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EFFECTS OF EXOGENOUSLY APPLIED MELATONIN ON GROWTH, PHOTOSYNTHESIS, ION ACCUMULATION AND ANTIOXIDANT CAPACITY OF CANOLA (*BRASSICA NAPUS* L.) UNDER CHROMIUM STRESS

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Abstract

The present investigation was performed to examine the positive role of exogenous application of melatonin on bio-mass production, photosynthetic pigments, total soluble proteins, nutrients uptake and oxidative defense on canola under chromium (Cr) stress. Two week old plants of a canola cultivar Ac-Excel were subjected to three stress levels of chromium (Cr) stress (0, 50 and 100 µM) and four levels of melatonin (MT) (0, 1, 5, and 10 µM) were applied foliarly. Cr stress significantly reduced the plant growth attributes, chlorophyll contents, total soluble proteins and total free amino acids, catalase (CAT), peroxidase (POD), superoxide dismutase (SOD) activity and yield attributes of canola. Foliar application of melatonin significantly increased plant growth status in terms of shoot height, number of leaves, fresh and dry biomass, enhance accumulation of total soluble proteins under Cr stress. Overall, foliar application of melatonin considerably improved stress tolerance of canola plants by up regulating the ROS scavenging enzymes (catalase, peroxidase). Chlorophyll *a* fluorescence measurements (F_o, F_m, F_v, F_o/F_m, F_v/F_o, F_v/F_m and other JIP-test parameters) revealed that chromium stress caused PSII photoinhibition by damaging donor end of PSII (oxygen evolving complex) and acceptor end of PSII (over reduction of Q_A). However exogenous application of melatonin protected the oxygen evolving complex of PSII and helped out in maintaining PSII activity. Overall melatonin induced growth improvement in canola cultivar under chromium stress was seemed to be associated with activation of antioxidant defense system which protected PSII from oxidative damage thereby resulting in enhanced photosynthetic capacity.

Key words: Antioxidants, Melatonin, Cr stress, Canola, Chlorophyll fluorescence, Chlorophyll contents.

Introduction

Heavy metals with density higher than 5.0 cm^{-3} such as chromium (Cr), cadmium (Cd), lead (Pb), silver (Ag) and mercury (Hg) etc., considered as a major environmental pollutants for agricultural soil (Banuraman & Meikandaan, 2013). Leather tanning industries and anthropogenic activities are main sources of soil pollution and now become the environmental concern for agricultural sector over few decades especially in developing countries like Pakistan (Joseph & Nithya, 2009). Heavy metal pollution adversely affects the soil biological activities, fertility, plant metabolism, biodiversity as well as human health at very large scale (Chakraborty & Newton, 2011). Heavy metals stress also causes detrimental effect on plants growth, develop phototoxic response and reduces crop productivity (Ali *et al.*, 2015).

Among all heavy metals chromium (Cr) is considered as one of the toxic element for agricultural crops and food safety. Cr accumulation to agricultural soil is of great concern and has some adverse effects on crop growth and productivity (Soares *et al.*, 2016). Chromium toxicity stimulates the production of reactive oxygen species (ROS) that consequences oxidative stress in plants and damage the plant photosynthetic pigment and protein contents (Nawaz *et al.*, 2016). Plants may develops antioxidative defense system to scavenge ROS by enhancing the production of antioxidant enzymes e.g., superoxide dismutase (SOD), peroxidase (POD), catalase (CAT) and ascorbate peroxidase (APX) under metal stress (Parlak *et al*., 2012).

A large number of approaches have been used to reduce Cr toxicity damage in agricultural crops. One such approach is foliar application of hormones to alleviate the metal stress environmental conditions (Tan *et al.*, 2007). Melatonin (tryptophan derivative) plays key role in protection and function of many organisms, including circadian rhythm, photoperiodic reactions, ROS scavengation (Rodriguez *et al.*, 2004). Melatonin inside the plant body acts as growth regulators and functions similar to indole acetic acid (IAA) involved in cell expansion and promotes the plant growth (Hasan *et al.*, 2015). Melatonin also involved in photoperiod and circadian rhythms of plants, which help in repairing of photosynthetic apparatus, by regulating the connectivity between antenna complex and light harvesting complex of photosystem second (PSII), prevents the degradation of chlorophyll contents during leaf senescence (Shi *et al.*, 2015). In addition melatonin also involved in activation of antioxidant defense system during the stress conditions in plants such as low temperature, drought, ultraviolet light UV-B (Hernandez‐Ruiz *et al.*, 2005; Li *et al.*, 2018).

Canola (*Brassica napus* L.) is well known and major source of edible oil throughout the world. Brassica species are considered as potential candidate against heavy metal stress because of its distinguishing

characteristics such as heavy metal absorption, rapid growth and greater biomass (Farooq *et al*., 2016). Canola plants have developed specific heavy metal tolerance mechanism that enables them to grow well in polluted soil. Thus, it is necessary to evaluate the *Brassica* species response or specific mechanism involved in metal tolerance. Hence, present study was carried out to analyze the canola crop (*B. napus)* tolerance against Cr stress by exogenously applied melatonin and its effects on plant growth, chlorophyll pigments, and enzymatic antioxidant system under metal toxicity.

Materials and Methods

Plant material and growth conditions: Present study was carried out to investigate efficiency of melatonin to alleviate the adverse effects of chromium stress on the growth of *Brassica napus* L. cultivar AC-Excel. Experiment was laid out at the Botanical Gardens of Bahauddin Zakariya University, Multan, Pakistan with 10/14 light/dark period at 800-1000 mmol m^{-2} s^{-1} PPFD, a day/night temperature cycle of 26/15°C, 60±5% relative humidity. Seeds of canola cultivar was obtained from Ayub Agriculture Research Institute (AARI), Faisalabad. River sand washed with water was used as a rooting medium. In experimentation 60 plastic pots with diameter of 28 cm having 8 kg sand were used and five seeds were sown in each pot, plants were watered on daily basis.

After germination plants were thinned, leaving three plants in each pot. After two weeks of germination, plants were supplied with different concentrations of Cr (0, 50, 100 µM) in full strength Hoagland nutrient solution. Melatonin (0, 1, 5, 10 µM) was exogenously applied as a foliar to the plants of canola cultivar growing at different concentrations of Cr. The experiment was designed in completely randomized design (CRD) with three Cr levels, four melatonin treatments, one cultivar and four replicates. After three weeks of treatment, below mentioned parammetrs were measured following standard laboratory protocols.

