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Cadmium Toxicity Alleviation through Exogenous Application of Gibberellic Acid (GA_3) in Mustard (*Brassica juncea* (L.) Czern.) and Rapeseed (*Brassica rapa* L.)

(Pengurangan Ketoksikan Kadmium melalui Penggunaan Eksogen Asid Giberelik (GA3) dalam *Mustard* (*Brassica juncea* (L.) Czern.) dan Biji Ragam (*Brassica rapa (*L.))

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ABSTRACT

An experiment was carried out by considering adverse impact of heavy metals on human health through consumption of crops. To alleviate the adverse effects of cadmium (Cd) toxicity through foliar application of gibberellic acid (GA_3), two varieties of Brassica including Indian mustard (*Brassica juncea* (L.) Czern.) commonly known as 'Raya' and rapeseed (*Brassica rapa* L.) as 'Toria' were studied. The Completely Randomized Design (CRD) was used with eight treatments including control in four replicates. Treatments were as following, T0 (control), T1 (150 μM $CdCl_2$), T2 (50 mg/L GA_3), T3 (75 mg/L GA_3), T4 (100 mg/L GA_3), T5 (150 μM $CdCl_2$ + 50 mg/L GA_3), T6 (150 μM $CdCl_2$ + 75 mg/L GA_3), and T7 (150 μM $CdCl_2$ + 100 mg/L GA_3). Gibberellic acid (GA_3), a plant growth regulator applied exogenously. The concentration of cadmium (150 μM $CdCl_2$) resulted in Cd toxicity affected adversely the morphological and biochemical parameters. Foliar application of GA_3 (50 mg, 75 mg and 100 mg) positively influenced the various growth parameters as root length (30 cm), shoot length (129.75 cm), number of leaves (14.5), pods per plant (88) and biochemical parameters like total chlorophyll (0.19 mg/g), protein content (0.70 mg/mL), carbohydrates (0.37 mg/mL) and CAT (0.56 units/mg). Outcome indicated that GA_3 reduces the harmful effects of Cd stress in both varieties. It was concluded that all growth and yield parameters of variety 'Raya' were better as compared to variety 'Toria', hence Raya recommended for large scale cultivation with GA_3 under Cd stress.

Keywords: Cadmium stress; gibberellic acid; mustard; toxicity

ABSTRAK

Satu uji kaji telah dijalankan dengan mempertimbangkan kesan buruk logam berat terhadap kesihatan manusia melalui penggunaan tanaman. Untuk mengurangkan kesan buruk ketoksikan kadmium (Cd) melalui penggunaan daun asid giberelik (GA_3), dua jenis Brassica termasuk *mustard* India (*Brassica juncea* (L.) Czern.) yang biasanya dikenali sebagai 'Raya' dan biji sesawi (*Brassica rapa* L.) 'Toria' dikaji. Reka Bentuk Rawak Sepenuhnya (CRD) digunakan dengan lapan rawatan termasuk kawalan dalam empat replikasi. Rawatan adalah seperti berikut, T0 (kawalan), T1 (150 μM $CdCl_2$), T2 (50 mg/L GA_3), T3 (75 mg/L GA_3), T4 (100 mg/L GA_3), T5 (150 μM $CdCl_2$ + 50 mg/L GA_3), T6 (150 μM $CdCl_2$ + 75 mg/L GA_3) dan T7 (150 μM $CdCl_2$ + 100 mg/L GA_3). Asid giberelik (GA_3), pengawal selia pertumbuhan tumbuhan digunakan secara eksogen. Kepekatan kadmium (150 μM $CdCl_2$) mengakibatkan ketoksikan Cd memberi kesan buruk kepada parameter morfologi dan biokimia. Penggunaan daun GA_3 (50 mg, 75 mg dan 100 mg) secara positif mempengaruhi pelbagai parameter pertumbuhan seperti panjang akar (30 cm), panjang pucuk (129.75 cm), bilangan daun (14.5), buah setiap tumbuhan (88) dan biokimia parameter seperti jumlah klorofil (0.19 mg/g), kandungan protein (0.70 mg/mL), karbohidrat (0.37 mg/mL) dan CAT (0.56 unit/mg). Keputusan menunjukkan bahawa GA_3 mengurangkan kesan berbahaya tekanan Cd dalam kedua-dua varieti. Disimpulkan bahawa semua parameter pertumbuhan dan hasil varieti 'Raya' adalah lebih baik berbanding varieti 'Toria', justeru Raya disyorkan untuk penanaman berskala besar dengan GA_3 di bawah tekanan Cd.

Kata kunci: Asid giberelik; ketoksikan; *mustard*; tekanan kadmium

Introduction

Brassica juncea L. and *B. rapa* L. are widely used species of family Cruciferae. *B. juncea* is commonly known as Indian mustard and Raya. Both are cultivated mostly under temperate climates but also in certain tropical and subtropical regions as a cold weather crop (Guo et al. 2019; Kumar et al. 2018). The family Cruciferae or Brassicaceae is consisting of 372 genera and more than 4060 species, which are among one of the most commonly consumed plants (Shankar et al. 2019). Economically various species are known as vegetables, fodder crops, edible seeds and industrial oil (Soengas et al. 2011). These are native to Asia and are extensively grown in China, Pakistan, India, Japan, Nepal, Russia, Canada and Europe since thousands of years (Iqbal & Akbar 2021).

Additionally, mustard and rapeseed exhibit economic uses as food crops, medicinal purposes, condiments and raw material for biodiesel production. These are one of the richest sources of iron, vitamin A, vitamin C, potassium, calcium, riboflavin thiamine, and β- carotene. Additionally applied as antiseptic, diuretic, emetic, and rubefacient (Kapoor, Kaur & Bhardwaj 2014). Presently, both agricultural crops and human health are facing devastating challenges due to contaminated food. Including several reasons, heavy metals (HMs) are posing severe risk to the sustainability of agriculture as well as humans, among extensively spread contaminants (Aziz et al. 2015). Heavy metals are known to be detrimental for several species of plants. Cadmium (Cd), one of the HMs, is a non-essential element, proved very toxic to plants (Nouairi et al. 2019). Nearly 20 million hectares of cultivable land is known to be contaminated worldwide by Cd (Liu et al. 2015). Cadmium has been ranked seventh among the top 20 toxins which affect the human health by entering in the food chain (Aprile et al. 2018).

