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Original Article

The Seed Priming using Putrescine Improves, Germination Indices and Seedlings morphobiochemical Responses of Indigo (*Indigofera tinctoria*) under Salinity Stress

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ABSTRACT

This research was done as a factorial experiment with 5 replications was carried out. The seeds were primed using putrescine at concentrations of 0 (hydropriming), 0.5, and 1 mM, and control were used as control**.** Salinity treatment was applied under normal, low, moderate and severe (0, 50, 100, and 150 mM NaCl, respectively) salinity stress. According to the findings, increasing salinity levels reduced germination percentage, (GP), germination rate, (GR), coefficient of velocity of germination, (CVG), seed weight vigour index, (SWVI), by 29, 66, 53 and 25%, respectively and seedling fresh weight, seedling dry weight, roots length and shoot length by 37, 14, 72 and 61%, respectively, while increasing mean germination time (MGT), percentage of dry matter, total soluble sugar (TSS), reactive oxygen species (ROS) and malondialdehyde (MDA) by 112, 53, 57,16 and 182%, respectively. When seeds were primed using putrescine reduced the effects of oxidative stress by reducing ROS and MDA levels by 11 and 47%, respectively compared to control and improved the GP, GR, CVG, SWVI, root length and shoot length by 31, 81, 67, 36, 27, 19%, and decreased MGT by 40%. Although seed priming using 1 mM putrescine was effective in some parameters, there was no significant difference when compared to 0.5 mM putrescine. As a result, seed priming indigo using 0.5 mM putrescine with increasing dry matter by 73% appears to be more practical and economical in terms of mitigating the negative effects of salinity stress.

INTRODUCTION

Salinity stress is an important abiotic stress that affects plant physiology and reduces agricultural plant growth and performance. From the germination stage to seed production, salinity affects all major processes such as growth, photosynthesis, protein biosynthesis, fat metabolism, and plant energy [1]. Salinity stress following drought stress is one of the most significant barriers to the production of medicinal plants in many areas, particularly dry areas. The highest level of salinity sensitivity in the plant life cycle has been observed during germination and the early stages of seed development [2].

Priming is a simple technology that hydrates seeds to the point where germination metabolic activity begins but no radicle emergence occurs [3]. Priming promotes germination and increases seed vigour by inducing various metabolic changes in the seed, resulting in rapid and uniform emergence and the establishment of a strong seed mass [4]. Furthermore, seed priming reduces abiotic stresses in plants by regulating antioxidant enzyme activity, ion balance, and photosynthetic characteristics [5]. Seed priming is a method that prepares the seed physiologically and biochemically for germination before placing it in the culture medium. This method causes physiological and biological changes in the seeds and seedlings produced, which improves the germination and seedling establishment in the cultivation environment [6].

Polyamines are important polycations with low molecular weight that produce defense responses in response to environmental stress. Polyamines' polycations properties allow them to react with proteins, phospholipids, and cell wall structures at physiological pH, which makes these molecules stable in plants [7]. Polyamines like putrescine, spermidine, and spermine are used in seed priming to increase the seedling resistance to environmental stresses such as salinity and drought [7]. Putrescine is an important factor in increasing salt stress resistance. Adding putrescine to plants under salinity stress prevents the destruction of macromolecules and lipid peroxidation and increasing glutathione and carotenoids, which play an antioxidant role in the plant [7].

Indigo plants (*Indigofera tinctoria* L.), belongs to the Fabaceae family, are famous for indigo dye, which is obtained from leaflets and branches of and is extracted through a fermentation process, and use as the natural blue colors and the coloring agent for traditional textiles and is widely distributed in tropical and subtropical areas [8]. The indigo plant is relatively sensitive to soil and water salinity in its early stages of development, if it can grow in this stage, it can tolerate moderate salinities [7].

The current study was designed to determine the effectiveness of putrescine in reducing the negative effects of salinity stress on indigo seed germination indices and biochemical responses of seedling.

