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Effect of Different Level of Auxin, Cytokinin and Gibberellin on Growth, Phenolic Compounds and Activity of Antioxidant Enzymes of *Opuntia cylindrica*

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Abstract

Cacti are among the most important and diverse plant families cultivated worldwide for various purposes, including food, medicine, cosmetic, and ornamental. To investigate the growth and physiological changes of *Opuntia cylindrica* under the treatment of auxin (IBA), cytokinin (Kin), and gibberellin (GA) hormones in concentrations of 250, 500, and 1000 mg/L, this study was conducted in a completely randomized design in the greenhouse of Islamic Azad University, Gorgan branch in 2020. The IBA treatment not only did not cause a significant change in the fresh weight (FW) and height, but also had no significant effect on the content of pigments, sugar, soluble protein, and phenolic compounds except flavonoids, the activity of phenylalanine ammonia-lyase (PAL) and superoxide dismutase (SOD) enzymes. But the treatment of cactus with Kin was caused a significant increase in FW, content of soluble protein, phenolic compounds, and increase the activity of PAL, SOD, and peroxidase (POD) enzymes. GA treatment caused a decrease in FW by 14 and 23%, a significant decrease in the content of pigments Chl. a by 31 and 70% and Chl. b by 37 and 68% and POD enzyme activity by 60 and 50% at 500 and 1000 mg/L, respectively. While the content of phenolic compounds and the activity of PAL and SOD enzymes were significantly enhanced. It seems that Kin at the concentrations of 500 and 1000 mg/L was the best treatment for reinforcement of the growth of *O. cylindrica* with high phenolic and antioxidant capacity. On the other hand, the decrease in FW and the content of chlorophylls in plant treated with GA, indicated the presence of stress in the plant, and the increase in the content of phenolic compounds and the activity of antioxidant enzymes specially at concentrations of 500 and 1000 mg/L probably moderated the stress.

Keywords: Antioxidant systems, Growth, Hormone, *Opuntia cylindrica*, Physiological parameters.

INTRODUCTION

The Cactaceae is one of the most remarkable and diverse families of flowering plants in nature, which mostly grow in hot, semi-arid, and arid regions of the world (Casas and Barbera, 2002; Hultine *et al*., 2023). This family has nearly 130 genera that have about 2000 species in different growth forms or shapes (Shedbalkar *et al*., 2010). These plants are often grown on sloping land as a barrier against soil erosion, for ecological restoration and land restoration, and also, they are also used commercially as fruits for humans and animal feed and are highly regarded as ornamental flowers (Lema-Ruminska and Kulus, 2014; Inglese *et al*., 2017; Stavi, 2022). The *Opuntia* genus is one of the most important known genera among the cactus family (Ritz *et al*., 2012). This genus is used in many regions especially in the Mediterranean-Central Asian area due to its edible fruit, leaf buds, nutritional and medicinal properties, and presence of valuable chemical compounds including phenolic compounds, flavonoids, carotenoids, saponins, steroids, terpenoids, vitamins, flavonoids and betalain (Inglese *et al*., 2017; Bouzroud *et al*., 2022). In addition, the *Opuntia* genus is traditionally used in the treatment of hypoglycemia, stomach ulcers, neuron protection through antioxidant activity, viral disease, diabetes, bronchitis burns, and asthma around the world (Madrigal-Santillán *et al*., 2022; Prisa, 2023).

The growth and chemical compositions of cactus plants are greatly influenced by environmental factors, growth conditions, plant age, and species (Bouzroud *et al*., 2022). Therefore, suitable inducers may improve the growth, medicinal value, and attractiveness of ornamental plants. Plant growth regulators including auxin, cytokinin, and gibberellin play a vital role in regulating growth processes in plants. Investigation of the impact of the plant hormone on the growth, nutrition, and ornamental value of cacti has a high economic value and is beneficial in many aspects (Cortés-Olmos *et al.*, 2023).

The current study has aimed to get more insight into the physiological responses of opuntia cylindrica as a species in the genus of opuntia to plant growth hormones including auxin (IBA), cytokinin (kinetin; Kin) and gibberellin (GA) at different concentrations in greenhouse condition. Fresh and dry weight and height were measured to evaluate the economic importance of different hormones in the growth of cactus cuttings. Antioxidant compounds and enzymes were analyzed to explore the possible impact of hormones on medicinal and nutritional value. On the other hand, the antioxidative defense of *O. cylindrica* to the possible negative effects of hormones at specific concentrations was studied.

MATERIALS AND METHODS

Growth conditions and plant harvesting

The same-shaped cactus (*O. cylindrica*) cuttings in terms of weight and height were obtained from the greenhouse of Islamic Azad University, Gorgan Branch. The pot (10 and 9 cm in diameter and height, respectively) experiment was done in autumn 2020. The cactus cuttings were grown in suitable soil including cocopeat, peat moss, and perlite in proportions of 10, 30, and 60% respectively. Some chemical and physical properties of growing media was tested (Table 1).

Table 1. Chemical and physical characteristics of individual media component.