Measurements: Plants were harvested after five weeks of growth under chromium stress. Data regarding shoot, root fresh and dry weight were determined.

Quantum yield of photosystem-II : Quantum yield of PSII were measured on youngest and fully matured leaves using FluorPen FP 100 –a hand held device. PSII photochemistry (Fv/Fm ratio under light adapted condition) we can determine the maximum quantum yield of primary Quinone (Q_A) reduction.

Chlorophyll *a* fluorescence (OJIP analysis and JIP-test): Chlorophyll *a* fluorescence data was recorded following nomenclature by Kodru *et al.,* (2015) and literature related to chlorophyll fluorescence available on its manufacturer website. Chlorophyll *a* fluorescence transients were observed on fully mature third leaf by using fluorescence meter, Fluor Pen FP 100. Before taking data plants were dark adapted for 30 minutes by using aluminum foil on leaf surface to be ensure that complete oxidation of electron transport chain and PSII with open reaction centers (RCs). Fluorescence light passes through 4 mm diameter area that faces the actinic light having intensity of 3000 µmol m^{-2} s^{-1}. Fluorescence kinetics were analyzed from 10 µs to 2 s and fluor pen has settings as: Fo the initial fluorescence as the first step O of 20 µs with all reaction centers are in open form, L step takes 150 µs, K step completes in 300 µs, J step completes in 2000 µs while I step completes in 30000 µs and P with 500000 µs time is the maximum fluorescence (Fm).

The original chlorophyll fluorescence transients without normalization of different treatments were plotted. For detailed analysis of fluorescence kinetics different normalization and ratios were taken. OJIP transients were double normalized between two fluorescence steps O (includes Fo) and P (includes Fm) and different variables of fluorescence between OP written as VOP (VOP = (Ft-Fo)/(Fm-Fo) were observed. Chlorophyll *a* fluorescence transients were also double normalized between Fo (30 ms) and FK (300 ms) expressed as VOK [VOK = (Ft-Fo)/(FK-Fo)] that describes the rise of fluorescence at early step on 300 ms. Chlorophyll *a* fluorescence transients were also double normalized between Fo and FJ written as VOJ [VOJ =(Ft-Fo)/(FJ-Fo)]. For the evaluation of O-I phase double normalization of fluorescence transients between Fo and FI written as VOI (<1) [VOI = (Ft-Fo)/ (FI-Fo)]. For the evaluation of I-P phase normalization two conditions occur, firstly fluorescence transients VOI between the time range of 30-300 ms written as VOI or PI (>1) [VOI = (Ft-Fo)/(FI-Fo)] and secondly transient normalization to the time range of 30-200 ms written as VIP [VIP = (Ft-FI)/(FM-FI)]. However differences in these transients were observed by L band (ΔVOK = VOK (salt stress) -VOK (control)) and K-band (ΔVOJ = VOJ (chromium stressed) –VOJ (control)). Similarly, ΔVOI and ΔVIP were observed for detailed analysis of chlorophyll fluorescence.

Chlorophyll contents: Chlorophyll contents were extracted in 80% acetone from the fully matured leaf sample of each pot plant. 0.2g of leaf sample was used, grinded in acetone 80% in pestle and mortar. Extract was filtered and maintain the volume 10 ml with 80 %acetone. Falcone tubes were covered with aluminum foil to prevents further absorbance of light and total chlorophyll content was determined at 663,652,645, and 470 nm by spectrophotometer (U-2900/2910 Hitachi) following (Arnon, 1949).

Determination of soluble protein: The soluble protein content was analyzed according to Bradford (1976) using Coomassie Brilliant Blue G-250 as dye and albumin as a standard.

Assay of antioxidant enzymes: Anti-oxidant enzymes such as superoxide dismutase (SOD), peroxidase (POD), catalase (CAT) were determined by spectrophotometrically.

Superoxide dismutase (SOD): Superoxide dismutase (SOD) activity was analyzed by nitroblue tetrazolium (NBT) method (Giannopolitis & Ries, 1977) at 560 nm by the calculation of photoreduction of NBT. The reaction mixture with total volume of 3 ml having 50 mM sodium phosphate buffer (pH 7.8), 13 mM methionine, 75 µM NBT, 10 µM EDTA, 2 mM riboflavin and 100 µl enzyme extract. Reaction started by placing test tubes containing reaction mixture below 15 W fluorescent lamp for 10 minutes.

Peroxidase (POD): Guaiacol peroxidase (POD) activity was analyzed by (Saeid *et al.*, 2014) with some modifications. The reaction mixture of total 3 ml with 100 µl enzyme extract, 100 µl Guaiacol (1.5% v/v), 100 µl H_2O_2 (300 mM) and 2.7 ml (25 mM) sodium phosphate buffer with 2 mM EDTA. Increase in absorbance due to oxidation of guaiacol was observed spectrophotometrically at 470 nm.

Catalase (CAT): Catalase activity was measured by method of Aebi (1984) Reaction mixture having total volume of 3 ml comprises of 100 µl enzyme extract, 100 µl H_2O_2 (300 mM) and 2.8 ml of 50 mM sodium phosphate buffer with 2 mM EDTA. CAT activity was observed by reduction in absorbance at 240 nm because of disappearance of H_2O_2.

Hydrogen peroxide (H_2O_2) contents: H_2O_2 was extracted by homogenizing 50 mg leaf or root tissues with 3ml of phosphate buffer (50 mM, pH 6.5). Then, the homogenate was centrifuged at 6,000×g for 25 min. To measure H_2O_2 content by following Bharwana *et al.,* (2013), 3 ml of extracted solution was mixed with 1 ml of 0.1% titanium sulphate in 20% (v/v) H_2SO_4 and the mixture was then centrifuged at 6,000 g for 15 min. The intensity of the yellow color of the supernatant was measured at 410 nm. H_2O_2 content was computed by using the extinction coefficient of 0.28 $\mu mol^{-1}cm^{-1}$.

