

Exogenous melatonin regulates chromium stress induced feedback inhibition of photosynthesis and antioxidative protection in Brassica napus cultivars

Ahsan Ayyaz (✉ ahsanayazmsc2015@yahoo.com)

Bahauddin Zakariya University <https://orcid.org/0000-0002-7121-700X>

Ahsan Farooq

Bahauddin Zakariya University

Zafar Ullah Zafar

Bahauddin Zakariya University

Habib ur Rahman

Bahauddin Zakariya University

Research Article

Keywords: OJIP, Chlorophyll fluorescence, Melatonin, Antioxidants, Chromium stress, Brassica napus

Posted Date: May 3rd, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-320997/v1>

License:  This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Version of Record: A version of this preprint was published at Plant Cell Reports on August 21st, 2021.
See the published version at <https://doi.org/10.1007/s00299-021-02769-3>.

Abstract

Developing heavy metal chromium (Cr) tolerant cultivars is mainly constrained due to poor knowledge of the mechanism behind Cr stress tolerance. In the present study, available germplasm of *Brassica napus* (canola) was screened out for Cr stress tolerance using Dual-PAM-100 and biochemical attributes and used to select one Cr stress tolerant (Ac-Excel) and one sensitive (DGL) under melatonin (MT) treatments for further studies. Plant oxidative enzymes and PSII activity were found to be key physiological discriminating factors. Changes in PSII and PSI efficiency, oxidative damage, higher proline content and antioxidants were further evaluated in selected Cr tolerant and sensitive cultivars. Cr stress reduced PSII efficacy and ETC in both canola cultivars. MT application increased NPQ and Y(NPQ), higher increase in NPQ and Y(NPQ) were observed in Cr stress tolerant cv AC-Excel, indicating that the MT treated tolerant cultivar have higher excitation of PSII by more heat dissipation via photo-protective component of NPQ under Cr stress. Reduced PSI efficiency and increased donor end limitation of PSI in both canola cultivars further confirmed the downregulation of electron transport from PSII. However, Cr stress sensitive cv DGL had poor efficacy to manage over-excitation of PSII via buildup of Y(NPQ) through increased oxidative stress. Tolerant cultivar exhibited higher proline content and antioxidant enzymes activity than Cr sensitive cultivar. Overall, MT induced tolerance in canola cultivars can be related to PSII activity, Y(NPQ), and antioxidants potential which can be effectively used for canola cultivars selection under Cr stress.

Introduction

Rapid urbanization and anthropogenic activities have been caused environmental deterioration and become major constraint to crop production due to higher accumulation of heavy metal in soil (Yang et al. 2019). Cr is non-essential toxic metal and its availability aggravated pollutant to agricultural soil and water which possess severe human health risks through its involvement to food chain (Ulhasan et al. 2019). Hyper-accumulation of Cr in plant tissues causes severe phytotoxicity by reducing growth and biomass accumulation, chlorophyll degradation as well as the increased production of reactive oxygen species (ROS) greatly inhibit the enzymatic activities especially in the tissues of leaves by the increment of malondialdehyde (MDA), hydrogen peroxide (H_2O_2) disorganized antioxidant defense system and photosynthetic efficiency (Ahmad et al. 2017). This increment in the production of ROS may damage the biological macromolecules and disrupt the antioxidant system in plant. When, plant undergo oxidative stress they activate their antioxidant defense machinery by the production of antioxidant enzymes such as superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), ascorbate peroxidase (APX) (Ahmad et al. 2020). Chromium (Cr) stress directly inhibits photosynthesis by affecting of photosystems (PSI, PSII), destruction of photosynthetic pigments, changes in electron transport (ETR) and carbon reduction pathway. Imbalance in electron transport at thylakoid membrane and consumption of reducing equivalent in Calvin cycle for CO_2 fixation leads to the generation of reactive oxygen species (ROS) (Chen et al. 2016). Metal-induced generation of ROS mainly occurs in chloroplast as superoxide around PSI via Mehler reaction, or singlet oxygen around PSII (Chaturvedi et al. 2020). A large number of approaches