Media components	pH	EC (dS m^{-1})	Moisture content (%)
Cocopeat	6.8	0.80	37.36
Peat moss	3.5	0.15	42.00
Perlite	7.1	1.50	24.50

The temperature of the greenhouse varied between 30 to 37 ºC, the humidity was reported by 80%, light intensity and photoperiod were 5000 lux and 12-14 hours, respectively. The cactus cuttings were pretreated with auxin hormone (IBA) at a concentration of 1500 mg/L for 8 seconds before being transferred to the pot. For the control sample, distilled water was used instead of hormonal pretreatment. Cacti were irrigated once a week with normal water in the amount of 50 ml. Four months after planting cactus cuttings in the pots, pretreatment cacti were treated with IBA (PT), cytokinin (kinetin; Kin), and gibberellin (GA) hormones each in three concentrations (250, 500, and 1000 mg/L) during four stages with an interval of five weeks. Furthermore, all treatments and controls were performed in four replicates (pots). Plant sampling was done five weeks after the last treatment. The shoots were separated from the roots and their fresh weight (FW) was weighed. To determine dry matter content (DMC; it was determined by dividing dry weight by fresh weight and multiplying by 100), total phenols, flavonoids, and flavonols content, the plant materials were air-dried at 35 °C (± 2) for 24 h. For measurement of the chlorophyll a (Chl. a), chlorophyll b (Chl. b), soluble protein, soluble sugar, anthocyanin content, and enzyme activity, the plant materials were frozen immediately in liquid N2 and stored at -80 °C.

Determination of photosynthetic pigments

The extraction of frozen plant samples was done by using 80% acetone (g/10 ml). Then the extraction was centrifuged at 3000 g for 20 min and subsequently the content of pigments was measured spectrophotometrically at 470, 646, and 663 nm (Lichtenthaler, 1987). The content of pigments was calculated using the following formula and expressed based on the µg/g FW of the plant.

$$Chl\ a = 12.7(A663) - 2.69\ (A645)$$
$$Chl\ b = 12.7\ (A663) - 4.78\ (A645)$$

Determination of soluble sugar content

Soluble sugar content was measured by the anthrone-sulfuric acid colorimetric method (Irigoyen *et al*.,1992). Air-dried shoots were extracted by 95% (v/v) ethanol (g/10 ml) using centrifugation at 3500 g for 10 min. The reaction mixture containing 0.1 ml of the plant ethanolic extract with 3 ml of freshly prepared anthrone solution (200 mg of anthrone + 100 ml of sulfuric acid) was incubated in a boiling water bath for 10 min. After cooling, the absorbance was recorded at 625 nm by using a spectrophotometer. The soluble sugar content was calculated based on the standard calibration curve of glucose and expressed as mg/g DW.

Determination of anthocyanin

The evaluation of anthocyanin content was done using the method of Masukasu *et al.* (2003). For the extraction of anthocyanin, fresh plant samples (0.2 g) were mixed with 3 ml of acidic methanol (V/V HCl 1%) and then filtered through filter paper. The extract was centrifuged at 6000 rpm for 20 min and subsequently kept overnight in the dark at 4°C. To measure the anthocyanin content, the absorbance of the supernatant was recorded at 550 nm by spectrophotometer, and the content of anthocyanin was calculated using the following formula and expressed based on mg/g FW of the plant.

$$Anthocyanin = \frac{(A550/3300)(1000)}{0.6}$$

Measurement of total phenols, flavonoids and flavonols

To extract phenolic compounds, 0.4 g air-dried plant samples were powdered and

homogenated with 10 ml of 70% methanol. The methanolic extract was sonicated and then centrifuged at 6000 rpm for 20 min (Thygesen *et al.*, 2007).

To evaluate total phenol, plant extract (125 µl) was mixed with distilled water (375 µl) and 10% Folin-Ciocalteu's reagent (2.5 ml). After 6 min, 2 ml of sodium carbonate (7.5% w/v) was added to the mixture. After incubation (90 min) at room temperature, the absorbance of the reaction mixture was recorded at 765 nm by a spectrophotometer. Finally, the content of total phenol was calculated using the standard curve of gallic acid (Singleton and Rossi, 1965).

To evaluate the total flavonoid content, 2 ml of plant extract was homogenated with 2.8 ml of distilled water, 100 µl of aluminum chloride (10%), and 100 µl of potassium acetate (1 M). The mixture was kept in the dark for 30 min and then the absorption of the mixture was determined at 415 nm by using a spectrophotometer (Akkol *et al*., 2008).

To determine total flavanols, plant extract (1 ml) was mixed with 1 ml of aluminum chloride (2%) and 3 ml of sodium acetate (5%). After 2.5 h. the absorbance of the mixture was recorded at 440 nm using a spectrophotometer. Finally, the content of the flavonoids and flavonols was calculated using the standard curve of quercetin (Akkol *et al*., 2008).

Measurement of soluble protein and enzymes activity

To determine the soluble protein and enzyme activity, frozen plant samples were extracted in 0.1 M potassium phosphate buffer with pH 7.0 (g FW/10 ml). The filtered extract was centrifuged at 16,000 g for 25 min at 4 ºC. The soluble protein content was determined by Bradford's method using bovine serum albumin as a standard (Bradford, 1976).