Malondialdehyde (MDA) content: The level of lipid peroxidation in the leaf tissue was measured in terms of malondialdehyde (MDA, a product of lipid peroxidation) content determined by the thiobarbituric acid (TBA) reaction using the method of (Heath & Packer, 1968). 0.25g leaf sample was homogenized in 5ml 0.1% TCA. The homogenate was centrifuged at 10,000 g for 5min. In 1ml aliquot of the supernatant, 4ml of 20% TCA containing 0.5% TBA was added. The mixture was heated at 95ºC for 30 min and then quickly cooled in an ice bath. After centrifugation at 10,000g for 10 min, the absorbance of the supernatant at 532 nm was read and the value of the nonspecific absorption at 600nm was subtracted.

Estimation of chromium (Cr): 0.1g dried ground plant material was taken in vials (digestion flask), added 2 ml of digestion mixture and kept for overnight. Then vials were placed on hotplate and temperature was increased gradually from 50-200^{0}C. By heating, when color of mixture changed into black then 0.5ml $HClO_3$ was added into each vial containing samples with the help of dropper and heated again. When color of mixture became clear (transparent), vials were removed from hotplate and cooled. Samples were diluted up to 50ml with distilled water. Then chromium concentrations were estimated (Ali *et al*., 2009).

Results

Plant biomass: Chromium stress significantly decreased (p<0.001) fresh and dry weight of both shoot and root of canola plants as shown in (Fig. 1). Increasing level of chromium stress caused a consistent decrease in fresh and dry weight of both shoot and root. But exogenous application of melatonin significantly improved the plant growth under Cr stress. Additionally, exogenous application of melatonin (10 µM) significantly improved the plant growth in terms of shoot and root biomass (fresh and dry) under Cr stress levels (50, 100 µM). Minimum biomass and ameriolating impact by Melatonin was recorded at 100 µM Chromium level (Table 1).

Quantum yield of photosystem II: Cr stress significantly decreased (p<0.001) quantum of photosystem II of the canola plants as shown in (Fig. 2). Canola plants differed significantly in their quantum yield of PSII with melatonin treated and non-treated plants under chromium stress. While melatonin application at 10 µM level improved to some extent PSII activity in chromium treated canola plants (Table 1).

Chlorophyll contents: Cr stress significantly (p<0.001) decreased photosynthetic pigments (chl *a*, chl *b*, total chl and carotenoids) concentrations in canola plants shown in (Fig. 3). 10 µM melatonin with 0 µM Cr stress enhanced chl *a* and chl *b* while 1 µM melatonin with Cr stress (50, 100 µM) could not enhance these attributes. Generally, chlorophyll contents were enhanced with foliar application of melatonin under Cr stress except Cr (100 µM) with Melatonin (1 µM) (Table 2).

Table 1. Mean square values from the analysis of variance of data for shoot fresh weight, root fresh weight, shoot dry weight, root dry weight and quantum yield of canola (*Brassica napus* L.) var. Ac-Excel, when MT (0, 1, 5 and 10 µM) was foliar applied on two week old plants grown under varying levels of Cr (0, 50, and 100 µM) for three weeks.

SOV	Df	Shoot F.wt	Shoot D.wt	Root F.wt	Root D.wt	QY
Cr	2	1669.43***	15.70***	35.89***	2.63***	0.056***
MT	3	58.51***	1.24***	3.03**	0.20***	0.003**
Cr × Mt	6	22.75***	0.38***	30.06***	0.09***	0.002**
Error	36	3.53	0.066	0.50	0.01	6.68
Total	47					

Table 2. Mean square values from the analysis of variance of data for chlorophyll contents in canola (*Brassica napus* L.) var. Ac-Excel, when MT (0, 1, 5 and 10 µM) was foliar applied on two week old plants grown at varying levels of Cr (0, 50, and 100 µM) for three weeks.

SOV	Df	Chl *a*	Chl *b*	Chl *a*/*b*	Total Chl	Carotenoids	Chl/Car
Cr	2	0.166*	0.026***	1.545**	0.509**	17.87**	8.55*
MT	3	0.248**	0.018**	0.199ns	0.398**	18.30***	1.63ns
Cr × Mt	6	0.337***	0.032***	0.523*	0.675***	29.38***	3.80ns
Error	36	0.040	0.003	0.202	0.066	2.83	1.86
Total	47						

