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Research Article

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Improvement in photosynthetic efficiency of *Brassica juncea* under copper stress by plant steroid hormone

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ABSTRACT

The present study was designed to investigate the effects of seed soaking with 24-epibrassinolide (24-EBL) on photosynthetic parameters and sugar content in 30 days old Brassica juncea plants under copper (Cu) metal stress. The results showed that increasing Cu concentration reduced the chlorophyll and carotenoid contents. However, Cu metal stress enhanced the reducing sugars, anthocyanin, flavonoid and xanthophyll contents. The gaseous exchange parameters like photosynthetic rate (Pn), stomatal conductance (Gs), intercellular CO_2 concentration (Ci) and transpiration rate (Et) were decreased under Cu stress, but application of 24-EBL has improved these parameters under Cu toxicity.

Key words: 24-epibrassinolide, copper stress, *Brassica juncea.*

INTRODUCTION

Environmental pollution caused by heavy metals is increasing and becoming a major problem in the modern world due to the development of the modern industry and agriculture. Amongst the heavy metals, copper is necessary for the normal growth and development of all higher plants and animals. Cu has become increasingly hazardous due to its involvement in pesticides, fungicides and fertilizers [1]. Excess of Cu can cause a wide range of harmful effects such as chlorosis, reduction in the biomass, lowering of chlorophyll content, altering the structure of chloroplast and composition of thylakoid membrane in leaves, inhibition of photosynthesis and pigment synthesis, functional changes, damage of plasma membrane and other metabolic disturbances [2, 3, 4, 5, 6, 7]. It has been reported that various hormones such as abscisic acid, ethylene, jasmonates, salicyclic acid and brassinosteriods (BRs) etc. are aided in the amelioration of oxidative stress [8, 9]. Among various plant hormones BRs are considered as a group of naturally occurring steroidal lactones which are ubiquitously distributed in the plant kingdom. BRs are known for their antistress and immunomodulatory properties that make them ideal candidate for new generation plant hormones. BRs are capable of providing resistance against various biotic and abiotic stresses to plants such as high or low temperature, moisture, drought, salinity, pesticide, and heavy metal stresses [10, 11, 12, 13, 14, 15]. *Brassica juncea* belongs to family brassicaceae and used as a main oil crop in India which has the potential to accumulate heavy metals. The present study was designed to study the effect of 24-Epibrassinolide (24-EBL) on photosynthetic pigments, gaseous exchange parameters and sugars of *Brassica juncea* L. plants under Cu metal stress.

EXPERIMENTAL SECTION

Seeds of *B. juncea* Var. RLC1 were procured from the seed technology unit of Punjab Agriculture University, Ludhiana, India. The seeds were dipped in 0.01% mercuric chloride ($HgCl_2$) solution for 1 minute for surface sterilization followed by 3-5 rinses with distilled water. The soil was added to pots having mixture of clay: sand: manure in the ratio of 3: 1: 1. Soil was treated with different concentrations of Cu metal solution (0, 0.25 mM, 0.50 mM, 0.75 mM). Before sowing, seeds were soaked for 8 hours in different concentrations of 24-EBL (0, 10^{-11}M, 10^{-9}M, 10^{-7}M). Photosynthetic parameters were estimated after 30 days of seed sowing.

Chlorophyll content was determined by method of Arnon et al. [16]. Carotenoid content was estimated according to the Maclachlan and Zalik [17]. Total Flavonoid content was determined following Kim et al. [18]. Anthocyanin content was estimated according to the method described by Mancinelli [19]. Xanthophyll content was performed as described by Lawrence [20].

Gaseous exchange parameters like photosynthetic rate (Pn), conductance rate (Gs), intercellular CO_2 concentration (Ci) and transpiration rate (Et) were measured using IRGA-6400XT at maximum light intensity.

Reducing Sugars were estimated by using the method of Nelson [21]. High performance liquid chromatography was used for qualitative analysis of sugars. Plant samples were dried at 80^0C in hot air oven and crushed to make fine powder. Powered sample (2 g) was mixed with double distilled water (5 ml) and then incubated at room temperature for 12 hours. Filtration was done by using 0.22 micron pore sized filter paper. Supernatant (20 µl) was used for the analysis of sugars by HPLC.

