	0.07 ^{ns}	241.21 ^{ns}	0.34 ^{ns}	1.23 ^{ns}	0.28 ^{ns}
concentration	3	156.28 ^{**}	0.94 ^{**}	0.94 ^{**}	0.85 ^{**}	11.20 ^{**}	19.28 ^{**}	0.85 ^{**}	213.20 ^{**}	19.28 ^{**}	4.33 ^{**}	11.20 ^{**}
salinity	3	24.54 [*]	0.04 [*]	0.20 [*]	0.04 [*]	0.20 [*]	0.40 [*]	0.04 [*]	77.3 [*]	0.40 [*]	0.04 [*]	0.20 [*]
polyamine × concentration	9	0.641 [*]	0.285 [*]	0.07	0.02	0.07	0.09	0.02	21.24 [*]	0.09	0.52 [*]	0.07 [*]
polyamine × salinity	9	14.51 [*]	0.015 [*]	12.14	9.45	12.14	12.14	9.45	0.80 ^{**}	12.14	10.23 ^{ns}	12.14 [*]
polyamine × concentration × salinity	3	213.07 [*]	0.04 [*]	0.20 [*]	0.04 [*]	0.20 [*]	0.40 [*]	0.04 [*]	0.26 [*]	0.40 [*]	0.22 [*]	13.1 [*]
error	9	239.2	0.017	0.07	0.02	0.07	0.09	0.02	0.12	0.09	3.01	1.98
CV	-	12.73	13.2	12.14	9.45	11.14	12.5	9.45	12.04	12	3.18	13

*and **: Significant at the 5% and 1% probability levels, respectively; "ns" is not significant.

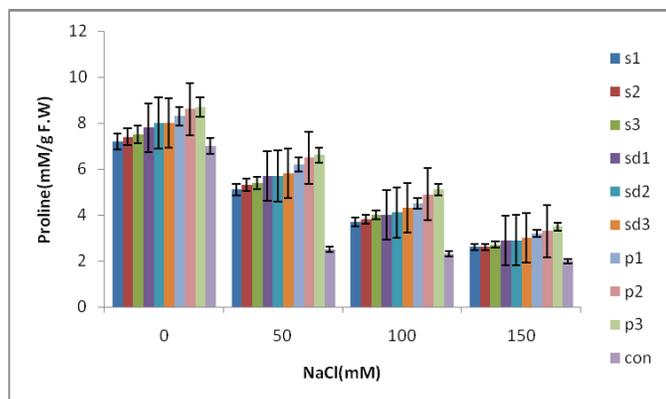


Figure 11: Changes in proline to polyamine application under salinity stress. p1: putrescine (50 mg), p2: putrescine (100 mg), p3: putrescine (150 mg), sd1: spermidine (50 mg), sd2: spermidine (100 mg), sd3: spermidine (150 mg), s1: spermine (50 mg), s2: spermine (100 mg), s3: spermine (150 mg); error bar indicate standard deviation.

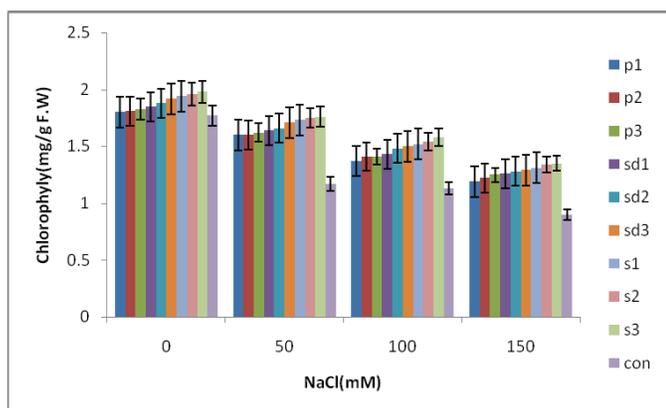


Figure 12: Changes in chlorophyll to polyamine application under salinity stress. p1: putrescine (50 mg), p2: putrescine (100 mg), p3: putrescine (150 mg), sd1: spermidine (50 mg), sd2: spermidine (100 mg), sd3: spermidine (150 mg), s1: spermine (50 mg), s2: spermine (100 mg), s3: spermine (150 mg); error bar indicate standard deviation.