Further, as a nonessential, heavy metal Cd had no beneficial biological role (Hou et al. 2019). Although Cd is a highly phytotoxic metal, it is easily taken up by plant roots growing on Cd-contaminated soils and transported to above ground plant parts (Benhamdi et al. 2021). Previous studies have shown that both varieties can easily absorb and accumulate multiple heavy metals including Cd and U from contaminated soils (Qi et al. 2014). Fortunately, GA_3, 6-benzyladenine (6-BA) and 24-Epibrassinolide (EBL) can enhance plant adaptation and resistance to various abiotic stresses and have protective effects against the toxicity of heavy metals (Aghbolaghi et al. 2022; Zulfiqar et al. 2022).

Additionally, Cadmium is a heavy metal pollutant that is continuously added to the soil from various natural as well as anthropogenic sources (Ahmad et al. 2015). Natural sources include excessive weathering of rocks, volcanic eruptions while anthropogenic sources comprise of industrial processes including smelting and mining, and overuse of phosphate fertilizer for agricultural purposes (Alyemeni et al. 2018). Toxicity of Cd impedes the photosynthetic rate through disturbing stomatal conductance, water balance of plants, CO_2 availability (Shahzad et al. 2021; Vetrano, Moncada & Miceli 2020), photosynthetic apparatus, structural integrity of membrane and chloroplast organization (Kluska, Adamczyk & Krężel 2018). Khan et al. (2016) described the decreased photosynthetic efficacy in *B. juncea* that have been facing Cd stress. Cadmium toxicity in plants has been reflected as stunted growth, leaf chlorosis and alteration in the activity of key enzymes of various metabolic pathways (Zhang et al. 2021). Once taken up by the plants, Cd retards growth, photosynthetic capacity and induces oxidative stress unexpectedly even at low doses (Qayyum et al. 2017). One reason of Cd toxicity in *B. juncea* B. *napus* is that these species are good at accumulating enough quantities of cadmium in their living cells and tissues (Irfan, Ahmad & Hayat 2014).

Owing to adverse effects of Cd toxicity on the physiology and growth of plants, attention has been focused on the plant growth regulators to alleviate the deleterious effects of cadmium stress on both varieties, gibberellic acid (GA_3) has given the importance in this respect (Irakoze et al. 2020). GA_3 has been reported for mitigating the adverse effects of Cd stress in brassica species (Masood et al. 2016). The role of gibberellins has been reported extensively in order to protect plant against the cadmium stress (Masood & Khan 2013). GA_3 is an essential growth hormone that is known to be actively involved in various physiological activities such as growth, flowering and ion transport. It is a phytohormone that is needed in small amounts at low concentration to accelerate plant growth and development including stem elongation and increase in dry weight and yield (Sharma et al. 2017).

B. juncea L. that has been exposed to Cd, gibberellins cause significant increase in growth rate (47.1 %) and overall rate of net assimilation (25.3 %), that has been attributed to GA-resulted decreased oxidative stress (Masood & Khan 2013). It was also found that GA can also be crucial for increasing protein content, carbohydrate metabolism, mitotic activity, and improve tolerance to Cd stress eventually (Asgher et al. 2015). The reports on the ameliorative role of GA in plants under Cd stress are also available. Gibberellins (GA) are plant hormones involved in diverse functions in plants, such as improving growth and photosynthetic enzymes (Hasan, Sehar & Khan 2020). The aims of the study were to investigate cadmium stress on morphological, physiological and biochemical attributes and its alleviation through the application of GA_3 on Toria and Raya varieties so that a variety can be selected and recommended for farmers use.

Materials and Methods

Experimental Design and Treatments

An experiment was conducted in the Botanical Garden of University of Gujrat, Punjab Pakistan during year 2019-2020. Pots used for this experiment were filled with 10 kg of sandy soil. The seeds of mustard plant were obtained from Punjab Seed Corporation, Gujranwala Center, Pakistan. Seeds of mustard were sown in October, 2019. Completely randomized design (CRD) was used for this experiment, along with 4 replicates. After germination, thinning was done to keep 5-6 plants per pot. Weeds and weak plants were eradicated, thinning helps plants to flourish further so that proper intake of the treatments can be possible and plants could gain proper vigor and height. After two weeks of germination, Cd was applied in the form of $CdCl_2$, subsequently foliar spray was done after 14 days of Cd treatment. The treatments that were applied include T0 = Control, T1 = 150 µM Cd, T2 = 50 mg GA_3, T3 = 75 mg GA_3, T4 = 100 mg GA_3, T5 = 150 µM Cd + 50 mg GA_3, T6 = 150 µM Cd + 75 mg GA_3, T7 = 150 µM Cd + 100 mg GA_3. Data for several morphological, physiological, biochemical and yield parameters were recorded after 21 days of treatments application.

Germination Percentage, Growth and Biomass Determination

Single plant was taken from each pot and washed using tap water. In order to estimate the percentage of germination, the number of seeds that germinated into seedlings were counted. Then, the number of seeds that germinated were divided by total number of seeds sown in each pot and multiplied by hundred to find the germination percentage (%). The length of roots and shoots were measured in cm with the help of meter scale. The fresh biomass of roots and shoots were measured in grams (g) by using electrical balance. To determine dry weight, plants were oven dried at 65 ºC. The total number of leaves were counted from each plant for the estimation of number of leaves per plant. For the measurement of leaf area of each plant, leaves were separated and placed on a graph paper. The boxes on the graph were outlined on the graph paper with the help of lead pencil. For measurement, 1 box = 1cm therefore leaf area was calculated in cm^2.

Gas Exchange Parameters

After three weeks of Cd treatment (0 or 150 µM Cd), gas exchange parameters such as net photosynthetic rate (A), intracellular CO_2 concentration (Ci), stomatal conductance (gs), transpiration rate (E), and water use efficiency (WUE) were measured on the youngest and fully expanded top leaves with the help of portable infrared gas analyzer (IRGA). The readings were recorded between 9.00 – 11.00 am under conditions of saturating light intensity, air relative humidity, leaf temperature and CO_2 concentration.

Determination of Photosynthetic Pigments

For estimating chlorophyll a, b, total chlorophyll and carotenoids, fresh leaves were taken and crushed in 80% acetone (Teas 2012). Extract was then transferred to falcon tubes, samples were incubated at -10 ºC for overnight. Readings were recorded for absorbance at five different wavelengths i.e., 480, 510, 645, 652, and 663 nm through Spectrophotometer. The content of chlorophyll a, b, total chlorophyll and carotenoids were measured by means of following formulas;

Chl. a (mg/g f. wt) – [12.7 (OD 663) – 2.69 (OD 645)] × V/1000 × W
Chl. b (mg/g f. wt) – [22.9 (OD 645) – 4.68 (OD 663)] × V/1000 × W
Total Chlorophyll [OD 652 × 1000/34.5] × V/1000 × W
Carotenoids (mg/g f. wt) – [7.6 (OD 480) – 1.49 (OD 510] × V/1000 × W
where V is the volume of leaf (mL); and W is the weight of fresh leaf tissue (g).