MATERIALS AND METHODS

This experiment was done in 2022 at Jiroft University's Faculty of Agriculture. A completely randomized design was used based on a factorial experiment, which included three replications. Salinity levels of 0, 50, 100, and 150 mM sodium chloride were used. Putrescine (1,4 Diaminobutane) (Merck) was used to pre-treat the seeds at concentrations of 0 (hydropriming), 0.5, and 1 mM, and unprimed seeds was used as control group. The seeds were prepared of the Roudbar ecotype. The seeds were disinfected using ethanol (70% for 10 seconds) and then sodium hypochlorite solution (70% for five minutes), followed by several washes times using distilled water. After that, they were primed for 4 hours at 25ºC using three times the volume of the desired solutions (specified concentrations of putrescine), and after the pre-treatment period, the excess solution was discarded and after 5 times of washing by benomyl at 3000 ppm fungicide solution, 50 seeds were transferred to sterile petri dishes containing filter paper, and the seeds in each petri dish were treated using 2 mL of the NaCl solution (0, 50, 100 and 150 mM) and were transferred to the germinator that had been set 16/8h, light/darkness photoperiod and 30/25ºC day/night temperatures and 75% relative humidity. The germinated seeds were counted after 24 hours, (seeds whose radicle length was 2mm or more were considered germinated seeds). The counting of germinated seeds was continued daily until there was no change in the number of germinated seeds three days after the last count, at which point the experiment was considered over. During the seed counting period, the petri dishes were checked for infection and humidity, and the petri dishes using low humidity were treated using relative salinity to achieve the desired humidity. The characteristics of seed germination were evaluated at the end of the experiment.

Measurement Seed Germination Responses

Germination Percentage (GP)

The germination percentage was calculated using the following equation.

$$PG = (Ni/N) \times 100$$

Where PG is the percentage of germination, Ni is the number of germinated seeds on the last day of counting and N is the total number of seeds [9].

Coefficient of Velocity of Germination (CVG)

It shows the germination speed. Its value increases when the number of germinated seeds increases and the time required for germination decreases. The CVG was calculated from the following equation:

$$CVG = (G1+G2\ldots+Gn)/ ((1\times G1) + (2\times G2) +\ldots(n\times Gn))$$

Where G1-Gn is the number of germinated seeds from the beginning to the end of the test [10].

Mean Germination Time (MGT)

It is an indicator of the speed and acceleration of germination and it calculates the mean time of

germination. The mean germination time was calculated using the following equation [11]:

$$MGT = (\sum (nd)) / (\sum n)$$

Where n: number of germinated seeds during d days, d: number of days, and ∑n: total number of germinated seeds

Seed Weight Vigour Index (SWVI)

Using the data of seedling dry weight and seed germination percentage, the seedling weight index was calculated using the following equation [7].

SWVI = dry weight of seedling × germination ability

Measurement of Seedlings Growth Parameters

Five seedlings were chosen at random from each petri dish, and the root length and shoot length was measured using a digital caliper. Twenty seedlings from each petri dish were weighed using a digital scale to determine their fresh weight (accuracy of 0.0001). Their dry weight was also determined after 24 hours in a 40 °C oven [7].

Measurement of Seedling's Biochemical Parameters

Measurement of Total Sugar Content

To determine the amount of total sugar, 0.02 g of leaf sample was ground in a Chinese mortar mixed using 2 mL of double distilled water and kept in a bain-marie at 100 ºC for 20 minutes before being centrifuged at 8000 rpm for 15 minutes. The optical absorption of the samples was measured at a wavelength of 315 nm after 1900 µL of concentrated sulfuric acid was added to 100 µL of the extract. The standard curve was created using glucose. Each sample's total sugar content was calculated and reported in g/fw [12].

ROS (Reactive Oxygen Species) Measurement

To measure ROS, 0.02g of leaf tissue was completely ground in a porcelain mortar on ice, then 2 mL of sodium phosphate buffer (50 mM, pH = 7.4) was added, and resulting homogeneous solution was poured into a micro tube and then centrifuged for 20 min at a 10000 rpm, at 4 ºC. The optical absorption of the resulting solution was measured at 560 nm after, 900 µL of fresh acidic orange xylenol reagent was added to 100 µL of the extract. Using different concentrations of 30% H_2O_2, a standard curve was drawn, and the results were calculated to µmol/g fw [7].

MDA (Malondialdehyde) Assay

To determine the content of MDA, which is known as a breakdown product of lipid peroxidation, (MDA-TCA) was used, 0.02 g of leaf tissue was rubbed 0 in 2 mL of trichloroacetic acid (TCA) 1% on an ice bath. The extract was centrifuged at 10,000 rpm for 20 min at 4 °C. 500 µL of supernatant was added to 500 µL of TCA-TBA (20% trichloroacetic acid and 0.5% thiobarbituric acid). The mixture was heated at 100 °C for 15 minutes in a hot bath and then cooled in an ice bath. Absorbance was recorded at wavelengths 532 and 600 nm. The extinction coefficient for calculating the MDA concentration was equal to 0.155 $mMcm^{-1}$ and the values were calculated and presented in terms of wet weight [13].

Data Analysis

Following data normalization, SAS software (9.4) was used to analyze data variance and compare averages using Duncan's multiple range test, and graphs were created using EXCEL software.