RESULTS AND DISCUSSION

Photosynthetic Pigments

Total chlorophyll content was reduced with increasing concentration of copper from 0.581 mg/g to 0.297 mg/g. The decline in chlorophyll content was possibly caused by interaction of Cu to –SH group of enzymes of chlorophyll biosynthesis. It has been suggested that heavy metals might hinder the biosynthesis of chlorophyll either through the direct inhibition of enzymatic steps or through the substitution of the central Mg ion [22, 23]. Similar findings were observed by Dey et al [24] with the increasing concentration of the Cu stress that gradually reduce the total chlorophyll content in both cultivars of *Camellia sinensis* (L.) O. Kuntze). 24-EBL treatment enhanced the total chlorophyll content when given in combination with metal treatments (Fig.1A). The content was enhanced from 0.529 mg/g to 0.573 mg/g and maximum increase was observed with 10^{-9}M EBL + 0.25 mM Cu concentration in comparison to 0.25 mM Cu treatment alone. This might have been due to stimulatory effect of EBR on the transcription of genes required for the biosynthesis of pigments [25]. Similar results were observed in *B. juncea* under Cd and Ni stress where application of BRs improved the levels of chlorophyll and rate of photosynthesis [26, 27]. Brassinolide (BL) in combination with heavy metals (Cd, Pb and Cu) enhanced the chlorophyll content in *C. vulgaris* cells. Carotenoid content decreased when only metal treatment was given to the plants. There was reduction in total carotenoid content upto 32.28% at 0.75 mM in comparison to untreated plants. These findings are in accordance with results of Singh and Malik [28], where carotenoid content was found to decrease in *B. juncea* under Hg stress. Similar results were observed in wheat under Zn and Cu stress [29]. The carotenoid content was increased when 24-EBL treatment was given along with metal treatment as shown in the Fig.1B. The content was enhanced from 0.207 mg/g to 0.228 mg/g and maximum enhancement was observed in case of plants treated with 10^{-9} M EBL + 0.25 mM Cu concentration as compared to 0.25 mM Cu treated plants alone. Anthocyanin content was enhanced with increasing concentration of metal from 0.276 µg/g to 0.739 µg/g (Fig.1C). Heavy metals might stimulate the synthesis of glutathione-S-transferase (GST) which further increases the synthesis of anthocyanin. This is due to the reason that heavy metal stress can stimulate the synthesis of GST. This enzyme plays an important role in the biosynthetic pathway of the anthocyanins [30]. It has been reported that Cu was the most effective metal in the accumulation of anthocyanin and they play an important role antioxidants in Cu stressed plants [31]. When seeds were treated with metal along with EBL, the anthocyanin content was raised (Fig.1C). BRs enhanced the total anthocyanin content which might have been due to increase in the regulation of downstream genes involved in the biosynthesis of anthocyanin [32]. Similarly total anthocyanin content was enhanced with the application of Castasterone under Cu toxicity in *B. juncea* [33]. When treatment of metal was given alone, total flavonoid content was found to increase from 0.167 mg/g to 0.196 mg/g. Flavonoid act as an antioxidant in response to heavy metal stress [34]. It has been reported that flavonoids accumulated up to 12-fold in response to $CuSO_4$ treatment in the cell cultures of *Ginkgo biloba* as compared to untreated cells [35]. With the application of 24-EBL supplemented with metal, the total flavonoid content was enhanced. In case of 0.50 mM Cu treated plants in combination with 24-EBL, the maximum increase in total flavonoid content was observed with 10^{-7} M EBL concentration from 0.184 mg/g to 0.227 mg/g (Fig.1D). Similarly total flavonoid content was enhanced with supplementation of EBL with to *B.*

juncea plants under Cu stress [36]. With increasing concentration of copper, the xanthophylls content was found to increase from 0.235 mg/g to 0.999 mg/g. When seeds were given metal treatment along with EBL, xanthophylls content was increased and 10-7 M EBL was found to be most effective concentration (Fig.1E). In *B. juncea*, Cd negatively affected chlorophyll and carotenoid contents and activated the xanthophylls cycle to protect the photosynthetic apparatus [37].

