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Alteration in photosynthetic pigments, osmolytes and antioxidants in imparting copper stress tolerance by exogenous jasmonic acid treatment in *Cajanus cajan*

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Jasmonic acid regulates various abiotic stress responses but its role in regulation of copper (Cu) homeostasis has been poorly studied. This present study was conducted to explore the potential of jasmonic acid (JA) to enhance copper (Cu) tolerance in *Cajanus cajan* seedlings in terms of photosynthetic pigments, osmolytes and antioxidants. Seeds treated with different concentration (0, 1 µM, 1 nM and 1 pM) of JA raised in toxic concentration of Cu for 15 days were taken for investigation. Results indicate that Cu stress led to the decreased growth in terms of shoot length, fresh and dry weight. Cu stress also increased Cu accumulation, lipid peroxidation, deteriorated chlorophyll b as well as carotenoids. Cu stress also diminished total proteins, vitamins A, C and B_2, and peroxidase activity which led to the deteriorated plant growth and metabolism. Seed priming treatment of JA on the other hand improved the protein content, sugar content and vitamins indicating the potential of JA for Cu stress tolerance. The JA supplementation to Cu stressed seedlings improved Cu tolerance of *C. cajan* to significant level.

Key words: Oxidative stress, lipid peroxidation, antioxidants, osmolyte, pigeon pea.

INTRODUCTION

Heavy metals pollution in the soil is a major threat to the ecosystem leading to deterioration of plant health by producing reactive oxygen species which are highly reactive and potentially toxic (Gjorgieva et al., 2013). Cu is probably the most common heavy metal contaminant of the soil. Copper loading of the agricultural soil due to anthropogenic activities is the major problem reducing crop productivity. Copper is an essential micronutrient and acts as a cofactor for Cu/Zn superoxide dismutase, cytochrome oxidase and polyphenol oxidase (Yruela. 2005). High availability of Cu in the soil results in growth inhibition and oxidative stress (Baryla et al., 2000;

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Hall 2002; Yruela, 2005). It also damages membrane integrity by binding to the sulphhydryl group of membrane proteins (El-Tyeb et al., 2006). Unfortunately, plant find an ample supply of Cu through their roots from soil and accumulated in system causing stress (Nicholls and Tarun, 2003; Agrawal and Sharma, 2006) along with triggering of certain physiological responses. The effect of their toxic influences on plants is largely a strong and fast inhibition of growth processes as well as decrease in activity of photosynthetic apparatus and senescence (Maksymiec and krupa, 2007). *Cajanus cajan* is the most important grain legume crop of rain-fed agriculture in semiarid tropics (Pal et al., 2011). It is the major legume crop of India and has great area under cultivation but the productivity is very low due to some exogenous and endogenous factors. Heavy metal toxicity is one of the major factors responsible for its low yield. A number of plant growth regulators available in literature are showing their potential effect on crop productivity. Jasmonic acid is one such plant growth regulator which is showing good potential in protecting plants from various types of stress conditions. JA is a plant signalling molecule responsible for regulating various morphological, physiological and biochemical processes within plants (Ueda and Saniewski, 2006; Norestenia et al., 2007). Promotion of leaf senescence estimated by a decrease in chlorophyll content and depression of photosynthesis related gene was one of the first reported physiological effects of jasmonates group (He et al., 2002). Cell division, plant growth, stomatal conductance and photosynthetic processes are all diversely affected by JA. It is reported from the studies carried out in previous years that application of JA could modulate the plant physiological processes towards abiotic stress tolerance (Walia et al., 2007) like salt stress (Kaur et al., 2013), and drought stress (Alam et al., 2014). Exogenous JA is effective in protecting plants from Cu stress by modulating photo-synthetic pigments (Sharma et al., 2013). This study is an addition to previous ones which is an effort to explore the mechanism laid behind tolerance capacity of JA. In the present study we analysed the effect of JA and Cu on pigments, osmolytes and enzymatic as well as non-enzymatic antioxidants in seedlings of *C. cajan* grown under Cu after JA treatment.

MATERIALS AND METHODS

Plant material and stress treatments

Seeds of *C. cajan* AL 201 (certified) were procured from Department of Plant Breeding and Genetics, Punjab Agriculture University, Ludhiana, India. Healthy seeds were selected and washed with running tap water and with 5% (w/v) tepol for 10 min followed by treatment with 5% hypochloride (v/v), a commercial fungicide for 5 min. Then surface sterilized seeds were soaked for pre-sowing treatments in different concentrations of JA and DW as control, for 6 h. Seeds of each treatment were sowed in triplicate. 10 mL of test solutions of respected Cu sulphate concentrations were given on first in each Petri plate. The soaked seed were grown in Petri plates in seed germinator at 24°C under controlled condition (200 PAR light, 80% humidity and 16±8 h photoperiod). Fifteen (15) days after sowing (DAS) seedlings were harvested for various biochemical and antioxidant analysis. Our experiment consisted of five treatments with three replication of each treatment. The treatment included control (DW), Cu (5 mM, copper sulphate), 1 µM JA and Cu, 1 nM JA and Cu, 1 pM JA and Cu.

Seed germination and growth

Seed germination was noted on 3rd day. The growth parameters in terms of root and shoot length were examined after 15 DAS of exposure to Cu stress.