To determine the activity of the phenylalanine ammonia-lyase (PAL), enzyme extract (800 µl) was homogenate with 600 µl of Tris-HCl (50 mM) and 900 µl of 2 mM l-1 henylalanine and the mixture was incubated at room temperature for 30 min. Then the reaction was stopped by 100 µL of HCl (2N). In the next step, the reaction was mixed with toluene (1.5 ml) and subsequently centrifuged at 5000 rpm for 5 min. The absorbance of the toluene phase containing trans-cinnamic acid was measured at 290 nm. Enzyme activity was expressed as mmol trans-cinamic acid/h/mg FW (Saunders and McClure, 1974).

To determine the activity of superoxide dismutase (SOD), enzyme extract (400 µl) was mixed with 1600 µl of potassium phosphate buffer (0.1 M), 0.1 mM EDTA, 13 mM methionine, 24 µM riboflavin, and 75 µM NBT. The reaction mixture was kept in the light at a distance of 10 cm from the light source for 10 min, and then the reaction was stopped by turning the lights off. The absorption of the reaction was determined with a spectrophotometer at 560 nm (Beauchamp and Fridovich, 1971). One unit of SOD activity was expressed as the amount of enzyme required to cause 50 % inhibition of NBT reduction under the experimental conditions. The activity of the enzyme was expressed as U/mg FW.

To measure the peroxidase activity, enzyme extract (200 µl) was mixed with 80 µl of guaiacol reagent (124.14 g/mol), 2.470 µl of potassium phosphate buffer (0.1 M), and 250 µl of hydrogen peroxide. After 1 min the absorbance of the reaction mixture was measured at 470 nm with a spectrophotometer (Nakano and Asada, 1981). The activity of the enzyme was expressed as U/mg FW.

Statistical analysis

To perform the statistical analyses, a one-way analysis of variance (ANOVA) was used. Tukey's HSD all-pairwise comparisons at the level of $P < 0.05$ as a post-hoc test was used to compare the treatment means. To analyze the data, the software of SPSS version 20 for windows 7 and to make the graph, the prism software (San Diego, CA, USA) was applied.

RESULT

Growth factors

There was not significant difference among the control, PT and different concentration of IBA in relation to FW and height. While in the case with Kin at different concentrations in comparison with control, and PT, there was only significant different in relation to FW. Likewise, there was significant difference among the control, PT and different concentration of GA in relation to FW, and height (Table 2).

Table 2. ANOVA table to indicate the significance difference among treatments or groups (pre-treatment with auxin at 1500 mg/L (PT) and PT along with IBA, Kin and GA) in relation to fresh weight (FW) and height.

Treatment (group)	Dependent variable	SS	df	MS	F	p-value
Control, PT and IBA (250, 500 and 1000 mg/L)	FW	1059.515	4	264.879	1.875	0.167
	Height	22.800	4	5.700	2.265	0.111
Control, PT and Kin (250, 500 and 1000 mg/L)	FW	1548.677	4	387.169	9.008	0.001
	Height	18.700	4	4.675	2.646	0.075
Control, PT and GA (250, 500 and 1000 mg/L)	FW	11742.627	4	2935.657	14.573	0.000
	Height	522.300	4	130.575	27.782	0.000

The mean of SS, df, MS, and F is the sum of squares, the degrees of freedom, the mean sum of squares, and the F-statistic, respectively. The value of $P < 0.05$ indicates a significant statistical difference between the treatments.

The results showed that the PT hardly affected the FW, and height of the cactus cuttings compared to the control. Similarly, the PT+IBA treatment at different concentrations did not change the growth parameters including FW and height. The FW of the plants treated with PT+Kin at all three concentrations were significantly enhanced compared to that of the plants treated with PT, although, the height of the plants was hardly changed. In plants exposed to PT+GA, the FW was reduced at 250, 500, and 1000 mg/L compared to that of the plant treated with PT by 16, 14, and 23%, respectively. While the height was enhanced by 17, 24, and 28 % in plants exposed to 250, 500, and 1000 mg/L PT+GA, respectively (Table 3).

Pigments content

There was not significant difference among the control, PT and different concentration of IBA in relation to both Chl. a and Chl. b. In contrast, in the case with GA there was significant difference in relation to both Chl. a and Chl. b. Similarly, the content of Chl. a was significantly changed between the groups control, PT and different concentration of Kin while that of the Chl. b was not significantly changed between the groups (Table 4).

The results showed that the content of Chl. a and Chl. b was hardly changed in plants exposed to PT. The PT+IBA treatment at any concentration hardly affected the content of both Chl. a and Chl. b. Likewise, the content of both Chl. was hardly changed under treatment of PT+Kin at all three concentrations compare with that of the plant exposed to PT. While the content of Chl. a and Chl. b in plant treated with PT+GA was reduced by 32 and 37 % at the concentration of 500 mg/L and by 69 and 68% at 1000 mg/L, respectively compared with that of Chl. a and Chl. b in plant exposed to PT (Fig. 1)

Table 3. Impact of pre-treatment with auxin at 1500 mg/L (PT) and PT along with IBA, Kin and GA hormones at different concentrations of 250, 500 and 1000 mg/L on FW and height.