Gaseous Exchange Parameters

The photosynthetic rate decreased with the application of Cu. Maximum decrease was observed in plants treated with 0.75mM Cu where the reduction was approximately 51% as compared to control untreated plants. The application of 24-EBL has led to increase in the photosynthetic rate as shown in the Fig.2A. The decrease in the transpiration rate under Cu stress was found to be 50% in the 0.75mM Cu treated plants, while the application of 24-EBL has improved the transpiration rate (Fig.2B). Similarly conductance rate was also decreased by the application of Cu metal. There was 42.78%, 53.87% and 77.09% decrease in the conductance with the treatment of various concentrations of Cu (0.25mM, 0.5mM and 0.75mM respectively). The application of 24-EBL helped to ameliorate the stress and enhanced the stomatal conductance rate. It has been described that the decrease in the photosynthesis due to copper stress might be because of its inhibitory role in whole chain electron transports as well as on photosystem II (PS II) catalysed electron transport [38]. In soybean plants, Cd and salt stress drastically reduced photosynthesis by reducing total chlorophyll content and stomatal conductance [39]. The intercellular CO_2 concentration followed the same trend where the decrease was recorded lowest (27.97%) in the 0.75mM Cu treated plants in comparison to control untreated plants. Similar results were found under copper stress in *Cucumis sativus* [40]. The increase was observed when 24-EBL was supplemented with Cu treatments (Fig.2C). Application of 100 nM 24-EBL had significantly enhanced net photosynthetic rate (Pn), stomatal conductance (Gs) and intercellular CO2 concentration (Ci) under salt stress in *Solanum melongena* L. [41]. The interactive effect of 24-EBL was observed under Cu to plants having more stomatal conductance, intercellular CO_2 concentration and transpiration rate and accordingly leading to the increased net photosynthetic rates as compared to stressed plants. Similar results were observed in *B. juncea* under Cu toxicity where co-application of castasterone improved the photosynthetic and transpiration rate [42]. It might has been due to improved synthesis or activation of chlorophyll biosynthesis enzymes as well as those associated with photosynthesis [43]. At higher concentration, Cu damages the photosynthetic machinery and the photosynthetic electron transport chain [7]. All these damaged processes finally lead into a severe loss in the rate of photosynthesis. Other than this, BRs have been suggested to positively affect the synthesis of proteins or enzymes which improves the overall metabolic activities of plants.

Reducing Sugars

Reducing sugars content increased with increasing concentration of Cu from 3.9 mg/g to 5.55 mg/g. During various stress conditions sugars play important role in the regulation of osmotic balance of cells [44]. Similar findings showed that reducing sugars increased in response to Lead, cadmium and nickel stress in *Phaselous vulgaris* [45]. Mannitol, a sugar alcohol is involved in osmoregulation, storage of carbon and energy, regulation of coenzymes and scavenging of free radicals [46]. Application of 24-EBL with Cu stress increased the level of reducing sugars (1F). Exogenous application of 28-homobrassinolide (28-HBL) and 24-EBL enhanced reducing sugars in *Raphanus sativus* [47]. In 30 days old untreated control plants of *B. juncea*, the sugars were analyzed qualitatively. The presence of glucose, dextrose and fructose was recorded in control plants (3A). At high concentration of Cu (0.75 mM) the presence of manitol, sorbitol, galactose, rahmnose, maltose was recorded (fig.3B). Application of 24-EBL with 0.75mM Cu indicated the presence of manitol, inositol, xylose, maltose, galactose, fructose, glucose and dextrose were recorded (Fig.3D).

