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Original article

Protective effect of jasmonic acid and potassium against cadmium stress in peas (*Pisum sativum* L.)

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ABSTRACT

A combination of mineral nutrients and plant growth regulators should be assessed to improve crop performance under various abiotic stresses. There is a need to include plant growth regulators in fertilization regime of various crops along with essential mineral nutrients, especially when they are irrigated with polluted water with higher levels of heavy metals. The performance of pea was evaluated under cadmium (Cd) stress coupled with potassium (K) and jasmonic acid (JA) supplementation. The Cd stress (50 μM) was applied to soil (sandy loam) grown pea plants as basal dose after a month of sowing. The control and stressed plants were then supplemented with K (5 M), JA (0.5 mM) and their collective application along with control as distilled water. Cd stress showed a marked reduction in growth pattern, however, the collective supplementation sufficiently improved the growth pattern of stressed peas plants as evidenced by improvement in shoot length (cm), root length (cm), number of leaves per plant, leaf area (cm^2), plant fresh and dry weight (gm). Potassium application under Cd stress significantly enhanced internodal distance (cm) while the number of seeds per pod and relative water contents remained non-significant. The applied treatment (JA + K) under Cd stress prominently improved enzymatic activities, which were measured as nitrate reductase activity (NRA), nitrite reductase activity (NiRA), superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT). Cd stress impacted the biochemical profile by enhancing antioxidant capacity (AC), antioxidant activity (AA), total phenols (TP), while reducing total soluble protein (TSP), chlorophyll 'a', chlorophyll 'b' and carotenoids. The combined application of JA and K under Cd stress enhanced AC, AA, TP, Chl a and b, TSP and carotenoids. The results indicate that foliar application of JA and K efficiently negated the harmful effects of Cd stress on peas.

1. Introduction

An important herbaceous and annual vegetable crop, protein-rich pea (*Pisum sativum* L.) from family Leguminosae is a self-pollinated crop susceptible to different stress factors (Sandalio et al., 2001). Abiotic stressors alter the growth and productivity pattern of vegetables. Cadmium (Cd) is a heavy metal that causes health hazards, even at low concentrations, when passed in the food chain (Shanmugaraj et al., 2019). Its main sources are anthro-

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pogenic activities including industrial effluents, manures and pesticides (Per et al., 2016; Ali et al., 2018). The roots are major Cd absorbing organ via the xylem and phloem, with different rates of absorption depending on plant species. Its absorption in plants interferes with plant metabolism, thus restricting root growth and photosynthesis and affecting the other nutrient absorption (Sandalio et al., 2001). Cd is a mobile element in the plant, which imposes serious alterations to its physiological, biochemical and molecular processes. It also disturbs the absorption and accumulation of other essential nutrients leading to deficiencies of Zn, Fe, Ca and Mg. Furthermore, it interrupts normal plant growth by causing oxidative stress through reactive oxygen species (ROS) production and degradation of photosynthetic apparatus (Shamsi et al., 2008a, 2008b, 2010).

Jasmonates (JA) belong to cyclopentanon compounds with linolenic acid as a precursor, synthesized through octadecanoic pathway. It is a phytohormone, widely researched against various abiotic and biotic stresses. During stress, it plays a chief role in the signaling network (Fujita et al., 2006). It has an inhibitive impact on plant growth, when applied at higher concentrations, however, supplementation to stressed plants at low concentration improves its stress tolerance ability (Keramat et al., 2009). The role of JA in plant growth has depicted a contradictory pattern. Jasmonates applied at high dose (≥100 μM) is reported to suppress germination and plant growth, chlorophyll contents and photosynthesis (Jubany-Marí et al., 2010). However, It accelerated plant growth, root development, dry matter formation, photosynthetic pigments and rate of CO_2 assimilation (Piotrowska et al., 2010) at lower doses. It is reported to support the pattern of physiological and biochemical mechanisms in plants. Its protective role against stress is related to improvement in antioxidant capacity and reduction in thiobarbituric acid reactive substances (TBARS) and hydrogen peroxide (Keramat et al., 2009; Singh and Shah, 2014). An important macronutrient, Potassium (K) is an essential nutrient that regulates the water and nutrient uptake and translocation, stomatal movements and carbohydrate storage. It activates different enzymes involved in ATP synthesis, carbohydrate and protein metabolism (Shamsi et al., 2008a, 2008b).

The farmers around the major cities are more interested in growing vegetables to meet the need of people in the cities who have no time and interest in growing vegetables. The major source of irrigation for market gardeners is untreated sewerage water which also contains effluents from industries having higher levels of heavy metals especially cadmium. The impact of cadmium stress on plants is devastating so there is a need to counteract its harmful impact as well as to improve the plant growth. The supplementation of nutrients like calcium, potassium, zinc, etc. and phytohormones like jasmonates, brassinosteroids, salicylic acid, etc. have been investigated against abiotic and biotic stresses in many plants. However, an interest in the interactive use of nutrients and phytohormone against stresses has recently developed. Therefore, our experiment was planned to investigate the combined effect of a plant growth regulator and a nutrient to suppress Cd stress. We hypothesize that PGRs will enhance plant growth under Cd stress conditions, while potassium will improve the quality and quantity of produce.