RESULTS AND DISCUSSION

Germination Responses

Salinity, seed priming, and their interaction all had a significant effect on germination percentage (GP), coefficient of velocity of germination (CVG), germination rate (GR), mean germination time (MGT), and seed weight vigour index (SWVI) (Table 1). Changes in GP have long been recognized as an important indicator of germination under stress conditions. GP reduced by 29% under severe salinity stress (150 mM) compared to normal conditions. Under severe salinity stress, seed priming using 0.5 and 1 mM putrescine increased GP by 25 and 31%, respectively (Table 2). According to Zeid [14] as salinity increased, GP decreased in *Phaseolus vulgaris*, and seed priming using putrescine increased GP under normal and salinity conditions, which may

be due to the activation of amylase and protease enzymes during germination period. Aslani *et al*. [15] discovered that applying spermidine on cucumber cultivars under salinity stress increased GP. CVG, GR, and MGT decreased in proportion to the increase in salinity concentration. When compared to the control, seed priming using 0, 0.5, and 1 mM putrescine increased CVG, and GR. Salinity levels of 50, 100, and 150 mM reduced CVG by 39, 43, and 53%, and GR by 42, 48, and 66%, respectively, when compared to normal conditions (Table 2). Seed priming using 0.5 mM putrescine under low, moderate, and severe salinity stresses, increased CVG by 61, 50 and 42% and GR by 81, 81 and 71%, and seed priming using 1 mM putrescine increased CVG by 67, 52 and 39%, and GR by 77, 81 and 105% compared to control, respectively. Seed priming can increase GP, germination rate, and uniformity by activating some enzymes in the seeds, making it easier to access nutrients during the germination phase. Primed seeds germinate faster and more tolerant stress conditions. Elkaco [16] found that GR increased by 10, 50, and 90% in primed seeds. Hossinifarahi *et al*. [17] reported that seed priming using putrescine increased GR by 20 and by 47% in two cucumber cultivars, respectively, when compared to the control. Seed priming using 0, 0.5, and 1 mM putrescine decreased MGT compared to the control. Salinity levels of 50, 100, and 150 mM significantly increased MGT by 65, 77, and 112%, respectively, compared to normal conditions (Table 2). Seed priming using 0.5 and 1 mM putrescine significantly reduced MGT by 38 and 40% under low, by 33% and 34%, under moderate, and by 29 and 28% under severe salinity, respectively, compared to the control (Table 2). Drought stress is caused by salinity because it reduces seed water absorption, and research showed that germination take longer in drought-stressed rape seeds and wheat seeds. SWVI decreased by 25% under severe salinity stress, compared to normal conditions. Under low, moderate, and severe salinity stresses, seeds priming using 1 mM putrescine increased SWVI by 18, 16, and 36%, respectively, compared to control (Table 2). Among the other compounds used in eggplant and sweet pepper, Hossinifarahi *et al*. [17] found that putrescine-primed seeds had the most effect on GP and root growth. Acceleration of the germination processes and faster emergence of seedlings from primed seeds compared to non-primed seeds are two of the most important reasons for the increase in seed germination. Among the most important reasons for the improvement of the primed seeds compared to non-primed seeds, are faster DNA repair, RNA production, enzyme activation, cell expansion, protein biosynthesis, removal of active oxygen radicals, and further progress in the germination stages [18].

Morphological Responses of Seedling

Root Length and Shoot Length

Salinity levels, seed priming, and their interaction all had a significant effect on the root length and shoot length (Table 1). In proportion to the increase in salinity concentration, the root length and shoot length, decreased. Salinity levels of 50, 100, and 150 mM reduced root length by 52, 54, and 72% and shoot length by 25, 34 and 61%, respectively, when compared to normal conditions (Table 3). Under normal conditions, concentrations 0 (hydropriming), 0.5, and 1 mM of putrescine increased root length by 45, 27 and 22% and shoot length by 12, 14 and 19% compared to control, respectively. Under low salinity stress, concentrations 0, 0.5, and 1 mM of putrescine increased root length by 25, 33 and 24%, respectively, compared to the control and did not have a significant effect on the shoot length. Under moderate salinity stress, seed priming not only did not have a significant positive effect on the root length and shoot length compared to control, but also decrease them. Under severe salinity stress, the hydroprimed seeds increased the root length and shoot length by 24 and 16%, respectively, compared to control, but primed seeds using 0.5 and 1 mM putrescine did not show a significant positive effect on them compared to control. Roots and shoots are the first organs that are negatively affected by salinity stress [7]. The general trend of plants, when faced with salt stress, is to reduce the length of roots and shoots [3,19,20]. The decrease in root growth under salinity stress can be due to the toxicity of sodium and chlorine ions and the imbalance in the absorption of nutrients and the decrease in water

absorption [21]. The increase in growth caused by putrescine priming under normal and stress conditions caused by sodium chloride may be due to the activation of amylase and protease during germination [14]. The reports showed polyamines play a role in plant defense against environmental stresses. The use of putrescine in wheat has been able to reduce plant growth inhibition under drought stress conditions and improve grain yield [22].