Determination of Glycine Betaine and Malondialdehyde (MDA)

To estimate the glycine betaine content, flag leaves were selected from plants grown both under normal and stress condition (Haider et al. 2021). In a 20 mL test tube 0.5 g of dry leaves were added and extracted by adding 5 mL of water toluene solution. After that filtrate was obtained by shaking all the tubes on a shaker in order to dry them at 250 ºC for 01 day. Filtrate (0.5 mL) was mixed with 2N HCL following the addition of 0.1 mL of KI. Then on ice water bath it was shake for 90 min, subsequently following the addition of 2 mL of cold water. It was then kept at room temperature for air dry and separation of layers for 2 min. Then, upper layer was removed and absorbance recorded at 360 nm.

The methods of Lalarukh and Shahbaz (2020) were followed to determine the level of liquid peroxidation with regard to the concentration of thiobarbituric acid reactive substances (TBARS) with some amendments. Fresh leaf was used and standardized in 3 mL 1.0 % (w/v) trichloroacetic acid (TCA) at 4 ºC. Then this homogenous mixture was centrifuged at 20,000 rpm for 15 min. Supernatant in the quantity (0.5 mL) was recovered and mixed with 3 mL 0.5 % (v/v) thiobarbituric acid (TBA) in 20 % trichloroacetic acid. The mixture was incubated at 95 ºC for 50 min in a shaking water bath, place them in an ice water bath for cooling the tubes. Absorbance of the supernatant was recorded at 532 nm after centrifugation at 10,000 × g for 10 min. Then, at 600 nm the subtracted value for nonspecific absorption was also noted. With the help of absorption coefficient of 155 $mmol^{-1}$ cm^{-1} the concentration of TBARS was measured.

Malondialdehyde peroxidation level (nmol) = Δ (A 532 nm - A 600 nm) /1.56 × 105

ANTIOXIDANT ENZYME ASSAY

Fresh leaves (0.5 g) were crushed in 5 mL of 50 mM cooled phosphate buffer (pH 7.8), which was then placed in ice bath for the extraction of antioxidant enzymes. The samples were centrifuged at 15,000 rpm at 4 ºC for 20 min. Supernatant was recovered and used for the determination of activities of different antioxidant enzymes.

Superoxide dismutase (SOD) activity was estimated by employing method of Hu et al. (2021). The SOD reaction solution (3 mL) consisted of 75 mM EDTA, 50 µM NBT, 1.3 µM riboflavin, 13 mM methionine, 50 mM phosphate buffer (pH 7.8), and 20 to 50 µL of enzyme extract. Test tubes were irradiated under the light (15 fluorescent lamps) at 78 μmol^{-2} s^{-2} for 15 min, then absorbance was measured at 560 nm through Spectrophotometer. The SOD activity of one unit was determined equally like the quantity of enzyme that inhibited photoreduction 50 % of NBT.

Following the approach of Perveen et al. (2019), the activities of both peroxidase (POD) and catalase (CAT) enzymes were measured. The composition of POD solution (3 mL) was as 40 mM H_2O_2, 50 mM phosphate buffer (pH 5.0), 20 mM guaicol, and 0.1 mL enzymes extract. Changes in absorbance of the solution were estimated at every 20 s, on 470 nm. A solitary POD action was characterized by utilizing an assimilation move of 0.02 units.

To determine the activity of catalase enzyme, CAT solution (3 mL) was prepared by adding 5.9 mM H_2O_2, 50 mM phosphate buffer (pH 7.0), and 0.1 mL enzyme extract. By the addition of enzyme extract in the solution, the reaction was started. After every 20 s, at 240 nm changes in the absorbance were measured. To describe the one unit activity of CAT, the absorbance change of 0.01 units per minute was used.

CARBOHYDRATES AND PROTEIN DETERMINATION

Fresh leaves (0.5 g) were grinded with 80% ethanol and distill water was added to make final volume of 10 mL. Concentrated H_2SO_4 (96 %) in the quantity of 5 mL was added in each falcon tube and then shaking the tubes. After that it was incubated at room temperature for 40 min. Phenol solution (5 %) of 1 mL was added to each tube and readings were recorded at 490 nm of absorbance by Spectrophotometer.

For determining protein content, 0.25 g of leaves were used. The final volume of each sample was made up to 10 mL with 5 mM phosphate buffer (pH 7.8) and centrifuged at 10,000 rpm at 4 ºC for 15 min (Bradford 1976). In each sample (aliquot) 2 mL of Bradford reagent was added and absorbance was recorded at 590 nm through Spectrophotometer.

ANTHOCYANIN AND ELECTROLYTE LEAKAGE

The concentration of anthocyanin was assessed by methodology proposed by Sharma et al. (2022). Leaves 0.2 g were homogenized with 3 mL of 1 % HCL-Methanol (99:1). The extract was centrifuged at 18000 rpm at 4 ºC for 30 min. The supernatant was then stored in a dark overnight at 5 ºC. Then, anthocyanin content were measured at 550 nm by using Spectrophotometer.

Anthocyanin = [OD 530 – 0.25 OD 657] × TV/ [d wt. × 1000]

where OD is the Optical density; TV is the Total volume of extract (mL); and d wt. is the weight of the dry leaf tissue (g).

Electrolyte leakage was measured by following Stefano et al. (2000), to estimate the membrane permeability. Electrolyte leakage was calculated according to the following formula:

Electrolyte Leakage (%) = (EC1/EC2) × 100

DETERMINATION OF YIELD ATTRIBUTES

To estimate the total number of pods per plant all, pods present on a single plant were calculated. Weight of hundred seeds per plant was calculated by taking 100 seeds from a plant through electrical balance. Likewise, total number of seeds per pod were recorded on a single plant basis per replication.

STATISTICAL ANALYSIS

Analysis of variance (ANOVA) for significance was carried out through Co-STAT software and means were compared at 5 % New Duncan's Multiple Range Test (DMRT) with *P* values ≤ 0.05 were considered to be significant.