Fresh weight and dry weight of seedlings

Fifteen (15) randomly selected fresh seedlings from each treatment were weighed, recorded and considered as fresh weight (FW) and dry weight (DW). Dry weight was determined after drying the seedlings at 60°C overnight.

Cu content

Cu content was calculated by (APHA, 1995 and AMC 1963 method). Dried plant sample was digested by heating in conc. H_2SO_4 and HNO_3. 0.5 ml of sample digest was diluted with 2.5 ml of double distilled water. 2.0% sodium dithiocarbamate was added and extracted in chloroform. Absorbance was measured at 435 nm in atomic absorption spectrophotometer.

Lipid peroxidation

The level of lipid peroxidation was measured by estimation of malondialdehyde (MDA) and other TBAR reactive molecules using thiobarbituric acid reaction method of Heath and Packer (1968).

Chlorophyll content

Leaf supernatant were extracted with 80%v/v acetone (centrifuging at 5,000 × *g*), absorbance were taken with UV-visible spectro-photometer at 663 and 645 nm; chlorophyll contents were calculated according to Lichtenthaler (1987).

Total soluble sugars, reducing and non-reducing sugars

Total sugars content was estimated by following Loewus (1952) method. Known weight of dried plant material was homogenised in 80% of ethanol and centrifuged at 3000x *g* for 15 min and the extract was collected for sugars estimation. For total sugars 0.05 ml of extract was diluted to 2 ml by distilled water and 3 ml cold anthrone reagent was added. Mixture was heated for 10 min and cooled. O.D. was recorded at 630 nm. Reducing sugars and non-reducing sugars were estimated according to method of Nelson and Somogyi (1952) method by taking absorbance at 620 nm.

Proline content

Proline was appraised according to Bates et al. (1973). 15 DAS seedlings were homogenized in 3% sulphosalicylic acid and

centrifuged at 11,500× *g*. The homogenate was then filtered and added with glacial acetic acid and acid-ninhydrin. After sample incubation at 100°C for 1 h toluene was added and absorbance at 520 nm was measured.

Glycine betaine

Plant material (0.5 g) is mechanically shaken with 20 ml of deionized water for 48 h at 25°C. Thawed extracts were diluted 1:1 with 2 N sulphuric acid. Cold potassium iodide-iodine reagent (0.2 ml) was added to the aliquat and stored at 0-4°C for 16 h. The supernatant was carefully removed after centrifugation and the periodite crystals were dissolved in 9 ml of 1,2-dichloro ethane (reagent grade). Absorbance was measured after 2.0-2.5 h at 365 nm.

Preparation of enzyme extract

Plant material was homogenized in 3 ml of pre-chilled phosphate buffer (pH-7.2), and centrifuged at 15,000 rpm for 15 min at 4°C. Supernatant prepared was used for the analysis of total protein, superoxide dismutase, catalase and peroxidase activity.

Total proteins

1 ml of ice cold 20% TCA was added to 0.5 ml of enzyme extract and kept for 18 h. Pellets were dissolved in 5 mL of 0.1 N NaOH. This was used for protein estimation. Total proteins were estimated by the method of Lowry et al. (1951) using BSA as standard.

Superoxide dismutase activity (EC 1.15.1.1)

The assay of superoxide dismutase was carried out based on the reduction of nitro blue tetrazolium (NBT) by Kono (1978) Method. To 0.5 ml of seed extract, 1.8 µl of 50 mM of sodium carbonate buffer (pH-10), 750 µl of 96 µM NBT, and 150 µl triton X-100 were added. The reaction was initiated by adding 0.4 ml of 1 mM hydroxylamine hydrochloride with recording absorbance at 540 nm.

Peroxidase activity (EC 1.11.1.7)

Peroxidase was assayed according to Putter et al. (1974) method by mixing 50 µl of guaiacol, 30 µl of H_2O_2 and 3 ml of potassium phosphate buffer and enzyme extract. Blank was prepared by adding all the reagents except enzyme extract.

Catalase activity (EC1.11.1.6)

The catalase activity was estimated using method of Aebi et al. (1984). 0.1 ml of enzyme extract was added to 1.9 ml of 50 mM phosphate buffer (pH 7.0). 1.0 ml of 30 mM hydrogen peroxide (H_2O_2) was added and a change in absorbance was followed for 1 min at 240 nm at 10 s intervals.

Vitamin A (retinol)

Vitamin A estimation was performed using the method described by Bayfield and Cole (1980). Homogenate (1.0 ml) was mixed with 1.0 ml of saponification mixture and refluxed for 20 min at 60°C in the dark and cooled with 20 ml of water. Vitamin A was extracted twice with 10 ml of (40 to 60°C) petroleum ether. TCA reagent (2.0 ml) was added rapidly, mixed and the absorbance was read immediately at 620 nm in a spectrophotometer.

Vitamin B_2 (Riboflavin)

To 5 g of sample powder, 150 ml of water and 5 ml of glacial acetic acid were added. The solution was boiled for 5 min and then cooled. After that, 30 ml of 1.0 M sodium hydroxide solution was added and diluted to 550 ml with water. The solution was filtered and absorbance was measured at 444 nm in Shimadzu UV-1201 spectrophotometer.

