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Castasterone regulated polyphenolic metabolism and photosynthetic system in *Brassica juncea* plants under copper stress

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Abstract
Cu is a micronutrient required for normal growth and development, but in excess amounts it became toxic. The present study investigated the effect of castasterone on regulation of polyphenolic metabolism and photosynthetic system along with effect on morphology of *Brassica juncea* plants under copper stress. Four concentrations of copper (0, 0.25, 0.50 and 0.75 mM) were used with or without castasterone. Copper treated plants significantly decreased the growth, biomass and photosynthetic pigments and photosynthetic characteristics. The copper toxicity also affected the total phenolic content, activity of polyphenol oxidase and caused variation in polyphenolic content. Application of castasterone significantly alleviated copper toxicity showing improvement in growth, biomass and photosynthetic systems. The activity of polyphenol oxidase and total phenolic content enhanced with castasterone supplementation, which improved the antioxidant potential of plants. These results showed that supplementation of castasterone can be useful for the enhancement of growth and biomass of plants growing in copper contaminated soils.

Keywords: Copper, photosynthesis, pigments, phenolic content, polyphenols.

Introduction
Increasing world population is leading towards a global challenge to mask the world's hunger by sustainable agriculture [1]. Various abiotic stresses such as drought, salinity and heavy metals affect productivity. Out of these, heavy metals toxicity in plants is a major limitation which reduces plant growth as well deteriorates the quality of food. Cu being an essential element plays an important role in various physiological processes like photosynthetic and respiratory electron transport chains and also a part of various key enzymes involved in different metabolic pathways [2]. Excess use of Cu in various agricultural practices it is becoming very hazardous to plants [3].

Plants show sensitivity to excess Cu concentration by altering plant morphology, and plant physiology. Reports indicate significant decrease in root and shoot length under Cu stress in various plants. Slight increase in Cu levels lead to the inhibition in seed germination, disturbance in nutrient uptake, reduced chlorophyll level, reduced photosynthetic functions in leaves, damage of the protein and lipid membrane constituents and functions of plasma membrane and tonoplast [4]. Reduction in chlorophyll biosynthesis is connected with either structural damage of photosynthetic apparatus at thylakoid level or interference of Cu with chlorophyll organization [5]. It is evident that enhanced Cu levels reduce the activity of PS II centers and leads to lowered photosynthetic electron transport activities [6]. The reduced photosynthesis is directly connected to the reduced growth of the plants under Cu stress.

Phytohormones are thought to play pivotal role in endogenous signaling under abiotic stress including metal stress and leads to the various adaptive strategies. Out of various phytohormones, BRs are plant steroids which are known to play important role in various physiological responses (stem elongation, induced synthesis of ethylene, activation of proton pump, synthesis of nucleic acid and proteins, activation of enzymes and photosynthesis etc.) of plants [7]. The ameliorative role of exogenously applied BRs under various biotic and abiotic stresses has been widely accepted. Among various classes of BRs, castasterone (CS) showed strong biological activities which make it important BR. It is identified and isolated more frequently in various plant species.

Plant polyphenols have shown wide variety of properties including plant resistance against microbial pathogens, solar radiation and metal stress. They play role in protecting plants not only by participating in constitutive agents but also by accumulating in plants under various environmental stresses. It has been found that phenol metabolism stimulates in response of metal stress in plants for the protection of plants and recovery from metal injury. Polyphenol oxidase a copper-containing enzyme catalyzes the oxidation of phenols to quinones. It is synthesized in cytoplasm and found in the chloroplast of healthy plant cells. The role of PPO as antioxidative enzyme under heavy metal stress has been established [8, 9]. Increase in the total phenolic content in *B. juncea* plants under cadmium stress has been reported [10]. Phenolic compounds act as antioxidants and help in scavenging free radicals or chelation of metals. Despite the reported role of polyphenols as effective antioxidant there is very scanty of information about the involvement of polyphenols in cellular responses of ROS generated under metal stress. Thus, by noticing the change in polyphenols under metal stress and BR supplementation can help to understand the role of polyphenols in defensive mechanism to avoid metal poisoning [11].

Thus, keeping in mind the ameliorative role of BRs in different abiotic stress and role of phenols as antioxidants the present work was formulated to evaluate: changes in growth and biomass of *B. juncea* plant, changes in photosynthetic machinery, activity of polyphenol oxidase and identification and quantification of polyphenolic compounds of plant extracts under the effect of CS in 30 DAS old *B. juncea* plants exposed to various levels of Cu.