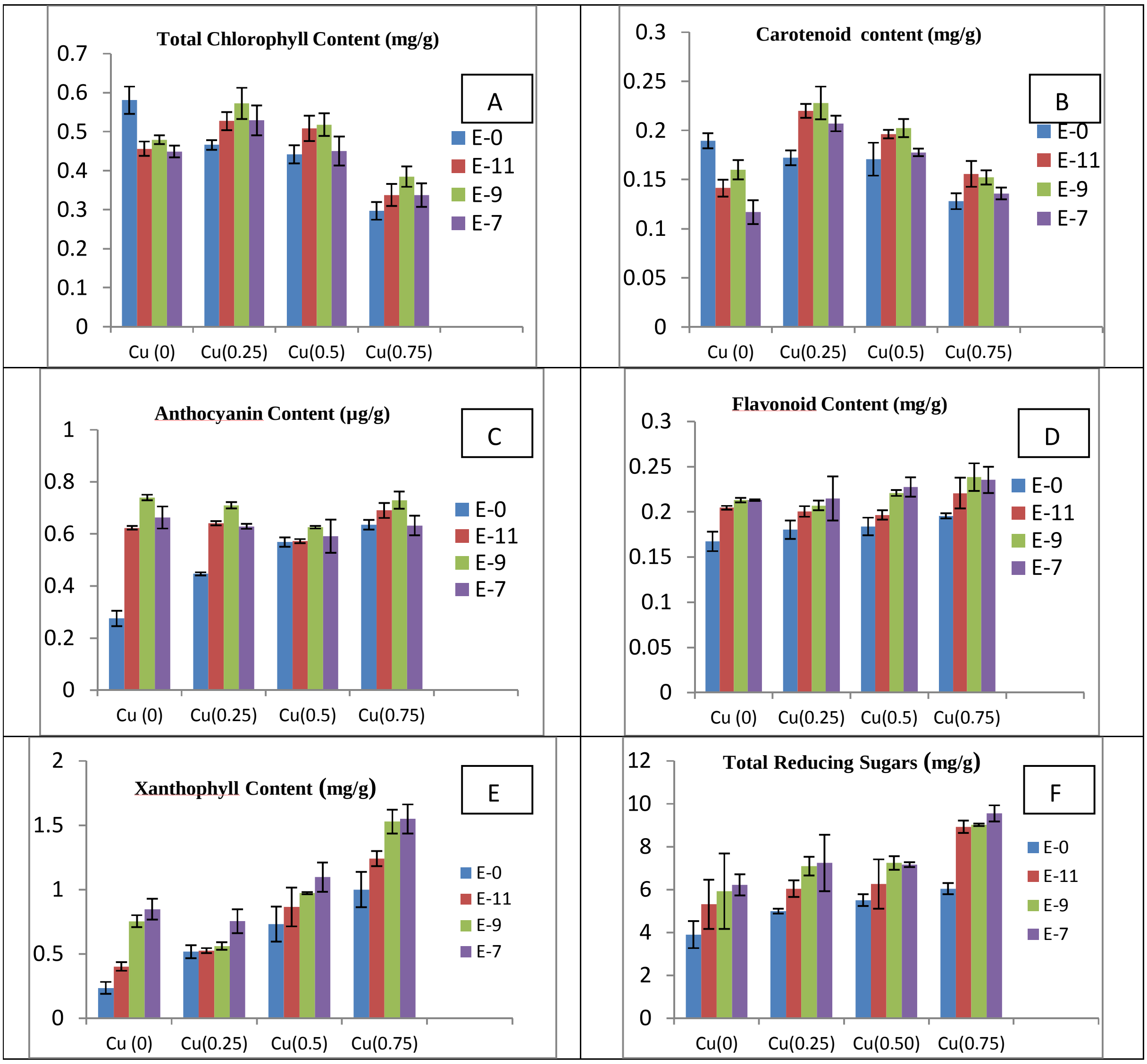
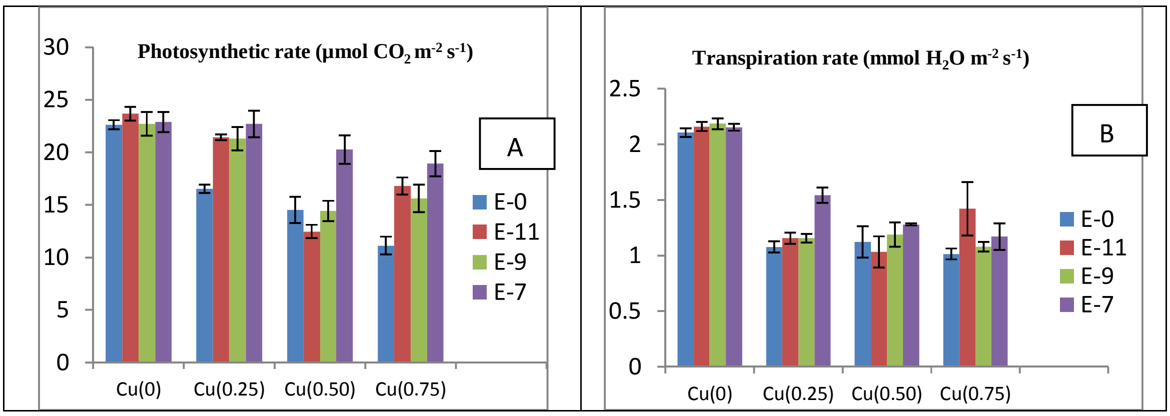