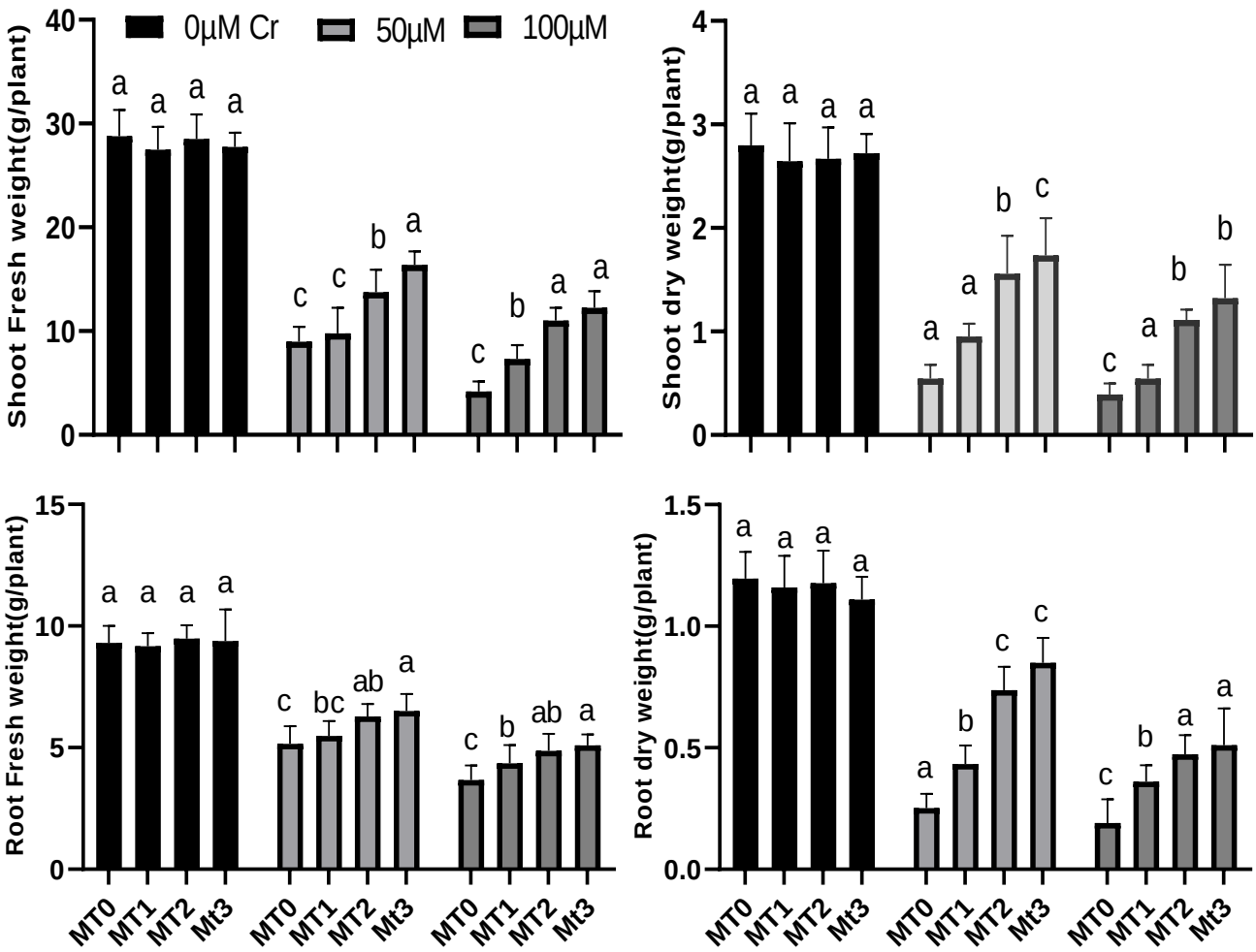

Fig. 1. Shoot fresh weight, root fresh weight, shoot dry weight and root dry weight of canola (*Brassica napus* L.) var. Ac-Excel, when MT (0, 1, 5 and 10 µM) was foliar applied on two week old plants grown under varying levels of Cr (0, 50, and 100 µM) for three weeks.

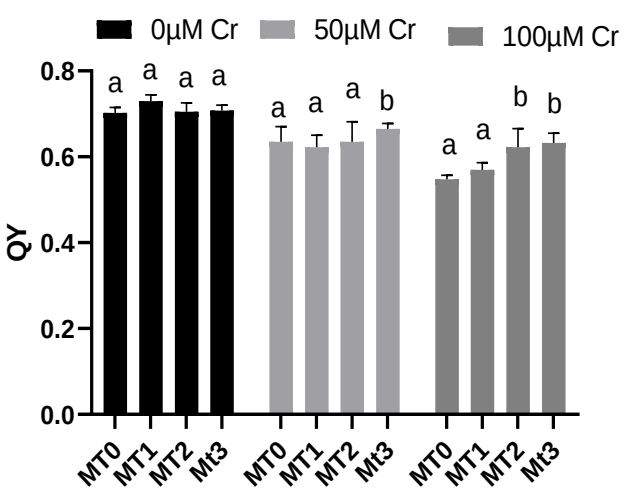

Fig. 2. Quantum yield of canola (*Brassica napus* L.) var. Ac-Excel, when MT (0, 1, 5 and 10 µM) was foliar applied on two week old plants grown under varying levels of Cr (0, 50, and 100 µM) for three weeks.

Antioxidants: Cr stress significantly increased antioxidant activities in canola plants. Low concentration of Superoxide dismutase (SOD) Peroxidase (POD), Catalase (CAT), in addition H_2O_2 and MDA contents were measured in plants with and without Cr stress. While increase in concentration of SOD, POD and CAT were observed with the increase of Cr stress and melatonin levels as shown in (Fig. 4). H_2O_2 production and MDA content was also lower in control condition as compared to Cr stressed condition. It was observed that increase level of Melatonin caused reduction in H_2O_2 and MDA concentration in Cr stressed plants (Table 3).

Total soluble proteins: Chromium (Cr) stress significantly (p<0.001) increased the total soluble proteins in canola as shown in (Fig. 5). Quantification of proteins contents can indicate protein synthesis/ protein degradation in plant under control and Cr stressed condition. Proteins contents were increased as the Cr stress level was increased. Exogenous application of melatonin also significantly increased total soluble protein contents by lowering the chromium induced proteins degradation under Cr stress as compared to the control plants (Table 3).

Fast chlorophyll *a* fluorescence: Chromium stress significantly reduced photosynthetic efficiency canola plants and Melatonin could not ameriolate toxic impact of Chromium. OJIP chlorophyll florescence transients

were measure from dark adapted leaves of canola under control and treatment conditions. Differences in relative variable fluorescence as a function of time were calculated as V_{OP}, V_{OK}, V_{OJ}, V_{OI} and V_{IP} . In order to assess Cr stress induced damages in PSII, changes in decrease electron transport, various phenomenological fluxes (ABS/RC, TRo/RC, ETo/RC and DIo/RC) were measured (Table 4). Comparing with control, absorption of energy (ABS/RC) or antenna size was decreased with increase in Cr stress and Melatonin level. Similalry, DIo/RC was also decreased with decrease in ET/RC beyond Quinone A in Cr stressed plants. Such ffects might be due to the damages in photosystem II. Quantum yiled, energy fluxes for absorption, energy fluxes for trapping, electron transport and energy dissipation fluxes indicated that canola plant absorbed lesser energy and has poor traaping, transport and ultization efficiency. It means that light harvesting, light energy trapping, energy transfer throught electron transport and its utuliztion was reduced with increased level of Cr stress as shown in (Fig. 6). It indicated that control plants have better PSII structural stability with its functional as compared to Cr stressed plants.

