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PACLOBUTRAZOL INDUCED NON-ENZYMATIC ANTIOXIDANTS AND POLYAMINE LEVELS IN SOYBEAN PLANTS GROWN UNDER SALINITY STRESS

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Abstract

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Paclobutrazol (PBZ) is a member of the triazole family of plant growth regulators and is known to protect crops from environmental stresses such as salinity and drought. It plays a role in maintaining water balance, photosynthesis capacity and enhancing antioxidant enzyme activities. Despite the well-documented effects of PBZ on antioxidant defence in soybean plants, the changes in non-enzymatic antioxidants and endogenous polyamines in the PBZ-treated plants grown under salinity stress are still not studied. Herewith, the study aimed to clarify the effects of PBZ on these molecules. For this purpose, the combined effect of salt (250 mM NaCl) and three different concentrations of PBZ (5, 10 and 20 ppm) on soybean (*Glycine max* L.) plants were studied. Proline, hydroxyl radical ($OH^{\cdot}$) scavenging capacity, non-enzymatic antioxidants (anthocyanin, flavonoid, phenolic and free thiol-containing compounds) and polyamines spermine, spermidine, and putrescine were investigated in the treated plants. We found that the PBZ (10 ppm) treatment was the most effective concentration to counteract oxidative stress due to NaCl in soybean seedlings. It significantly increased hydroxyl radical scavenging activity, proline, total phenolics, flavonoids, and conjugated polyamine content compared to those found in salt-treated plants only. For the first time, our study showed that PBZ could induce the non-enzymatic antioxidant defence in soybean plants grown under salt stress conditions.

Keywords: paclobutrazol, phenolics, polyamines, salinity stress, soybean.

INTRODUCTION

Triazole compounds are synthetic plant growth regulators that act as antigibberellins and retard shoot growth in plants. Paclobutrazol (PBZ) is a triazole compound used as a growth retardant that inhibits shoot length elongation (Mackay et al., 1990; Jaleel et al., 2007). PBZ inhibits ent-kaurene oxidase activity, a key enzyme in the GA biosynthetic pathway that catalyses the oxidation of ent-kaurene to ent-kaurenoic acid (Kondhare et al., 2014). Besides this, triazole compounds protect plants from different stress factors (drought, waterlogging, salt, chilling) (Fletcher et al., 2000). They effectively maintain membrane stability, enhance osmolytes and hormones, photosynthesis activity and maintain (enhance) yield under stress conditions (Soumya et al., 2017).

Inhibition of growth and development, reduction in photosynthesis, imbalance in water status, overproduction of free radicals, and finally, crop yield losses occur due to soil salinity worldwide (van Zelm et al., 2020). The reactive free radicals are byproducts of normal metabolism, which in excess can induce oxidative damage to DNA, proteins, membrane lipids and carbohydrates (Hasanuzzaman et al., 2020). Reactive oxygen species (ROS) include superoxide anion (O_2^-), hydroxyl radical ($OH^·$), singlet oxygen, hydrogen peroxide (H_2O_2). Plants accumulate low-weight molecular antioxidant compounds under stress conditions to mitigate oxidative injury and detoxify ROS molecules (Hasanuzzaman et al., 2020). These are so-called non-enzymatic antioxidants (phenolic compounds, flavonoids, ascorbic acid and reduced glutathione). Anthocyanins are water-soluble pigments derived from flavonoids – a group of secondary metabolites of phenolic nature, which are synthesised *via* the phenylpropanoid pathway (Samanta et al., 2011). These compounds accumulate in tissues and organs such as flowers, leaves, stems, shoots, and grains under the influence of environmental stimuli (Ishtiaq et al., 2020). They have antioxidant properties and can function as ROS scavengers because of hydroxyl groups in their structure (Chutipaijit et al., 2009).

Polyamines, putrescine (Put), spermidine (Spd), and spermine (Spm) are low molecular aliphatic polycations found in a wide range of living organisms ranging from bacteria to plants and animals. It is known that polyamines play roles in plant growth and development and programmed cell death, but they affect plants' defence under abiotic stress (Bouchereau et al., 1999; Alcázar et al., 2020). In addition, PAs are considered ROS scavenger compounds because of their pH maintaining capabilities and hydrophilic properties (Kova'cs et al., 2010).

Soybean (*Glycine max* L.) is worldwide grown as an oilseed and protein-rich crop (Wang & Komatsu, 2017). It is considered a salt-sensitive glycophyte. All developmental stages of soybean are adversely affected by salinity stress (Phang et al., 2008). In addition, soil salinity provokes critical alterations in soybean metabolism, including changes in antioxidant defence machinery (Phang et al., 2008; Sadak et al., 2020).

The present study aimed to investigate the effects of paclobutrazol on soybean salt tolerance, which possibly is related mainly to non-enzymatic antioxidants. Many studies report that paclobutrazol has positive effects on water balance, nutrient uptake, photosynthetic pigments, and antioxidant enzymes in different crops (Kamran et al., 2020; Desta & Amare, 2021). However, there is no study on the non-enzymatic antioxidants in soybean plants treated with PBZ under salinity stress. Therefore, we aimed to determine phenolics, flavonoids, anthocyanins, hydroxyl radical scavenging activity, low-molecular thiols, polyamines, and proline content applying different doses of PBZ to salt-treated soybean plants. We hypothesised that PBZ might positively influence the non-enzymatic antioxidant defence of salt-treated soybean.

MATERIALS AND METHODS

Experimental design and plant material

Soybean (*Glycine max* (L.) Merr.) SA88 seeds were obtained from a commercial provider (Agrova, Adana, Turkey). The surface of seeds was sterilised in 5% sodium hypochlorite for 10 min, rinsed five times with distilled water, and then allowed to germinate. The seeds were sown in plastic pots, each of which (width 20 cm, length 12 cm, height 18 cm) contained five seeds and the mixture of soil: clay: clay-loam in the ratio of 2:1:1, pH 6.5. Soil composition contained organic matter mix 78–79%, electrical conductivity (EC) 0.45 mm hos cm^{-1}, $CaCO_3$ 0.08%, cation exchange capacity (CEC) 68.04 mEq 100 g^{-1}, ash content was around 5%. In addition, its other properties were humidity 58%, water holding capacity 360.85%, specific gravity 1.23 gr cm^{-3}, porosity 84.07% (Cokuysal et al., 2006). After germination, seedlings were grown in a growth room at 25°C (16 h day/8 h night photoperiod), a light intensity of 350 µmol m^{-2} s^{-1} and watered with Hoagland solution, 25 ml per pot, for eight days (Hoagland & Arnon, 1950).