Material and Methods

Seeds of *B. juncea* L var. RLC1 were procured from Punjab Agriculture University, Punjab (India). After surface sterilization with commercial detergent Cween -20, seeds were dipped in 3 concentrations of CS (0, 10^{-11}, 10^{-9} and 10^{-7} M) for 8 hours. The seeds were then raised in pots with different concentrations of Cu metal (0, 0.25, 0.50 and 0.75 mM). Plants were harvested on 30 DAS and analyzed for following parameters.

Plant growth analysis: Plants were removed carefully from the soil and washed. The plants were blotted to remove extra water and their root/shoot length and fresh weight was recorded. The plants were then dried in oven at 70°C for 24 hours and weighed to observe the dry weight.

Chlorophyll Content: The chlorophyll content of fresh leaves was estimated using spectrophotometric method given by Arnon *et al.* (1949) [12]. Following equations were used to calculate total chlorophyll, chlorophyll a and chlorophyll b:

Total chlorophyll content (mg/g FW)

$$= [(Abs_{645} \times 20.2) + (Abs_{663} \times 8.3)] \times (V/ 1000 \times W)$$

Chlorophyll a content (mg/g FW)

$$= [(Abs_{663} \times 12.7) + (Abs_{645} \times 2.29)] \times (V/ 1000 \times W)$$

Chlorophyll b content (mg/g FW)

$$= [(Abs_{645} \times 22.9) + (Abs_{663} \times 4.68)] \times (V/ 1000 \times W)$$

Where V is volume of solvent and W is weight of plant tissue used.

Carotenoids content: Carotenoid content was estimated by using the method provided by Maclachlan and Zalik (1963) [13]. The carotenoids content was calculated using the following equation:

Total carotenoid content (mg/g)

$$= [7.6 (O.D_{480}) – 1.49 (O.D_{510})] \times (V/ d \times W \times 1000)$$

Anthocyanin content: Anthocyanin content was determined by procedure proposed by Mancinelli (1984) [14]. Anthocyanin content was calculated by the following equation:

Total anthocyanin content (mg/g)

$$= Absorbance_{530} – 0.25(Absorbance_{657})$$

Photosynthetic measurements: Various photosynthetic measurements like net photosynthetic rate (P_N), internal carbon dioxide (Ci), transpiration rate (trmmol), and water use efficiency (P_N/trmmol) were measured using LI-COR 6400 portable photosynthesis system. All the measurements were done during 1100 hrs to 1200 hrs of the day.

Polyphenol oxidase (PPO): Activity of PPO was measured by using the method provided by Kumar and Khan (1982) [15]. Reaction mixture containing 1 ml of phosphate buffer (100 mM, pH-6.0), 0.5 ml of 100 mM catechol and 0.25 ml enzyme extract was incubated for 2 min. Reaction was stopped by adding 1 ml of 2.5 N H_2SO_4. The increase in absorbance was recorded at 495 nm for 1 min at 3 sec interval at 25 °C.

Phenolic content estimations: Total phenolics were estimated using the method of Singleton and Rossi (1965) [16]. Absorbance was measured at 765 nm against blank prepared with distilled water. Total phenolic content was calculated from standard curve prepared by using gallic acid as standard.

Phenol profiling: The profiling of phenolic compounds was done using UHPLC (ultrahigh pressure liquid chromatography). For profiling, extracts were prepared by homogenizing 1 g of plant samples in 4 ml methanol. The extracts were allowed to incubate for 24 hours at 4°C. The homogenate was centrifuged at 13,000 rpm for 10 minutes at 4 °C. The supernatant was collected and filtered through micro filters. 10 µL of samples was injected in the UPLC Nexara system (Shimadzu, USA) for the identification and quantification of various polyphenols.

Statistical analysis: The data obtained was analyzed statistically using two way ANOVA test and Tukey's HSD.

Results

Plant growth and biomass

Increasing Cu concentrations resulted in the decrease in plant growth parameters such as root and shoot length, fresh and dry weight and dry matter content while the application of different concentrations of CS has improved the plant growth parameters (table 1). Maximum 58% decrease in root length and 34% reduction in shoot length was observed in the 0.75 mM Cu in comparison to control untreated plants. The maximum enhancement in root length (approx. 125%) was observed in 0.75 mM Cu treated plants supplemented with

10^{-11} M CS while in shoots maximum improvement was seen in 0.50 mM Cu treated plants with co-application of 10^{-9} M CS (table 1). Similarly maximum reduction in fresh weight (25%), dry weight (31%) and dry matter content (47%) was observed in 0.75 mM Cu treated *Brassica* plants in comparison to control untreated plants. Maximum improvement in terms of fresh and dry weight (60% and 18% respectively) was observed in 0.50 mM Cu treated *Brassica* plants with co-application of 10^{-7} M CS while in case of dry matter content maximum enhancement (62.5%) was observed in 0.75 mm Cu and 10^{-11} M CS treated *Brassica* plants (table 1).