2. Materials and methods

The pot 12 cm diameter, 15 L) experimental units (sandy loam) with pea (Orion variety) were set in the Department of Horticulture, Bahauddin Zakariya University, Multan, in a completely randomized design (CRD) with a factorial arrangement having three replications per treatment. The plant population was maintained as 10 plants per pot and was irrigated with Hoagland's solution (half strength) after germination. After a month of sowing, cadmium stress (50 μM) was applied to selected pots, while control was maintained with the application of distilled water as basal dose as 500 mL/pot. After 45 days of the initiation of stress treatment, the solutions of K (5 M), JA (0.5 mM), their collective application (5 M potassium + 0.5 mM JA) and distilled water (control) were sprayed, followed by a subsequent application after a week. Data was collected after a week of chemicals spray as:

2.1. Plant growth

Physical growth parameters included plant height (cm), root length (cm), number of leaves per plant, leaf area (cm^2), internodal distance (mm), Fresh biomass (g), dry biomass (g) and relative water contents. Reproductive growth parameters included the number of pods/plant and the number of seeds/pod.

2.2. Enzymes

These sampled leaves were cleaned and ground in 5 mL phosphate buffer (pH 7.8, 50 mM) and centrifuged at 15,000*g* for 20 min and supernatant was reserved to be used for estimation of enzymatic activities. SOD activity (IU min^{-1} mg^{-1} protein) was measured by the rate of inhibition of photochemical reduction of Nitroblue tetrazolium. The reaction mixture contained 50 μL of enzyme extract, 1 mL of 50 μM NBT, 1 mL of 1.3 μM riboflavin, 500 μL of 13 mM methionine, 950 μL of 50 mM of phosphate buffer, 500 μL of 75 mM EDTA and was placed under 30 W fluorescent lamp, in order to start the reaction. The lamp was turned off after 5 min, it stimulated blue formazan formation, which is measured at 560 nm and compared with the sample which remained in dark (Giannopolitis and Ries, 1977). The POD activity was measured through guaiacol oxidation expressed as 0.01 absorbance change min^{-1} mg^{-1} protein. The reaction mixture included 2 mL phosphate buffer (50 mM), 500 μL H2O2 (40 mM) and 400 μL guaiacol (20 mM) and 100 μL enzyme extract. After every 20 s up to 5 min, the changes in absorbance at 470 nm of the reaction mixture were recorded (Chance and Maehly, 1955). The activity of catalase was estimated from a reaction mixture having 900 μL (5.9 nM) of hydrogen peroxide, 2 mL (50 mM) phosphate buffer and 100 μL enzyme extract. The H_2O_2 putrefaction was measured and H_2O_2 concentration dilution was recorded after every 30 sec for 5 min via a UV–visible spectrophotometer. Using various concentrations of H_2O_2, standard curves were made and CAT activity was estimated as μmol of H_2O_2 min^{-1} mg $protein^{-1}$ (Chance and Maehly, 1955).

The nitrate reductase activity (NRA) and nitrite reductase activity (NiRA) of peas were estimated according to the methods given by Sym (1984) and Ramarao et al. (1983). For NRA, the reaction mixture containing chopped pea leaves, 2.5 mL phosphate buffer (pH 7.0) and 0.5 mL of 20 mM KNO3 was incubated at 32 °C for 1 h in the dark. A 0.5 mL sulfanilamide and 0.5 mL N-(1-naph thyl)-ethylenediamine dihydrochloride was mixed with 1 mL aliquot of the incubated reaction medium, which resulted in pink diazo colored complex development. 5 mL distilled water was added to it to dilute color after 20 min and the solution was centrifuged at 2000 rpm for 5 min. The absorbance of centrifuged and a set of $NaNO_2$ standards was measured at 542 nm. A blank reading was obtained by performing the above procedure without adding the sample. For NiRA assessment, 0.5 g pea leaf sample was added to a test tube containing 4.5 mL phosphate buffer (pH 5.0) and 0.5 mL 20 mM $NaNO_2$ and incubated at 30 °C for 150 min. The samples were then transferred into boiling water for 2 min and cooled. The reaction mixture was comprised of 0.5 mL, 1% sulfanilamide and 0.5 mL 0.02% N-(1-Naphthyl)-ethylenediamine

dihydrochloride. The optical density was observed at 540 nm and a standard curve was developed using known $NaNO_2$ solutions.

2.3. Biochemical parameters

Antioxidant capacity (mM Trolox/100 mL) was recorded following the method of Brand-Williams et al. (1995). Sample extract (30 μL) was added to 2.97 mL of 2,2-diphenyl-1-picrylhydrazyl (DPPH) solution (0.1 mM), incubated in dark for 30 min and absorbance was measured at 515 nm using a spectrophotometer. The antioxidant capacity of samples was estimated by comparing with standard curves of Trolox (10–100 μmol/L). The antioxidant activity (%) was as a difference between absorbance of control and sample, divided by absorbance of the control, multiplied by 100.