Treatment	FW (g)	Height (cm)
Control	247±10 a	41±1.2 a
PT	237±10 a	42±1.5a
PT + IBA (250 mg/L)	250±7 a	42.5±1 a
PT + IBA (500 mg/L)	258±14 a	44±2 a
PT + IBA (1000 mg/L)	239±16 a	41.2±2.2 a
Control	247±4.9 ab	41±1.2 a
PT	237±4.2 b	42±1.5 a
PT + Kin (250 mg/L)	263±2.5 a	43±1.6 a
PT + Kin (500 mg/L)	255±2 a	41.5±1.9 a
PT + Kin (1000 mg/L)	256±4 a	42.7±0.5 a
Control	247±4.9 a	41±1.2 c
PT	237±4.2 a	42±1.5 c
PT + GA (250 mg/L)	199±18 b	49±2.4 b
PT + GA (500 mg/L)	204±13 b	52 ±2.5 ab
PT + GA (1000 mg/L)	183±18 b	54±2.9 a

Data on growth parameters represent the mean of four biological replicates (± SD). Different letters indicate significant differences between treatments (P<0.05; One-way ANOVA, Tukey's HSD all-pairwise comparisons as a post-hoc test). The letter 'a' shows the highest value and the rest of the english alphabet shows lower values, respectively. FW is the abbreviation of fresh weight.

Table 4. ANOVA table to indicate the significance difference among treatments or groups (pre-treatment with auxin at 1500 mg/L (PT) and PT along with IBA, Kin and GA in relation to Chl. a and Chl. b.

Treatment (group)	Dependent variable	SS	df	MS	F	p-value
Control, PT and IBA (250, 500 and 1000 mg/L)	Chl. a	361.126	4	90.282	1.823	0.177
	Chl. b	305.380	4	76.345	1.037	0.420
Control, PT and Kin (250, 500 and 1000 mg/L)	Chl. a	879.835	4	219.959	3.595	0.030
	Chl. b	353.462	4	88.366	2.217	0.116
Control, PT and GA (250, 500 and 1000 mg/L)	Chl. a	11687.378	4	2921.844	81.081	0.000
	Chl. b	1548.531	4	387.133	14.464	0.000

The value of P < 0.05 indicates a significant statistical difference between the treatments.

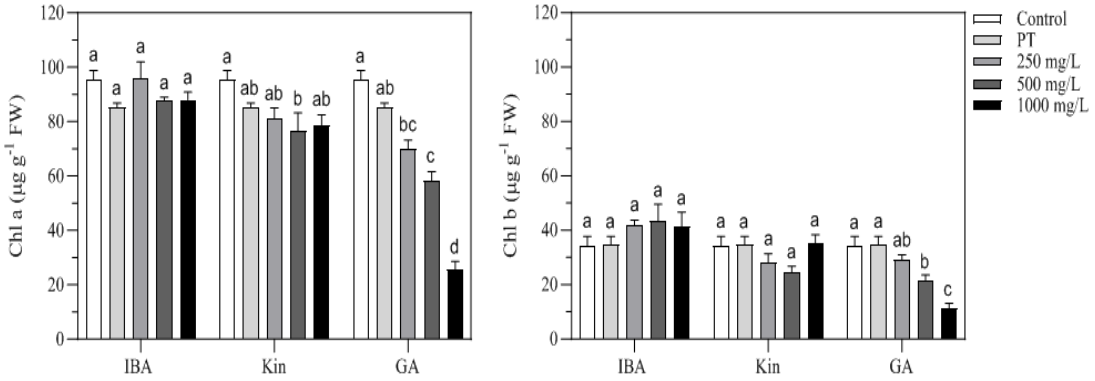

Fig. 1. Impact of pre-treatment with auxin at 1500 mg/L (PT) and PT along with IBA, Kin and GA hormones at different concentrations of 250, 500 and 1000 mg/L on the content of Chl. a and Chl. b. Data on pigment content represents the mean of four biological replicates (± SD). Different letters indicate significant differences between treatments (P < 0.05; One-way ANOVA, Tukey's HSD all-pairwise comparisons as a post-hoc test).

Soluble sugar and soluble protein content

The results of analysis of variance showed that there was not significant difference among the control, PT and different concentration of IBA in relation to soluble sugar and soluble protein content. While in the case with Kin at different concentrations in comparison with control, and PT, there was only significant different in relation to soluble protein content. Likewise, there was only significant difference among the control, PT and different concentration of GA in relation to soluble sugar (Table 5).

Table 5. ANOVA table to indicate the significance difference among treatments or groups (pre-treatment with auxin at 1500 mg/L (PT) and PT along with IBA, Kin and GA in relation to soluble sugar and soluble protein content.

Treatment (group)	Dependent variable	SS	df	MS	F	p-value
Control, PT and IBA (250, 500 and 1000 mg/L)	Soluble sugar	0.132	4	0.033	0.379	0.820
	Soluble protein	0.610	4	0.153	0.808	0.539
Control, PT and Kin (250, 500 and 1000 mg/L)	Soluble sugar	0.339	4	0.085	0.766	0.564
	Soluble protein	5.056	4	1.264	5.493	0.006
Control, PT and GA (250, 500 and 1000 mg/L)	Soluble sugar	2.320	4	0.580	5.426	00.007
	Soluble protein	1.263	4	0.316	1.480	0.258

The value of $P < 0.05$ indicates a significant statistical difference between the treatments.