Fig.1 Effect of 24-EBL on photosynthetic pigments and reducing sugars of 30 days old *B. juncea* plants under Cu metal stress (A= total chlorophyll content, B= Carorenoid content, C= Anthocyanin content, D= Flavonoid content, E= Xanthophylls content and F= Reducing sugars)



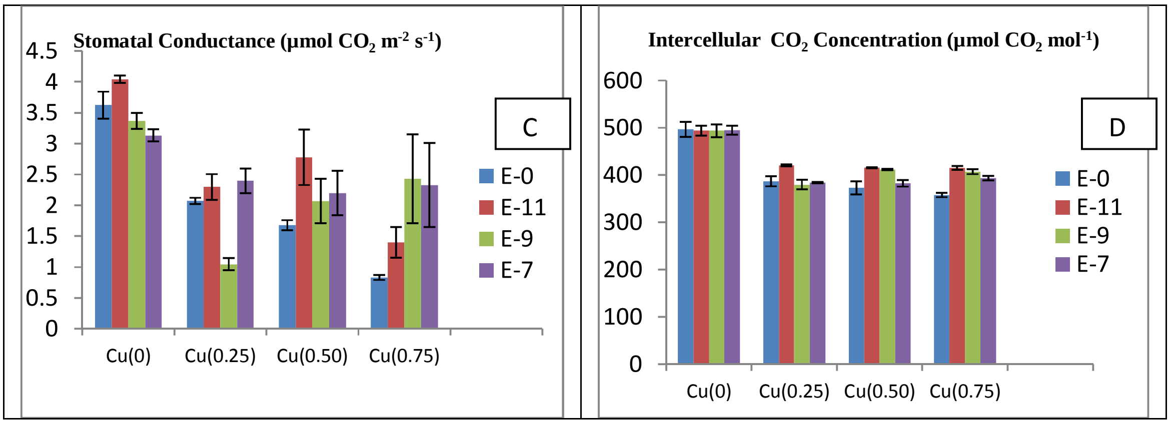
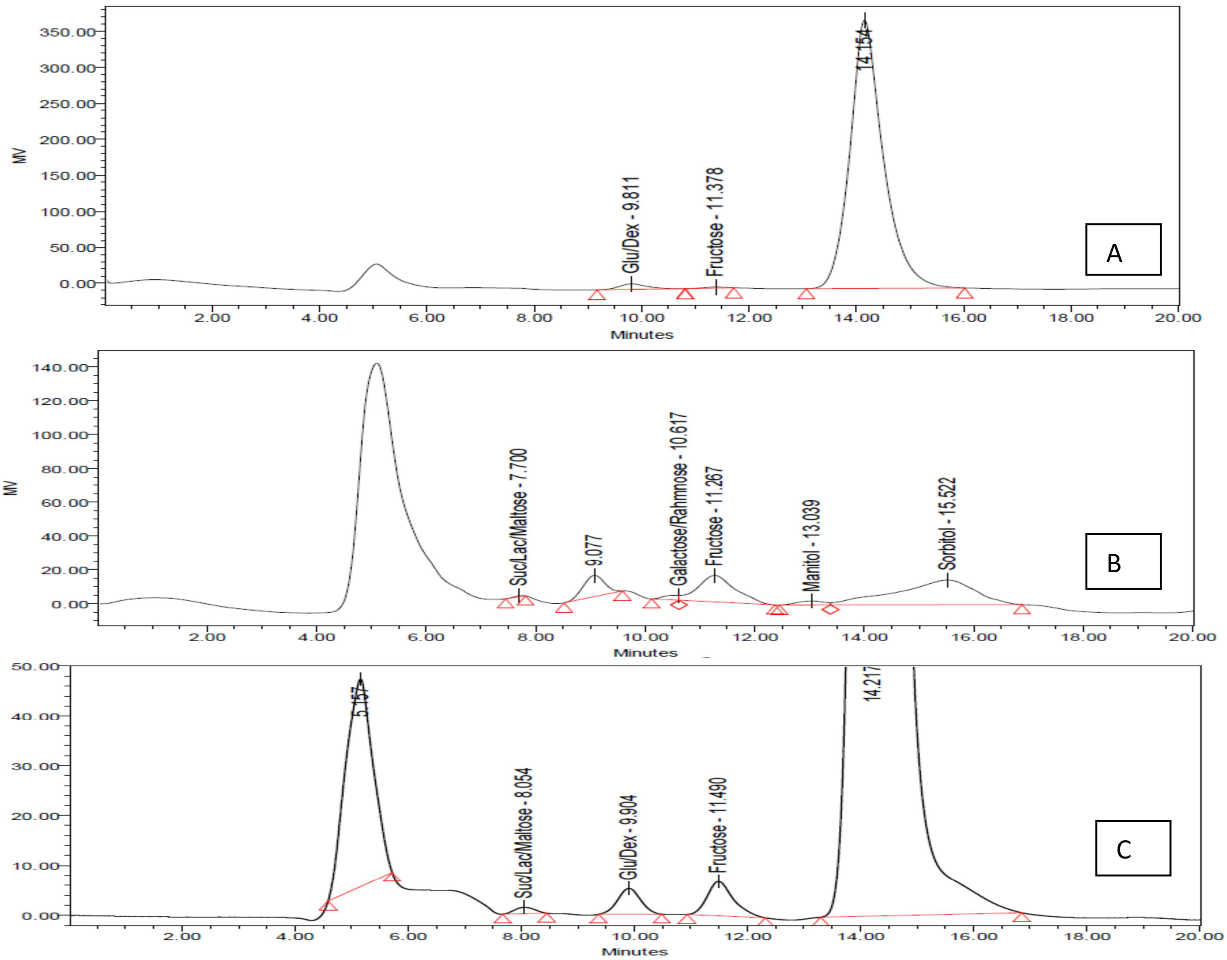