Vitamin C (ascorbic acid)

Vitamin C estimation was done according to the Chinoy et al. (1976) method. To 2 ml of plant extract, 8 ml of 2,6-dichlorophenol indophenols dye was added. Blank was prepared by using 2 ml of distilled water instead of extract and OD was recorded at 530 nm.

Vitamin E (α-tocopherol)

The estimation of vitamin E (α-tocopherol) was performed using the method described by Rosenberg (1992). Sample was mixed slowly with 0.1 N sulphuric acid and incubated at room temperature for overnight and then filtered. To the 1.5 ml of tissue extract, 1.5 ml of xylene was added and centrifuged. Then 1.0 ml of xylene was separated and mixed with 1.0 ml of 2,2-pyridyl and the absorbance was noted at 460 nm. In the beginning, 0.33 ml $FeCl_3$ was added with blank and mixed well. After 15 min, the test and standard was read against the blank at 520 nm.

Statistical analysis

All analysis was done on a completely randomized design. All data obtained was subjected to one way analysis of variance (ANOVA) using Graphpad Prism 5.0 software. Each data was the mean of three replicates (n=3) except for shoot and root length of *C. cajan* L. seedlings (n=5) and comparisons of p-values <0.05 were considered significant and different from control.

RESULTS

Effect of JA and Cu stress on growth

Germination of seedlings in presence of Cu showed 3% decrease in germination rate from that of control seeds grown in distilled water (Table 1). When seeds treated with different concentration of JA were grown in Cu stress, 1 µM concentration of JA showed inhibitory effect and up to 18% decrease in seed germination percentage was observed. Contrary to this, 1 nM and 1 pM JA concentrations proved beneficial for increasing germination rate and up to 17% increase in seed germination was observed in 1 pM JA treated seeds grown in Cu stress. Lower concentration of JA as seed priming treatment neutralize damaging effect of Cu by operating in antagonist

Table 1. The effect of JA on morphological and physiological parameters of *Cajanus cajan* L. Millsp. seedlings induced by JA under Cu stress conditions.

Treatment	Seed germination (%)	Root length (cm)	Shoot length (cm)	Fresh weight (mg/15 seedlings)	Dry weight (mg/15 seedlings)
Control	$82 \pm 1_c$	$4.26 \pm 0.83_a$	$10.17 \pm 0.76_a$	$3308 \pm 18_a$	$680 \pm 25_a$
5 mM	$80 \pm 2_c$	$0.83 \pm 0.05_b$	$7.23 \pm 0.2_{ab}$	$2792 \pm 95_b$	$653 \pm 14_a$
1 µM ± 5JA	$66 \pm 1_d$	$0.83 \pm 0.83_b$	$7.40 \pm 0.95_{ab}$	$2748 \pm 73_{bc}$	$672 \pm 19_a$
1 nM ± 5JA	$88 \pm 1.5_b$	$1.3 \pm 0.26_b$	$8.75 \pm 0.68_a$	$2120 \pm 48_e$	$527 \pm 14_{bc}$
1 pM ± 5JA	$94 \pm 2_a$	$1.1 \pm 0.36_b$	$8.27 \pm 0.21_{ab}$	$2353 \pm 15_e$	$558 \pm 27_b$

Mean (± SD) was calculated from three replicates for each treatment. Values with different letters a to e are significantly different at P> 0.05 applying DMRT.