Table 1: Effect of Cu and castasterone on growth parameters (Root and shoot length, fresh and dry weight and dry matter content) in 30 days old *Brassica juncea* plants.

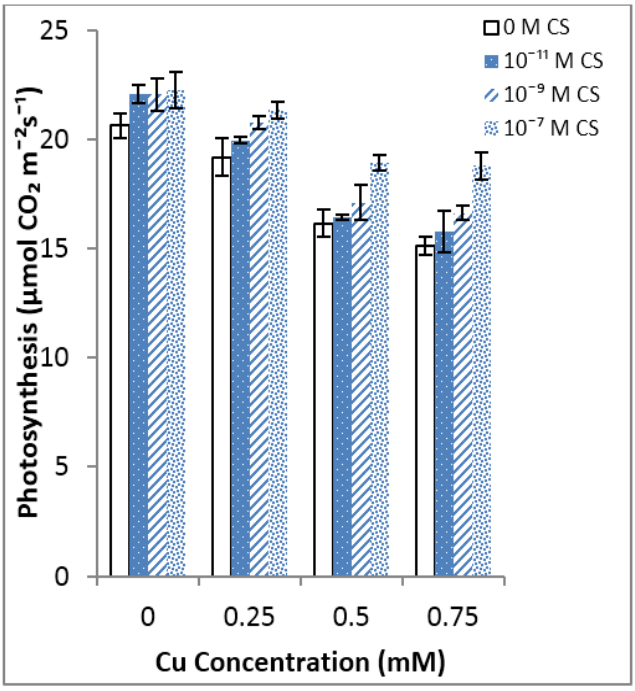
Parameters / Concentration	Root length (in cms.)	Shoot length (in cms.)	Fresh Weight (mg/plant)	Dry Weight (mg/plant)	Dry matter content (in %age)
Control	9.57±0.47	17.00±0.61	0.6143±0.0030	0.0467±0.0015	7.59±0.203
0.25mM Cu	6.50±0.40	13.00±0.93	0.6097±0.0039	0.0363±0.0012	6.04±0.156
0.5 mM Cu	5.47±0.58	11.53±0.61	0.5197±0.0097	0.0340±0.0015	4.70±0.165
0.75 mM Cu	4.00±0.75	11.27±0.37	0.4603±0.0200	0.0323±0.0090	4.00±0.078
10^{-11} M CS	11.23±0.41	17.23±0.62	1.0650±0.0051	0.0643±0.0015	5.96±0.160
10^{-9} M CS	11.30±0.68	18.03±0.55	0.9797±0.0026	0.0460±0.0015	5.52±0.176
10^{-7} M CS	13.47±0.09	16.77±0.43	1.1833±0.0033	0.0473±0.0090	5.63±0.233
0.25 mM Cu +10^{-11} M CS	8.17±0.98	14.47±0.54	0.7190±0.0040	0.0397±0.0012	4.38±0.249
0.25 mM Cu +10^{-9} M CS	10.73±0.47	16.00±0.76	0.7107±0.0055	0.0400±0.0017	6.55±0.342
0.25 mM Cu +10^{-7} M CS	11.10±0.60	14.80±0.93	0.9280±0.0038	0.0407±0.0022	6.03±0.162
0.5 mM Cu +10^{-11} M CS	10.83±0.27	12.70±0.15	0.6417±0.0066	0.0387±0.0012	5.78±0.208
0.5 mM Cu +10^{-9} M CS	11.07±0.32	15.03±0.84	0.6797±0.0064	0.0393±0.0018	4.86±0.091
0.5 mM Cu +10^{-7} M CS	11.00±1.15	13.57±1.79	0.8310±0.0141	0.0403±0.0007	7.05±0.336
0.75 mM Cu +10^{-11} M CS	9.03±0.27	14.33±0.84	0.5640±0.0147	0.0367±0.0012	6.50±0.092
0.75 mM Cu +10^{-9} M CS	8.87±0.32	12.77±2.04	0.6360±0.0089	0.0377±0.0015	5.92±0.212
0.75 mM Cu +10^{-7} M CS	9.40±0.35	13.80±1.21	0.7340±0.0363	0.0363±0.0017	4.948±0.261
F-ratio:(3,32)	26.112	57.299	280.14	91.386	8.972
F-ratio:(3,32)	55.936	21.056	1180.24	18.37	80.706
F-ratio:(9,32)	3.040	3.346	75.119	9.477	5.760
HSD	3.013	2.621	0.043	0.007	1.097