have been used to reduce metal toxicity damage in agricultural crops. One such approach is foliar application of hormones to alleviate the metal stress environmental conditions (Bücker-Neto et al. 2017). Melatonin (MT) plays key physiological functions and protects plants against certain environmental stressors. Previous studies suggested that exogenously applied MT under Cr stress considerably enhanced plant growth, photosynthetic efficiency, antioxidants activity along with reduced the chromium toxicity damage to crop plants (Altaf et al. 2020). In another study foliar application of MT in Brassica against chromium stress showed increased performance index (PI), indicating higher energy conservation of excited electron to reduce PSI (Ayyaz et al. 2020). Brassica napus is the main cash crop that is widely cultivated in both the north and south hemispheres during annuals or biennials seasons and has an annual value of USD 41 billion. (Lohani et al. 2020). *B. napus* is considered as very interesting crop plant because of its higher stress tolerance response it is known as hyperaccumulator for metal and involved in phytoremediation at very large scale. *Brassica* plants has developed higher metal stress tolerance mechanism which enables it to grow well in metal contaminated soil. The study's main goal is to understand (1) Chromium stress intervention in *B. napus* cultivar growth; (2) Melatonin-induced differential antioxidative protection and against oxidative stress; and (3) Melatonin-associated PS(II) and PS(I) efficiency regulation and evaluation using chlorophyll fluorescence measurements, as well as physiological characteristics that may indicate plant chromium tolerance.

Materials And Methods

Plant material and growth conditions

Present experiment was performed at research station of Bahauddin Zakariya University, Multan, Pakistan with field conditions (30°N and 71°28E). Seeds of canola cultivars Ac-Excel and DGL were taken from Ayyub Agriculture Research Institute (AARI) Faisalabad. In this study 40 plastic containers having diameter of 28 cm and 7kg sand, 10 seeds were sown in each pot. Thinning was carried out leaving 4 healthy plants in each pot. Plants were sprayed twice a day with foliar application of Melatonin MT (10 µM) on lower and upper surface of leaves until runoff before one week of Cr treatment. Based on our previous study (Ayyaz et al. 2020), 28 days old canola plants were applied with chromium (K₂Cr₂O₇) salt solution (50 µM) with Hoagland's nutrients for 14 days. Two cultivars, Three Cr levels and four melatonin levels having five replicates for each treatment were used in the CRBD (completely randomized block design) experiment. After harvesting plants were separated into root and shoot, data regarding fresh and dry biomass was measured.

Quantum yield (Fv/Fm) measurements

For Fv/Fm measurement mature leaves of canola plants were selected and kept in dark for about 30 minutes by using a handheld device Fluor Pen FT100.

Leaf relative water content (RWC) measurement

For RWC evaluation, full mature leaves were randomly harvested and weighed for fresh weight measurement. After the leaves had been thoroughly submerged in distilled water for 6 hours, the leaf TW (turgid weight) was calculated. Then, after 24 hours at 70°C leaf DW (dry weight) was measured. Following the given formula RWC was determined:

$$\text{RWC} = [(\text{FW}-\text{DW}) / (\text{TW}-\text{DW})] \times 100$$

Chlorophyll content:

Photosynthetic pigments were observed by using (Hitachi F-4600) spectrophotometer following the methodology of (Zheng et al. 2020).

Proline, hydrogen peroxide (H₂O₂), Malonaldehyde (MDA) and TSP analysis

Proline content was determined by following Bates et al (1973). Reagent toluene-containing chromophores were aspirated from the aqueous process, and spectrophotometric readings at 520 nm were taken. Using Chen et al. (2010) methods the quality of H₂O₂ was determined. The H₂O₂ content was determined using a normal curve and the absorbance was estimated at 390 nm. Lipid peroxidation was observed in terms of Content of MDA was measured by using TCA and TBA as reagents, at 532 and 600 nm according to Hong et al (2010). The total soluble protein (TSP) contents were measured using the Bradford (1976) procedure and bovine-serum albumin as a reference.

Biochemical analysis of enzymatic activities

Superoxide dismutase activity was observed in terms of inhibition of photochemical reduction according to the methodology of Farooq et al. (2016). In addition, riboflavin, methionine and EDTA used, reading was observed at 560 nm by spectrophotometer. The behaviour of the CAT was evaluated by following (Aebi et al. 1984). POD behaviour was detected by the following approach (Chance et al.1955) by measuring a shift in absorbance at 470 nm for 1 min. The APX activity was determined as per reduction of ascorbic acid (AsA) with H₂O₂ (Zhang 1992).

OJIP fast chlorophyll transient test

Mature leaves of canola were kept in dark for about 20 minutes covering with aluminum foil around them until saturating light pulse of 3000 mol m⁻² s⁻¹ light intensity for 0.8 sec length exposed over a 4 mm leaf area by using the Fluor Pen FP-100.