RESULTS

PLANT GERMINATION PERCENTAGE, GROWTH AND BIOMASS

The effect of cadmium (Cd) stress on the germination percentage of mustard was found highly significant ($P \leq 0.001$), whereas the exogenous application of gibberellic acid (GA_3) positively influenced the germination percentage of *B. juncea* (Figure 1). Cd stress reduced

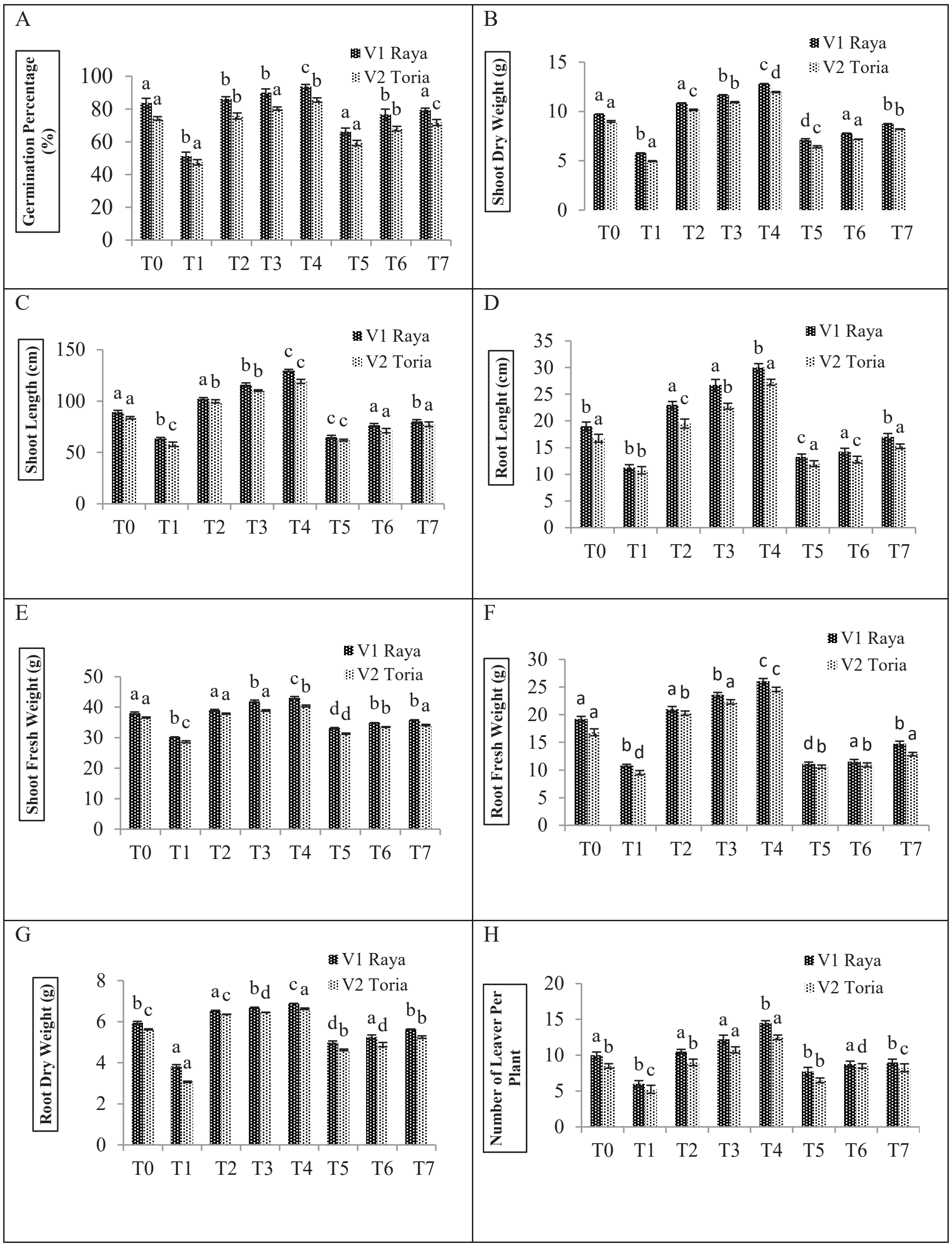

FIGURE 1. Effect of GA_3 on germination, growth, and biomass production of mustard (*Brassica juncea* L.) under Cd stress (at 150 µM $CdCl_2$) and without stress. Values are the means of four replicates. ± SE. Different letters along with the values indicate significant and highly significant differences at $P \leq 0.05$ and highly significant at $P \leq 0.001$ probability levels

the germination percentage to about 51.25% and 47.5% in both varieties, respectively. Highly significant effects ($P \leq 0.001$) of GA_3 were found on root and shoot length, as well as on fresh weight of root and shoot. It indicates that maximum root length was 30 cm and 27.25 cm in V1 and V2 correspondingly. Fresh and dry weight of root and shoot decreased significantly at 150 µM Cd stress and root dry weight of V1 was increased to 26.08 g as the level of GA_3 increases to 100 mg/L. Cd treatment affected number of leaves per plant by reducing them and had highly significant results. Maximum number of leaves and leaf area were observed at concentration of 100 mg GA_3/L. Highest leaf area was found to be 376.3 cm^2 and 350.8 cm^2 at 100 mg and 75 mg GA_3, respectively, in variety Raya of mustard.

GAS EXCHANGE ATTRIBUTES

Photosynthesis is a significant characteristic of plant that is decreased considerably under Cd toxicity. Figure 2 indicates a substantial decline of nearly 24.05 µmol