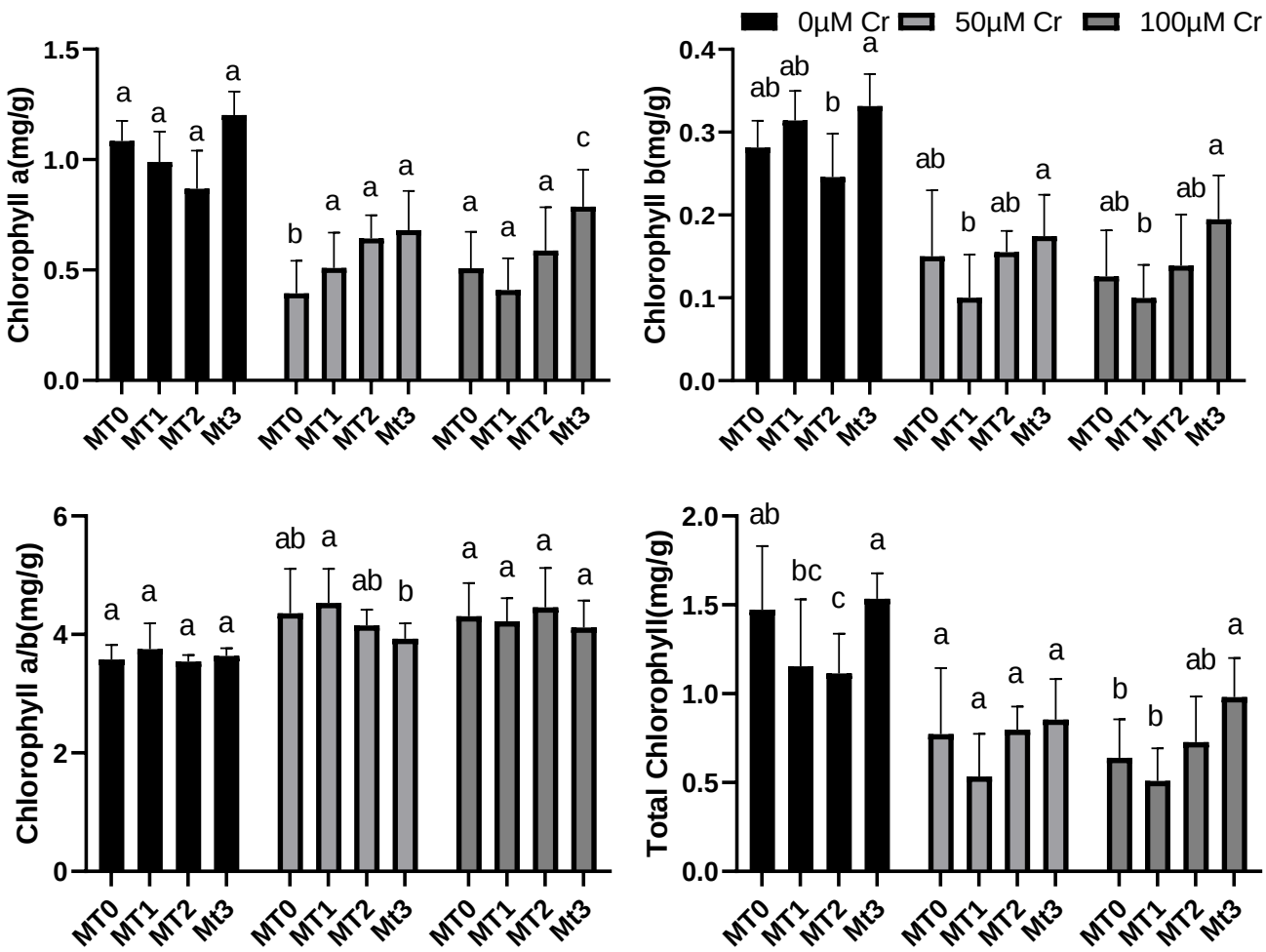

Fig. 3. Chlorophyll contents of *Brassica napus* L. var. Ac-Excel, when MT (0, 1, 5 and 10 µM) was foliar applied on two week old plants grown under varying levels of Cr (0, 50, and 100 µM) for three weeks.

Table 3. Mean square values from the analysis of variance of data for Superoxide dismutase, Peroxidase, Catalase of *Brassica napus* L. var. canola (Ac-Excel), when MT (0, 1, 5 and 10 µM) was foliar applied on two week old plants grown under varying levels of Cr (0, 50, and 100 µM) for three weeks.

SOV	Df	Superoxide dismutase	Peroxidase	Catalase	MDA	H_2O_2	T. soluble proteins
Cr	2	0.084***	0.138***	0.026***	0.020***	0.019***	97.74***
MT	3	0.036***	0.039***	0.014***	0.086***	0.088***	17.11***
Cr × Mt	6	0.008**	0.007***	0.002*	0.304**	0.301***	1.001ns
Error	36	0.001	0.001	9.65	0.002	0.002	2.49
Total	47						