Fresh and Dry Weight and Percentage of Dry Matter of Seedlings

Salinity levels, seed priming, and their interaction significantly affected fresh and dry weight and percentage of dry matter of seedlings (Table 1). The fresh weight of seedlings increased by 19% under low salinity stress compared to the control, while under severe salinity stress decreased by 37%. The dry weight of seedlings under low, moderate and severe salinity stress, increased by 9, 14 and 5% compared to control, respectively, in control (Table 3). Concentrations 0 (hydropriming), 0.5 and 1 mM of putrescine increased the fresh weight of seedlings under low salinity conditions by 9, 24 and 21%, and under severe salinity stress by 67, 38 and 24% compared to control, respectively (Table 3). Under low and moderate salinity stress, the seed priming using 1 mM putrescine increased the dry weight of seedlings by 8 and 14% compared to control, respectively. Under moderate and severe salinity stress, increased the dry matter percentage of seedlings by 40 and 53% compared to normal conditions. Under normal conditions, seed priming using 1 mM putrescine increased dry matter of seedling by 16% compared to control. In addition, the seed priming using 0.5 and 1 mM putrescine increased the dry matter under low salinity stress by 33 and 73% and under moderate salinity stress by 11 and 22% compared to control. In addition, under severe salinity stress, seed priming using 0.5 mM putrescine increased dry matter of seedling by 9% compared to control (Table 3). One desirable features for evaluating the effect of salinity stress on plant seedlings is to determine the fresh and dry weight of the plant. The general trend in salinity-stressed seedlings is a loss of fresh and dry weight [19-20]. According to our findings, under severe salinity stress, the fresh weight of the seedlings under control decreased significantly compared to normal conditions, whereas under low salinity stress, the fresh weight of these seedlings did not decrease, but also increased significantly, which indicates that this plant is interested in the salinity level of 50 mM. Salinity stress significantly reduces photosynthetic pigments in chloroplasts, which reduces photosynthetic efficiency and causes a serious decrease in final productivity [23,24]. In the salinity stress of 150 mM, the fresh weight of seedlings using 0, 0.5 and 1 mM putrescine increased significantly. This finding is consistent with previous findings that seed priming of medicinal pumpkin seeds [19] and wheat [22] using putrescine increases plant growth by increasing the fresh weight of roots, shoot and seedling dry matter percentage under stress conditions. Plant growth caused by the use of external putrescine in saline conditions can reduce the accumulation of sodium ions in the root, increase putrescine and grafted polyamine
biosynthesis, and thus plant growth, as reported in a salt-sensitive rice variety [25].

Biochemical Responses

Total soluble sugars, ROS, and MDA contents were affected by salinity levels, seed priming, and their interaction (Table 4). The salinity increased total soluble sugars, ROS, MDA. Under the low, moderate, and severe salinity stresses, the amount of total soluble sugar increased by 22, 57, and 39%, respectively, compared to the normal conditions (Table 4). Plants respond to salinity stress by increasing the content of organic solutes such as sugars and amino acids for osmotic regulation [14]. Plants that are exposed to salinity accumulate sugar in their tissues due to osmosis regulation, osmosis protection, and carbon storage. As a result of the increase in sugar using increasing salinity levels, indigo is most likely a salinity-tolerant plant. Under low salinity stress, seed priming using all concentrations of putrescine reduced the amount of total soluble sugar by 7%, and under moderate salinity stress, seed priming using 0.5 mM putrescine reduced the soluble sugar by 6% compared to control.