The content of soluble sugar was hardly changed in plants exposed to PT compared with that of the soluble sugar in control plants. Similarly, the content of soluble sugar in plants treated with different concentrations of PT+IBA and PT+Kin was hardly changed compared to that of the soluble sugar in plant exposed to PT. likewise, soluble sugar was not affected by PT+GA at 250 mg/L, while the content of soluble sugar in plants treated with PT+GA at 500 and 1000 mg/L was enhanced by 37 and 36.7 %, respectively compared with that of the soluble sugar in plants exposed to PT (Fig. 2).

The content of total protein in plants pretreated with auxin (PT) was hardly changed compared to that of the control plant. Similarly, the content of protein was not significantly affected by PT+IBA and PT+GA at any concentrations compared with that of the protein in pre-treated plants with auxin. While the content of soluble protein in plants treated with PT+Kin at 500 and 1000 mg/L was enhanced by 14 and 8.4 %, respectively compared with that of the soluble protein in plants exposed to PT (Fig. 2).

Total phenols, flavonoids and flavanols content

The results of ANOVA showed that there was a significant difference among the control, PT and different concentration of IBA in relation to flavonoids and anthocyanin. In the case with Kin there was significant difference in relation to phenols, flavonoids, and anthocyanin. Similarly, there was a significant difference among the control, PT and different concentration of GA in relation to the content of phenols, flavonoids, flavanols, and anthocyanin (Table 6).

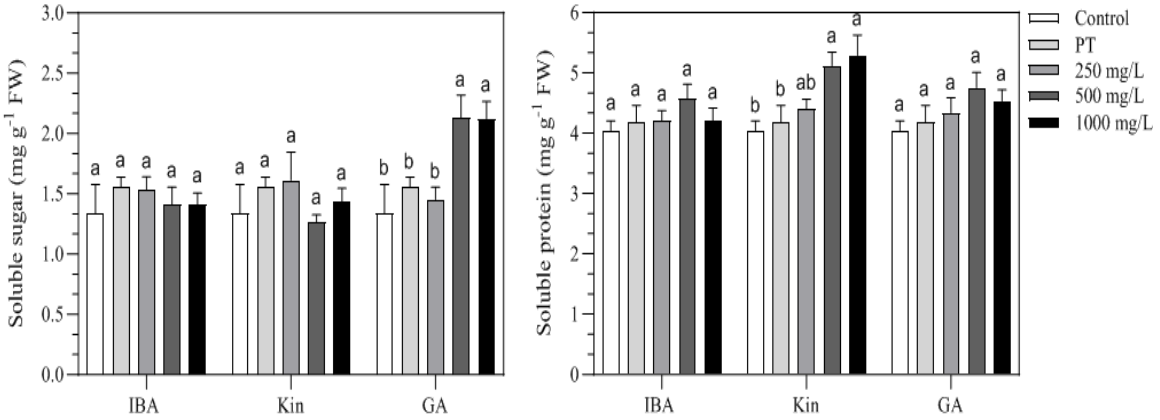

Fig. 2. Impact of pre-treatment with auxin at 1500 mg/L (PT) and PT along with IBA, Kin and GA hormones at different concentrations of 250, 500 and 1000 mg/L on the content of soluble sugar and soluble protein. Data on soluble sugar and soluble protein represent the mean of four biological replicates (± SD). Different letters indicate significant differences between treatments ($P < 0.05$; One-way ANOVA, Tukey's HSD all-pairwise comparisons as a post-hoc test).

Table 6. ANOVA table to indicate the significance difference among treatments or groups (pre-treatment with auxin at 1500 mg/L (PT) and PT along with IBA, Kin and GA in relation to the content of phenols, flavonoids, flavanols, and anthocyanin.

Treatment (group)	Dependent variable	SS	df	MS	F	p-value
Control, PT and IBA (250, 500 and 1000 mg/L)	Phenols	0.884	4	0.221	0.594	0.672
	Flavonoids	6.376	4	1.594	10.048	0.000
	Flavanols	0.124	4	0.031	0.665	0.626
	Anthocyanin	0.000	4	0.000	4.856	0.010
Control, PT and Kin (250, 500 and 1000 mg/L)	Phenols	7.127	4	1.782	17.038	0.000
	Flavonoids	26.212	4	6.553	32.686	0.000
	Flavanols	0.200	4	0.050	2.227	0.115
	Anthocyanin	0.000	4	0.000	3.493	0.033
Control, PT and GA (250, 500 and 1000 mg/L)	Phenols	12.267	4	3.067	13.609	0.000
	Flavonoids	13.451	4	3.363	10.506	0.000
	Flavanols	0.988	4	0.247	10.779	0.000
	Anthocyanin	0.000	4	0.000	8.035	0.001

The value of $P < 0.05$ indicates a significant statistical difference between the treatments.

The content of total phenols, flavonoids, and flavanols in plants pretreated with auxin (PT) was hardly changed compared with the content of these compounds in control plants. Likewise, the content of total phenols, flavanols, and anthocyanin was not significantly affected by PT+IBA at any concentrations except the content of flavonoids was enhanced by 43 and 44% in plant exposed to 500 and 1000 mg/L PT+ IBA, respectively compared with that of the compounds in pre-treated plant with IBA (Fig. 3).