Fig.2. Effect of 24-EBL on gaseous exchange parameters of 30 days old *B. juncea* plants under Cu metal stress. (A= Photosynthetic rate, B= Transpiration rate, C= Stomatal conductance rate and D= Intercellular CO_2 concentration)



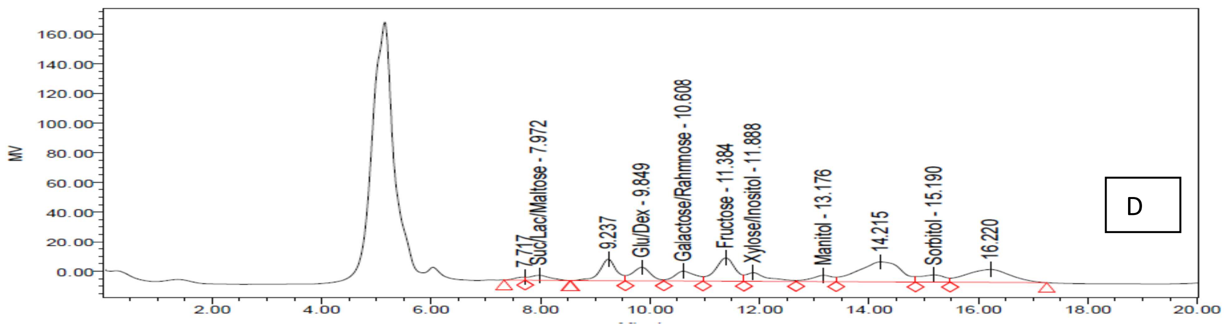


Fig.3 HPLC analysis showing effect of 24-EBL on sugars in 30 days old *B. juncea* plants under Cu metal stress. (A=control plants, B=0.75 mM Cu treated plants, C=10^{-9} M 24EBL treated plants, D=0.75 Mm+10^{-9} M 24EBL treated plants

CONCLUSION

The present study revealed that seed soaking with 24-EBL enhanced the gaseous exchange parameters along with the contents of photosynthetic pigments and reducing sugars in *B. juncea* plants under Cu toxicity.

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