Photosynthetic pigments

Decrease in photosynthetic pigments like total chlorophyll, chlorophyll 'a', chlorophyll 'b' and carotenoids and increase in anthocyanin content was observed (table 2). Maximum decrease in total chlorophyll and chlorophyll 'a' was observed in 0.75 mM Cu treated *B. juncea* plants (approximately 27% and 29% respectively) while maximum decrease in chlorophyll 'b' was observed in 0.5 mM Cu treated plants (approximately 25%) in comparison to control untreated plants. It has been observed that application of CS has improved the total chlorophyll, chlorophyll 'a', chlorophyll 'b' and carotenoids. The maximum improvement of total chlorophyll, chlorophyll 'a' and carotenoids (14%, 17% and 78% respectively) was observed in 0.50 mM Cu treated *Brassica* plants supplemented with 10^{-9} M CS while for chlorophyll 'b' maximum (16%) improvement was seen in 0.75mM Cu and 10^{-7} M CS treated *Brassica* plants. It has been seen that anthocyanin content increased with metal toxicity. Maximum enhancement was observed in the 0.75 mM Cu. The supplementation of CS has further lead to the increase in the anthocyanin content where the maximum content was observed in the 0.75 mM Cu treatment with the co-application of 10^{-9} M CS.

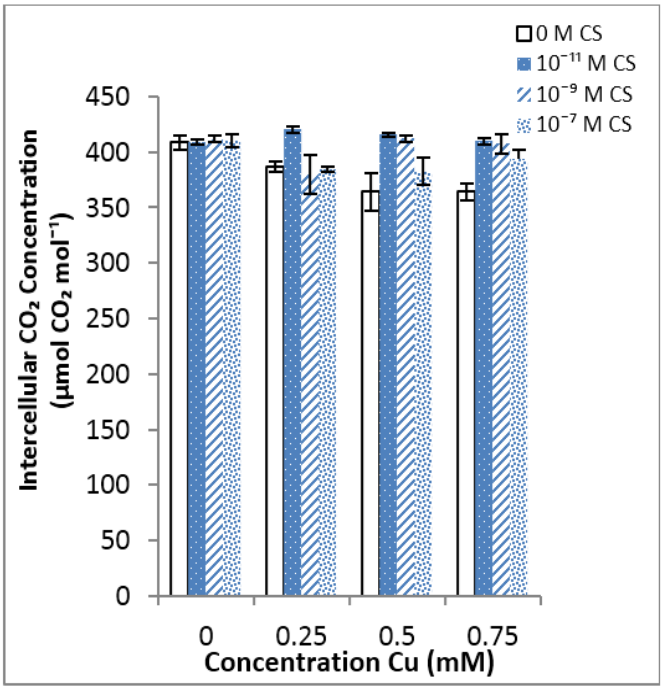
Photosynthetic attributes

Significant decrease in photosynthetic attributes resulted with the increasing concentration of Cu was recorded. Maximum decrease in photosynthetic rate (approx. 27%), C_i (12%), transpiration rate (33%) was observed in the 0.75 mM Cu treated *Brassica* plants as compared to control untreated plants (Fig. 1 a,b,c). Co-application of CS with Cu toxicity improved the photosynthetic and transpiration rate. Maximum improvement (24% and 8% respectively) was observed in the 0.75 mM Cu treated plants with supplementation of 10^{-7} M CS while in terms of C_i maximum enhancement was noticed in 10^{-11} M CS. Increase in WUE was observed with the treatment of Cu but at 0.75 mM Cu treatment decrease in value of WUE was recorded. The co-application of CS has showed a varied effect on WUE (Fig. 1d).