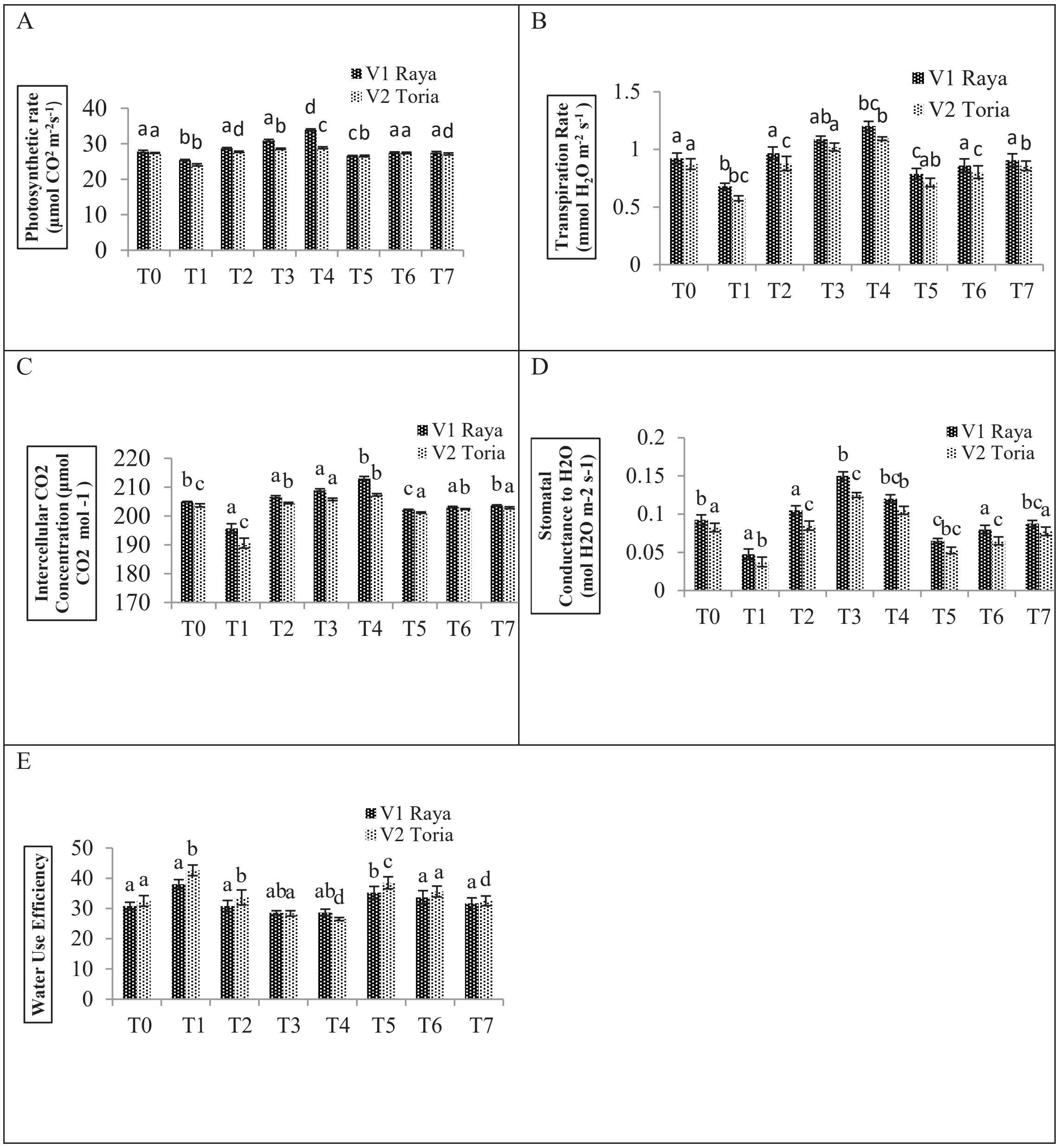

FIGURE 2. Effect of GA_3 on gas exchange parameters (photosynthetic rate, stomatal conductance, transpiration rate, intercellular CO_2 concentration and water use efficiency) of mustard (*Brassica juncea* L.) under Cd stress (at 150 µM $CdCl_2$) and without stress. Values are the means of four replicates. ± SE. Different letters along with the values indicate significant and highly significant differences at $P \leq 0.05$ and highly significant at $P \leq 0.001$ probability levels

CO_2 m^{-2} s^{-1} in photosynthetic rate of mustard at 150 μM $CdCl_2$; however, a prominent increase was noticed when treated with GA_3. Varietal response is highly significant ($P \leq 0.001$), whereas interaction between Cd and varieties is non-significant. Cadmium (Cd) toxicity has significant influence on rate of transpiration but the varietal response was non-significant as displayed from the graph. Foliar spray of gibberellic acid increases the transpiration rate to 1.20 mmol H_2O m^{-2} s^{-1} and intercellular CO_2 concentration to 209 μmol CO_2 mol^{-1} at 100 mg GA_3 concentration. Cd has significant ($P \leq 0.05$) impact on the stomatal conductance of mustard while the varietal response is highly significant. Results indicated that the stomatal conductance was highest approximately 0.15 mol H_2O m^{-2} s^{-1} at 75 mg GA_3/L concentration in variety Raya (V_1). Effect of Cd as well as GA_3 on water use efficiency of mustard was observed to be non-significant, however the interaction between Cd and GA_3 was highly significant. It was found that water use efficiency was highest at concentration of 150 μM $CdCl_2$.

PHOTOSYNTHETIC PIGMENTS

All photosynthetic pigment including; chlorophyll a, b, total chlorophyll and carotenoids content were enhanced through application of GA_3 hormone (Figure 3). Highly significant effect of GA_3 was noted on chlorophyll a, while significant effects were shown from interaction of GA_3 and varieties on carotenoid content of mustard. Content of chlorophyll b was lowest at 150 μM $CdCl_2$ while its value was highest at 100 mg GA_3 (0.067 mg/g) and combined treatment of 100 mg GA_3/L + 150 μM $CdCl_2$ (0.063 mg/g). Total chlorophyll content of mustard was reduced to 0.07 mg/g in variety Toria with the increase in Cd stress particularly at 150 μM $CdCl_2$. Harmful effects of cadmium were observed at higher levels (150 μM $CdCl_2$), particularly in Toria. Gibberellic acid (GA_3) is known to enhance carotenoid content of mustard predominantly at 100 mg GA_3.