In the plant treated with PT+Kin, the content of flavanols and anthocyanin was hardly changed compared with the content of these compounds in pre-treated plants with auxin. While the content of total phenols and flavonoids was enhanced by 21, 19, and 94, 93% at 500 and 1000 mg/L Kin along with PT (Fig. 3).

The content of total phenols, flavonoids, and flavanols in plants exposed to GA was

enhanced by 27, 26 and 79, 65 and 26, 27 % at 500 and 1000 mg/L, and the content of anthocyanin was enhanced by 33% at 1000 mg/L along with PT compare with the content of these compounds in pre-treated plant with auxin (Fig. 3).

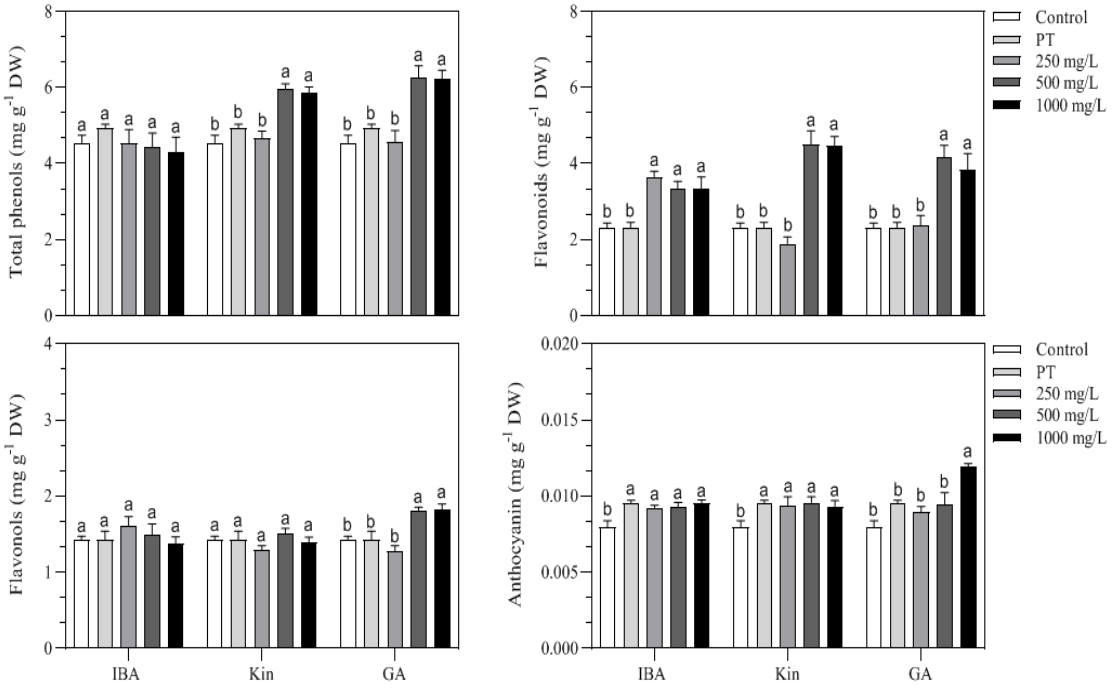

Fig. 3. Impact of pre-treatment with auxin at 1500 mg/L (PT) and PT along with IBA, Kin and GA hormones at different concentrations of 250, 500 and 1000 mg/L on the content of total phenols, flavonoids, flavonols, and anthocyanin. Data on phenolic compounds represent the mean of four biological replicates (± SD). Different letters indicate significant differences between treatments ($P < 0.05$; One-way ANOVA, Tukey's HSD all-pairwise comparisons as a post-hoc test).

Enzyme activity

The results of analysis of variance showed that there was a significant difference among the control, PT and different concentration of IBA in relation to PAL and POD activity. Similarly, in the case with Kin and GA at different concentrations in comparison with control, and PT, there was significant different in relation to PAL, SOD and POD activity (Table 7).

The activity of the PAL enzyme in plants pretreated with auxin (PT), was hardly changed compared with that of the control plant. Similarly, the activity of the PAL enzyme was hardly changed in pretreated plants with IBA exposed to any concentrations of IBA (Fig. 4).

The activity of the PAL enzyme was enhanced in pretreated plants with auxin exposed to Kin by 25, 28 and 27% and also exposed to GA by 26, 27 and 28 % at concentrations of 250, 500, and 1000 mg/L, respectively compared with the plant only pretreated with IBA (Fig. 4).

The activity of the SOD and POD enzymes in plants pretreated with auxin (PT), was hardly changed compared with that of the control plant. In pretreated plants with IBA exposed to Kin at concentrations of 500 and 1000 mg/L, the activity of SOD was enhanced by 3.7 and 3-fold and the activity of POD was enhanced by 2.8 and 2.6- fold, respectively (Fig. 4).

The activity of SOD was enhanced in pretreated plants with auxin exposed to GA by 2, 2, and 3-fold at concentrations of 250, 500, and 1000 mg/L, respectively. While the activity of POD was reduced in pretreated plants with auxin exposed to GA by 55, 59 and 49 % at concentrations of 250, 500 and 1000 mg/L, respectively (Fig. 4).