The pots with germinated seedlings were divided into eight treatments. The experimental design included the eight treatment groups: (1) Control – watered with Hoagland solution; (2) NaCl – watered with NaCl 250 mM; (3) PBZ_1 – watered with PBZ 5 ppm alone; (4) PBZ_2 – watered with PBZ 10 ppm alone; (5) PBZ_3 – watered with PBZ 20 ppm alone; (6) PBZ_1 + NaCl – watered with PBZ_1 and NaCl; (7) PBZ_2 + NaCl – watered with PBZ_2 and NaCl; (8) PBZ_3 + NaCl – watered with PBZ_3 and NaCl. Each variant contained three pots (a total of 40 plants). The pots were watered with 25 ml of respective solutions for ten days. Then, the seedlings were harvested on the 10th day, and the samples were preserved at -80°C. Finally, the PBZ (5, 10, 20 ppm) concentrations and NaCl were applied with Hoagland's solution in the soil medium. All chemicals used in the experiment were purchased from Sigma-Aldrich Co.

Biochemical Analysis

Content of free thiol-containing compounds. Approximately 20 mg of dry leaf material was ground with 0.1% (w/v) trichloroacetic acid, and homogenates were centrifuged for 30 min (15 000 × g, 4°C). The supernatant was used for the determination of the content of free thiol-containing compounds using Ellman's reagent (5,5′-dithiobis-(2-nitrobenzoic acid) according to the original method of Ellman (1959). The absorbance was recorded at 412 nm, and the quantity was calculated using a molar extinction coefficient of 13.6 mM^{-1} cm^{-1}.

Anthocyanin and flavonoid analysis. Fresh leaf material (0.1 g) was homogenised by adding 6 ml of MeOH:HCl:H_2O solution (79:1:20, v:v:v). The homogenate was centrifuged at 10000 rpm at 4°C for 20 minutes, and the resulting supernatant was used to determine anthocyanins and flavonoids. Leaf anthocyanin content was assessed by absorbance measurements at 530 nm, corrected for chlorophyll absorbance at 657 nm (Mancinelli et al., 1975). The results are expressed as anthocyanin content per gram of fresh weight.

The supernatant absorbance values were read on UV spectrophotometer at 300 nm (Mirecki & Teramura, 1984). The number of flavonoids in leaf tissues was compared to the control and was calculated as a percentage.

Proline content. The proline contents of roots were determined according to Bates et al. (1973). For each treatment, 0.5 g leaf sample was ground in a mortar with 5 ml of a 3% (w/v) aqueous sulfosalicylic acid solution. The homogenate was centrifuged for 30 min (15.000 × g, 4°C). The supernatant was mixed with glacial acetic acid, and ninhydrin reagent and the reaction mixture was incubated in a boiling water bath for 1 h. After terminating the reaction in ice, the absorbance was read at 520 nm. The proline content was determined by a standard curve and calculated on a fresh weight basis.

The scavenging ability of hydroxyl radical. Hydroxyl radical scavenging activity was measured according to the modified method given by Chung et al. (1997). The method is based on the ability of fusiformis extracts to scavenge the hydroxyl radical generated by the Fenton reaction. Competition between deoxyribose and the sample for $OH^{\cdot}$ generated from the Fe^{3+}/ascorbate/EDTA/H_2O_2 system was measured to determine the $OH^{\cdot}$ scavenging activity. The reaction mixture contained 0.3 ml of 20 mM Na-phosphate buffer (pH 7.0), 0.15 ml of 10 mM 2-deoxyribose, 0.15 ml of 10 mM $FeSO_4$, 0.15 ml of 10 mM EDTA, 0.15 ml of 10 mM H_2O_2, 0.525 ml H_2O, and 0.075 ml sample. The mixture was incubated at 37°C for 2 h. A mixture of 0.75 ml of 2.8% (w/v) trichloroacetic acid and 0.75 ml of 1.0% (w/v) thiobarbituric acid in 50 mM NaOH was added to the test tubes and boiled for 20 min. After the mixture cooled, absorbance was measured at 520 nm against a blank solution. The scavenging activity on hydroxyl radicals was calculated as follows: $[(A0 - A1)/A0 \times 100]$, where A0 is the absorbance of the control reaction and A1 is the absorbance in the presence of the resveratrol sample.

Total phenolics content. Approximately 20 mg of dry leaf material was ground with 0.1% (w/v) trichloroacetic acid, and homogenates were centrifuged for 30 min (15.000 × g, 4°C). The supernatant was used to determine the content of total phenolics by incubation with Folin-Ciocalteu reagent and sodium carbonate following the experimental procedure of Swain & Goldstein (1964). The absorbance of the reaction mixture was read at 725 nm. The total phenolic content was calculated as gallic acid equivalents using a standard curve prepared with known amounts of gallic acid.

Determination of polyamine levels. The content of free putrescine, spermidine and spermine was measured in the supernatant by direct dansylation after extraction with 5% TCA according to Smith & Best (1977) method. Conjugated PAs were measured in HCl hydrolysed supernatants (soluble conjugated PAs) and in HCl hydrolysed pellets (insoluble conjugated PAs) according to Torrigiani et al. (1989) method. After hydrolysis, both conjugated fractions of PAs were dansylated. The polyamines were separated by thin-layer chromatography (TLC) using cyclohexane: ethylacetate (3:2 v/v) solvent system on precoated plates of Silicagel G 60 (Merck). Spots were visualised using

UV light, scraped off and eluted in 2 ml anhydrous acetone. The fluorescence was measured (excitation 360 nm, emission 505.5 nm) on Spectrofluorophotometer RF-1601 (Shimadzu, Japan). The data were compared with dansylated polyamine standards loaded on the same TLC plate, treated as extracted samples and expressed as nmol per g DW of plant material.