Table 1 Variance analysis of salinity effect, seed priming using putrescine and their interaction on seed germination indices and morphobiochemical parameters of seedling in indigo plants

Sources of variation	Degrees freedom	GP	CVG	GR	MGT	SWVI	Root length	Shoot length
Salinity (S)	3	635.64 **	0.246 **	840.11 **	1.82 **	4.49 **	420.73 **	905.68 **
Putrescine (P)	3	224.08 **	0.094 **	413.71 **	0.92 **	2.81 **	11.38 **	3.45 **
S × P	9	39.46 **	0.01 **	38.07 *	0.11**	0.62 *	4.95 **	13.92 **
Error	30	9.64	0.003	3.15	0.011	0.21	0.23	0.46
C.V.	-	3.73	7.84	5.70	6.46	6.74	5.23	3.56
Sources of variation	Degrees freedom	Seedling FW	Seedling DW	Dry matter	Total sugar	ROS	MDA	
Salinity (S)	3	0.0508 **	0.0002 **	77.95 **	0.022 **	354.93*	0.500 **	
Putrescine (P)	3	0.0196 **	0.0001 ns	36.84 **	0.003 **	59.17 ns	0.552 **	
S × P	9	0.0193 **	0.00006 *	10.28 **	0.005 **	420.33**	0.874 **	
Error	30	0.0013	0.00002	0.77	0.0012	82.697	0.0231	
C.V.	-	5.70	5.80	5.74	11.28	5.71	12.27	

ns, * and **, non-significant and significant at 5% and 1% level of probability.

Table 2 Effect of seed priming of indigo plants using putrescine on seed germination indices under salinity stress conditions

Treatments		Seed germination indices				
Salinity (S)	Putrescine (P) (mM)	GP (%)	CVG	GR	MGT (day)	SWVI
Control	Control (unprimed)	85.33 bcd	0.80 ab	37.61 bc	1.24 hi	6.51 cd
	0 mM (hydropriming)	86.00 bc	0.73 be	35.28 cd	1.38 fgh	6.53 cd
	0.5 mM	92.67 a	0.85 a	42.90 a	1.19 hi	7.17 bc
	1 mM	92.67 a	0.85 a	42.89 a	1.17 i	7.20 bc
Low (50 mM)	Control (unprimed)	82.00 cde	0.49 fg	21.86 f	2.05 cd	6.84 cd
	0 mM (hydropriming)	84.67 be	0.77 ad	36.50 bcd	1.31 ghi	6.66 cd
	0.5 mM	89.33 ab	0.79 abc	39.63 b	1.28 ghi	7.06 cd
	1 mM	85.33 bcd	0.82 a	38.78 b	1.23 hi	8.08 a
Moderate (100 mM)	Control (unprimed)	80.00 def	0.46 fgh	19.43 fg	2.19 bc	6.99 cd
	0 mM (hydropriming)	90.67 ab	0.65 e	33.68 d	1.55 f	7.92 ab
	0.5 mM	88.00 ab	0.69 de	35.17 cd	1.46 fg	6.90 cd
	1 mM	85.33 bcd	0.70 cde	35.16 cd	1.45 fg	8.04 a
Severe (150 mM)	Control (unprimed)	60.67 g	0.3 8h	12.88 h	2.63 a	4.87 e
	0 mM (hydropriming)	74.67 f	0.44 gh	18.02 g	2.26 b	6.17 d
	0.5 mM	76.00 f	0.54 f	22.00 f	1.86 e	6.51 cd
	1 mM	79.33 def	0.53 fg	26.43 e	1.89 de	6.62 cd

Different letters within the same columns indicate significant differences according to Duncan's multiple range test. GP, germination percent. CVG, coefficient of velocity of germination. GR, germination rate. MGT, mean germination time. SWVI, seed weight vigour index.

Table 3 Effect of seeds priming of indigo plants using putrescine concentrations on morphological responses of seedlings under salinity stress conditions

Treatments		Morphological responses of seedlings				
Salinity (S)	Putrescine (P) (mM)	Root length (mm)	Shoot length (mm)	Seedling FW (g)	Seedling DW (g)	Dry matter (%)
Control	Control (unprimed)	14.45 c	27.55 c	0.67 bcd	0.0763 e	11.80 h
	0 mM (hydropriming)	21.01 a	30.88 b	0.66 cde	0.0760 e	12.04 h
	0.5 mM	18.33 b	31.49 b	0.70 bc	0.0773 de	11.98 h
	1 mM	17.61 b	32.84 a	0.61 def	0.0777 de	13.66 g
Low (50 mM)	Control (unprimed)	6.99 e	20.65 d	0.80 a	0.0833 bcde	10.78 h
	0 mM (hydropriming)	8.75 d	20.33 d	0.73 b	0.0786 cde	11.71 h
	0.5 mM	9.33 d	20.69 d	0.61 def	0.0790 bcde	14.39 fg
	1 mM	8.66 d	20.08 d	0.63 def	0.0946 a	18.63 abc
Moderate (100 mM)	Control (unprimed)	6.62 e	18.28 e	0.65 cde	0.0873 abc	16.47 e
	0 mM (hydropriming)	6.75 e	14.56 f	0.59 efg	0.0876 ab	15.45 ef
	0.5 mM	6.21 ef	14.62 f	0.53 gh	0.0783 de	18.32 bc
	1 mM	5.69 fg	11.42 gh	0.54 gh	0.0943 a	20.04 a
Severe (150 mM)	Control (unprimed)	4.11 i	10.77 h	0.42 i	0.0803 bcde	18.11 cd
	0 mM (hydropriming)	5.08 gh	12.54 g	0.70 bc	0.0826 bcde	14.33 fg
	0.5 mM	4.21 hi	10.30 h	0.58 fg	0.0856 bcd	19.78 ab
	1 mM	4.83 hig	9.06 j	0.52 h	0.083 bcde	16.72 de