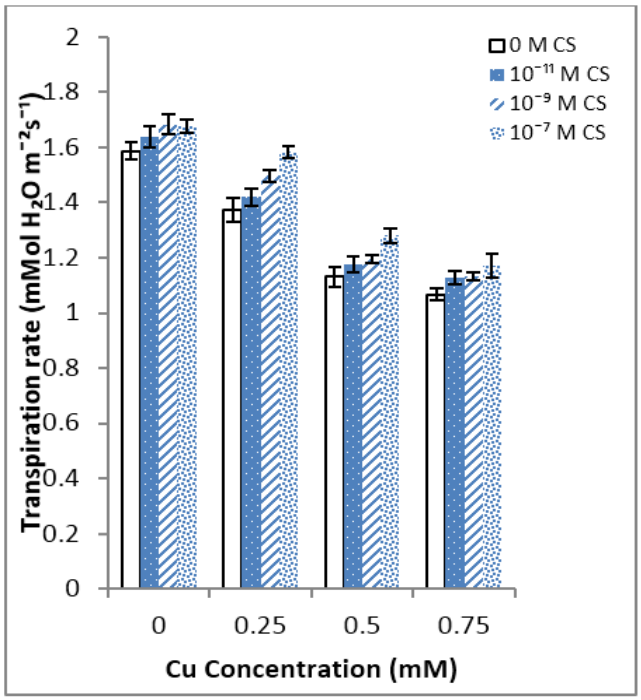
F-ratio:(3,32): 214.22, F-ratio:(3,32): 40.02
F-ratio:(9,32): 2.68, HSD: 1.784

Fig. 1 a



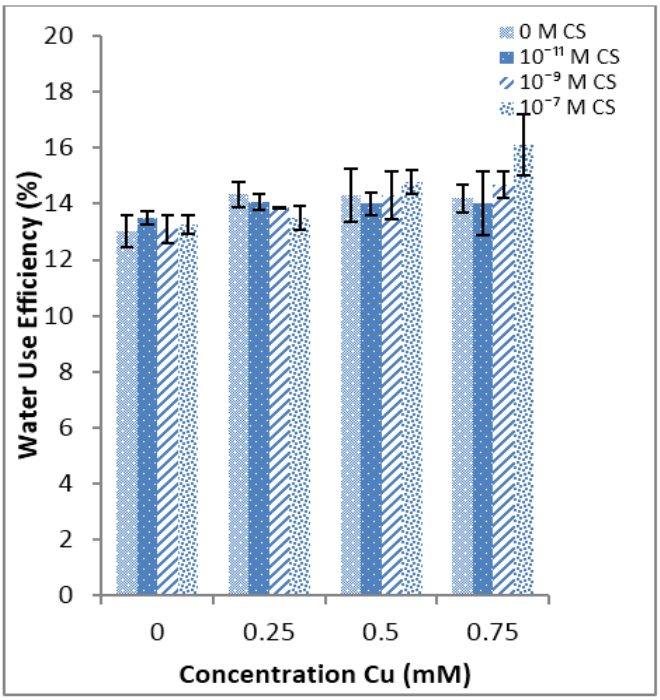
F-ratio:(3,32): 12.75, F-ratio:(3,32): 37.64
F-ratio:(9,32): 9.85, HSD: 24.962

Fig. 1 b



F-ratio:(3,32): 243.92, F-ratio:(3,32): 23.39
F-ratio:(9,32): 2.58, HSD: 0.148

Fig. 1 c



F-ratio:(3,32): 13.25, F-ratio:(3,32): 1.73
F-ratio:(9,32): 2.43, HSD: 1.877

Fig. 1 d

Fig 1: Effect of Cu and castasterone on net photosynthetic rate (a), Intercellular CO_2 Concentration (b), transpiration rate (c), and water use efficiency (d) 30 days old *Brassica juncea* plants.

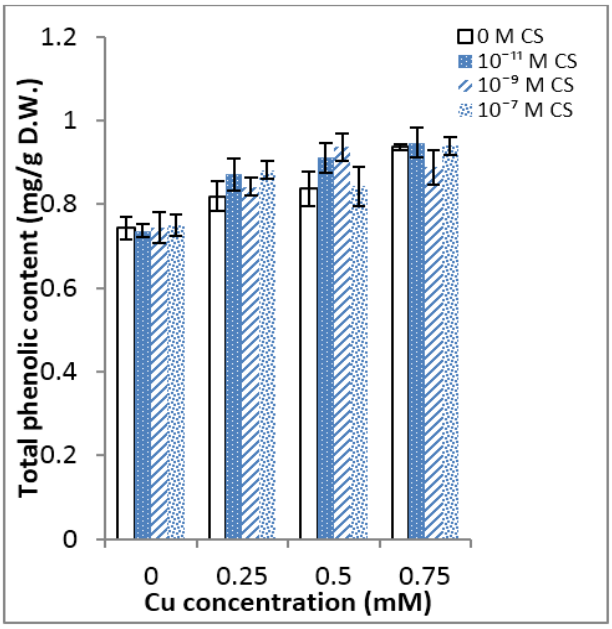
Polyphenol oxidase: Increase in the activity of polyphenol oxidase was observed under the increasing concentration of Cu and maximum activity was observed in 0.75 mM Cu treated plants (6.33 to 11.04 UA mg^{-1} protein). The application of CS has further enhanced the activity and maximum enhancement (31%) was observed in 0.75 mM Cu treated plants when supplemented with 10^{-9} M CS in comparison to alone 0.75 mM Cu treatment (Fig. 2 b).