Statistical analysis

The experiment was conducted in a completely randomised design, and measurements were performed with six replicates (n = 6). Statistical variance analysis of the data was performed using ANOVA, and differences among treatments were compared using Tukey's honestly significant difference post hoc analysis at 5% level. One-Way Analysis of Variance (ANOVA) was performed to compare the values of the parameters studied in each group. The statistical significance level (a) was taken as 5% in the calculations, and the SPSS (IBM SPSS for Windows, ver.24) statistical package programme was used for analysis.

RESULTS

Proline content. All treatments provoked an increase in proline quantity in soybean leaves. Application of 250 mM NaCl increased proline content by 43%, although PBZ$_3$ (20 ppm) application significantly augmented its content compared to the control (88.5%) (Table 1). In the present study, the PBZ treatments under salt stress conditions led to a significant increase in proline content (by 44.7%, by 80.2% and by 53.2%, respectively) compared to the salt treatment alone (Table 1).

Table 1. NaCl and paclobutrazol (PBZ) effects on free proline content, $^{\bullet}$OH radical scavenging capacity, and the content of free thiol groups containing compounds (SH) in soybean leaves (*Glycine max* L.). Treatments: Control, NaCl 250 mM, PBZ$_1$ 5ppm, PBZ$_2$ 10 ppm, PBZ$_3$ 20 ppm, NaCl + PBZ$_1$ 5 ppm, NaCl + PBZ$_2$ 10 ppm, NaCl + PBZ$_3$ 20 ppm. Different letters within columns represent significantly different mean values ($p < 0.05$)

Test variant	Proline mkmol/gDW	Hydroxyl radical scavenging capacity	SH mkmol/ gDW
Control	14.1 ± 0.9^{h}	1.1 ± 0.2^{h}	7.05 ± 0.06^{b}
NaCl	20.2 ± 0.7^{g}	5.3 ± 0.2^{e}	5.04 ± 0.04^{c}
PBZ$_1$	20.3 ± 1.0^{f}	5.4 ± 0.2^{c}	6.32 ± 0.10^{c}
PBZ$_2$	25.2 ± 0.8^{e}	4.5 ± 0.2^{g}	3.56 ± 0.10^{g}
PBZ$_3$	26.6 ± 0.6^{d}	4.6 ± 0.3^{f}	4.21 ± 0.10^{f}
PBZ$_1$ + NaCl	29.2 ± 0.7^{c}	5.4 ± 0.2^{d}	4.78 ± 0.06^{e}
PBZ$_2$ + NaCl	36.1 ± 0.5^{a}	7.7 ± 0.3^{a}	8.42 ± 0.22^{a}
PBZ$_3$ + NaCl	30.9 ± 0.8^{b}	6.4 ± 0.1^{b}	3.66 ± 0.16^{g}

Hydroxyl radical scavenging capacity. The NaCl application increased hydroxyl radical scavenging activity by 4.7 fold. PBZ$_1$ (5 ppm) also increased significantly (4.8 fold) this parameter compared to the control (Table 1). Moreover, the PBZ application under salt stress caused an additional increase in hydroxyl radical scavenging activity (by 1.7, 45.1, and 19.6%, respectively) compared to the salt treatment alone (Table 1).

Total thiol-containing compounds content. Free thiol-containing (SH) compounds content (Table 1) was decreased by the NaCl application (28.5%). The application of PBZ, both alone and in combination with NaCl, also led, albeit to varying degrees, to a decrease in the SH content compared to the control, except the combination of PBZ$_2$ (10 ppm) and NaCl. The PBZ$_2$ (10 ppm) treatment led to 67.1% increase in SH content under salt stress compared to the salt treatment alone.

Total phenolic content. Our results showed that after all single treatments, total phenolic content was increased, and the effect of NaCl was most pronounced (by 67.4%) compared to the control (Table 2). On the other hand, compared to salt stress only, the PBZ$_1$ (5 ppm) and PBZ$_3$ (20 ppm) treatments decreased phenolics by 21.2% and by 14.6%,

Table 2. NaCl and paclobutrazol (PBZ) effects on the content of total phenolics, anthocyanins, and flavonoids in soybean leaves (*Glycine max* L.). Treatments: Control, NaCl 250 mM, PBZ$_1$ 5 ppm, PBZ$_2$ 10 ppm, PBZ$_3$ 20 ppm, NaCl + PBZ$_1$ 5 ppm, NaCl + PBZ$_2$ 10 ppm, NaCl + PBZ$_3$ 20 ppm. Different letters within columns represent significantly different mean values ($p < 0.05$)

Test variant	Phenolics mkmol/gDW	Anthocyanins mg/gFW	Flavonoids %
Control	11.6 ± 0.2^{h}	0.084 ± 0.001^{a}	100.0 ± 0.5^{b}
NaCl	19.4 ± 0.2^{b}	0.068 ± 0.002^{bc}	74.7 ± 0.9^{e}
PBZ$_1$	16.1 ± 0.2^{d}	0.085 ± 0.001^{a}	88.8 ± 0.8^{c}
PBZ$_2$	12.7 ± 0.2^{g}	0.071 ± 0.001^{bc}	50.0 ± 0.5^{g}
PBZ$_3$	12.9 ± 0.2^{f}	0.073 ± 0.000^{bc}	119.7 ± 0.5^{a}
PBZ$_1$ + NaCl	15.3 ± 0.3^{e}	0.074 ± 0.001^{bc}	52.1 ± 0.5^{f}
PBZ$_2$ + NaCl	23.7 ± 0.2^{a}	0.067 ± 0.002^{c}	85.5 ± 0.5^{d}
PBZ$_3$ + NaCl	16.5 ± 0.2^{c}	0.069 ± 0.002^{bc}	48.6 ± 0.7^{h}

respectively, while the PBZ$_2$ (10 ppm) application additionally increased phenolic quantity by 22.6% under the salinity conditions.

Anthocyanin content. Plants treated with NaCl showed lower anthocyanin content than the control (by 15%), while PBZ$_1$ (5 ppm) caused an increase by 6.2% compared to the control group. The rest of the PBZ treatments either applied alone or in combination with salinity stress, did not significantly change the leaf's anthocyanin content (Table 2).

Flavonoid content. Most treatments decreased the flavonoid content in soybean leaves compared to the control (Table 2). However, only the PBZ$_3$ (20 ppm) application increased it significantly (by 20%). Nevertheless, the PBZ$_2$ (10 ppm) treatment alleviated (14.4%) the reduction in flavonoid content caused by salinity in soybean, while the PBZ$_1$ (5 ppm) and PBZ$_3$ (20 ppm) applications additionally decreased it (by 30.3% and by 35.1%) compared to salt stress only (Table 2).