For leaf total protein contents, the leaf sample was crushed in liquid nitrogen. 0.5 g of the crushed sample was added to test tubes containing 1 mL of 2 mM potassium phosphate buffer solution (pH 7.2), 2.7 mM of potassium chloride, 1.37 mM sodium chloride, and 10 mM sodium phosphate, mixed thoroughly and centrifuged at 10,000 rpm for 5 min. The solution was stored in a refrigerator (Sambrook and Russell, 2006). 200 μL of supernatant was taken and mixed with 780 μL water and 20 μL dye and optical density was observed at 595 nm (Bradford, 1976). Harborne et al.'s (1973) method was used for estimation of Chlorophyll "a", "b" and carotenoids. 0.5 g grounded leaf sample was centrifuged with 80% acetone (10 mL) at 12000 rpm for 5 min. The absorbance of Chl a, b and carotenoids was recorded at 645 nm, 663 nm and 480 nm in UV-1900 spectrophotometer. Arnon (1949) formula was used for Chl estimation. For Chl a, the optical density measured at 663 and 645 was multiplied with factors 0.0127 and 0.00269, respectively. Their difference was recorded and divided by 0.5 and multiplied by 100. For Chl b, the optical density measured at 645 and 663 was multiplied with factors 0.0229 and 0.00468, respectively. Their difference was recorded and divided by 0.5 and multiplied by 100. The total chlorophyll was estimated as the sum of the product of the optical density of 645 and 663 with 20.2 and 8.02, respectively, multiplied by the volume of extract, divided by 1000 and multiplied by the weight of the sample. Carotenoid contents was estimated as $A^{car}/Em \times 100$.

$$\text{Where } A^{car} = OD\ 480 + 0.114(OD\ 663) - 0.638(OD\ 645)E^{100}\%\ cm = 2500$$

For total phenolics, as described by Julkenen-Titto, (1985), 0.5 g leaf sample was taken and homogenized with 10 mL of 80% acetone, using a centrifuge at 10,000g for 10 min. 100 μL of supernatant was added to 2.0 mL of distilled water and mixed with 1 mL of Folin Ciocalteau's phenol reagent and 5.0 mL of 20 % Na_2CO_3 solution. Volume was made up to 10 mL using distilled water. The reaction mixture was shaken and observed at optical density of 750 nm on the Spectrophotometer (Hitachi-120, Japan).

2.4. Statistical analysis

The collected data for each parameter was analyzed using statistix 8.1 software and treatment means were compared using least significant difference test at 0.01% significance level. Fisher's analysis of variance technique was used to mark the significance of applied chemicals on studied parameters of peas.

3. Results

3.1. Growth parameters

The pea plants which received Cd stress followed by supplementation of K, JA and their combination, showed a marked influence on their growth (Table 1). The effect can be summarized in terms of various physical growth parameters. The Cd stressed plants attained less shoot length (16.67 cm), however, the stress effect was negated to some extent when these plants were subjected to JA and K (22.33). The collective application of JA and K was also found to influence positively the root length (13.33 cm) and the number of leaves per plant (54.66) of salt-stressed pea plants, followed by K and JA separate applications, respectively. JA + K improved leaf area (1.55 cm^2) while K applied alone influenced internodal distance (1.96 cm) in stressed conditions. Plant fresh (11.33 g) and dry biomass (0.78 g) and the number of pods per plant (6.33) were found to be superior when the combination of JA and K, K alone and KA + K, respectively were applied to Cd stress. The number of seeds per pod and relative water contents remained non-significant in relation to the applied treatments.

3.2. Enzymatic parameters

The ANOVA presented in Table 2 represented the significant impact of applied nutrient and PGR on enzymatic activities of peas under Cd stress. Cadmium stress and applied PGR and nutrients significantly affected the enzymatic attributes of pea plants (Fig. 1). With the reference NRA (nitrate reductase activity), an increase of 2.60% was noted in stressed plants as compared to control. An increase of 2.46%, 1.76% and 5.89% was observed when stressed pea seedlings were treated with K, JA and collective application of JA and K as compared to Cd stressed plants (without any PGR/nutrient supplementation). However, there was an increase of 6.64%, 4.91% and 10.71% in NRA, when K, JA and K + JA applied under stressed conditions were compared with their respective controls (K, JA and K + JA without cadmium stress). NiRA (Fig. 1) increased by 17.76% in stressed pea plants. An increment of 6.87%, 22.22% and 17.76% was recorded with the application of K, JA and K + JA, respectively when compared with their respective non-stressed treatments. When NiRA was compared under stressed conditions, it increased by 9.39%, 19.97% and 24.67%, as a result of K, JA and JA + K application, respectively. SOD of Cd stressed plants increased by 8.3 % when compared to control (Fig. 1). Under stressed conditions, K improved SOD by 17.17%, JA and JA + K caused an increase of 14.11% and 22.39%. When treatments were compared with their respective nonstressed plants, K and JA stimulated SOD activity by 2.96% and 12.82%. However, a combined application of JA and K improved SOD by 9.31%. Stressed conditions increased POD and CAT activity by 52.27% and 17.35%, respectively (Fig. 1). However, the application of K, JA and JA + K applied to stressed plants improved POD by 4.47%, 79.10% and 76.11%, and CAT by 12.84%, 25.68% and 11.24%, respectively when compared with stressed plants. The comparison of stress applied K, JA and JA + K with non-stressed application revealed an increase of POD by 9.94%, 34.76% and 1.72%, respectively. The CAT activity of stressed plants applied with K, JA represented an increase of 11.96% and 8.6%, while JA + K application decreased CAT by 2.38%, when compared with their respective control plants (nutrient and PGR applied but without stress).