Fast chlorophyll fluorescence (Light curve measurements)

Pulse amplitude modulated Dual PAM 100 fluorometer (Walz, Effiltech, Germany) was used to record the chlorophyll fluorescence (Hanelt et al. 2018). For the measurement of Light response curve, darkly adapt the leaves for 10 min (Maxwell and Johnson 2000).

Estimation of chromium (Cr)

Take 0.1 g of dried soil plant material in vials (digestion flask) and add 2 ml of digestion mixture for 10 hours. Vials were heated by using hotplate at 50-200°C. Appropriate amount of HClO₃ (0.5ml) added into each vial having samples using a plastic dropper. Vials cool down when the color of the mixture becomes clear (transparent). Dilute up to 50ml to each sample with deionized water.

Statistical analysis

Data of rapeseed oil varieties from various parameters were subjected to completely randomized (three-way ANOVA) and LSD 0.05 level of confidence by using CO-STAT (cohort software 6.4). All the data contains mean values with standard error.

Results

Genotypic variation of physiological responses to chromium stress

Plant growth attributes

Chromium (Cr) toxicity considerably ($P < 0.001$) declined the plants growth attributes including fresh and dried biomass of plants (Table 1). Although, under control and melatonin (MT) treatment against (0 and 50 μ M) Cr stress conditions effectively increased plants biomass (shoot dry weight) accumulation with maximum increase of (16%, 63%) and (33%, 104%) in tolerant Ac-Excel cultivar relative to (8%, 73%) and (45%, 83%) in sensitive DGL cultivar respectively (Fig. 1). However, our results suggested that cultivar Ac-Excel exhibited highest dry weight, while DGL exhibited lowest dry weight among all cultivars. Overall, Ac-Excel and was found tolerant relative to DGL including plant biomass in Cr toxicity (Fig. 1A, B, C, D).

Chlorophyll content, Leaf number, Relative water content (RWC) and Quantum yield (Fv/Fm)

Photosynthetic pigments dramatically ($P < 0.001$) declined in both *B. napus* cultivars in chromium stress (Table 1). Under chromium stress MT treated plants of tolerant cultivar Ac-Excel showed higher photosynthetic pigments relative to sensitive DGL cultivar with different range of chlorophyll a (20%, 89%) and (48%, 92%), chlorophyll b (28%, 60%) and (11%, 78%) and total chlorophyll (14%, 64%) and (33%, 79%) in AC-Excel that of DGL with maximum increase of chlorophyll a (15%, 53%) and (7%, 59%), chlorophyll b (15%, 57%) and (18%, 64%) and total chlorophyll (4%, 56%) and (37%, 63%) respectively (Fig. 2A, B, C, D). In addition, carotenoids content under were high in Ac-Excel with different range (8%, 53%) and (40%, 91%) that DGL (14%, 49%) and (36%, 58%) whereas, under Cr stress MT treated and non-treated plants did not show significant effect on Xanthophyll content as in Ac-Excel (7%, 9%) and (3%, 12%); but increased somehow in DGL with maximum increase of (2%, 8%) and (2%, 19%) (Fig 2 E, F). According to (Table 1) Cr stress considerably reduced number of leaves of both Brassica cultivars. However, MT treated plants of Ac-Excel showed significant increase in number of leaves (7%, 45%) and (18%, 66%) relative to DGL with maximum increase of (4%, 34%) and (20%, 62%) respectively (Fig. 3A). Results (Table 1) Cr caused significant reduction in RWC and were observed higher under MT treatment in Ac-Excel as (17%, 56%) and (18%, 62%) relative to DGL (23%, 42%) and (10%, 32%) respectively (Fig. 3B).

Chromium stress considerably reduced in yield of photochemistry of PS(II) in terms of quantum yield (Fig. 3C). Although, under MT treatment (Fv/Fm) values were significantly ($P < 0.001$) increased under control and chromium stress as (7%, 63%) and (35%, 104%) in Ac-Excel relative to (20%, 59%) and (11%, 78%) in DGL respectively (Table 1). Higher Fv/Fm values in Ac-Excel shows higher PSII efficacy including primary photochemistry that was found to be lowest in DGL cultivar under chromium stress. **Chromium (Cr) concentration**

Chromium content in leaves were found to be increased in Cr stress treatments. Increased Cr uptake significantly reduced the plant growth (root, shoot) by directly damaging the roots' structure and function consequence reduced nutrients uptake in chromium treated plants (Table 1). Higher chromium content accumulation was observed in sensitive cultivar "DGL" compared to tolerant cultivar "Ac-Excel leaves which consequences more damage to roots structure. While MT treated plants showed significant increase in plants growth along with reduced Cr ions uptake and translocation resulting reduced chromium toxicity damage in *Brassica* plants (Fig.3D).