Polyamine content. The changes in the content of different polyamine fractions in soybean leaves varied depending on the treatments (Tables 3–5). The content of free PAs was not altered significantly, except for free putrescine, which raised two times above the control after the NaCl application (Table 3), compared to soluble- and insoluble-conjugated fractions. No soluble-conjugated putrescine (Table 3) was found after the NaCl treatment. Salt stress also caused a substantial reduction in the soluble-conjugated fraction of spermidine by 36% (Table 4). There was a remarkable increase in insoluble-conjugated PA content (Tables 3–5), especially after PBZ$_2$ (10 ppm), which augmented spermine by 80% compared to the control level. Meanwhile, most of the soluble-conjugated polyamines (Tables 3–5) were lower than the respective controls after the PBZ alone applications. The highest decline by 72% due to PBZ$_2$ (10 ppm) was detected in soluble-conjugated putrescine (Table 3), while PBZ$_1$ (5 ppm) raised soluble-conjugated spermine by 35.5% (Table 5). Besides this, the combination treatments of

Table 3. NaCl and paclobutrazol (PBZ) effects on the content of free, soluble-conjugated and insoluble-conjugated putrescine in soybean leaves (*Glycine max* L.). Treatments: Control, NaCl 250 mM, PBZ$_1$ 5 ppm, PBZ$_2$ 10 ppm, PBZ$_3$ 20 ppm, NaCl + PBZ$_1$ 5 ppm, NaCl + PBZ$_2$ 10 ppm, NaCl + PBZ$_3$ 20 ppm. Different letters within columns represent significantly different mean values ($p < 0.05$)

Test variant	Free putrescine nmol/gDW	Soluble-conjugated putrescine nmol/gDW	Insoluble-conjugated putrescine nmol/gDW
Control	642 ± 33^{a}	610 ± 22^{d}	146 ± 2^{ab}
NaCl	1280 ± 89^{b}	n.d.	127 ± 8^{a}
PBZ$_1$	658 ± 32^{a}	310 ± 23^{b}	168 ± 8^{bcd}
PBZ$_2$	541 ± 2^{a}	172 ± 3^{a}	168 ± 8^{bcd}
PBZ$_3$	611 ± 34^{a}	525 ± 51^{c}	180 ± 6^{bc}
PBZ$_1$ + NaCl	637 ± 14^{a}	503 ± 14^{c}	159 ± 3^{bcd}
PBZ$_2$ + NaCl	633 ± 22^{a}	709 ± 12^{e}	170 ± 6^{bcd}
PBZ$_3$ + NaCl	629 ± 41^{a}	478 ± 25^{c}	184 ± 12^{d}

Table 4. NaCl and paclobutrazol (PBZ) effects on the content of free, soluble-conjugated and insoluble-conjugated spermidine in soybean leaves (*Glycine max* L.). Treatments: Control, NaCl 250 mM, PBZ$_1$ 5 ppm, PBZ$_2$ 10 ppm, PBZ$_3$ 20 ppm, NaCl + PBZ$_1$ 5 ppm, NaCl + PBZ$_2$ 10 ppm, NaCl + PBZ$_3$ 20 ppm. Different letters within columns represent significantly different mean values ($p < 0.05$)

Test variant	Free spermidine nmol/gDW	Soluble-conjugated spermidine nmol/gDW	Insoluble-conjugated spermidine nmol/gDW
Control	2019 ± 59^{b}	1360 ± 8^{d}	432 ± 16^{a}
NaCl	2199 ± 13^{b}	867 ± 44^{a}	408 ± 20^{a}
PBZ$_1$	2052 ± 55^{b}	1308 ± 16^{c}	526 ± 23^{b}
PBZ$_2$	1743 ± 44^{a}	1115 ± 23^{b}	585 ± 24^{bc}
PBZ$_3$	2007 ± 49^{b}	1233 ± 46^{c}	528 ± 26^{b}
PBZ$_1$ + NaCl	2014 ± 93^{b}	1393 ± 12^{d}	462 ± 16^{a}
PBZ$_2$ + NaCl	2002 ± 34^{b}	1716 ± 84^{e}	544 ± 16^{b}
PBZ$_3$ + NaCl	2073 ± 29^{b}	1258 ± 94^{c}	644 ± 15^{c}

Table 5. NaCl and paclobutrazol (PBZ) effects on the content of free, soluble-conjugated and insoluble-conjugated spermine in soybean leaves (*Glycine max* L.). Treatments: Control, NaCl 250 mM, PBZ$_1$ 5 ppm, PBZ$_2$ 10 ppm, PBZ$_3$ 20 ppm, NaCl + PBZ$_1$ 5 ppm, NaCl + PBZ$_2$ 10 ppm, NaCl + PBZ$_3$ 20 ppm. Different letters within columns represent significantly different mean values ($p < 0.05$)

Test variant	Free spermine nmol/gDW	Soluble-conjugated spermine nmol/gDW	Insoluble-conjugated spermine nmol/gDW
Control	3404 ± 117^{bc}	2747 ± 104^{b}	739 ± 45^{a}
NaCl	3744 ± 164^{c}	2549 ± 150^{b}	797 ± 39^{ab}
PBZ$_1$	3244 ± 64^{ab}	3725 ± 64^{c}	1001 ± 34^{c}
PBZ$_2$	2885 ± 92^{a}	2249 ± 92^{a}	1330 ± 92^{d}
PBZ$_3$	3147 ± 164^{ab}	2284 ± 77^{a}	953 ± 28^{bc}
PBZ$_1$ + NaCl	3244 ± 235^{ab}	4194 ± 161^{d}	724 ± 22^{a}
PBZ$_2$ + NaCl	3532 ± 191^{bc}	3909 ± 66^{d}	944 ± 31^{bc}
PBZ$_3$ + NaCl	3653 ± 39^{bc}	3678 ± 43^{cd}	1234 ± 33^{d}

PBZ and salinity increased soluble-conjugated and insoluble-conjugated (Tables 3–5) polyamine content significantly compared to NaCl alone. While insoluble-conjugated polyamines increased in a dose-dependent manner due to the PBZ treatments under salinity, PBZ_2 (10 ppm) was the most effective concentration on soluble-conjugated putrescine (Table 3) and spermidine (Table 4) in combination with NaCl, compared to salinity stress alone.