Table 7. ANOVA table to indicate the significance difference among treatments or groups (pre-treatment with auxin at 1500 mg/L (PT) and PT along with IBA, Kin and GA in relation to the activity of PAL, SOD and POD activity.

Treatment (group)	Dependent variable	SS	df	MS	F	p-value
Control, PT and IBA (250, 500 and 1000 mg/L)	PAL activity	7.384	4	1.846	5.755	0.005
	SOD activity	12.431	4	3.108	2.802	0.064
	POD activity	1.425	4	0.356	9.440	0.001
Control, PT and Kin (250, 500 and 1000 mg/L)	PAL activity	6.579	4	1.645	3.803	0.025
	SOD activity	300.849	4	75.212	96.162	0.000
	POD activity	4.905	4	1.226	15.268	0.000
Control, PT and GA (250, 500 and 1000 mg/L)	PAL activity	6.645	4	1.661	3.938	0.022
	SOD activity	49.626	4	12.407	34.032	0.000
	POD activity	0.488	4	0.122	10.799	0.000

The value of $P < 0.05$ indicates a significant statistical difference between the treatment.

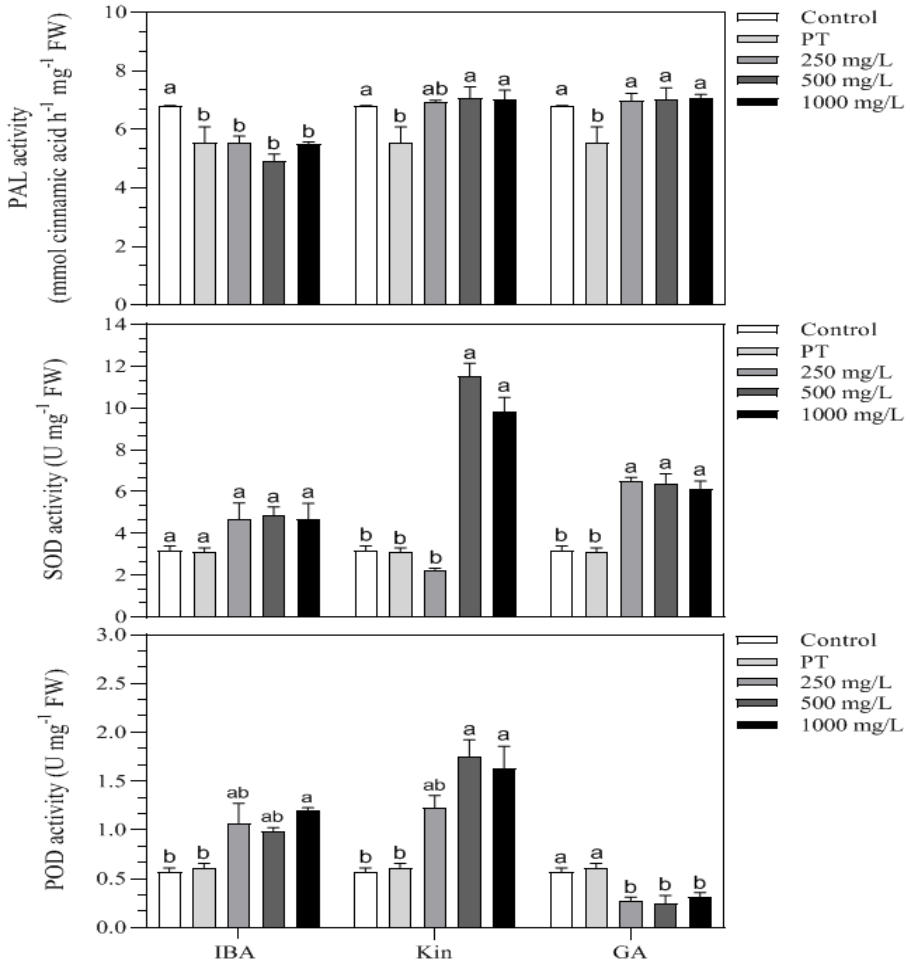

Fig. 4. Impact of pre-treatment with auxin at 1500 mg/L (PT) and PT along with IBA, Kin and GA hormones at different concentrations of 250, 500 and 1000 mg/L on the activity of PAL, SOD and POD enzymes. Data on enzyme activity represent the mean of four biological replicates (± SD). Different letters indicate significant differences between treatments ($P < 0.05$; One-way ANOVA, Tukey's HSD all-pairwise comparisons as a post-hoc test).

DISCUSSION

The commercial use of plant growth regulators like hormones including auxin, Kin, and GA which improve ornamental plants' morphophysiological traits and performance has received much attention nowadays (Ahmad *et al*., 2020; Krzemińska *et al*., 2023). However, plants' response to these compounds' use is largely influenced by the concentration, type of plant, growth stage, and the exposure of the plant to treatments (Miceli *et al*., 2019). Cytokinin stimulates cell division and mesophyll cell elongation which subsequently can affect plant growth (Sosnowski *et al*., 2023). Similarly, the present results showed that the application of Kin led to an increase in the biomass of *O. cylindrica* at all three different concentrations. While GA decreased the fresh weight and increased the height of the plant in all three concentrations compared to the plant treated with PT. It seems that applying GA caused the allocation of energy and organic matter for the longitudinal growth of the plant. Due to the impact of GA on the meristem cells in the internodes of the stem, it may increase the longitudinal growth and as a result, increase the height of the plant (Ahmad *et al*., 2020).