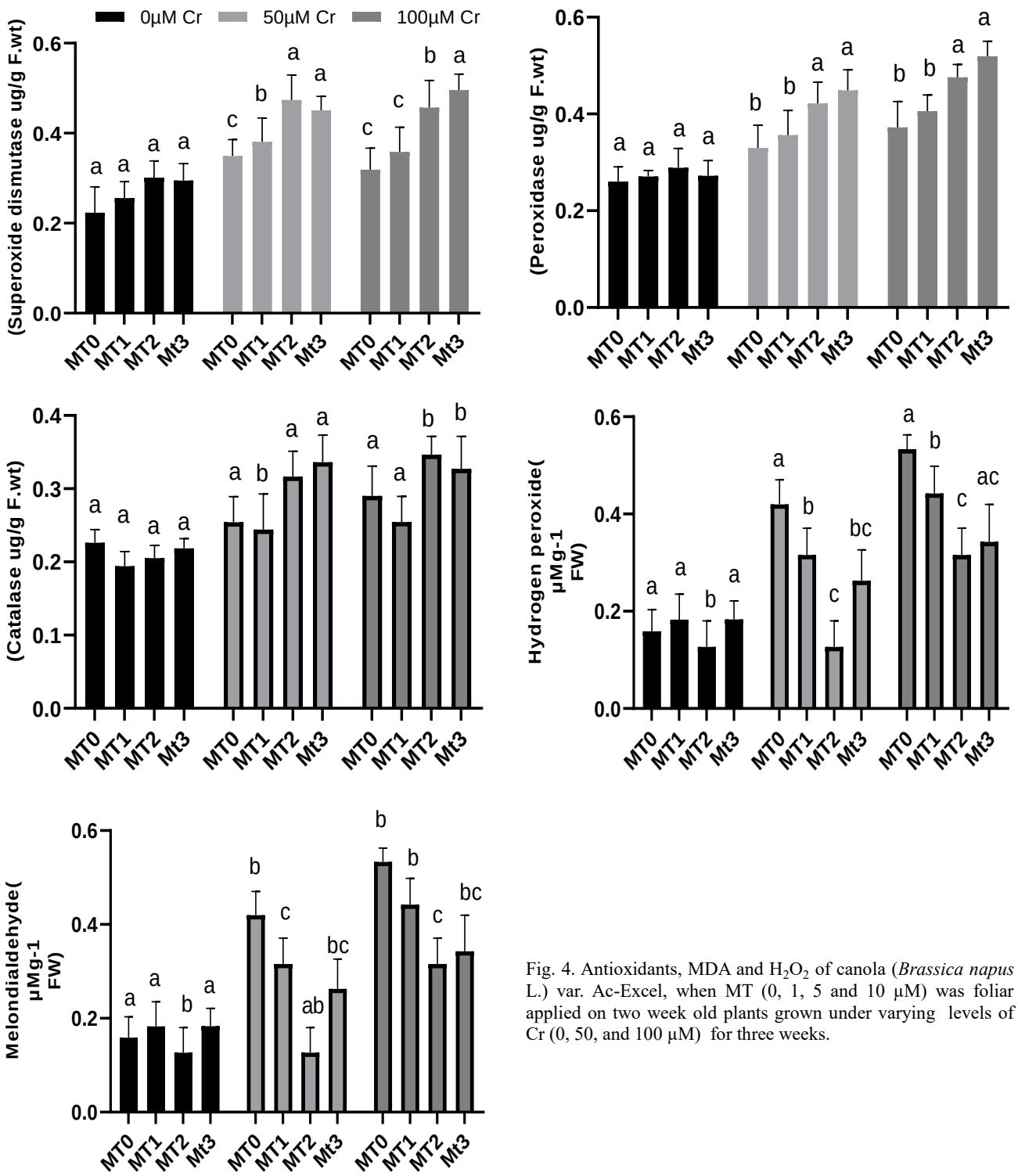

Fig. 4. Antioxidants, MDA and H_2O_2 of canola (*Brassica napus* L.) var. Ac-Excel, when MT (0, 1, 5 and 10 µM) was foliar applied on two week old plants grown under varying levels of Cr (0, 50, and 100 µM) for three weeks.

Table 4. Mean square values from the analysis of variance of data for ABS/RC, TRo/RC, ETo/RC and DIo/RC of canola (*Brassica napus* L.) var. Ac-Excel, when MT (0, 1, 5 and 10 µM) was foliar applied on two week old plants grown under varying levels of Cr (0, 50, and 100 µM) for three weeks.

SOV	Df	ABS/RC	TRo/RC	ETo/RC	DIo/RC
Cr	2	0.713***	0.450***	0.197***	0.030*
MT	3	0.117ns	0.086ns	0.085***	0.010ns
Cr × Mt	6	0.027ns	0.017ns	0.005ns	0.005ns
Error	36	0.064	0.040	0.007	0.006
Total	47				

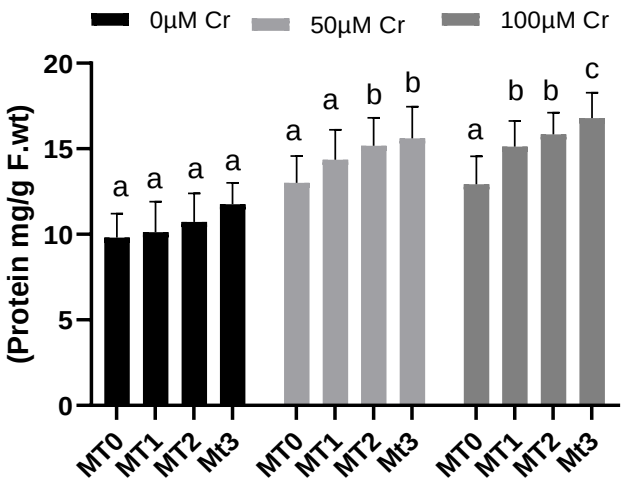

Fig. 5. Total soluble proteins of canola (*Brassica napus* L.) var. Ac-Excel, when MT (0, 1, 5 and 10 µM) was foliar applied on two week old plants grown under varying levels of Cr (0, 50, and 100 µM) for three weeks.

Chromium concentration: Chromium concentration in leaf and root as shown in (Fig. 7), was found to be increased with increase in Cr stress level. Increased Cr uptake significantly reduced the plant growth by damaging cell structure and disturbing cell functions. Cr does not have spcefic transporter therefore itcan easily enter through specific or non specific channels of essential elements. Therefore cr accumulates usually in roots of plant with limited translocation towards shoots. In our study, it measured that chromium accumulation was also increased both in leaf and roots with increase in Cr stress level.Foliar application of Melatonin retarted the accumulation of Cr both in leaf and root. Foliar application of Melatonin (5 µM) significantly reduced Cr accumulation both in leaf and root under Cr stressed (50, 100 µM) in canola plants (Table 5).

Table 5. Mean square values from the analysis of variance of data for leaf Cr and Root Cr of canola (*Brassica napus* L.) var. Ac-Excel, when MT (0, 1, 5 and 10 µM) was foliar applied on two week old plants grown under varying levels of Cr (0, 50, and 100 µM) for three weeks.

SOV	Df	Leaf chromium	Root chromium
Cr	2	0.45***	0.38***
MT	3	0.35***	0.073***
Cr × Mt	6	0.009**	0.018***
Error	36	0.02	0.002
Total	47		