MDA, H₂O₂ and Proline Contents

Chromium stress considerably affected the membrane lipid peroxidation and increased MDA level ($P < 0.001$), while MT treated plants considerably reduced MDA content formations under Cr stress (Table 1) with maximum decrease of (25%, 20%) and (53%, 33%) in Ac-Excel that of (7%, 4%) and (74%, 41%) in DGL (Fig. 4A). While, under Cr stress H₂O₂ content were considerably ($P < 0.001$) increased in both canola plants (Table 1), while reduced under MT treatments as (36%, 26%) and (56%, 41%) in Ac-Excel while, (12%, 9%) and (83%, 38%) in DGL (Fig. 4B). Proline content significantly ($P < 0.001$) enhanced under Cr and MT treatment in *B. napus* cultivars that of control plants (Table 2). Proline content were observed higher (14%, 74%) and (51%, 130%) in Ac-Excel while, decreased (39%, 74%) and (56%, 88%) in DGL (Fig. 4C).

Total soluble protein and antioxidative enzyme assay

Chromium stress induced changes in total soluble protein and antioxidative enzymes activities among *B. napus* cultivars including SOD, POD, CAT, APX. Generally, it was observed that under chromium stress total soluble protein and antioxidant activates increased in Ac-Excel that of DGL as shown in (Table 1). Ac-Excel showed higher total soluble protein content under MT treatments with and without chromium stress increased with different range of (27%, 35%) and (43%, 60%) relative to DGL (11%, 24%) and (27%, 41%) shown in (Fig. 4D). However, SOD activity enhanced in a range of (51%, 66%) and (77%, 89%) in Ac-Excel that of (25%, 20%) and (35%, 55%) in DGL as compared to their respective control plants respectively (Fig. 5A). Results (Table 1) indicated that POD activity was increased (21%, 65%) and (75%, 91%) in Ac-Excel that of (31%, 45%) and (58%, 71%) in DGL as compared to respective control plants respectively (Fig. 5B). Similarly, CAT activity was increased (6%, 38%) and (87%, 132%) in Ac-Excel that of (40%, 58%) and (58%, 71%) in DGL plants respectively (Fig. 5C) (Table 2). While, APX activity showed maximum increase of (10%, 72%) and (98%, 140%) in Ac-Excel that of (32%, 49%) and (58%, 96%) in DGL plants respectively (Fig. 5D). However, data suggested that Ac-Excel possess greater antioxidative activity indicates higher chromium stress tolerance than DGL.

Fast chlorophyll fluorescence kinetics (OJIP)

Chromium stress remarkably reduced the PSII efficiency in terms of quantum yield observed as measured ratio of chlorophyll fluorescence parameters in two *Brassica* cultivars. Exogenously applied MT treatment under Cr stress clearly showed considerable difference in chlorophyll fluorescence kinetics as compared to their respective control conditions. However, Cr stress induced reduction in fluorescence kinetics was more obvious in sensitive cultivar DGL relative to tolerant cultivar Ac-Excel. Meanwhile, MT and Cr treatments showed significant increase in amplitude of OP band (FT-Fo/Fm-Fo) as compared to respective control plants in both tolerant Ac-Excel and sensitive DGL cultivars (Fig. 6A, B, C, D) respectively. According to our results under Cr stress reduced maximum number of turns over for QA reduction until Fm reached (N), rate of QA reduction (Mo) and maximum turnover of QA reduction until Fm reached (N). In addition, MT application against Cr stress substantially improved energy flux for absorption (ABS/RC), Trapping energy per absorbance (TRo/RC) and Electron transport per absorbance (ETo/RC) in both *Brassica* cultivars (Fig. 7). While, in contrast dissipation energy flux per reaction center (Dlo/RC) was remarkably enhanced in both genotypes under Cr stress. Although, decrease of energy flux per reaction center was more obvious in sensitive cultivar DGL relative to tolerant cultivar Ac-Excel. Moreover, both cultivars showed significant decrease in efficiency of electron donation to PSI (Fv/Fo) and maximum quantum yield of PSII (Fv/Fm) under Cr stress. Cr toxicity considerably decreased the area between 'Fo' and 'Fm' which indicated the electron transport inhibition from reaction center (RC) to plastoquinone (PQ) and performance index on absorption basis (PI_{ABS}) in both cultivars. While, under MT and Cr treatments tolerant cultivar "Ac-Excel" greater photosynthetic characteristics that of sensitive cultivar "DGL" (Fig. 7A, B).