3.3. Biochemical attributes

The significance of biochemical parameters with respect to applied chemicals under stress conditions is depicted in Table 2.

Table 1
Effect of Jasmonic acid and potassium on physical growth parameters of cadmium stressed peas seedlings.

	Shoot length (cm)			Root length (cm)			No. of leaves/plant			Leaf area (cm^2)			Internodal distance (cm)		
Treatments	0 μM Cd	50 μM Cd	Mean	0 μM Cd	50 μM Cd	Mean	0 μM Cd	50 μM Cd	Mean	0 μM Cd	50 μM Cd	Mean	0 μM Cd	50 μM Cd	Mean
Control	20.66 D	16.667F	18.663 D	11.33C	10.33 D	10.83C	35.33F	33.66 G	34.49 D	1.38C	1.25 D	1.31C	2.66C	1.93 D	2.30 BC
Potassium (5 M)	24.667B	18.333 E	21.5B	12.66B	11.66C	12.16B	43.33 E	48.66 D	46.00C	1.09 E	1.23 D	1.16 D	2.93 A	1.96 D	2.45 A
JA (0.5 mM)	21.333 D	20.667 D	21.000C	13.33B	9.33 E	11.33C	62.66 A	42.66 E	52.66B	1.70 A	1.22 D	1.46B	2.86 AB	1.83 E	2.35B
K (5 M) + JA (0.5 mM)	25.667 A	22.333C	24.000 A	15.66 A	13.33B	14.50 A	57.33B	54.66C	55.99 A	1.65 AB	1.56B	1.60A	2.83B	1.63F	2.23C
Mean	23.083 A	19.500B		13.25 A	11.16B		49.66 A	44.91B		1.45 A	1.31B		2.82 A	1.84B	
LSD ≤ 0.05	0.964			0.619			1.07			0.107			0.102		
CV (%)	2.59			2.90			1.29			4.41			2.50		
	Fresh Biomass (g)			**Dry Biomass (g)**			**No. of pods/plant**			**No. of Seeds/pod**			**Relative water contents (%)**		
Treatments	**0 μM Cd**	**50 μM Cd**	**Mean**	**0 μM Cd**	**50 μM Cd**	**Mean**	**0 μM Cd**	**50 μM Cd**	**Mean**	**0 μM Cd**	**50 μM Cd**	**Mean**	**0 μM Cd**	**50 μM Cd**	**Mean**
Control	12.33 A	9.33C	10.83 A	0.78C	0.65 D	0.72C	8.66 A	5.66CD	7.16 A	8.66	6.66	7.66 A	253.97	310.00	281.99
Potassium (5 M)	11.33B	8.66CD	10.00 BC	0.87B	0.77C	0.82B	5.66CD	5.33 D	5.50B	7.66	5.33	6.50B	272.22	245.50	258.86
JA (0.5 mM)	10.66B	8.33 D	9.50	0.67 D	0.79C	0.72C	6.66B	5.33 D	6.00B	6.66	5.33	6.00B	223.03	236.36	229.70
K (5 M) + JA (0.5 mM)	9.33C	11.33B	10.33 AB	0.94 A	0.74C	0.85 A	8.13 A	6.33 BC	7.33 A	8.66	7.33	8.00 A	293.65	271.43	282.54
Mean	10.91 A	9.41B		0.82 A	0.74B		7.33 A	5.6667B		7.91 A	6.16B		260.72	265.82	
LSD ≤ 0.05	1.04			0.07			0.97			NS			NS		
CV (%)	5.88			5.59			8.56			8.27			9.60		

Means followed by different letters within a column or a row significantly ($p < 0.05$) differ from each other.

Table 2
Mean sum of squares of growth, enzymatic and biochemical parameters cadmium stressed peas seedlings.