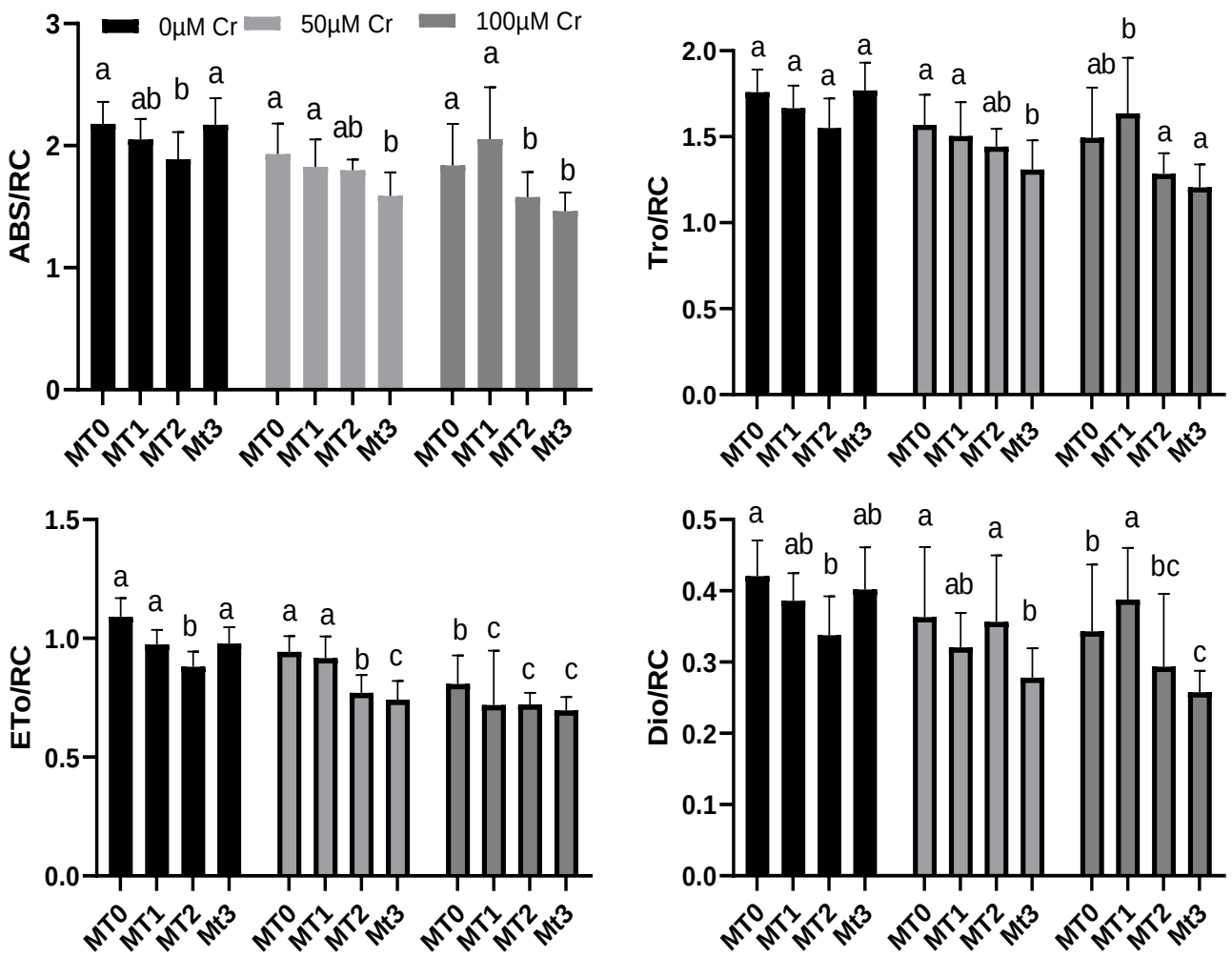

Fig. 6. Phenomenologial fluxes from fast Chlorophyll fluorescence of canola (*Brassica napus* L.) var. Ac-Excel, when MT (0, 1, 5 and 10 µM) was foliar applied on two week old plants grown under varying levels of Cr (0, 50, and 100 µM) for three weeks.

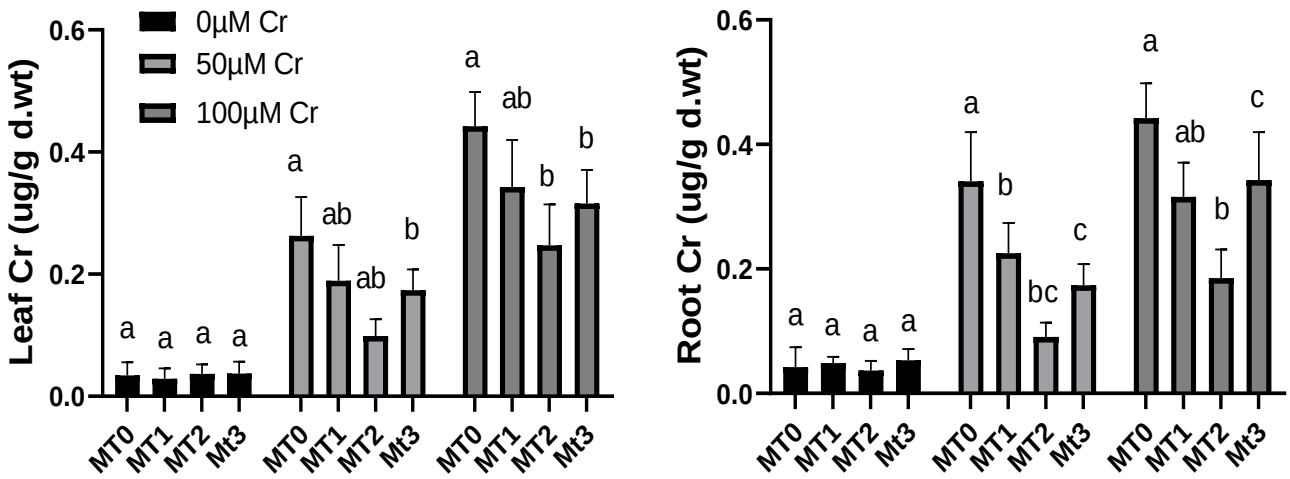

Fig. 7. Cr concentration in canola (*Brassica napus* L.) var. Ac-Excel, when MT (0, 1, 5 and 10 µM) was foliar applied on two week old plants grown under varying levels of Cr (0, 50, and 100 µM) for three weeks.

Discussion

Despite of significant efforts and advancements in abiotic stress research, still metal toxicity is one of the detrimental factors for agricultural crops (Farooq *et al*., 2016). In the present study we analyzed melatonin induced reduction of Cr toxicity in canola plants. Foliar application of melatonin is considered as effective strategy for the alleviation of metal stress (Farouk and Al-Amri, 2019), in this study improved plant growth of melatonin treated plants in Cr stress confirmed the effective role of melatonin in plant growth regulation. Exogenously applied melatonin increases Cr tolerance and plants antioxidants defense capacity at significant level may be through phyto-chelatins and compartmentalization of Cr in cell wall and vacuole (Roychoudhury *et al.*, 2012). Several studies suggested melatonin induced increased SOD, POD, CAT, APX and GR activities, which might involve plants antioxidative defense system (lowering oxidative stress) against Cr stress (Figs. 4,5). Melatonin pretreatment prevents chlorophyll degradation, increased photosynthesis, antioxidants ability and drought tolerance in cucumber seedlings (Zhang *et al.*, 2013). It is assumed that melatonin induced photosynthetic ability in plants is because of some unusual bio-stimulating pathway through regulation of photosystem II efficiency in certain light and dark conditions (Zhao *et al.*, 2019).