DISCUSSION

Salinity stress affects various physiological and metabolic processes, which leads to inhibited plant growth and decreased crop production. Soil salinity disturbs plant metabolism in the form of osmotic stress, which is then followed by ion toxicity (Gupta & Huang, 2014). Our recent study (unpublished data under review) showed that the PBZ application caused a reduction in salt-induced damages and an increase in biomass yield, water status, chlorophyll content of salt-treated soybean seedlings. PBZ regulated enzymatic antioxidants and alleviated the oxidative damages under salinity through induction of Na^+/H^+ antiporter *GmNHX1* and a chloride channel *GmCLC1* gene expressions and substantial reduction of Na^+ and Cl^- toxic ion levels. Along with the antioxidant enzymes, the biosynthesis of non-enzymatic antioxidants is another part of the antioxidant defence that higher plants have developed to reduce oxidative damage (Gill & Tuteja, 2010). This study expands our investigation on the effect of PBZ applied to salt-treated soybean by determining non-enzymatic antioxidants content.

Compounds that contain free thiol groups possess antioxidant properties and are an important part of a plant defence system. Their accumulation, particularly glutathione, the most abundant low-molecular-weight thiol in plants (Zagorchev et al., 2013; Khunpon et al., 2018), is regarded as a favourable response, while their decline is accounted as an adverse effect of stress. We found that free SH content was decreased by salinity stress in soybean, which probably indicates impaired antioxidant machinery (Table 1). In the present study, the PBZ_2 (10 ppm) treatment under salt stress increased the free SH content compared to the salt treatment alone, which possibly assisted plants in coping with ROS damages caused by salinity. Similar findings were reported by Jaleel et al. (2008), who showed a significant decrease in glutathione content after salt treatment of *Withania somnifera* plants. This decrease was mitigated by triazole derivative, which provided a sufficient redox state of cells to cope with salinity-induced ROS damages. Accordingly, salt stress caused a decline in the content of reduced glutathione in rice and pea plants, while the PBZ pretreatment also increased GSH content under salt stress, as it has recently been reported (Khunpon et al., 2018; Sofy et al., 2020).

Phenolics are secondary metabolites that play an essential role under salt stress conditions (Waśkiewicz et al., 2013). Flavonoids are a significant class of phenolic compounds in plant cells that accumulate in response to salinity stress in different plant species (Sharma et al., 2019; Kiani et al., 2021). It has been shown that salinity increases anthocyanin content (Dkhil & Denden, 2012). We found that salinity increased total phenolic levels compared to the control while reducing flavonoids and anthocyanins content (Table 2). It could be suggested that other members of the phenolic compounds, rather than flavonoids and anthocyanins, could also be increased in soybean leaves under salinity. Similarly, PBZ applied alone increased phenolics, but reduced flavonoids (except PBZ_3) and anthocyanins (except PBZ_1). Otherwise, only the PBZ_2 treatment combined with NaCl increased total phenolics and flavonoids content compared to salinity stress alone. In contrast, the PBZ application under salt stress did not cause a significant change in anthocyanins content. Our results parallel Hassanpour et al. (2013) that various triazolic compounds could enhance the number of phenolic compounds in plant cells. In addition, it has been reported that triazole treatment increases d anthocyanin content in maize grown under water deficit compared to drought-stressed plants. Still, the increase is slightly, and the anthocyanin level remains below the control (Rajasekar et al., 2016).

It had been widely discussed earlier that the increase of proline was a marker of stress. Nowadays, it has been accepted that its accumulation ensures membrane stability and mitigates the adverse effects of abiotic stresses like water deficit and NaCl (Dar et al., 2016). In our study, salinity increased proline levels of soybean (Table 1). Similarly, previous investigations have reported that salt treatment

(150 mM) significantly increases proline content in soybean compared to control (Dinler et al., 2016). We found that the PBZ application alone considerably increased proline concentration in leaves. The PBZ treatment led to an additional increase in proline content under salt stress compared to the salt treatment alone. This suggests an enhanced ability of the PBZ-treated plants to counteract the adverse effects of salinity. Similarly, Forghani et al. (2020) have found that sweet sorghum plants had an increased proline level under the PBZ and salt treatment. Most likely, proline as compatible solute is accumulated due to the PBZ application under salinity to stabilise the cellular water potential and water uptake.

Besides proline, other nitrogen-containing endogenous protectors such as PAs are included in the responses of plants to mitigate oxidative stress. PAs occur in a free form, but can also conjugate with small molecules such as phenolic acids (soluble-conjugated) or biomacromolecules like proteins and nucleic acids (insoluble-conjugated) (Todorova et al., 2013). The conjugation of polyamines (especially soluble-conjugated fraction) is discussed as a regulatory mechanism of the free PAs pool and as a contribution in the control of various cellular functions as scavenging of free radicals and antioxidant activity under stress conditions. In addition, the insoluble-conjugated polyamines ensure the membrane stability of diverse cell constituents (Minocha et al., 2014; Alcázar et al., 2020).

The accumulation of PAs is considered to take part in the plants' tolerance to abiotic and biotic stress. In this study, there was no remarkable change in free PAs content after the PBZ treatments. However, a significant accumulation of free putrescine (Table 3) was detected after the alone NaCl treatment. In contrast, no soluble-conjugated putrescine fraction was detected, suggesting its complete liberation from soluble conjugates with phenolic acids. Often the accumulation of free diamine is accepted to indicate stress injury in different plants subjected to environmental stress (Todorova et al., 2007; Minocha et al., 2014; Pál et al., 2015; Nahar et al., 2016).