Different letters within the same columns indicate significant differences according to Duncan's multiple range test. FW, fresh weight. DW, dry weight.

Table 4 Effect of seed priming of indigo plants using putrescine concentrations on biochemical responses of seedlings under the salinity stress conditions

Treatments		Biochemical responses of seedlings		
Salinity (S)	Putrescine (P) (mM)	Total sugar (mg/g FW)	ROS (μmol/g FW)	MDA (μmol/g FW)
Control	Control (unprimed)	0.23 e	143.10 f	0.71 h
	0 mM (hydropriming)	0.38 a	171.86 ab	1.27 efg
	0.5 mM	0.30 bcd	157.00 f	1.93 bc
	1 mM	0.23 e	164.06 ae	1.49 de
Low (50 mM)	Control (unprimed)	0.28 cde	165.56 ad	2.07 b
	0 mM (hydropriming)	0.26 de	151.06 f	1.67 cd
	0.5 mM	0.26 de	147.38 f	0.96 gh
	1 mM	0.26 de	156.28 f	1.21 efg
Moderate (100 mM)	Control (unprimed)	0.36 ab	162.10 f	1.30 ef
	0 mM (hydropriming)	0.32 a-d	148.74 f	1.34 ef
	0.5 mM	0.34 abc	170.53 ab	1.04 fg
	1 mM	0.36 ab	143.88 f	1.41 de
Severe (150 mM)	Control (unprimed)	0.32 a-d	165.95 ad	2.00 b
	0 mM (hydropriming)	0.36 ab	178.69 a	2.65 a
	0.5 mM	0.36 ab	156.99 a	1.06 fg
	1 mM	0.37 ab	167.03 abc	1.25 efg

Different letters within the same columns indicate significant differences according to Duncan's multiple range test. ROS, reactive oxygen species and MDA, malondialdehyde

Under severe salinity stress, seed priming using 0.5 and 1 mM putrescine increased soluble sugars content by 16 and 18%, respectively. Stress causes an increase in metabolites related to adaptation, such as soluble sugars, in order to regulate osmosis and prevent the leaf's relative water content from decreasing [26]. The destruction and hydrolysis of larger molecules such as starch and their conversion into sugar compounds such as sucrose and then smaller molecules such as glucose and fructose as a result of salinity and drought stress cause the water potential in the cell membrane to become negative, and regulate osmosis [27]. Salinity stress affects the carbohydrates synthesis during photosynthesis as well as the transfer and use of these compounds in plant tissues and increases carbohydrate synthesis [28]. Accumulation of sugars under salt stress conditions is an adaptive mechanism that can improve salt tolerance in plants [29]. Despite the decrease in chlorophyll at salinity levels due to increased sugar, our findings suggest that indigo is likely a salinity-tolerant plant. According to some researchers [26,29], sugar accumulation under salt stress conditions is an adaptation mechanism that can improve salt tolerance in plants.

ROS increased by 16, 13, and 16%, under the low, moderate, and severe salinity stresses, respectively, compared to normal conditions (Table 4). Seed priming using 0.5 mM putrescine reduced ROS by 11 and 6% under the low and severe salinity stress, respectively and seed priming using 1 mM putrescine decreased ROS by 11% under the moderate salinity stresses, compared to control. Salinity stress causes the stomata to close, preventing carbon dioxide (CO2) from entering the leaves. This inhibits CO2 fixation and allows the chloroplast to stimulate extremely high levels of energy which increase ROS [30,31]. Reactive oxygen species (ROS) play a central role in the regulation of dormancy, germination, and seed decay [32,33]. Salinity stress through ROS damages the main molecules such as lipids, proteins and nucleic acids, enzymes, and DNA to molecular and cellular components [34]. Salinity levels of 50, 100, and 150 mM increased MDA by 192, 83, and 182%, respectively, when compared to normal conditions (Table 4). Seed priming using 0.5 mM putrescine decreased MDA by 54, 20, and 47%, respectively, under, low moderate and severe salinity stress when compared to control. Under salinity stress, an increase in ROS content causes peroxidation of lipid membranes and an increases in MDA [35]. Previous research also found that salinity stress (100 mM NaCl) increased the amount of MDA by 35 and 68% in wheat after 5 and 10 days of stress exposure [36]. Putrescine not only aids in growth and development, but also helps to tolerance of various abiotic stresses such as salinity, drought, high temperature, and cold. The main described mechanisms are related to free radical's inhibition, ABA regulation, lipid peroxidation prevention, cellular pH and ion balance maintenance, and cation channel regulation [37].