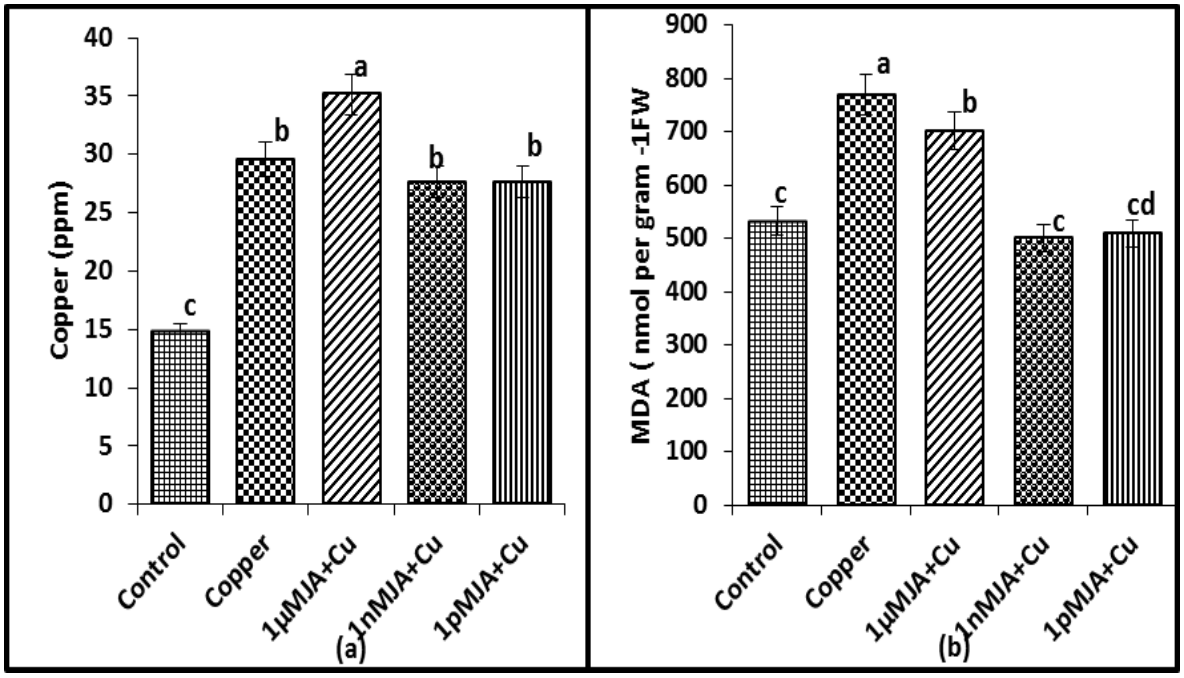

Figure 1. The effect of JA on **(a)** copper content, and **(b)** lipid peroxidation in *Cajanus cajan* L. Millsp. Seedlings under Cu stress conditions. Mean (± SE) was calculated from three replicates for each treatment. Values with different letters a to e are significantly different at P> 0.05 applying DMRT.

way. Seedling growth in terms of root and shoot length also showed synergistic mechanism of negative effect on growth particularly on shoot length. Root length also affected negatively in presence of Cu stress and up to 81% decrease in root length was observed which was also seen in 1µMJA treated seedlings grown in Cu stress; But, lower concentrations of JA showed modulating effect in root length and up to 56% increase in root length was observed. Shoot length was decreased up to 29% in Cu stressed seedlings as compared to the control but JA treatment showed increase in shoot length up to 21% in Cu stressed seedlings as compared to the non JA treated seedlings. Fresh weight and dry weight was decreased in Cu stressed seedlings as compared to the control. 1 µM JA supplementation improved dry weight up to 2% only.

Cu uptake and lipid peroxidation

Exposure of *C. cajan* to Cu stress increased Cu content (Figure 1a) in the seedlings up to 100%. JA supplementation showed dual effect on Cu accumulation in the seedlings as the 1 µM JA treatments increased Cu accumulation up to 138% while 1 nM and 1 pM JA treatments showed decreased accumulation of Cu. Lipid peroxidation in the form of MDA and other reacting molecules shown in (Figure 1b) was increased up to 44% in seedlings grown in Cu stress conditions as compared

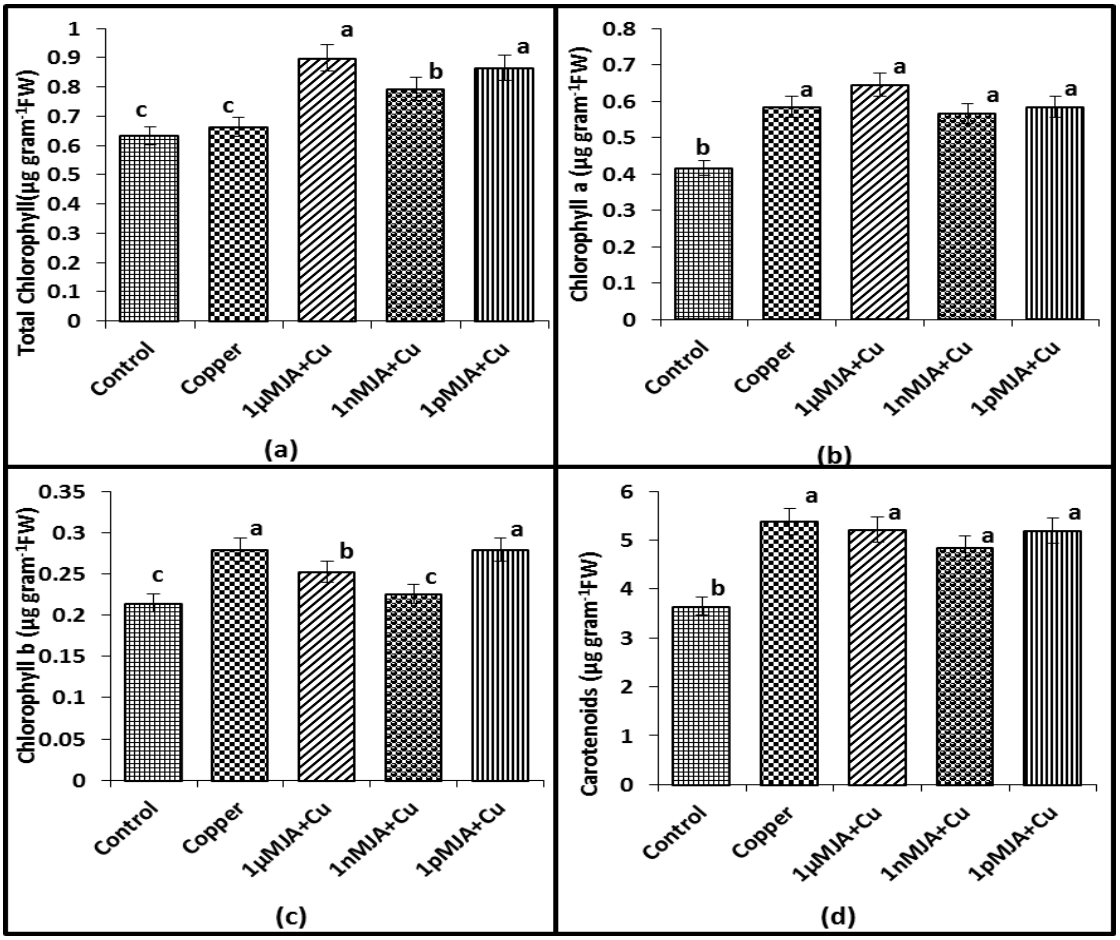

Figure 2. The effect of JA on **(a)** total chlorophyll, **(b)** chlorophyll a, **(c)** chlorophyll b, and **(d)** total carotenoids content in *Cajanus cajan* L. Millsp. seedlings under Cu stress conditions. Mean (± SD) was calculated from three replicates for each treatment. Values with different letters a to e are significantly different at P> 0.05

to the control untreated ones. JA supplementation decreased lipid peroxidation and up to 35% decrease was observed in 1 nM JA treatments.