Table 2: Effect of Cu and castasterone on photosynthetic pigments in 30 days old *Brassica juncea* plants

Parameter / Concentration	Total chlorophyll content(mgg^{-1})	Chlorophyll A (mg g^{-1})	Chlorophyll B (mg g^{-1})	Carotenoids Content (mg g^{-1})	Anthocyanin Content (mg g^{-1})
Control	2.55±0.06	1.91±0.08	0.670±0.03	0.093±0.079	0.293±0.013
0.25mM Cu	2.07±0.10	1.53±0.08	0.556±0.05	0.042±0.003	0.313±0.005
0.5 mM Cu	1.86±0.11	1.38±0.09	0.503±0.04	0.036±0.001	0.296±0.011
0.75 mM Cu	1.85±0.08	1.35±0.03	0.513±0.07	0.034±0.001	0.326±0.045
10^{-11} M CS	2.50±0.04	1.87±0.04	0.660±0.06	0.056±0.003	0.378±0.019
10^{-9} M CS	2.41±0.05	1.80±0.06	0.633±0.02	0.047±0.003	0.415±0.028
10^{-7} M CS	2.49±0.11	1.85±0.04	0.660±0.08	0.049±0.003	0.434±0.037
0.25 mM Cu +10^{-11} M CS	2.27±0.01	1.70±0.07	0.588±0.03	0.057±0.001	0.436±0.015
0.25 mM Cu +10^{-9} M CS	2.24±0.05	1.67±0.04	0.596±0.02	0.061±0.009	0.433±0.003
0.25 mM Cu +10^{-7} M CS	2.21±0.10	1.65±0.06	0.587±0.05	0.059±0.008	0.444±0.015
0.5 mM Cu +10^{-11} M CS	2.09±0.11	1.56±0.03	0.545±0.10	0.047±0.008	0.503±0.005
0.5 mM Cu +10^{-9} M CS	2.12±0.09	1.61±0.04	0.538±0.05	0.064±0.017	0.561±0.018
0.5 mM Cu +10^{-7} M CS	2.07±0.09	1.50±0.04	0.582±0.05	0.053±0.007	0.658±0.035
0.75 mM Cu +10^{-11} M CS	2.05±0.10	1.51±0.06	0.560±0.05	0.055±0.002	0.723±0.057
0.75 mM Cu +10^{-9} M CS	2.05±0.08	1.52±0.07	0.563±0.03	0.054±0.008	0.747±0.020
0.75 mM Cu +10^{-7} M CS	1.96±0.06	1.39±0.07	0.594±0.09	0.047±0.009	0.711±0.016
F-ratio:(3,32)	79.68	115.25	9.71	2.33	83.04
F-ratio:(3,32)	6.28	9.39	1.34	14.02	35.28
F-ratio:(9,32)	2.29	3.61	0.42	2.21	4.82
HSD	0.271	0.177	0.169	0.021	0.042