In the present study, putrescine also increased proline and reduced the effects of salinity stress. This can be due to the role of polyamines in proteins and enzymes involved in proline synthesis, photosynthesis, and nutrient modification. Proline content can be obtained through the pathway synthesize from Pyrroline [16].

10. CHLOROPHYLL CONTENT

Salinity stress reduced chlorophyll content. In plants treated with 150 mg/l spermine, had increased chlorophyll compared to control treatment with 150 mM NaCl (negative control) (Fig. 12).

The results of this study are consistent with Cohen et al. [17], who showed that polyamines prevent chlorophyll degradation and increase chlorophyll content in rice under stress condition.

There are many studies reporting chlorophyll degradation due to stress [17]. Salinity increases the activity of chlorophyllase enzymes, which lead to a decrease in chlorophyll content. Under

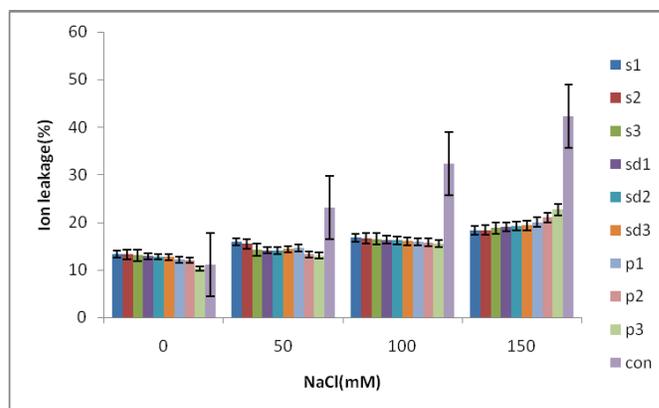


Figure 13: Changes in ion leakage to polyamine application under salinity stress. p1: putrescine (50 mg), p2: putrescine (100 mg), p3: putrescine (150 mg), sd1: spermidine (50 mg), sd2: spermidine (100 mg), sd3: spermidine (150 mg), s1: spermine (50 mg), s2: spermine (100 mg), s3: spermine (150 mg); error bar indicate standard deviation.

stress conditions, some plant growth regulators such as abscisic acid and ethylene increase, which can stimulate chlorophyllase synthesis and activity. Salinity causes thylakoid degradation and decreases chlorophyll content in some plants.

According to Table 1, the content of chlorophyll declined in response to salinity stress compared to the control. The effect of combined polyamine and saline treatment was more intense and was highest when compared to control. The decrease in the chlorophyll content in *Pineda ovata* and *Nicotiana rustica* under saline conditions was caused by increased chlorophyll degradation in the leaf and, finally, led to the reduction of chlorophyll efficiency in photosynthesis [18].

11. ION LEAKAGE

Salinity increased the ionic leakage under stress compared to control plants. The control plants treated with polyamines had reduced ionic leakage compared to untreated plants (Fig. 2), but in plants under salt stress of 50 mM with 150 mg/l spermine had reduced ion leakage (14%) compared to negative control (43.34%) (Fig. 13).

High salt concentration damages the integrity and function of the cell membrane. In bean roots, the presence of salt in the growing medium leads to ions leakage (Na^+ , K^+) in addition to structural changes which happened across membrane metamorphism and vesiculation. Increasing salt concentration can change the calcium binding to the plasma membrane and other internal cell membranes and induce a decrease in the $\text{Ca}^{2+}/\text{Na}^+$ ratio, which is a result of integrity of cell membrane and its function [19–22].

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