Pigments

An increase in total chlorophyll content up to 4% was observed on exposure of *C. cajan* seedlings to Cu stress (Figure 2a). JA treatment also increased the chlorophyll content in Cu stressed seedlings as compared to non-treated seedlings and maximum increase of 35% is increased in 1 µM JA treated seedlings grown in Cu stress. Chlorophyll a content (Figure 2b) was increased up to 40% when *C. cajan* seedlings without JA priming treatment were grown in Cu stress; but, more increase up to 10% is seen in JA primed seedlings grown in Cu stress as compared to the non-treated ones grown in Cu stress. Chlorophyll b content (Figure 2c) also increased in JA treated and untreated seedling grown in Cu stress as compared to the control and highest increase up to 29% has been seen non treated seedlings grown in Cu stress. An increase in total carotenoids content (Figure 2d) was observed in Cu stressed seedlings in presence and absence of JA and highest increase up to 47% was observed in non JA treated seedlings grown in Cu stress. JA supplementation further decreased the total carotenoids.

Osmolytes

Total soluble sugars (Figure 3a) increased up to 54% in Cu stressed seedlings and JA supplementation showed dual effect on total soluble sugars. 5% increase was seen in 1nM JA treated seedlings grown in Cu stress as compared to the non-treated ones while 1µM and 1pM JA treatments decreased soluble sugars up to 13% and 6%