SOV	DF	SL	RL	NLP^{-1}	LA	ID	PFB	PDB	NP $plant^{-1}$	NS pod^{-1}	RWC	NRA
Cd Stress (S)	1	77.04**	26.04**	135.37**	0.12**	5.80**	13.50**	0.034**	13.5**	18.37**	156.32 NS	40.43**
Treatment (T)	3	28.70**	15.81**	540.04**	0.21**	0.05**	1.88*	0.024**	3.83**	5.37**	3736.5 NS	0.842**
S × T	3	8.15**	3.04**	174.04**	0.09**	0.05**	8.27**	0.028**	1.38**	0.37NS	2210.47 NS	2.864**
SOV	**DF**	**NiRA**	**SOD**	**POD**	**CAT**	**AC**	**AA**	**TP**	**TSP**	**Chl a**	**Chl b**	**Car**
Cd Stress (S)	1	864.12**	0.531**	1.05**	0.382**	0.09**	20836223**	4.465**	5.704E-04**	1.667E-05**	0.022**	7.94E-05**
Treatment (T)	3	248.793**	0.593**	4.92**	0.559**	0.326**	2373557**	0.279**	7.699E-04**	0.0497**	2.390**	5.067E-04**
S × T	3	80.989**	0.0365**	0.173**	0.0272**	0.005**	201067**	0.666**	5.002E-04**	4.056E-04**	0.5537**	6.757E-06*

Here, SOV = source of variance, DF = degree of freedom, SL = Shoot Length, RL = Root Length, NLP^{-1} = Number of Leaves per Plant, LA = Leaf Area, ID = Internodal Distance, PFB = Plant Fresh Biomass, PDB = Plant Dry Biomass, NP $Plant^{-1}$ = Number of Pods per Plant., NS Pod^{-1} = Number of Seeds per Pod, RWC = Relative water contents, NRA = Nitrate reductase activity, NiRA = Nitrite reductase activity, SOD = Superoxide dismutase, POD = peroxidase, CAT = Catalase, AC = Antioxidant capacity, AA = Antioxidant activity, TP = Total Phenolics, TSP = Total soluble protein, Chl a = Chlorophyll *a*, Chl b = Chlorophyll *b*, Car = Carotenoids, NS = non-significant, ** = significant at $p \leq 0.01$.