CONCLUSION

Today, the use of different substances such as amino acids can overcome the negative effects of salinity stress in plants. In this study, with increasing salinity levels, decreased the indices of seed germination and seedling growth, while increased total soluble sugars, ROS, and MDA. The seeds priming using putrescine 0.5 mM under saline conditions improved seed germination and seedling growth and had a reducing effect on the amount of MDA and ROS, improving seedlings biochemical status against oxidative stress.

REFERENCES

1. Salek Mearaji H., Tavakoli A, Ghanimati S, Kathyrlo P. The effect of salinity stress on traits related to Chenopodium quinoa germination. Plant Ecol. 2019;15:59-69.
2. Ganjali A.R., Ajorlo M., Khaksafidi A. The effect of drought and salinity stress on seed germination of (Alyssum homalocarpum). J. Crop Breed. 2017;21:133-146.
3. Tania S.S., Rhaman M.S., Hossain M.M. Hydro-priming and halo-priming improve seed germination, yield and yield contributing characters of okra (Abelmoschus esculentus L.). Trop Plant Res. 2020;7:86–93.
4. Zhu ZH., Sami A., Xu Q Q, Wu L.L., Zheng W.Y., Chen Z.P., Jin X.Z., Zhang H., Li Y., Yu Y. Effects of seed priming treatments on the germination and development of two rapeseed (Brassica napus L.) varieties under the co-influence of low temperature and drought. PLos One. 2021;16, e0257236.
5. Rhaman M.S., Imran S., Rauf F., Khatun M., Baskin C.C., Murata Y., Hasanuzzaman M. Seed priming with