Rapid light response curve analysis

Chromium stress induced excessive energy loss or photochemistry of PSII and PSI was assessed by using DUAL-PAM-100. Chromium stress remarkably reduced the PS(I), PS(II) quantum yield expressed in terms of [Y(I)], [Y(II)] (Fig. 8 A, B, C, D) and electron transport rate ETR(I), ETR(II) (Fig. 8 E, F, G, H) in both *Brassica* cultivars respectively. However, tolerant cultivar Ac-Excel showed maximum increase in Y(II) and ETR(II) under Cr and MT treatments, relative to sensitive cultivar DGL as compared to their respective control plants respectively. Similarly, under Cr and MT treatment increase in NPQ was more obvious in cultivar DGL relative to cultivar Ac-Excel (Fig. 9 A, B). While, the quantum yield of photo-protective heat dissipation [Y(NPQ)] was increased under Cr and MT stress in the both *Brassica* cultivars although, cultivar DGL showed higher [Y(NPQ)] values than Ac-excel (Fig. 9C, D). Moreover, non-photoprotective heat dissipation [Y(NO)] was increased because of Cr toxicity damage to PSII which was clearly decreased by MT application in Ac-Excel that of DGL relative to their control plants (Fig. 9E, F). To assess the exact damage site of PSI; limitations of donor or acceptor sites of PSI were observed. According to our results, Cr stress considerably decreased the Y(ND) (the donor side limitation of PSI), while MT application affectively increased the Y(ND), maximum increase was observed in tolerant cultivar Ac-Excel relative to sensitive cultivar DGL (Fig. 9G, H).

Discussion

In the present work, remarkable variation for Cr stress tolerance was observed in canola cultivars. However, based on our previous study (Ayyaz et al. 2020) two canola cultivars Ac-Excel and DGL were further evaluated. It is presumed that such variability in Cr stress tolerance in canola cultivars might have been because of variation in physio-biochemical attributes (Farooq et al. 2013). Based on some physiological parameters, such as chlorophyll content, TSP and Fv/Fm, and PSII and PSI efficiency Ac-Excel considered as Cr stress tolerant and DGL as Cr stress sensitive. Thus, difference between the canola cultivars for Cr stress tolerance based on several physiological parameters along with growth attributes seems to be better. Although, Cr stress tolerance in canola cultivar is possibly related to improved plant growth, higher photosynthesis pigments, TSP, enhanced antioxidant potential and Fv/Fm and PSII activity. In our study foliar application of melatonin considered effective strategy for the alleviation of metal stress (Farouk and Al-Amri et al. 2019). Our results suggested that Cr toxicity reduced plant growth including fresh and dry weight in both canola cultivars. Against MT and Cr treatments reduction in biomass was more obvious in sensitive cultivar “DGL” relative to tolerant cultivar “Ac-Excel”. Present findings were in correspondence with (Hasan et al. 2015a) Which suggested that Cr stress induced reduction in canola plants growth attributes associated with inhibition of plant cell turgidity, cell division, biosynthesis of cell wall, cell elongation and reduced relative water content. Similarly, data suggested that Cr stress significantly declined chlorophyll pigments in all canola cultivars (Table 1). It may happen because of higher chlorophyllase enzyme activity, oxidative pigments degradation, distortion of thylakoidal membrane, and improper uptake of nutrients. Protochlorophyllid reductase decreased activity further lowers the chlorophyll synthesis under Cr stress, that is supposed the main reasons of reduction in photosynthetic pigments (Zhang et al. 2013, Zhao et al. 2017). Meanwhile, (Zhao et al. 2019) reported that exogenous application of MT prevents chlorophyll degradation, increases photosynthesis, antioxidant’s ability and Cr stress tolerance in cucumber seedlings. 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 (Zhang et al. 2013b). Cr stress has significant influenced on photosynthetic efficiency of plant, and Fv/Fm supposed as key indication of photosynthetic efficacy. Our results indicated that Cr stress considerably reduced the plants quantum yield (Fv/Fm) ratios and reduction in QY was more pronounced in sensitive cultivar “DGL” relative to tolerant cultivar “Ac-Excel” (Fig. 2F). Generally, Fv/Fm often confirmed by chlorophyll contents and cell shape, which may affect by many factors which influences the PSII activity and nutrient status (Wang et al. 2013).