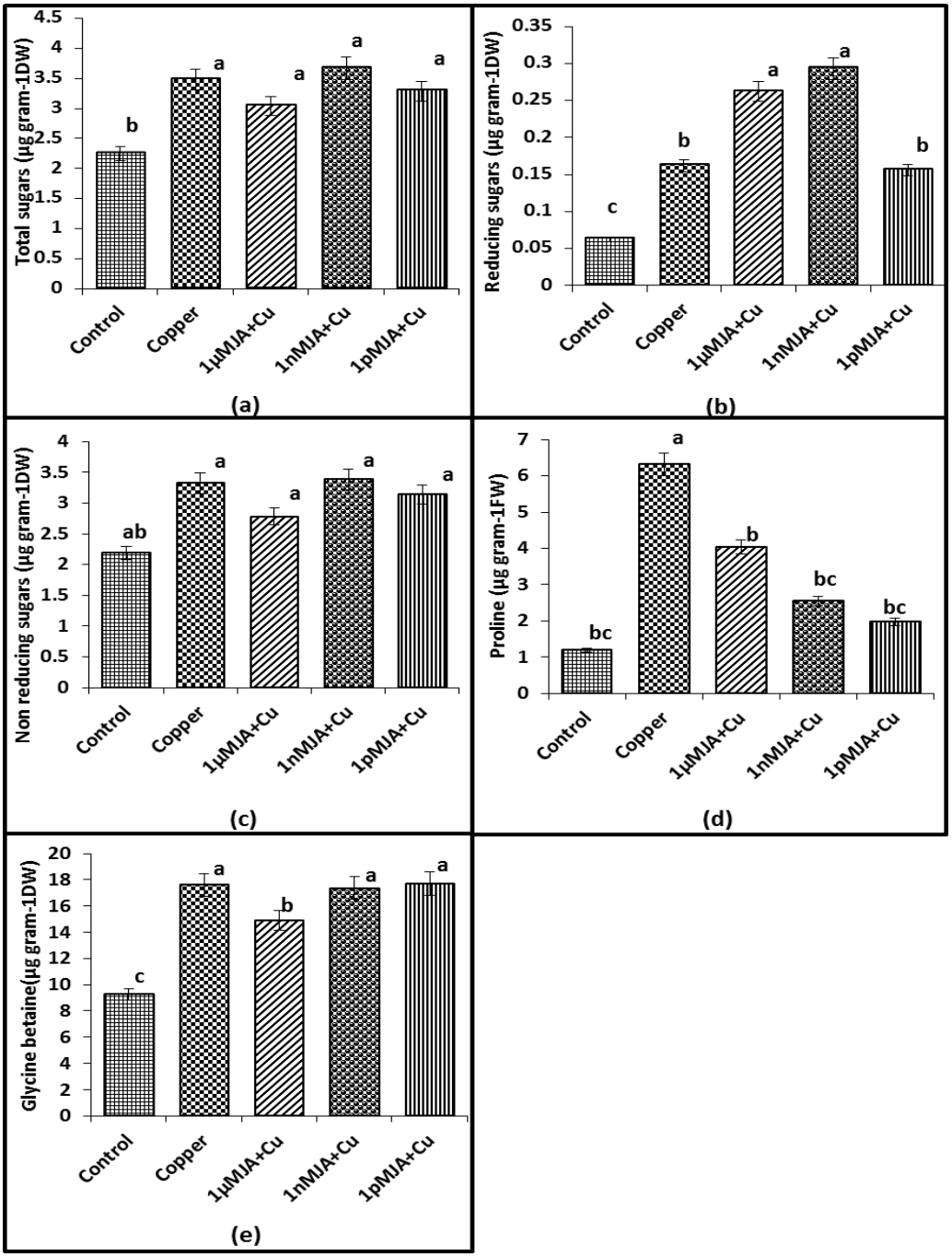

Figure 3. Showing the effect of JA on **(a)** total sugars, **(b)** reducing sugars, **(c)** non-reducing sugars, **(d)** proline, and **(e)** glycine betaine content in *Cajanus cajan* L. Millsp. seedlings under Cu stress condition. Mean (± SD) was calculated from three replicates for each treatment. Values with different letters a to e are significantly different at P> 0.05 applying DMRT

respectively as compared to the non-treated ones grown in Cu stress. Reducing sugars (Figure 3b) increase up to 159% while non-reducing sugars (Figure 3c) show up to 51% increase in Cu stress. When JA supplementation was given to Cu stressed seedlings, reducing sugars increased highest up to 319% while non-reducing sugars showed less increase only up to 54% as compared to the control seedlings. When compared to the Cu stressed seedlings, JA treated seedling grown in Cu stress showed increase in reducing sugars highest up to 80%

while only 1% increase was observed in non-reducing sugars in 1 nM JA. Proline content (Figure 3d) was increased up to 428% in seedlings grown in Cu stress as compared to the control untreated ones. JA supplementation increased the proline content in seedlings grown in Cu stress as compared to the control but decreased proline content was seen as compared to the JA non-treated seedlings grown in Cu stress. Glycine betaine content (Figure 3e) was increased up to 90% in seedlings grown in Cu stress as compared to the control untreated ones. JA supplementation decreased glycine betaine content in the seedlings as compared to the JA non-treated seedlings grown in Cu stress.

Antioxidant enzymes and non-enzymes

Total protein

Cu stress decreased total protein content (Figure 4a) up to 22% and higher concentrations of JA further decreased the protein content but interestingly an increase in protein content up to 26% is seen in 1 pM JA treated seedlings grown in Cu stress. Activity of the antioxidant enzyme SOD (Figure 4b) was increased (129%) in seedlings grown in Cu stress conditions and when JA supplementation was SOD activity was also increased but more increase was observed in JA alone. POD activity (Figure 4c) was decreased in seedlings grown in Cu stress conditions up to 67% in control seedlings. When JA supplementation was done, an increase up to 233% was observed in 1 nM JA primed seedlings grown in Cu stress conditions. Activity of the antioxidant enzyme CAT (Figure 4d) was increased (169%) in seedlings grown in Cu stress conditions and when JA supplementation was given, CAT activity was decreased in seedlings grown in Cu stress. Exposure of JA treated seeds to Cu stress showed antagonist effect on vitamin A content (Figure 4e) and up to 9% decrease was observed in *C. cajan* seedlings grown in Cu stress. JA priming treatments also decreased vitamin A content and highest decrease was up to 29%. Figure 4f shows decrease in Vitamin B_2 content up to 26% in seedlings grown in Cu stress as compared to the control; while, 1 nM JA treatments resulted to 3% increase in vitamin B_2 content in Cu stress grown seedlings as compared to non-treated seedlings grown in Cu stress. Vitamin C content (Figure 4g) was decreased up to 23% in seedlings grown in Cu stress as compared to the control untreated ones but when JA supplementation was given, an increase up to 67% was seen in 1 nM. Contrary to this, 1 µM JA and 1 pM JA primed seeds grown in Cu stress conditions showed decreased vitamin C content. Vitamin E content (Figure 4h) was increased up to145% in seedlings grown in Cu stress as compared to the control untreated ones but when JA supplementation was given an increase was seen which was highest up to 220% in 1 µM JA primed seeds grown in Cu stress conditions.

DISCUSSION

Copper stress caused by 5 mM copper sulphate containing growing medium was responsible for creating osmotic stress in *C. cajan* seedlings and it was found that all morphological parameters starting from seed germination, root length, shoot length, fresh weight and dry weight was reduced while lipid peroxidation enhanced as compared to control untreated plants (Table 1 and Figure 1b). Heavy metal stress reduced plant biomass as well as growth which is very common effect caused by heavy metal as studied in many researches (Karimi et al., 2012; Weckx and Clisters 1996). In our results, enhancement in Cu uptake by 15 days old seedlings of *C. cajan* was observed in 5 mM Cu which was attributed to Cu stress in plants. Higher Cu accumulation resulted in overproduction of ROS which causes damage to membrane as observed in increased MDA content. Exogenous application of JA to Cu stressed plants helped in restoring the MDA content as well as morphological attributes representing growth of seedlings to significant level but in dose dependent manner. Many studies are available that revealed JA as plant protector by effectively restoring fresh weight, shoot length, root length as well as seed germination. Anjum et al. (2011) and Mahmood et al. (2012) revealed in their studies that JA is an effective restorer of plant water content and growth in water deficit conditions.