In addition, the decrease in the plant biomass was in line with the decrease in the content of chlorophyll content in *O. cylindrica* upon exposure to GA. It seems there is a limitation in biosynthesis and an increase in the degradation of photosynthetic pigments upon exposure of plants to GA hormones. It has been observed GA prevents the expression of genes involved in the biosynthesis of chlorophyll through the inhibition of DELLA transcription factors (Liu *et al*., 2017). Kin modulates the content of photosynthetic pigments by regulation of chlorophyll biosynthesis (Cortleven and Schmülling, 2015) or by delaying the degradation of chlorophyll (Hönig *et al*., 2018). However. It depends on cytokinin structure and concentration, the age of the plant (Zubo *et al*., 2008), and environmental conditions (Vlckova *et al*., 2006). In the current study, Kin showed an ambiguous effect on *O. cylindrica*. Exposing the plant to Kin resulted in no impact at 250 and 1000 mg/L or even a significant reduction of the content of Chl. a at 500 mg/L Kin.

Sugars are an important source of energy and carbon skeleton for the growth and development of plants. However, they also act as signaling molecules that can influence growth and metabolic processes (Rolland *et al*., 2006). It has been shown that plant hormones cause changes in the content of soluble sugars in different plants by modulating the expression of genes involved in the biosynthesis of sugar (Loreti *et al*., 2008). The findings showed that GA treatment increases sugar accumulation, adjusts the size of the sugar sink, and prevents sugar unloading from the sink (Li *et al*., 2024). This was in line with our finding which showed GA enhanced the content of soluble sugars at 500 and 1000 mg/L.

Phenolic compounds including anthocyanins, flavonoids, and flavonols as plant's secondary metabolites not only play a role in plant defense against herbivores, bacteria, fungi, and abiotic stress but also act as attractor of pollinators that lead to the distribution of plants (Lin *et al*., 2016). However, the presence of plant phenolic compounds is also interesting nowadays because of their positive impact on human health in the control of many chronic diseases due to their antioxidant potential and free radical-scavenging activity (Sardoei *et al*., 2014). Therefore, an increment of phenolics content in *O. cylindrica* induced by Kin and GA treatments would show its positive role in plant antioxidative potential. Indeed, cytokinin increases the level of phenolic compounds through a positive effect on its biosynthetic pathway, however, the type and concentration of cytokinin have different effects on the biosynthesis of the phenolic compounds (Grzegorczyk-Karolak *et al*., 2020). Interestingly, the content of phenolic compounds was in line with PAL enzyme activity. PAL as a kay enzyme is responsible for the biosynthesis of trans-cinnamic and subsequently other phenolic compounds from L-phenylalanine. PAL activity is

stimulated by a variety of environmental factors, including pathogenic factors, mechanical damage, ultraviolet radiation, heavy metals stress, low temperature, and low levels of nitrogen and phosphate. However, the regulation of PAL activity in response to plant hormones seems to have complex mechanisms (Zhang and Liu, 2015). The current study showed that external application of Kin and GA induced the activity of PAL in *O. cylindrica*, while auxin hormone hardly affected the activity of this enzyme compared with that of the PT plants.

Superoxide dismutase and peroxidase enzymes are among the most important enzymes of the plant's antioxidant defense system. The superoxide dismutase enzyme catalyzes the conversion of superoxide anion into hydrogen peroxide, which is an important step in neutralizing and reducing the level of oxygen-free radicals in plants. Peroxidase also plays an active role in neutralizing hydrogen peroxide (Rajput *et al*., 2021). The increase of POD enzyme activity following the rise of SOD enzyme activity in the plant treated with Kin hormone enforces the positive role of this hormone in increasing the antioxidant property of the cactus plant. While the significant decrease in the activity of the POD enzyme and instead the increase in the activity of SOD may indicate the presence of stress caused by GA hormone due to the possible accumulation of hydrogen peroxide.

CONCLUSION

In the present study, the role of hormones auxin, cytokinin and gibberellin on growth and antioxidant properties of the *O. cylindrica* cactus was investigated. The current study showed that cytokinin specially at concentrations of 500 and 1000 mg/L had resulted in the increase of fresh weight, the amount of total phenol and flavonoids, PAL enzyme activity as well as the increase in the activity of antioxidant enzymes SOD and POD. While the decrease in fresh weight and the content of chlorophyll a and b as well as the decrease in POD enzyme activity under gibberellin treatment is a possible sign of stress caused by this hormone. Therefore, an increase in the activity of antioxidant enzymes like POD and an increase in the content of phenolic compounds probably play an important role in the adaptation of plants to the adverse impact of gibberellin. It can be concluded that cytokinin had a positive and best effect on improving the antioxidant capacity and growth of *O. cylindrica* cactus and it is recommended to use cytokinin in cultivation of *O. cylindrica*.

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