Previous studies suggest the destructive effect of Cr on reaction center and ETC which consequence reduced net PSII quantum yield (Oukarroum et al. 2015). While, according to (Ayyaz et al. 2020) foliar application of MT under Cr stress prevents photosynthetic pigment degradation along with enhanced overall photosynthetic process in *B. napus*. The formation of ROS can also play a potential role in protein synthesis and gene expression regulation accompanied with plant stress defense. On the other hand, tremendous ROS generation supposed to be cytotoxic which, can affect the cell macromolecules such as phospholipids, protein, DNA, as well as other cell bodies (Hasan et al. 2015b). Present study suggests the

higher H₂O₂ contents in sensitive cultivar which leads more lipid peroxidation, ultimately causing increased MDA content which consequence more cellular toxicity which causes cell death. This kind cellular effect was observed by Ding et al. (2017) where higher H₂O₂ contents altered cellular structure and production and elimination of active oxygen radicles which cause cell death (Meng et al. 2014). Increased production of free radicals against Cr stress was also reported in *B. napus* species, which can cause severe membrane damage to Cr sensitive crop plants. The generation of malondialdehyde (MDA) is the potential indicator of free-radical formation in plant tissues under stress conditions. The present study indicated that elevated levels of MDA under Cr stress may lead oxidative stress in *B. napus* plants causing membrane leakage and loss of DNA which consequences severe damage to plant cell membrane. An increased lipid peroxidation against Cr stress has been reported in maize cultivars (Ding et al. 2017). This study also showed higher malonaldehyde (MDA) content formation especially in DGL relative to Ac-Excel, suggesting membrane damage of *B. napus* plants in response to Cr stress (Table 1). It has been reported that under Cr stress the integrity of plasma membranes damaged by alteration in lipoxy gene activity in different crop plants, which ultimately causes cell death in plant tissues (Gill et al. 2015). However, previous studies suggested that MT pretreated cucumber seedlings showed reduced ROS formation with enhanced antioxidants activity under Cu (Zhang et al. 2013).

The present study indicated that Cr stress remarkably increased the plant antioxidative defense system among all *B. napus* cultivar. Cr stress toxicity stimulated potential antioxidative defense enzymes such as SOD, POD, CAT and APX activities to reduce the oxidative stress in all *B. napus* cultivars. CAT and APX considered as potential antioxidative enzymes accompanied with H₂O₂ removal in leaves (Jahan et al. 2019a). Specifically, CAT splits H₂O₂ into water and oxygen (Tang et al. 2018a), and counteracts H₂O₂ generation in peroxisomes by GOX activity during photorespiration. While, APX protects chloroplasts membrane integrity by reducing H₂O₂ to water conversion through ascorbate oxidation (Li et al. 2018a). Reduced ascorbate regeneration is necessarily required for H₂O₂ quenching and enzymes like APX participates in ascorbate glutathione pathway, the central redox site in plants (Jahan et al. 2019b). These findings are also supported by previous studies (Chen et al. 2018) who suggested that *B. napus* species exhibits higher CAT activities when exposed to salt stress. The free radical's formation under Cr stress decreased in tolerant cultivar Ac-Excel, showing higher CAT activity compared to the sensitive cultivar DGL. While, surprisingly SOD activity significantly increased in canola plants under Cr stress (Table 1). In plants, most of SOD activity occurs in chloroplast, mitochondria and cytosol and responsible for the catalysis of highly oxidative anions to less harmful H₂O₂ (Ni et al. 2018) Apparently, against Cr stress increased SOD activity is independent of genotypes, though representing common shared ROS generating mechanism and in turn can be scavenged by genotype response and effective antioxidative responses. Previous studies suggested that MT application improved SOD activity in pepper seedlings when exposed to severe Cr stress. Enhanced SOD activity reveals significant protection against excessive ROS production under metal stress. Similarly, increased POD activity suggests the enzymatic antioxidative protection against Cr stress in *B. napus* cultivars. However, present study indicated higher POD activity against Cr stress. Similar results were experienced by (Tang et al. 2018b) in *Brassica* species against Cr stress.