On the contrary, the PBZ applications to salt-treated plants diminished NaCl-induced accumulation of free putrescine, suggesting fewer stress injuries. Accordingly, Wang & Steffens (1985) and Forghani et al. (2020) have also shown that the increased free polyamine levels in mango and sweet sorghum due to water stress or NaCl, respectively, are reduced near to control by the PBZ treatment. Nowadays, it is believed that higher aliphatic polyamines spermidine and spermine play a role in plant defence. Their accumulation (particularly their conjugated fractions) is associated with the plant tolerance to salinisation (Todorova et al., 2013; Gupta & Huang, 2014). We found that the NaCl treatment did not change considerably conjugated spermidine and spermine fractions (Tables 4 and 5), which probably indicates the impaired ability of salt-treated soybean to counteract stress. In contrast, the application of PBZ led to a significant increase in the soluble- and the insoluble-conjugated polyamines in NaCl-treated plants. As endogenous protectors, Spd and Spm could stabilise cell membranes and/or affect cell ionic balance through activation of H^+-ATPases (Liu et al., 2006; Todorova et al., 2013). In addition, conjugated polyamines were involved indirectly scavenging ROS at salt stress because their molecules are positively charged (Todorova et al., 2013; Saha et al., 2015). Respectively, the induced accumulation of conjugated polyamine fractions by the PBZ treatment in soybean subjected to NaCl could ensure the increased capacity of these plants to counteract stress injuries.

As one of the reactive oxygen species, the hydroxyl radical can attack all types of biomolecules, including enzymes. Many reports state that increased tolerance to stress is related to higher antioxidant capacity. This study showed that the PBZ treatments alone induced the hydroxyl radical scavenger capacity 4.8 fold, and it was even higher when PBZ was combined with NaCl (Table 1). This finding confirms that PBZ could reduce the oxidative damage due to high salinity by increasing the phenolic, flavonoid, proline, SH and conjugated polyamine contents in soybean plants.

CONCLUSION

This study showed that PBZ_2 (10 ppm) was the most effective dose, specifically increasing free SH, phenolics, flavonoids, proline, conjugated polyamine levels, and hydroxyl scavenging activity in soybean plants grown under salinity conditions. Furthermore, we demonstrated that PBZ could induce the non-enzymatic antioxidant defence in salt-treated soybean leaves. The study complements our earlier research on enzymatic antioxidants in the same model system, which is helpful in the elucidation of the plant stress tolerance mechanisms.

REFERENCES

Alcázar R., Bueno M., Tiburcio A.F., 2020: Polyamines: Small amines with large effects on plant abiotic stress tolerance. – Cells, 9(11): 2373. https://doi.org/10.3390/cells9112373

Bates L., Waldren R., Teare I., 1973: Rapid determination of free proline for water-stress studies. – Plant and Soil, 39: 205–207. https://doi.org/10.1007/BF00018060

Bouchereau A., Aziz A., Larher F., Martin-Tanguy J., 1999: Polyamines and environmental challenges: recent development. – Plant Science, 140(2): 103–125. https://doi.org/10.1016/S0168-9452(98)00218-0

Chung S.K., Osawa T., Kawakishi S., 1997: Hydroxyl radical scavenging effects of spices and scavengers from brown mustard (*Brassica nigra*). – Bioscience Biotechnology and Biochemistry, 61: 118–123. https://doi.org/10.1271/bbb.61.118

Chutipaijit S., Cha-um S., Sompornpailin K., 2009: Differential accumulations of proline and flavonoids in indica rice varieties against salinity. – Pakistan Journal of Botany, 41: 2497–2506.

Cokuysal B., Cakici H., Erbas E., 2006: The effect of salinity and fertilizer applications on leaf nutrient status and some quality characteristics of *Ficus benjamina*. – International Journal of Botany, 2: 107–112. https://dx.doi.org/10.3923/ijb.2006.107.112

Dar M.I., Naikoo M.I., Rehman F., Naushin F., Khan F.A., 2016: Proline accumulation in plants: Roles in stress tolerance and plan development. – In: Iqbal N., Nazar R., Khan N.A. (eds), Osmolytes and Plants Acclimation to Changing Environment: Emerging Omics Technologies: 155–166. – New Delhi. https://doi.org/10.1007/978-81-322-2616-1_9

Desta B., Amare G., 2021: Paclobutrazol as a plant growth regulator. – Chemical and Biological Technologies in Agriculture, 8(1): 1–15. https://doi.org/10.1007/s40502-017-0316-x

Dinler B.S., Gunduzer E., Tekinay T., 2016: Pre-treatment of fulvic acid plays a stimulant role in protection of soybean (*Glycine max* L.) leaves against heat and salt stress. – Acta Biologica Cracoviensia Series Botanica, 58(1): 29–41. https://doi.org/10.1515/abcsb-2016-0002

Dkhil B.B., Denden M., 2012: Effect of salt stress on growth, anthocyanins, membrane permeability and chlorophyll fluorescence of okra (*Abelmoschus esculentus* L.) seedlings. – American Journal of Plant Physiology, 7: 174–183. https://dx.doi.org/10.3923/ajpp.2012.174.183

Ellman G.L., 1959: Tissue sulfhydryl groups. – Archives of Biochemistry and Biophysics, 82: 70–75. https://doi.org/10.1016/0003-9861(59)90090-6

Fletcher R.A., Gilley A., Sankhla N., Davis T.D., 2000: Triazoles as plant growth regulators and stress protectants. – Horticultural Reviews, 24: 55–138. https://doi.org/10.1002/9780470650776.ch3

Forghani A.H., Almodares A., Ehsanpour A.A., 2018: Potential objectives for gibberellic acid and paclobutrazol under salt stress in sweet sorghum (*Sorghum bicolor* [L.] Moench cv. Sofra). – Applied Biological Chemistry, 61(1): 113–124. https://doi.org/10.1007/s13765-017-0329-1

Forghani A.H., Almodares A., Ehsanpour A.A., 2020: The role of gibberellic acid and paclobutrazol on oxidative stress responses induced by in vitro salt stress in sweet sorghum. – Russian Journal of Plant Physiology, 67: 555–563. https://doi.org/10.1134/S1021443720030073

Gill S.S., Tuteja N., 2010: Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. – Plant Physiology and Biochemistry, 48: 909–930. https://doi.org/10.1016/j.plaphy.2010.08.016

Gupta B., Huang B., 2014: Mechanism of salinity tolerance in plants: physiological, biochemical and molecular characterization. – International Journal of Genomics, 1–18. https://doi.org/10.1155/2014/701596

Hasanuzzaman M., Bhuyan M.H.M., Parvin K., Bhuiyan T.F., Anee T.I., Nahar K., Fujita M., 2020: Regulation of ROS metabolism in plants under environmental stress: A review of recent experimental evidence. – International Journal of Molecular Sciences, 21(22): 8695. https://doi.org/10.3390/ijms21228695

Hassanpour H., Khavari-Nejad R.A., Niknam V., Najafi F., Razavi K., 2013: Penconazole induced changes in photosynthesis, ion acquisition and protein profile of *Mentha pulegium* L. under drought stress. – Physiology and Molecular Biology of Plants, 19(4): 4894–98. https://doi.org/10.1007/s12298-013-0192-4

Hoagland D.R., Arnon D.I., 1950: The water-culture method for growing plants without soil (2nd ed.). – Agricultural Experiment Station, California.