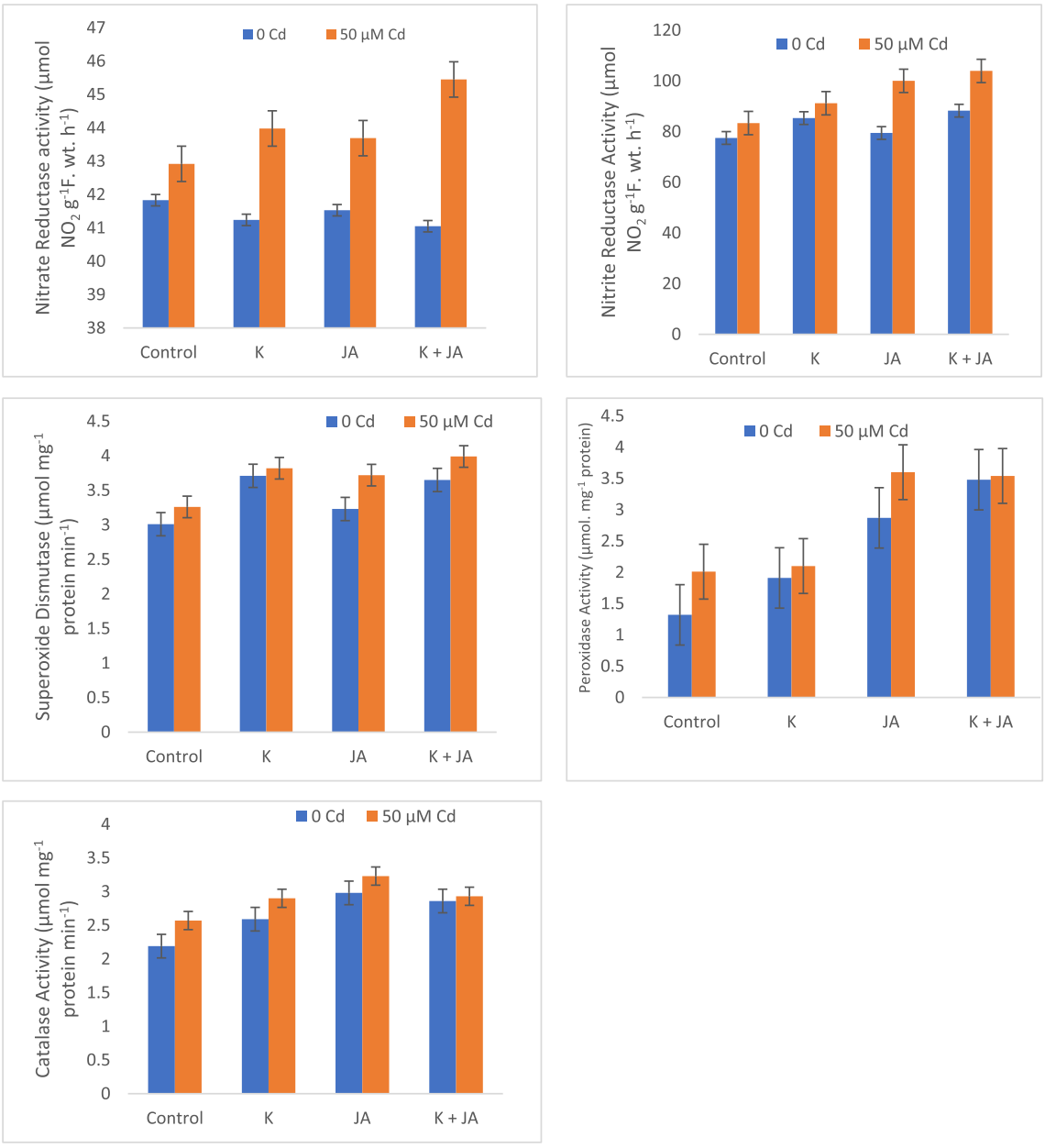

Fig. 1. Effect of Jasmonic acid and potassium on enzymatic attributes of cadmium stressed peas seedlings. *Each value in the above figures is the mean of 3 replicates and the vertical bars give the standard error (SE) of the mean. Least significant difference test for stress and treatments were significant at P = 0.01.*

The antioxidant capacity (AC) of Cd stressed pea seedlings increased by 34.54%, when compared with control (Fig. 2). A respective increase of 9.45%, 13.51% and 20.27% was observed in AC of K, JA and JA + K supplemented stressed pea plants compared with stressed plants. The comparison of stressed plants with application of K, JA and JA + K with control (non-stressed plants without chemical treatment), showed an increase of 9.45%, 11.11% and 21.98%, respectively. An increase of 4.02% was noted in antioxidant activity (AA) of Cd-stressed plants as compared to control. Antioxidant activity of Cd stressed pea plants subjected with K, showed an increment of 54.98% as compared to stressed plants and 35.89% when compared with non-stressed plants (Fig. 2). An improvement in AA by 94.12% and 109.83% (compared with stressed plants) and by 28.0% and 36.10% (compared with non-stressed plants) was noted in plants supplied with JA alone and JA + K, respectively. Total phenolic contents (Fig. 2) of Cd stressed plants increased by 60.83%, while the stressed plants supplemented by K, JA and JA + K experienced an increase of 26.08%. 10.86% and 29.13%, respectively, when compared with stressed plants. When Cd stressed plants with K, JA and JA + K was compared with their respective plants with no stress, an increase of 128.34% and 32.758% was observed with K and JA application, however the response of JA + K was remained at par. Total soluble protein (Fig. 2) was reduced by 15.78% in Cd stressed plant as compared to control. Application of K, JA and JA + K under Cd stress improved total soluble protein contents by 12.5%, 31.25% and 12.5%, as compared with stressed pea plants. Cd stress reduced Chl a and Chl b by 33.33% and 30.96%, however when stressed plants were subjected to K, JA and JA + K, an increase of 65%, 100% and 350% was noted in Chl content and an increase of 84.11%, 95.32% and 190.65% was recorded in Chl b contents when compared with stressed plants (Fig. 2). Stressed conditions reduced