Melatonin induced regulation of photosynthetic attributes in canola cultivars was observed specifically in tolerant cultivar "Ac-Excel" relative to sensitive cultivar "DGL" might involve its genetic potential although, its effects on the exact site of photosynthetic apparatus is still unclear. Data of present study suggested that both *Brassica* cultivars of present study explained that primary photochemistry and photochemical quenching at O-J and J-I step reduced under Cr stress in both canola cultivars, While MT application enhanced compensation reduction rate of PS(I) at I-P phase which consequences improved PSII quantum yield in both brassica cultivars. While, decreased fluorescence values suggest Cr stress induced reduction of energetic connectivity in L-band. Increased chlorophyll fluorescence curve at I-P phase suggested that MT application against Cr stress remarkably increased electron transport rate from PQH₂ to secondary electron acceptor site of PS(I) and maintains plastoquinone redox state by transferring electrons to PS(I). These findings were in correspondence to (Li et al. 2018b), who suggested that MT considerably enhanced PS(I) efficiency in wheat plants under cold stress. While, Cr treated plants showed decreased fluorescence curve at I-P phase suggesting reduced PQ pool size resulting in decreased redox potential which may be because of sharp decline in plant water status as compared to control plants. However, this increase in I-P band was more pronounced in "Ac-Excel" which suggests higher redox potential and quantum yield of PS(I) that of "DGL". Similarly, (Oukarroum et al. 2015) observed that under salt stress higher ROS accumulation causes reduction in quantum yield of PS(I) with enhanced photochemistry in *Limna gibba* plant.

Plants photosynthetic efficiency considered very crucial as it directly contributes in plant growth and productivity against certain abiotic stressors (Oukarroum et al. 2015). Photosynthetic capacity of plants Chlorophyll fluorescence measurement under control and stressed conditions are considered as best method for the evaluation of PSII efficiency (Maxwell and Johnson 2000). Cr stress severely affected the PSII functional and structural constancy which resulted in reduced plant photosynthetic attributes in both *B. napus* cultivars. Our results were in correspondence with previous study which suggests the increased metal toxicity adversely affects the photosynthetic efficiency of crop plants (Goussi et al. 2018). Against Cr stress decreased Fm values indicates the denaturation of antenna complex of PSII and reduced PSII efficiency which consequences maximum energy dissipation. This energy dissipation may occur due to reduced electron transport or poor quantum yield of PSII and PSI in both *Brassica* cultivars under Cr stress. However, MT application against Cr stress remarkably improved the electron transport rate ETR(I), ETR (II) and quantum yield Y(I), Y(II) of PS(I), PS(II) in both *Brassica* cultivars respectively (Fig.). Although, this increase was more pronounced in tolerant cultivar "Ac-Excel" relative to "DGL" indicating MT induced structural and functional modulation of photosystem under Cr stress. While, in contrast poor electron transport rate and quantum yield consequences excessive energy dissipation in sensitive cultivar "DGL" with increased NPQ and Y(NPQ) values depicts the higher energy loss and reduced PS(II) and PS(I) efficiency. This might be happened when photosynthetic system is damaged at Calvin cycle end and all ferredoxins are reduced. Then, Y(ND) of Cr stressed plants decreased and Y(NA) was enhanced. It caused photo-damage by the production of ROS which damage the D1 protein of PSII, PSI and oxygen evolving complex (Ruban et al. 2012). Generally, non-photochemical quenching responds quickly and obstruct ROS formation by dissipating the light energy as heat from antenna complex (Lambrev et al. 2012).

However, excessive light energy if not dissipated can be used in photosynthesis to cause photodamage in PSII. Hence, photoprotection is required for the efficient regulation of light harvesting to maintain optimum photosynthesis. So, in the antenna complex of PSII, non-photochemical quenching (NPQ) carefully dissipates the excessive light energy. Whereas, PSII is also protected from extra heat by non-photoprotective heat dissipation Y(NO) (Niyogi and Truong 2013). In the current study, Y(NO) enhanced in "DGL" under Cr stress to protect the plants from photo-damage.

Conclusion

The melatonin induced observed differences suggest that metal tolerance capability of *Brassica* plants under Cr stress. Cr toxicity reduced the plant growth, chlorophyll content and photosynthetic activity in canola plants. To overcome these stressful conditions exogenous application of melatonin effectively can increase the plant growth, development, PSII efficiency, reduce ROS generation and regulates stress tolerance by increasing antioxidants enzymes 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.

Declarations

Author Contributions

HAR and MAH Conceptualizes, wrote and edited the manuscript and supervised the work AA performed experiments ZUZ helped in analyzing physiological parameters and provided technical and helpful discussions. All authors read and approved the manuscript.

Conflicts of interest

The authors have no conflict of interest to declare.