Free amino acid proline, glycine betaines are well established osmolytes which acts in plant as osmotic balancer as well as signaling molecule (Hayat et al., 2012). In present experimental studies, profound increase in level of proline and glycine betaines along with sugars (total soluble sugar, reducing and non-reducing sugars) was observed under Cu stress. Supplementation of JA to Cu stressed seedlings of *C. cajan* reduced proline content to significant level which was detrimental with the dilution of JA concentration and minimum in 1 pM JA to Cu stress conditions. Glycine betaines also showed reduction but not to significant levels in Cu stress conditions (Figure 3a to e). Sugars cell under any stress condition was also revealed in present experiment and it was reducing sugars which performed more significantly to the exogenous application of JA. Many researchers have reported enhancement in osmolyte accumulation under physiological stress conditions (Subbarao et al., 2001; Allakhvertdieva 2001). Increase in osmolytes under Cu stress in this study supported the notion of these molecules responsible for maintaining the homeostasis of the as osmoprotectant under any stress condition. Exogenous application of JA responsible for reduction

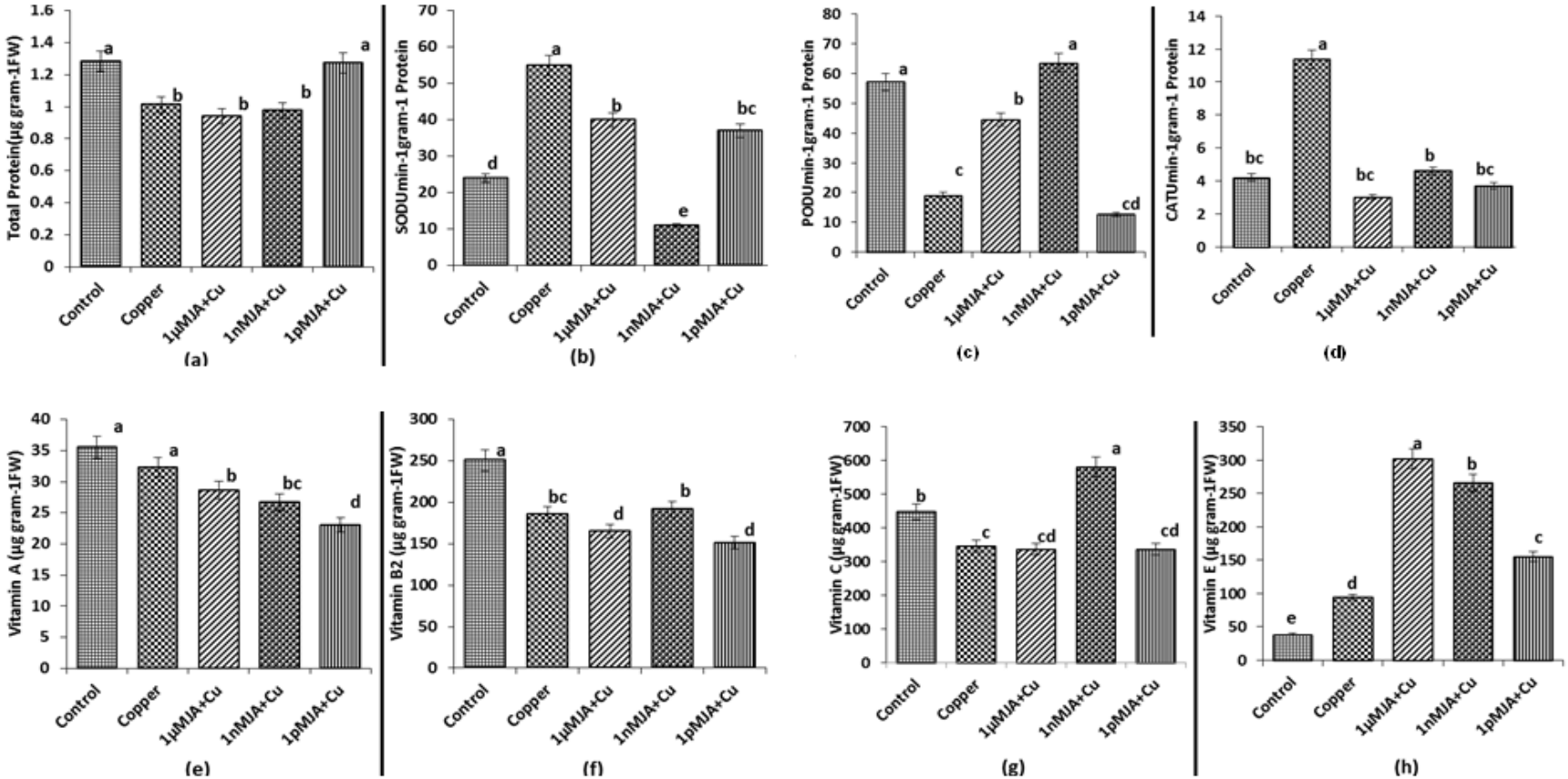

Figure 4(a-h). The effect of JA on **(a)** total protein, **(b)** SOD activity, **(c)** POD activity, **(d)** CAT activity, **(e)** vitamin A, **(f)** vitamin B_2, **(g)** vitamin C, and **(h)** vitamin E content on *Cajanus cajan* L. Millsp. Seedlings under Cu stress condition. Mean (± SD) was calculated from three replicates for each treatment. Values with different letters a to e are significantly different at P> 0.05 applying DMRT