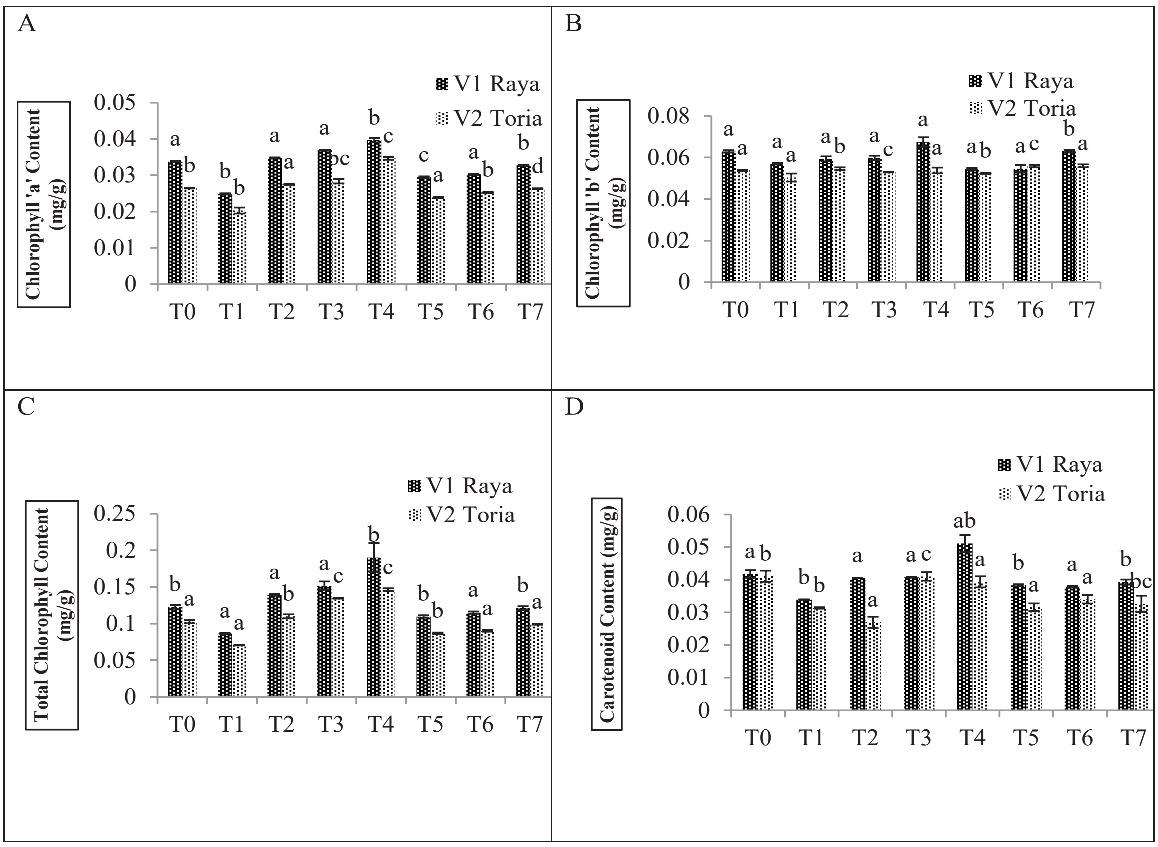

FIGURE 3. Effect of gibberellic acid GA_3 on photosynthetic pigments (Chlorophyll a, chlorophyll b, total chlorophyll and carotenoids) of mustard (*Brassica juncea* L.) under Cd stress (at 150 μM $CdCl_2$) and without stress. Values represent the means of four replicates ± standard error (SE)

GLYCINE BETAINE, MDA, CARBOHYDRATES, PROTEIN, ANTHOCYANIN AND ELECTROLYTE LEAKAGE

Highly significant response of GA_3 was observed on all these parameters. Further, harmful impact of Cd on biochemical parameters was also highly significant ($P \leq 0.001$). The mean values showed highly significant effects of Cd on total protein content of mustard. Glycine betaine and MDA were known to increase in response to exogenous application of GA_3 (Figure 4). Glycine betaine content was highest nearly 1.35 µ mol/g dry wt. at 150 µM $CdCl_2$ among all treatments. According to results the carbohydrate content decreased with the increase in cadmium level, whereas it was maximum about 0.35 mg/mL and 0.37 mg/mL at 75 mg and 100 mg GA_3. Gibberellic acid (GA_3) decreased the percentage of electrolyte leakage to nearly 20.76 % and 15.21 %, on the other hand, Cd treatment enhanced it to 30.42 % and 28.41 % in both varieties, respectively.

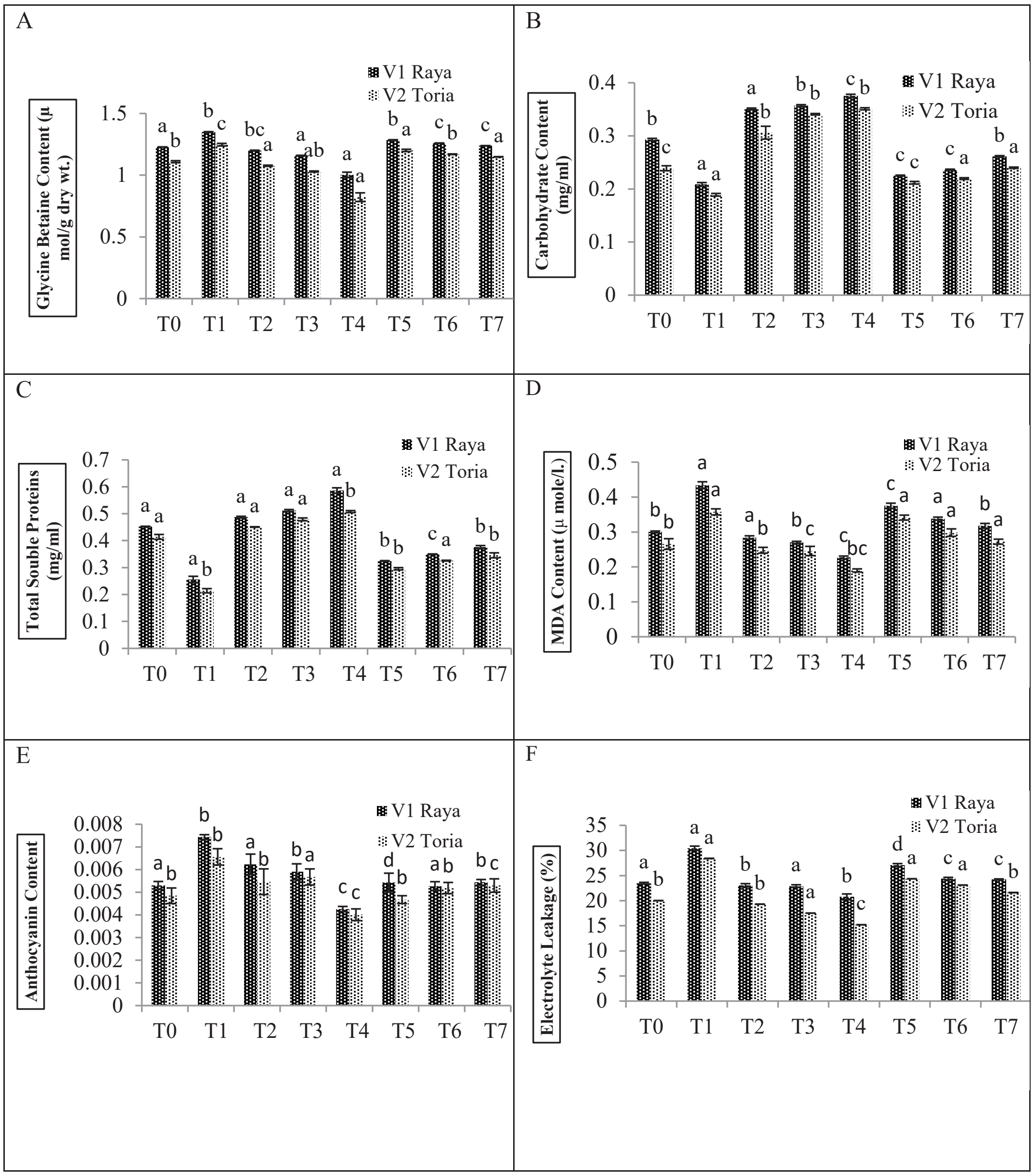

FIGURE 4. Effect of GA_3 on different biochemical parameters (glycine betaine, carbohydrates, protein, MDA, anthocyanin and electrolyte leakage) of mustard (*Brassica juncea* L.) under Cd stress (at 150 µM $CdCl_2$) and without stress. Values represent the means of four replicates ± standard error (SE). Different letters along with the values indicate significant and highly significant differences at $P \leq 0.05$ and highly significant at $P \leq 0.001$ probability levels