Inside plants metal toxicity can be reduced by their binding with metalloid ligands such as proteins, polysaccharides and organic acids (Andresen *et al.*, 2018), until ratio of chelated and non-chelated metal ions changed into other form in cellular organelles such as nucleus, chloroplast and mitochondria. These freely available metal (metalloids) ions toxicity directly damage structure and function of these cellular organelles. Metallic ions immobilization assumed to be co-related with melatonin induced biosynthesis of thiol compounds. Metallic ions competes with mineral nutrients for the same transport system from root to shoot resulting ionic imbalance and disturbed plasma membrane stability (Nazar *et al.*, 2012). H^+-ATPase of plasma membrane translocate organic compound and ions across the plasma membrane (Gevaudant *et al.*, 2007). Metallic ions sequestration in root cortex and endodermis takes place because of decreased transportation of metallic ions from root to shoot of plants body (Song *et al.*, 2017). In our study melatonin treated plants showed decreased transportation of Cr ions in cell wall and vacuole, results in reduced Cr toxicity damage suggesting that melatonin acts as cell wall barrier and reduced transportation of Cr ions in Cr treated plants. Melatonin improves ionic balance by transportation of H^+-ATPase and its conversion into 5-methoxytryptamine that stimulates H^+-ATPase activity and protects plasma membrane by reducing reactive oxygen species generation and enhancing antioxidants enzymes activity (Jiang & Zhang, 2003). However, improved membrane stability, ions transportation and Cr tolerance in melatonin treated plants might be due to improved H^+-ATPase activity under Cr stress.

Cr toxicity affects plant photosynthesis process at very large scale, where Fv/Fm considered key indicator of plant photosynthesis ability. Generally Fv/Fm always verified as a re sult of different pigment concentrations and PSII structure that considered being sensitive against environmental factors such as light, nutrients, temperature and certain chemicals that can alter PSII efficiency (Li *et al*., 2018). Several studies explained that Cr toxicity alters the structure and function of reaction centers by affecting electron transport system consequencing reduced Fv/Fm. Melatonin application in Cr stress prevents pigment degradation that helps in improvement of overall photosynthetic process (Ayyaz *et al*., 2020).

In has been reported that under stressful conditions endogenous melatonin biosynthesis increases that reduces metal toxicity damages in pea plants under copper stress (Zhang *et al*., 2013). Several previous studies focused on the phytoremediation ability of plants by exogenous application of melatonin exposed to metal stress by enhancing antioxidant activity, root growth, photosynthesis, organic acid anion exudation by reducing metal contents (Reiter *et al*., 2015), by increasing antioxidants related gene expression and by reactivating

the micro RNA mediated redox homoeostasis in different crops. Melatonin enables plants in cadmium sequestration and transformation from cytosol to vacuole and cell wall (Ismael *et al*., 2019).

However, melatonin induced improvement in photosynthetic efficiency of Ac-Excel cultivar is because of its genetic potential but its effects on the exact site of photosynthetic apparatus is still unclear. In a semi-quantitative observation of melatonin treatment with and without Cr stress on different parts of photosynthetic apparatus of canola plants. Whole OJIP normalized transients of Cr stressed and non-stressed plants were measured. All the transient data of canola plants of present study explained that primary photochemistry fluorescence and photo electrochemical quenching at O-J and J-I step reduced due Cr stress in canola plants, where melatonin application increased photosynthetic activity by compensating reduction rate at PSI and electron acceptor at step I-P site in Canola plants. In addition Fo normalized transient and relative variable fluorescence transients of Fo and Fm verified our results, for detailed analysis whole difference of kinetics at each step from OJ-JI-IP was performed. Low fluorescence values in L-band in Cr stress conditions showed that loss of energetic connectivity to some extent due to Cr toxicity.

Meanwhile, fluorescence curve at I-P step that indicates the electron transfer rate from PQH_2 to electron accepter end of PSI, melatonin treated plants showed positive increase in fluorescence transient values at I-P step in Cr stress, while decrease in Cr treated plants suggesting that exogenous melatonin application enhanced PQ redox rate ultimately lowering the Cr stress and increased PQ pool size in both the canola cultivars. Decreased fluorescence transient curve at I-P phase eventually happens because of sharp decline of leaf water status in Cr stress that might reaches to maximum tolerance level of pants. Chlorophyll fluorescence transients and their different ratios at each step of OJIP considered as key indicators for PSII efficiency evaluation.

Cr stress significantly reduces Fo (minimum fluorescence level) that eventually increases energy excitation and transfer rate from antenna complex to reaction center ultimately leads to low Fo. Foliar application of melatonin enables the plants to maintain balance of Plastoquinone redox state by transferring electron to PSI. Melatonin treated plants showed reduction in ABS/RC values suggested, increased size of antenna complex of active reaction centers. However, PI most sensitive parameter of OJIP indicates the conformational changes and confirms the vitality canola plants of PSII. While exogenously applied melatonin in Cr stress increased the PI values and possible link between ETo/RC and log PI_{ABS} that suggests the utilization of PAR which reduces the CO_2 into sugars in natural environmental conditions.

Conclusion

The melatonin induced difference observed suggests that metal tolerance capability of canola plants under Cr stress. Cr toxicity reduced the plant growth, chlorophyll contents and photosynthetic activity in canola plants. To overcome these stressful conditions exogenous application of melatonin effectively can increase the plant growth, development and tolerance against Cr stress. There is need to focus on exogenous application of growth enhancing agents that enables plants especially agricultural crops to increase their yield and tolerance against toxic elements. Given that melatonin showed positive effect on plants and it is expected that in future melatonin could have potential role in developing photosynthetically efficient stress tolerant transgenic crops.

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