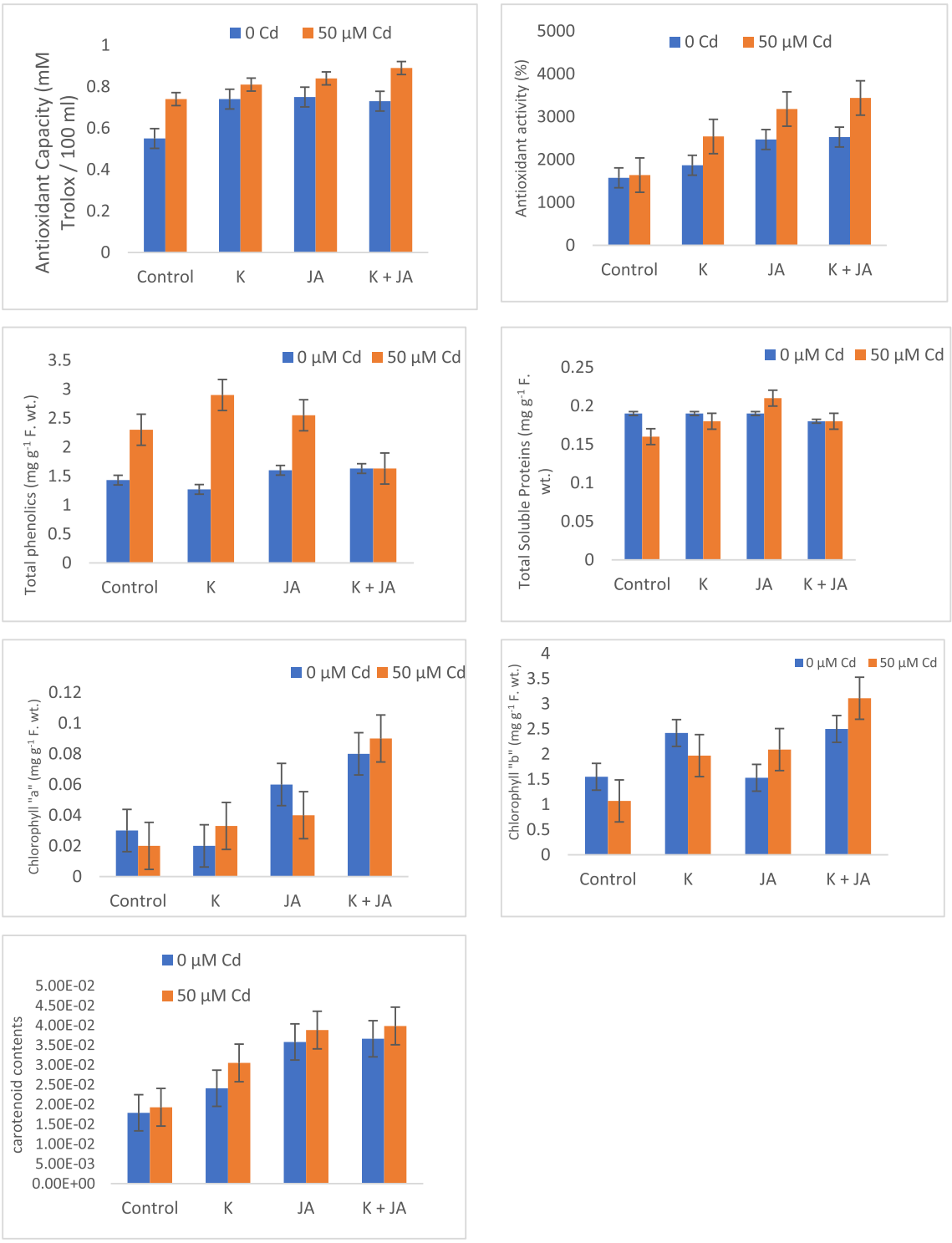

Fig. 2. Effect of Jasmonic acid and potassium on biochemical parameters of cadmium stressed peas seedlings. Each value in the above figures is the mean of 3 replicates and the vertical bars give the standard error (SE) of the mean. Least significant difference test for stress and treatments were significant at P = 0.01.

carotenoid contents (Fig. 2) by 30.49%, the increase of 58.16%, 95.40% and 94.89% was recorded when K, JA and JA + K applied to stressed plants were compared to stressed plants receiving no chemical spray.

4. Discussion

Cadmium stress, being a hazardous growing condition, negatively impact plant growth and development. It is a common nonessential heavy metal that has found its way in polluting irrigation water when industrial effluents and sewage wastes are being disposed of in the running water of rivers and canals (Shanmugaraj et al., 2019). In the current research experiment, Cd caused a considerable decrease in plant growth attributes due to its ability to disturb root growth and limit moisture availability to plant (Silva et al., 2012). The high mobility rate of Cd implements drastic effects even at its low concentration (Barceló and Poschenrieder, 1990). The rate of absorption of Cd by plant roots is quick and

adversely impacted the xylem in leaves, disrupting the photosynthesis and nutrient uptake by roots (Sandalio et al., 2001). Roots are the foremost plant organ, which is directly affected by cadmium toxicity (Andresen and Küpper, 2013), which is obvious from the reduction in root length and also plant fresh biomass. Shoot length also reduces as a result of Cd stress, characterized by rolling of leaf sheaths, chlorosis and stunted appearance. The stunted or low growth rate can also be attributed to cell division or elongation inhibition, poor root growth and less nutrient absorption and translocation to above-ground plant parts (Mondal, 2013). The expression of Cd stress on the apparent growth of peas in current research coincides with Sandalio et al. (2001) and Silva et al. (2012). The loss of green color in leaves may be attributed to decline in iron absorption, phosphorus availability or less manganese mobility (Benavides et al., 2005). These nutrient imbalances due to Cd negatively impact photosynthesis (Alcantara et al., 1994). The negative impact of Cd on pea growth was negated to a considerable extent through sole applications of K, JA and their combination. An antagonistic response was observed between potassium and cadmium, as reported by Shamsi et al. (2008a, 2008b). It can also be related to an observation made by Wang et al. (2017), according to which potassium is responsible for reduced uptake of Cd by the plants. The augmentation observed in potassium applied Cd stressed pea growth might be attributed by its crucial functions including maintenance of energy level of plant and cell water relations, assimilate translocation, enzyme activation, guarding stomatal movements, transfer of nutrients and protein and starch synthesis. Methyl jasmonate is an important growth regulator, which act as a stress signaling molecule, when applied at low concentrations (Keramat et al., 2009). It has also equipped stressed plant with better growth attributes. However the combine application of K and JA produced superior results in many growth attributes, leading to confer that both JA and K have direct or indirect influence in stabilizing plant growth under stress.