phytohormones: An effective approach for the mitigation of abiotic stress. Plant. 2021;10:37.
6. Mousavi S.M., Omidi H., Mousavi S.A. The effect of biological pretreatments on growth and physiological germination indicators of fenugreek seedlings under natural salinity stress. Iran seed sci technol. 2022;11:117-145.
7. Khodabakhshi L., Seyedi A., Mazaheri-Tirani M., Parsa Motlagh B. Morphological and physiological responses of Indigofera tinctoria L. to putrescine under drought stress. Russ J Plant Physiol. 2023;70:1-22.
8. Bahrasemani S., Seyedi A., Fathi SH., Jowkar M. Improvement of germination indices and morphophysiological characteristics of indigo seedlings due to priming of seeds with pistachio wood vinegar under salinity stress. Iran J Plant Biolog. 2023; Accepted.
9. Salimi H., Ghorbani M. A study on seed germination of Avena ludoviciana and the effective factors in seed dormancy breaking, Bot J Iran. 2001;2:37-40.
10. Ranal M.A., De Santana D.G. How and why to measure the germination process? Revista Brasil, Botani. 2006;29:1-11.
11. Khajeh-Hosseini M., Lomholt A., Matthews S. Mean germination timein the laboratory estimates the relative vigour and field performance of commercial seed lots of maize (Zea mays L.). Seed Sci Technol. 2009;37:446-456.
12. Albalasmeh A.A., Berhe A.A.., Ghezzehei T.A. A new method for rapid determination of carbohydrate and total carbon concentrations using UV spectrophotometry. Carbohydr Polym. 2013;97:253-261.
13. Heath R.L., Packer L. Photoperoxidation in isolated chloroplasts: I. Kinetics and stoichiometry of fatty acid peroxidation. Arch Biochem Biophys. 1968;125:189-198.
14. Zeid I.M. Responses of been (Phaseolus vulgaris) to exogenous putrescine treatment under salinity stress. Pak. J. Biol. Sci. 2004;7:219-225.
15. Aslani L., Mobli M., Soleimani M. The effect of different concentrations of spermidine on seed germination of three cultivars of cucumber (Cucumis sativus) at low temperature. Ira Seed Res J. 2014;2:53-64.
16. Elkaco E. Osmo- and hydropriming enhance germination rate and reduce thermal time requirement of pea (Pisum sativum L. cv. Winner) seeds. Akademik Ziraat Dergisi. 2014;3:1-12.
17. Hossinifarahi M, Moazen H.A., Amiri A., Jowkar M.M., Mottaghipisheh J. Evaluation of seed priming and culture media to improve the germination performance and quality of sweet pepper and eggplant seedlings. Int J Hortic Sci Techno. 2022;l9:415-428.
18. Nascimento W.M., West SH. Muskmelon transplant production in response to seed priming. Horti technol. 1999;9:35-55.
19. Farsaraei S., Mehdizadeh L., Moghaddam M. Seed priming with putrescine alleviated salinity stress during germination and seedling growth of medicinal pumpkin. J Soil Sci Plant Nutr. 2021;21:1782–1792.
20. Malik J.A., AlQarawi A.A., AlZain MN, Dar BA, Habib MM, Ibrahim SNS. Effect of salinity and temperature on the seed germination and seedling growth of desert forage grass lasiurus scindicus henr. Sustainability. 2022;14:8387.
21. Çirka M., Kaya A.R., Eryiğit T. Influence of temperature and salinity stress on germination and seedling growth of soybean (Glycine max L.). Legum Res seed. 2021;44:1053-1059.
22. Aydin M., Hossein Pur A., Haliloglu K., Tosun M. Effect of putrescine application and drought stress on germination of wheat (Triticum aestivum L.). J Agric Facult. 2015;46:43-55.
23. Afzal I., Rauf S., Basra S.M.A., Murtaza G. Halopriming improves vigor, metabolism of reserves and ionic contents in wheat seedlings under salt stress. Plant Soil Environ, 2008;54:382–388.
24. Guo R., Yang Z., Li F., Yan C., Zhong X., Liu Q., Xia X., Li H., Zhao L. Comparative metabolic responses and adaptive strategies of wheat (Triticum aestivum L.) to salt and alkali stress. BMC Plant Biol. 2015;15:170.
25. Quinet M, Ndayiragije A, Lefevre I, Lambillotte B, Dupont-Gillain C.C., Lutts S. Putrescine differently influences the effect of salt stress on polyamine metabolism and ethylene synthesis in rice cultivars differing in salt resistance. J Exp Bot. 2010;61:2719–2733.
26. García-Caparrós P., Teresa Lao M. The effects of salt stress on ornamental plants and integrative cultivation practices. Sci Hort. 2018;240:430-439.
27. Bartels D., Sunkar R. Drought and salt tolerance in plants. Crit Rev Plant Sci. 2005;24:23–58.
28. Gupta B., Huang B. Mechanism of salinity tolerance in plants: physiological, biochemical, and molecular characterization. Int J Genomics. 2014;701596.
29. Karimian Z, Samiei L. Mitigation of salt stress by mycorrhizal inoculation on Nitraria schoberi as a native landscape plant in the arid regions. Desert. 2021;26:16-27.
30. Hasanuzzaman M., Nahar K., Fujita M. Plant response to salt stress and role of exogenous protectants to mitigate salt-induced damages. Ecophysiology and responses of plants under salt stress. 2013;25–87.
31. Farooq M., Hussain M., Wakeel A., Siddique KH.M. Salt stress in maize effects resistance mechanisms and management: A review. Agron Sustain Dev. 2015; 35: 461–48.
32. Kurek K., Plitta-Michalak B., Ratajczak E. Reactive oxygen species as potential drivers of the seed aging process. Plants Basel. 2019;8:174.

33. Considine M.J., Foyer CH. Stress effects on the reactive oxygen species-dependent regulation of plant growth and development. J Exp Bot. 2021;72:5795–5806.

34. Sachdev S., Ansari S.A., Ansari M.I., Fujita M., Hasanuzzan M. Abiotic stress and reactive oxygen species: generation, signaling, and defense mechanisms. Antioxidunts. 2021;10:277.

35. Ebrahimi A., Naqvi M.R., Sabokdast M. Comparison of different species of barely landraces in terms of chlorophyll, carotenoids, protein and enzyme. Crop Sci. 2010;41:57-65.

36. Zou P., Li K., Liu S., He X., Zhang X., Xing R., Li P. Effect of sulfated chitooligosaccharides on wheat seedlings (Triticum aestivum L.) under salt stress. J Agri Food Chem. 2016;64:2815–2821.

37. Gill S.S., Tuteja N. Polyamines and abiotic stress tolerance in plants. Plant Signal Behav. 2010;5:26–33.