Ishtiaq S., Hanif U., Shaheen S., Bahadur S., Liaqat I., Awan U.F., Meo M., 2020: Antioxidant potential and chemical characterization of bioactive compounds from a medicinal plant *Colebrokea oppositifolia* Sm. – Anais da Academia Brasileira de Ciências, 92(2) :1–15. https://doi.org/10.1590/0001-3765202020190387

Jaleel C.A., Gopi R., Manivannan P., Panneerselvam R., 2007: Responses of antioxidant defense system of *Catharanthus roseus* (L.) G. Don. to paclobutrazol treatment under salinity. – Acta Physiologiae Plantarum, 29(3): 205–209. https://doi.org/10.1007/s11738-007-0025-6

Jaleel C.A., Lakshmanan G.M.A., Gomathinayagam M., Panneerselvam R., 2008: Triadimefon induced salt stress tolerance in *Withania somnifera* and its relationship to antioxidant defense system. – South African Journal of Botany, 74(1): 126–132. https://doi.org/10.1016/j.sajb.2007.10.003

Kamran M., Ahmad S., Ahmad I., Hussain I., Meng X., Zhang X., Han Q., 2020: Paclobutrazol application favours yield improvement of maize under semiarid regions by delaying leaf senescence and regulating photosynthetic capacity and antioxidant system during grain-filling Stage. – Agronomy, 10(2): 187. https://doi.org/10.3390/agronomy10020187

Khunpon B., Cha-um S., Faiyue B., Uthaibutra J., Saengnil K., 2018: Paclobutrazol mitigates salt stress in indica rice seedlings by enhancing glutathione metabolism and glyoxalase system. – Biologia, 73(12): 1267–1276. https://doi.org/10.2478/s11756-018-0132-4

Kiani R., Arzan A., Mirmohammady Maibody S.A.M., 2021: Polyphenols, flavonoids, and antioxidant activity involved in salt tolerance in wheat, *Aegilops cylindrica* and their amphidiploids. – Frontiers in Plant Science, 12: 493. https://doi.org/10.3389/fpls.2021.646221

Kondhare K.R., Hedden P., Kettlewell P.S., Farrell A.D., Monaghan J.M., 2014: Use of the hormone-biosynthesis inhibitors fluridone and paclobutrazol to determine the effects of altered abscisic acid and gibberellin levels on pre-maturity amylase formation in wheat grains. – Journal of Cereal Science, 60: 210–216. https://doi.org/10.1016/j.jcs.2014.03.001

Kovács Z., Simon-Sarkadi L., Szűcs A., Kocsy G., 2010: Differential effects of cold, osmotic stress and abscisic acid on polyamine accumulation in wheat. – Amino acids, 38(2): 623–631. https://doi.org/10.1007/s00726-009-0423-8

Liu J., Yu B.J., Liu Y.L., 2006: Effects of spermidine and spermine levels on salt tolerance associated with tonoplast H^+-ATPase and H^+-PPase activities in barley roots. – Plant Growth Regulation, 49(2): 119–126. https://doi.org/10.1007/s10725-006-9001-1

Mackay C.E., Hall J.C., Hofstra G., Fletcher R.A., 1990: Uniconazole-induced changes in abscisic acid, total amino acids, and proline in *Phaseolus vulgaris*. – Pesticide Biochemistry and Physiology, 371: 74–82. https://doi.org/10.1016/0048-3575(90)90110-N

Mancinelli A., Yang C.-P.H., Lindquist P., Anderson O., Rabino I., 1975: Photocontrol of anthocyanin synthesis III. The action of streptomycin on the synthesis of chlorophyll and anthocyanin. – Plant Physiology, 55(2): 251–257. https://doi.org/10.1104/pp.55.2.251

Minocha R., Majumdar R., Minocha S.C., 2014: Polyamines and abiotic stress in plants: a complex relationship. – Frontiers in Plant Science, 5: 175. https://dx.doi.org/10.3389/fpls.2014.00175

Mirecki R.M., Teramura A.H., 1984: Effects of ultraviolet-B irradiance on soybean v. the dependence of plant sensitivity on the photosynthetic photon flux density during and after leaf expansion. – Plant Physiology, 74(3): 475–480. https://doi.org/10.1104/pp.74.3.475

Nahar K., Hasanuzzaman M., Rahman A., Alam M.M., Mahmud J-A., Suzuki T. Fujita M., 2016: Polyamines confer salt tolerance in mung bean (*Vigna radiata* L.) by reducing sodium uptake, improving nutrient homeostasis, antioxidant defense, and methylglyoxal detoxification systems. – Frontiers in Plant Science, 7: 1104.

https://dx.doi.org/10.3389%2Ffpls.2016.01104

Pál M., Szalai G., Janda T., 2015: Speculation: Polyamines are important in abiotic stress signalling. – Plant Science, 237: 16–23. https://doi.org/10.1016/j.plantsci.2015.05.003

Phang T.H., Shao G., Lam H.M., 2008: Salt tolerance in soybean. – Journal of Integrative and Plant Biology, 50(10): 1196–1212. https://doi.org/10.1111/j.1744-7909.2008.00760.x

Rajasekar M., Rabert G.A., Manivannan P., 2016: The effect of triazole induced photosynthetic pigments and biochemical constituents of *Zea mays* L. (Maize) under drought stress. – Applied Nanoscience, 6(5): 727–735. https://doi.org/10.1007/s13204-015-0482-y

Sadak M.S., Abd El-Hameid A.R., Zaki F.S.A., Dawood M.G., El-Awadi M.E., 2020: Physiological and biochemical responses of soybean (*Glycine max* L.) to cysteine application under sea salt stress. – Bulletin of the National Research Center, 44(1): 1–10. https://doi.org/10.1186/s42269-019-0259-7

Saha J., Brauer E.K., Sengupta A., Popescu S.C., Gupta K., Gupta B., 2015: Polyamines as redox homeostasis regulators during salt stress in plants. – Frontiers in Environmental Science, 3: 21. https://doi.org/10.3389/fenvs.2015.00021

Samanta A., Das G., Das S., 2011: Roles of flavonoids in plants. – International Journal of Pharmacology Science Technology, 6 (1): 12–35.