ANTIOXIDANT ACTIVITIES OF ENZYMES

The activities of all antioxidant enzymes including SOD and POD increased with Cd treatment, while CAT activity was reduced through Cd application (Figure 5). Results showed that hormonal impact was highly significant on all antioxidant enzymes. On the other hand, their interaction with each other and varieties was non-significant. Highest SOD activity was observed at 150 µM $CdCl_2$ which was about 0.40 (units/mg protein) in Raya (V1) when concentration of Cd is increased. The activity of POD was also maximum at elevated concentration of Cd (150 µM $CdCl_2$), while minimum POD activity, nearly 0.392 (units/mg protein) and 0.291 (units/mg protein) was observed at 75 mg GA_3 and 100 mg GA_3 in Toria (V_2), respectively. On the other hand, CAT activity was maximum generally 0.564 (units/mg protein) at 100 mg GA_3/L and is reduced with the rise in the concentration of Cd as indicated. The non-significant interaction was observed between hormone and Cd.

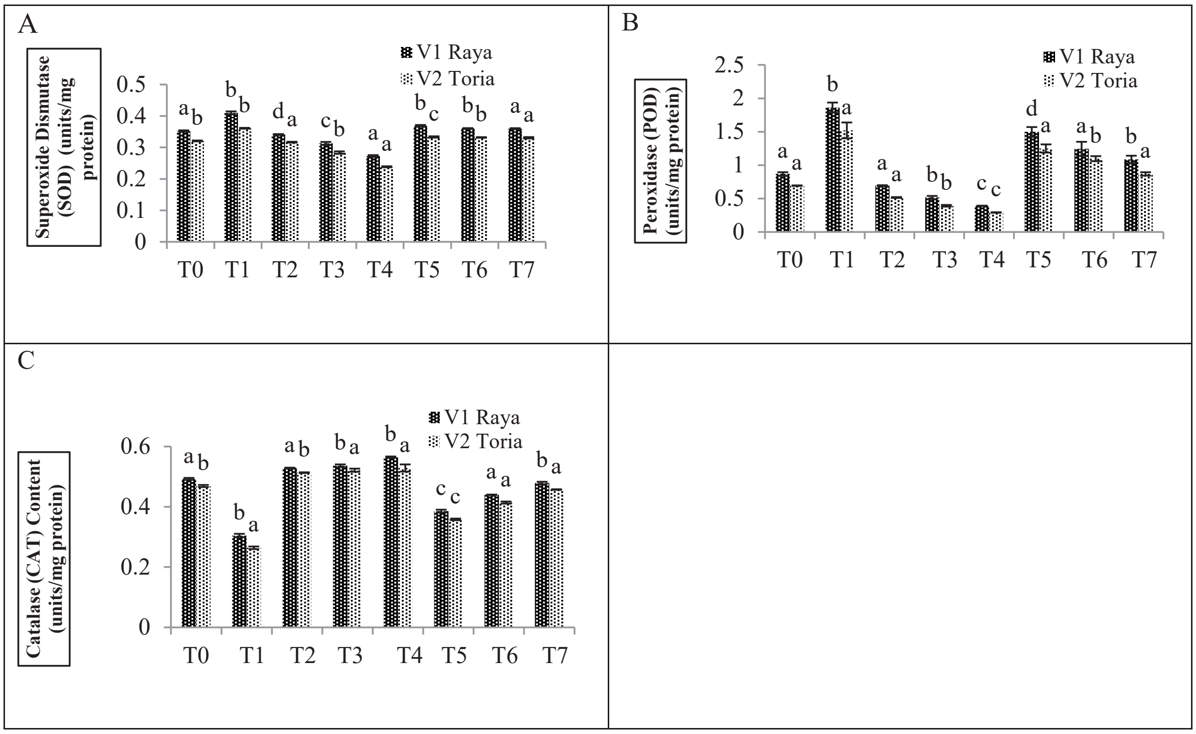

FIGURE 5. Effect of GA_3 on activity of antioxidant enzymes (SOD, POD and CAT) of mustard (*Brassica juncea* L.) under Cd stress (at 150 µM $CdCl_2$) and without stress. Values represent the means of four replicates ± standard error (SE). Different letters along with the values indicate significant and highly significant differences at $P \leq 0.05$ and highly significant at $P \leq 0.001$ probability levels

YIELD PARAMETERS

Data showed that all yield parameters exhibited highly significant results on application of hormone as well as the response to Cd was also highly significant. Maximum number of pods were observed in V1 (Raya) at concentration of 100 mg GA_3, while maximum number of seeds/plant were noticed at 75 mg GA_3 (Figure 6). Maximum seed weight of 1.645 g was observed at 100 mg GA_3, while minimum seed weight was observed at 0.488 g at 150 µM $CdCl_2$. The varietal response of all yield parameters was significant ($P \leq 0.05$) except weight of seeds that showed non-significant response. Decline in the number of seeds/pod and number of seeds/plant were noticed at higher Cd level such as 150 µM $CdCl_2$.