Being a leguminous crop, pea has an active mechanism of nitrogen assimilation. As heavy metal stress, particularly Cd toxicity leads to imbalance in nutrient absorption and its translocation within plant body. Cd stress minimizes the nitrate absorption through altering the nitrate reductase activity and its mobility from root system to aerial part (Hernandez et al., 1996). This nitrogen fixation inhibition and assimilation of ammonia was also noted in soybean with Cd stress (Balestrasse et al., 2003). A collective supplementation of K and JA supported the mechanism of nitrogen fixation and resulted due to improved NiRA and NRA enzyme activity.

The antioxidant defense system of plants follows a disturbed pattern in response of Cd stress, thus favoring oxidative stress (Romero-Puertas et al., 1999) by reactive oxygen species (ROS) and free radicals generation (Sandalio et al., 2001). These ROS confer adverse impacts on plant cell structure and hereditary material. Plants have an internal mechanism (enzymatic and nonenzymatic) to counteract the ROS effect. Protein antioxidants as ROS scavengers include superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APOX), glutathione reductase (GR) (Mittler, 2002). SOD is the main scavenging molecules that convert the radical superoxide to hydrogen peroxide. The APX or CAT then converts hydrogen peroxide to water and oxygen. The excessive amount of H_2O_2 is removed by CAT (Mourato et al., 2012). Cadmium stress improved the antioxidant activity, as in line with current observation regarding SOD, POD and CAT (Cho and Seo, 2005). The stress-induced increased enzymatic activity might be due to enhanced H_2O_2 generation (Li et al., 2016). It can also be inferred that peas can acclimatize to Cd stress to some extent by increasing the activity of their defense system. Potassium application enhanced SOD and POD in Cd stressed soybean (Haider et al., 2008) and gladiolus (Zaheer et al., 2018). The increase of enzymatic defense enzyme activity as a response to K application could be due to its role in reducing the NAD(P)H oxidases activity and supporting the electron transport chain (Siddique et al., 2012). Moreover, K also supports protein synthesis of thioredoxin, glutaredoxin, cyclophilin, which plays a significant role in reduced peroxiredoxins regeneration, which further leads to reduced ROS formation (Tripathi et al., 2009; Siddique et al., 2012). Methyl jasmonate improved the antioxidant defense system of Cd stressed *O. sativa* and *Capsicum frutescens* (Singh and Shah, 2014). In this study, the collective application of JA and K in Cd-stressed plants surpassed their sole application in terms of enhanced antioxidant enzymes activity.

The negatively impacted antioxidant capacity and antioxidant capacity of Cd stressed peas were noticed to be relieved through JA application (Singh and Shah, 2014). In the current investigation, the combined application of nutrient and plant hormones augmented the antioxidant capacity of stressed peas as compared to their separate application. Cadmium stress leads to alteration in biochemical attributes of plants. As Cd stress diminishes the ATPase activity, thus induces changes in membrane functionality, disturbs the chloroplast metabolism (Fodor et al., 1995; Haider et al., 2008) and chlorophyll biosynthesis inhibition (Parmar et al., 2013), which is also observed in the current investigation. JA application resulted in improved photosynthetic pigments which can be linked with antioxidant enzymes activation (Per et al., 2016). K also serves to protect the chlorophyll molecules andaminolevulinic acid (ALA) (Siddique et al., 2012). The application of JA along with K served to prevent the disorganization of chloroplast structure, thus helping improve chlorophyll contents of stressed plants. Cd stress also reduced total soluble proteins, total phenolics and carotenoid contents. The role of potassium has been listed in enhancing protein contents of stressed plants by enhancing its synthesis, reducing the rate of proteolysis and enzyme denaturation (Levitt, 1980). The potassium application to Cd stressed gladiolus plants improved total phenolic (Zaheer et al., 2018) which is in line with current observation. JA application is also observed to improve the secondary metabolites including alkaloids and phenolics (Kim et al., 2007) and carotenoids (Czerpak et al., 2006), which can offer protection to photosynthetic pigments (Memelink et al., 2001). The increased phenolic compounds may also serve to antagonist the uptake of heavy metals thus protecting the plant. They have antioxidant properties due to their ability of electron donation (Michalak, 2006). Carotenoids belong to the lipophilic antioxidant group, serve to detoxify ROS (Mourato et al., 2012) and act as a precursor to signaling chemicals which improves plant growth response under stressed conditions (El-Beltagi and Mohamed, 2013). In the current experiment, carotenoid contents of peas increased when experienced collective application of K and JA, under Cd stress. Therefore, the hypothesis was found to be supported by the findings of this research making it clear that JA and K was helpful in relieving the negative impacts of Cd stress in peas.

5. Conclusion

Exogenously applied K and JA stimulated the plant growth performance under Cd stress by improving morphological growth, biochemical and enzymatic activities of peas. Therefore, exogenously applied K and JA can be used to improve the productivity of pea under Cd stress.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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