in stress effects required the support of Alam et al. (2014). Increase in proline and glycine betaines considered as indication of stress and exogenous JA reduced significantly in the present study which affirmed protective role of JA in Cu stressed C. cajan seedling. Cu is an integral structural and catalytic component of photosynthetic pigments but its concentration must be tightly regulated. The excess supply of Cu interfere at various levels of chlorophyll biosynthesis by acting as competitors of other metal ions as Fe^{2+} and Mg^{2+} which are also important components of photosynthetic pigments (Agarwal et al.,1977; Siedlecka and Krupa, 1999). Our findings evidenced that exogenous application of 5 mM Cu ameliorate the biosynthetic pathway of photo-synthetic pigment synthesis (Figure 2a to d). These experimental results are contradicting the results found

in water stressed plants where water stress causes decline in photosynthetic pigment which might be due to oxidation of pigments or impaired pigment biosynthesis. Interaction of Cu and JA in present research point upvariable effects on photosynthetic pigments, significant positive effect on total chlorophyll and chlorophyll a, little decline in chlorophyll b and carotenoids. These results also evidenced by variable effect of interaction of JA with drought stress in terms of photosynthetic pigments in different plant species (Alam et al., 2014; Anjum et al., 2011; Mahmood et al., 2012; Bideshki and Arvin 2013).

Increase in antioxidant enzyme activity is assumed as reason for stress tolerance provided by JA and Cu independently or in combination in *C. cajan* seedlings (Figure 4b to d). Higher level of CAT activity in Cu stressed *C. cajan* seedlings might be due to higher MDA level which must be controlled to protect plant by mitigating the oxidative stress. Different researches have been performed showing a redox balance in the plant which might be regulated by higher level of antioxidant enzyme activity. Our study reveals that exogenous application of JA enhanced POD activity to many folds which helped in maintaining the homeostasis of the cell during oxidative stress caused due to higher concentration of Cu. SOD is the prime enzyme responsible for production of H_2O_2 which is the substrate for CAT and POD. Our findings show that Cu causes higher oxidative stress producing more superoxide radicals which are mitigated by regulated activity of SOD and CAT. The SOD activity is vital to sustain superoxide radicals under control by mitigating into H_2O_2 under Cu stress. Supplementation of JA to Cu stressed seedlings reduced SOD and CAT activity to significant level showing that JA has beneficial role in decreasing the Cu stress by modulating the enzymatic and non-enzymatic machinery of the plants. This was further evidenced by DPPH in which Cu stressed plants showed significant positive effect on ROS mitigation activity by antioxidants both enzymatic and non-enzymatic. Supplementation of JA further enhanced antioxidant potential in Cu stressed seedlings of *C. cajan.* Karpet et al. (2014) also discussed increase in SOD and other antioxidant enzymes in presence of JA in wheat coleoptiles.

Vitamins such as ascorbate (vitamin C), tocopherol (vitamin E), retinol (vitamin A), riboflavin (vitamin B_2) are important non enzymatic antioxidants of plants and major redox compounds that protect the plant from environmental stress. In the present study, the response of these vitamins to Cu stress is variable. Vitamin E increased its level to significant level in presence of Cu as compared to control untreated seedlings. Cu stressed plants showed decline in level of vitamins A, B_2 and C. Supplementation of JA at various concentrations in the same stress treatment varied the level of vitamins. Highest accumulation of vitamins C was found in 1 nM JA treated plants under Cu stress, vitamin E maintain its highest level in Cu stressed plants at 1 µM treatment under Cu stress. JA acts as an inducer of signal that leads to the regulation and maintenance of ascorbate, along with glutathione which has an important role for acquisition of water stress tolerance (Shan and Liang 2010). The variation in the level of different vitamins in JA treated plants might be due to difference in the capacity of synthesis or accumulation of these vitamins under Cu stress. Different species of Brassica respond to drought stress in reference to ascorbate varied as compared to control seedlings which might be due to difference in the capacity of different species under drought stress (Pazoki et al., 2010; Din et al., 2011; Rad 2012; Alam et al., 2014) like other abiotic stresses. Cu stress also causes increased production of ROS consequence to deterioration in macromolecules of the plants specially proteins, which also happened in our present study where total protein significantly deteriorated in Cu stress. In response to JA treatment with Cu, total proteins tried to maintain their level equal to or higher than Cu stressed seedlings of *C. cajan.*

Conclusion

The present study and the available literature reviewed in the paper lead to the conclusion that seed priming treatment of JA might be very effective agent for eliciting Cu tolerance in *C. cajan* by altering photosynthetic pigments, osmolytes and antioxidants. JA reduced the oxidative stress and improved the physiological tolerance mechanism in *C. cajan* under Cu stress. The best response was observed in 1 nM JA treatments which showed less damaging effect in Cu stress. More pronounced increase in antioxidant activities of SOD and POD in Cu stress showed the active involvement of Cu in many physiological processes. However very little work has been done in abiotic stress tolerance particularly Cu and information regarding effect of JA on osmolytes and antioxidants in literature is also very rare. There is further need to explore JA mechanism for stress tolerance using various omics and it might be further area of research.

Conflict of interest

The authors declared there is no conflict of interest.

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