Sharma A., Shahzad B., Rehman A., Bhardwa R., Landi M., Zheng B., 2019: Response of phenylpropanoid pathway and the role of polyphenols in plants under abiotic stress. – Molecules, 24(13): 2452. https://doi.org/10.3390/molecules24132452

Smith T., Best G., 1977: Polyamines in barley seedlings. – Phytochemistry, 16: 841–843. https://doi.org/10.1016/S0031-9422(00)86676-5

Sofy M.R., Elhindi K.M., Farouk S., Alotaibi M.A., 2020: Zinc and paclobutrazol mediated regulation of growth, upregulating antioxidant aptitude and plant productivity of pea plants under salinity. – Plants, 9(9): 1197. https://doi.org/10.3390/plants9091197

Soumya P.R., Kumar P., Pal M., 2017: Paclobutrazol: a novel plant growth regulator and multi-stress ameliorant. – Indian Journal of Plant Physiology, 22(3): 267–278. https://doi.org/10.1007/s40502-017-0316-x

Swain T., Goldstein J.L., 1964: The quantitative analyses of phenolic compounds. – In: Pridham J.B. (ed.), Methods in Polyphenol Chemistry: 131–146. – Oxford.

Todorova D., Sergiev I., Alexieva V., Karanov E., Smith A., Hall M., 2007: Polyamine content in *Arabidopsis thaliana* (L.) Heynh during recovery after low and high temperature treatments. – Plant Growth Regulation, 51(3): 185–191. https://doi.org/10.1007/S10725-006-9143-1

Todorova D., Katerova Z., Sergiev I., Alexieva V., 2013: Role of polyamines in alleviating salt stress. – In: Ahmad P., Azooz M.M., Prasad M.N.V. (eds), Ecophysiology and Responses of Plants Under Salt Stress: 355–379. – New York. https://doi.org/10.1007/978-1-4614-4747-4_13

Torrigiani P., Altamura M., Copitani F., Serafini-Fracasini D., Bagni N., 1989: De novo root formation in thin cell layers of tobacco: changes in free and bound polyamines. – Physiologia Plantarum, 77(3): 294–301. https://doi.org/10.1111/j.1399-3054.1989.tb05644.x

van Zelm E., Zhang Y., Testerink C., 2020: Salt tolerance mechanisms of plants. – Annual Review of Plant Biology, 71(1): 403–433. https://doi.org/10.1146/annurev-arplant-050718-100005

Wang S.Y., Steffens G.L., 1985: Effect of paclobutrazol on water stress-induced ethylene biosynthesis and polyamine accumulation in apple seedling leaves. – Phytochemistry, 24(10): 2185–2190. https://doi.org/10.1016/S0031-9422(00)83007-1

Wang X., Komatsu S., 2017: Improvement of soybean products through the response mechanism analysis using proteomic technique. – Advances in Food and Nutrition Research, 82: 117–148. https://doi.org/10.1016/bs.afnr.2016.12.006

Waśkiewicz A., Muzolf-Panek M., Goliński P., 2013: Phenolic content changes in plants under salt stress. – In: Ahmad P., Azooz M., Prasad M. (eds), Ecophysiology and Responses of Plants under Salt Stress, 11: 283–314. – New York. http://dx.doi.org/10.1007/978-1-4614-4747-4_11

Zagorchev L., Seal C.E., Kranner I., Odjakova M., 2013: A central role for thiols in plant tolerance to abiotic stress. – International Journal of Molecular Sciences, 14(4): 7405–7432. https://dx.doi.org/10.3390/ijms14047405

PAKLOBUTRAZOLO SUKELTŲ NEFERMENTINIŲ ANTIOKSIDANTŲ IR POLIAMINO KONCENTRACIJA SOJOSE, AUGINAMOSE DRUSKOS SUKELTO STRESO SĄLYGOMIS

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Santrauka

Paklobutrazolas yra augalų augimą reguliuojantis triazolo grupės junginys. Žinoma, kad šis junginys apsaugo kultūrinius augalus nuo aplinkos veiksnių, tokių kaip dirvožemio druskingumas ir sausra, sukelto streso. Paklobutrazolas reguliuoja vandens pusiausvyrą augale, fotosintezės greitį ir sustiprina antioksidacines fermentų savybes. Antioksidacinis paklobutrazolo poveikis sojoms gana gerai ištirtas, tačiau iki šiol nebuvo tyrinėta, kaip šis junginys pakeičia druskos sukelto streso sąlygomis augančių augalų nefermentinių antioksidantų ir endogeninių poliamidų veikimą. Tyrimu buvo siekiama nustatyti, kaip paklobutrazolas veikia šių junginių molekules.

Tirtas natrio chlorido (250 mM NaCl) ir trijų koncentracijų paklobutrazolo tirpalo poveikis gauruotosios sojos (*Glycine max* L.) augalams. Tirtas minėtomis medžiagomis apdorotuose augaluose susidariusių hidroksilo radikalų, prolino, nefermentinių antioksidantų (antocianinų, flavonoidų, fenolinių ir laisvojo tiolio turinčių junginių), poliaminų (spermino ir spermidino) ir putrescino aktyvumas. Nustatyta, kad sojų daigai, apdoroti tirpalu, kurio milijonui tirpiklio dalių tenka 10 dalių paklobutrazolo, geriausiai ištvėrė NaCl sukeltą stresą. Augaluose reikšmingai padidėjo hidroksilo radikalų aktyvumas, taip pat prolino, bendroji fenolių, flavonoidų ir konjuguotų poliaminų koncentracija, palyginti su augalais, kurie buvo apdoroti tik NaCl tirpalu. Tyrimo rezultatai patvirtino, kad paklobutrazolas skatina nefermentinių antioksidantų aktyvumą sojose, auginamose druskos sukelto streso sąlygomis.