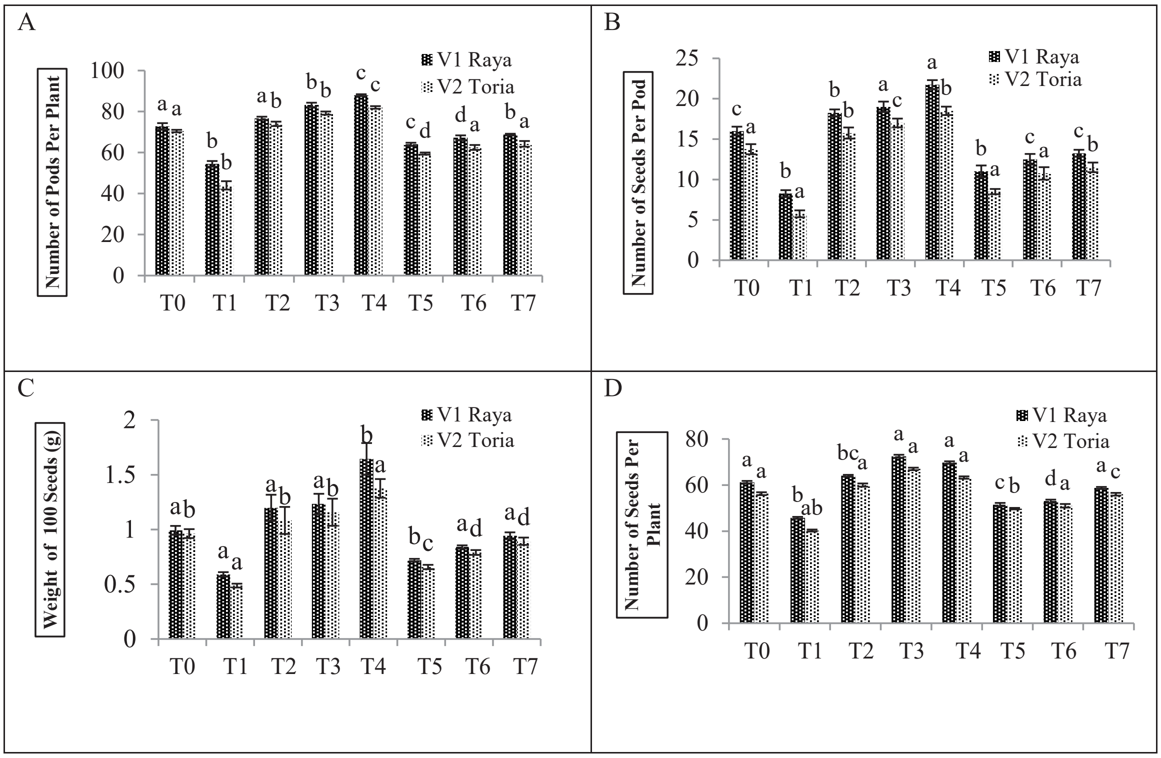

FIGURE 6. Effect of GA_3 on yield parameters (number of pods/plant, weight of 100 seeds, number of seeds/pod and number of seeds/plant) of mustard (*Brassica juncea* L.) under Cd stress (at 150 µM $CdCl_2$) and without stress. Values represent the means of four replicates ± standard error (SE). Different letters along with the values indicate significant and highly significant differences at $P \leq 0.05$ and highly significant at $P \leq 0.001$ probability levels

DISCUSSION

Plant life depends on several factors to provide healthy production, therefore, if plant characters grow uniformly without any stress, plant as well as crop yield and yield related traits flourish very well and end user would be able to get healthy food and feed for livestock. Contaminant free and non-toxic raw material and ingredients would also be a good preference for industrial products. Heavy metals including Cd have adverse impact not only on plants but also humans by consumption. Current findings reported that leaf area was affected although the value was in negative, likewise number of leaves were also declined by Cd treatment. Nagarajan, Varatharajan and Gandhimeyyan (2021) reported that minimum numbers of leaves were found at higher Cd level. Consistent to the results of current research, Cd foliar application decreased fresh biomass of roots and shoots to about 50% and dry biomass of roots and shoots to nearly 35% (Guo et al. 2019; Kapoor et al. 2019).

Further, photosynthetic activity of both of the varieties was adversely affected and declined under Cd stress, it is extremely sensitive response of plants against stress, as majority of heavy metals are recognized to prevent the photosynthetic process at varying levels (Khan et al. 2016). Likewise, it is also indicated by Nouairi et al. (2019) that values of NP, Ci, gs, E, and WUE considerably decreased when treated with 50 µM Cd compared to the control, therefore, it is necessary to require serious attention to overcome adverse effects and environmental pollution of heavy metals (Kapoor, Kaur & Bhardwaj 2014). In the present outcome, the content of glycine betaine is accumulated in response to Cd stress, however, GA_3 proved beneficial in reducing the content of glycine betaine. As important osmoregulator glycine betaine enhances growth, photosynthesis, antioxidant enzymes activities, nutrients uptake and reduces excessive heavy metal up take and oxidative stress.

Further results showed that activities of antioxidant enzymes (SOD and POD) were increased whereas CAT is decreased under Cd stress. It was also found that the activity of SOD is significantly increased under extreme Cd stress in *B. juncea* (Al-Mahmud et al. 2019). The findings of Guo et al. (2019) confirmed that treatment of cadmium (Cd) resulted in an increase in superoxide dismutase (SOD) and peroxidase (POD) activity in *B. juncea* plants, whereas a reduction was found in activity of catalase (CAT) in *Phragmites australis*. While, it is confirmed from the findings of Shahzad et al. (2021) that activities of antioxidant enzymes SOD and POD declined in maize and *Vigna radiata* through exogenous spray of GA_3. On the other hand, Sharma et al. (2012) noticed that activity of CAT was increased after foliar application of GA_3 in broad bean and lupin. As per function, antioxidant enzymes protect cellular components by stabilizing and/or deactivating free radicals and in addition interrupt the oxidizing chain reaction from free radicals from probable damage.

Additionally, it was observed that the content of total soluble carbohydrates and proteins were decreased when treated with Cd in *B. juncea* (Kapoor et al. 2019). However, MDA, anthocyanin level and electrolyte leakage were increased as advocated by Ahmad et al. (2015), Kapoor et al. (2019), and Nouairi et al. (2019). In contrary to Cd toxicity, Alyemeni et al. (2018) reported that foliar spray of GA_3 reduced the percentage of electrolyte leakage in tomato. Reduction in electrolyte leakage can protect cell membrane permeability and save plants from cellular damage, thus provides tolerance to the cells against temperature fluctuations. Furthermore, it was also found that Cd stress decreases all of the yield parameters of both varieties (Wang, Yang & Shan 2022). In the same context, treatment with exogenous GA_3 enhanced leaves number, highest number of pods per plant and more seeds per plant and seed weight (Sharma et al. 2017). Likewise, foliar spray of GA_3 in various concentrations enhanced the quality and yield attributes of both of the varieties.

On the basis of current research work, it is evident that exogenous application of gibberellic acid (GA_3) lessened the toxic impact of Cd stress in both varieties of *Brassica* particularly in Raya (V1) i.e., *B. juncea*. Among various concentrations of exogenously applied GA_3, 100 mg/L was proved to be more effective and beneficial in improving plant growth. Applications of GA_3 increased germination percentage up to 93.75 % and root and shoot length to 30 cm and 129.75 cm respectively, similarly other parameters were also enhanced.

Conclusions

GA_3 not only improves plant growth but also mitigates the harmful effects of Cd toxicity in both varieties. Conclusively, among both varieties of Brassica, V1 (Raya) (*B. juncea*) exhibited promising results in terms of growth and yield as compared to V2 (Toria) *B. rapa*. The best results were obtained at concentration of 75 mg/L GA_3 and 100 mg/L GA_3, therefore these concentrations are recommended for the alleviation of Cd stress or toxicity in *B. juncea*. Variety 1 *B. juncea* (Raya), hence recommended for cultivation at farmers field.

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