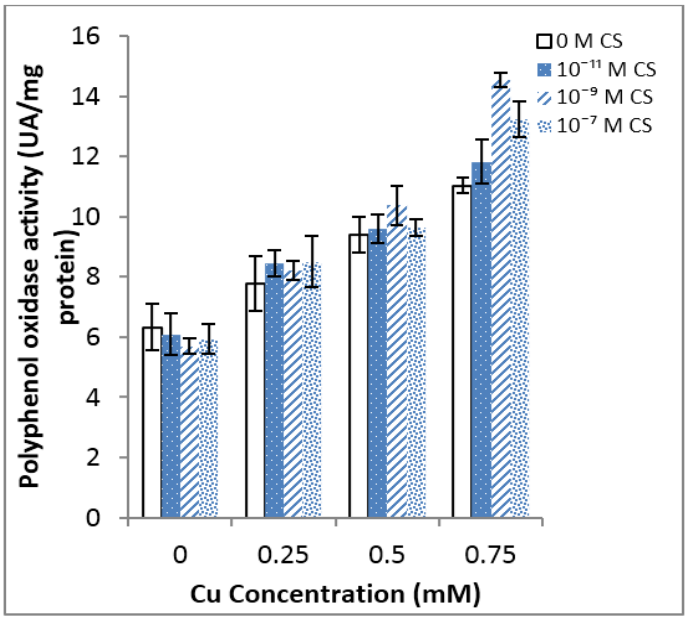
Phenolic content

The increasing concentration of Cu has led to the enhanced total phenolic content in 30 days old *B juncea* plants. The application of CS with Cu has led to production of more phenolic content. Maximum enhancement in the content was observed in the 0.75 mM Cu treated plants when supplemented with 10^{-11} M CS (0.94 mg g^{-1} DW) compared to its control (0.84 mg g^{-1} DW) (Fig. 2 a). The interactions of CS and Cu for the total phenolic content were found to be significant.



F-ratio:(3,32): 70.49 F-ratio:(3,32): 2.068
F-ratio:(9,32): 3.16, HSD: 0.098

Fig. 2 a



F-ratio:(3,32): 282.68, F-ratio:(3,32): 7.25
F-ratio:(9,32): 5.80, HSD: 4.359

Fig. 2 b

Fig 2: Effect of Cu and castasterone on total phenolic content (2a) and polyphenol oxidase activity (2b) in 30 days old *Brassica juncea* plants.

Polyphenolic profiles

The presence of polyphenols in *B. juncea* was done by comparing the RTs (retention times). The change in phenolic content in *B. juncea* plants exposed to copper and CS is shown in table 3. Chlorogenic acid and caffeic acid were predominant phenols identified. In comparison to control plants increase in various phenols like catechin, chlorogenic acid, caffeic acid, coumaric acid, rutin, quercetin has been noticed under 0.5 mM Cu stress. With the supplementation of CS (10^{-7} M) to the plants treated with 0.5 mM Cu, reduction in all phenols except Kaempferol has been noticed which showed increase both in only CS treated plants as well as treatment in combination of Cu and CS.

Table 3: Effect of Cu and castasterone on polyphenolic content in 30 days old *Brassica juncea* plants.

Polyphenolic compound	Control	10^{-7} M CS	0.5mM Cu	0.5mM Cu+10^{-7} M CS
Catechin[a]	0.803	1.452	1.882	6.7
Chlorogenic acid[a]	19.661	14.68	12.547	36.374
Caffeic acid[a]	39.917	34.696	60.189	83.751
Coumaric acid[a]	0.083	nd	0.229	0.294
Rutin[a]	5.087	3.411	3.673	7.064
Quercetin[a]	4.388	0.028	0.33	5.283
Umbelliferone[a]	nd	nd	0.767	nd
Ellagic acid[a]	4.298	1.432	1.748	2.932
Kaempferol[a]	10.756	117.248	43.86	nd
Gallic acid[a]	1.009	0.485	0.682	nd
tert-butyl hydroquinone[a]	nd	nd	0.569	3.82
Sum[b]	86.002	173.432	126.476	146.218

nd not detected

[a] ppm of phenolic compound present in plant extract

[b] ppm of total phenolic compounds in plant extract

Discussion

In the present study, it has been seen that Cu severely affected the plant growth viz. root/shoot length, fresh/dry biomass and dry matter content. The stressful conditions, lower the metabolic activity of plants thus reducing growth. Our results are in accordance with the Fariduddin *et al.* (2013) [17], and Feigl *et al.* (2015) [18], who validated that under Cu stress decrease in growth parameters occur in *B. juncea*, *Cucumis sativus* and *B. napus* respectively. Cu toxicity also reduces mitotic activity of plants thereby affecting cell elongation [19].Cu toxicity is also linked with the membrane permeability and leads to reduced nutrient content mainly Fe, P, K, Ca and Mg [20]. However it has been seen that application of castasterone improved the growth parameters of *B. juncea* plants. These results are in accordance with the Fariduddin *et al.* 2009 [7], who also observed that application of EBL improved root/shoot length and fresh/ dry biomass of the *B. juncea* plants under Cu stress. This might be due to the role of brassinosteroids in the cell elongation or due to modification of plasma membrane permeability under stressful condition.

In present study it has also been observed that Cu affects the photosynthetic apparatus by significantly reducing the levels of various pigments as well other photosynthetic parameters. The decrease in chlorophyll content under Cu stress in leaves might be due to change in structure of chloroplast as well in thylakoid membrane. It has been suggested by Azmat and Riaz (2012) [21] that Cu interferes with the photosynthetic machinery by modifying the pigment and thylakoid protein composition which results in decrease in internal CO_2 concentration (Fig.). Feigl *et al.* (2015) [18], has also reported decrease in pigment concentration in *B. juncea* and *B. napus* under Cu stress. At toxic levels, copper replaces the Mg^{2+} alongwith inhibiting synthesis of aminolevulinic acid (precursor of chlorophylls) and protochlorophyllide reductase (enzyme to catalyze the reductive formation of chlorophyllide). Copper has inhibitory role on chain electron transport and photosystem II catalyzed electron transport resulting into reduced photosynthesis [22]. The collective effect of all these reformed processes leads to reduced photosynthetic rate (Fig.1). However, the supplementation of castasterone in interaction to Cu resulted in more values of stomatal conductance, internal CO_2 concentration and water use efficiency thus, causing enhanced photosynthetic rate (Fig.1). BR mediated improved photosynthesis is linked with the improved biosynthesis of pigments and activation of enzymes related with photosynthesis [23]. BRs are also involved in the synthesis of proteins or enzymes and thus improve overall metabolic activities of plants.

Increase in the PPO activity under metal stress as well with the application of CS has been observed in present study. PPO acts as an antioxidative defense enzyme under various metal stresses. Devi Chinmayee *et al.* (2014) [9] have reported enhanced enzymatic activity along with PPO under various heavy metals in *Jatropha curcas* (Cd, Cr and Hg). Similarly, Kebeish *et al.* 2014 [24] has also found enhancement in PPO activity in *Chlorella vulgaris* under Cu stress. The activity of PPO was improved with the supplementation of CS. These results are in accordance with the Sharma *et al.* (2014) [8] who reported increase in PPO activity under heavy metal stress as well under 28-HBL in *Raphanus sativus*. Similarly, Hayat *et al.* (2007) [25] has observed protective role of 28-HBL on various growth as well antioxidative enzymes under Cd metal stress in *B. juncea* plants. It was seen that this brassinosteroids reduce the toxicity of Cd and increase growth and antioxidant enzymes.

Plants produce a variety of secondary metabolites with known functions in protection against various biotic and abiotic stresses. Compounds with phenol group are one of these secondary metabolites with known importance in stress management. It has been said that polyphenols are better in free radical scavenging due to their chemical structure and show better capacity than vitamin E and C. Increase in the total phenolic content has been seen in wheat plants under Cu and Zn stress [26]. Similarly Hamid *et al.*, (2010) [27] have also reported that total phenolic content increases under heavy metal stress. Our results are in accordance with them. Total phenolic content further increased with the application of CS which shows that this brassinosteroid help in the efficient removal of free radicals produced and ameliorating Cu toxicity.

Role of polyphenolic compounds in different biological functions like UV protection, pollen tube growth, antimicrobial activity, biotic and abiotic stress resistance make them important for plants. Simple phenols (trans-cinnamic acid, coumaric acid) act as precursor for the formation of complex phenolic compounds like flavonoids, tannins, lignins and anthocyanin [28]. A group of phenolic acids such as caffeic acid, trolox, rosmarinic acid has been recognized as good antioxidants. The level of identified polyphenols has varied with the treatment of Cu as well by supplementation of CS. This can be assumed from this behavior that increase in total phenolic content as well as

changes in various polyphenols levels lead to the enhanced antioxidant capacity against metal stress.

Conclusion

Increasing copper pollution due to various agricultural practices is posing threat for growth and reproduction of plants. It has been revealed in the present study that excess copper decreases the root/shoot length and fresh/dry weight in a dose-dependent manner. The photosynthetic pigments and photosynthetic attributes (net photosynthetic rate, internal carbon dioxide, transpiration rate, and water use efficiency) also decreased with increasing copper toxicity. At the same time castasterone has been found to effective copper stress alleviator through enhancing antioxidant system and thus improved the growth and photosynthetic machinery. Enhanced polyphenol oxidase activity help in the activation of defense system and reduced toxic effects of Cu was observed. In this study, we have also found that polyphenols act as potent antioxidants under copper stress as there is a significant increase in their total content. It has been found that content of various polyphenols has shown increase under effect of Cu and CS contributing to the increased antioxidant potential. The studies indicate that like other BRs, castasterone is also playing important role in defense responses in *B. juncea* plants.

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