Ethics approval

This work complies with ethical standards.

Consent for publication

All authors have read and approved the final manuscript.

Funding

No funding from any institutions received for this study.

References

Aebi H (1984) [13] Catalase in vitro. In: Methods in enzymology. Elsevier, pp 121-126.

Ahmad R, Ali S, Abid M, Rizwan M, Ali B, Tanveer A, Ahmad I, Azam M, Ghani MA (2020) Glycinebetaine alleviates the chromium toxicity in *Brassica oleracea* L. by suppressing oxidative stress and modulating the plant morphology and photosynthetic attributes. Environ. Sci. Pollut. Res 27(1): 1101-1111.

<https://doi.org/10.1007/s11356-019-06761-z>

Ahmad R, Ali S, Hannan F, Rizwan M, Iqbal M, Hassan Z, Akram NA, Maqbool S, Abbas F (2017) Promotive role of 5-aminolevulinic acid on chromium-induced morphological, photosynthetic, and oxidative changes in cauliflower (*Brassica oleracea* L.). Environ. Sci. Pollut. Res 24(9): 8814-8824.

<https://doi.org/10.1007/s11356-017-8603-7>

Altaf MA, Shahid R, Ren MX, Mora-Poblete F, Arnao MB, Naz S, Anwar M, Altaf MM, Shahid S, Shakoor A (2020) Phytomelatonin: An overview of the importance and mediating functions of melatonin against environmental stresses. Physiol. Plant. <https://doi.org/10.1111/ppl.13262>

Ayyaz A, Amir M, Umer S, Iqbal M, Bano H, Gul HS, Noor Y, Javed M, Athar HR, Zafar ZU (2020) Melatonin induced changes in photosynthetic efficiency as probed by OJIP associated with improved chromium stress tolerance in canola (*Brassica napus* L.). Heliyon 6(7):

<https://doi.org/10.1016/j.heliyon.2020.e04364>

Bates L, Wadern R, Teare I (1973) Rapid estimation of free proline for water stress determination. Plant Soil 39(1): 205-207. <https://doi.org/10.1007/BF00018060>

Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72(1-2): 248-254.

[https://doi.org/10.1016/0003-2697\(76\)90527-3](https://doi.org/10.1016/0003-2697(76)90527-3)

Bücker-Neto L, Paiva ALS, Machado RD, Arenhart RA, Margis-Pinheiro M (2017) Interactions between plant hormones and heavy metals responses. Genet. Mol. Biol 40(1): 373-386. <https://doi.org/10.1590/1678-4685-GMB-2016-0087>

Chaturvedi R, Talwar L, Malik G, Paul MS (2020) Heavy Metal-Induced Toxicity Responses in Plants: An Overview from Physicochemical to Molecular Level. Cellular and Molecular Phytotoxicity of Heavy Metals: 69-88.

Chen YE, Mao JJ, Sun LQ, Huang B, Ding CB, Gu Y, Liao JQ, Hu C, Zhang ZW, Yuan S (2018) Exogenous melatonin enhances salt stress tolerance in maize seedlings by improving antioxidant and photosynthetic capacity. Physiol. Plant 164(3): 349-363. <https://doi.org/10.1111/ppl.12737>

Chen Z, Song S, Wen Y, Zou Y, Liu H (2016) Toxicity of Cu (II) to the green alga *Chlorella vulgaris*: a perspective of photosynthesis and oxidant stress. Environ. Sci. Pollut. Res 23(18): 17910-17918.

[https://doi: 10.1007/s11356-016-6997-2](https://doi.org/10.1007/s11356-016-6997-2)

Ding F, Liu B, Zhang S (2017) Exogenous melatonin ameliorates cold-induced damage in tomato plants. *Sci. Hortic*219: 264-271. <https://doi.org/10.1016/j.scienta.2017.03.029>

Farooq MA, Ali S, Hameed A, Ishaque W, Mahmood K, Iqbal Z (2013) Alleviation of cadmium toxicity by silicon is related to elevated photosynthesis, antioxidant enzymes; suppressed cadmium uptake and oxidative stress in cotton. *Ecotoxicol. Environ. Saf* 96: 242-249. [https://doi: 10.1016/j.ecoenv.2013.07.006](https://doi.org/10.1016/j.ecoenv.2013.07.006)

Farooq MA, Gill RA, Islam F, Ali B, Liu H, Xu J, He S, Zhou W (2016) Methyl Jasmonate Regulates Antioxidant Defense and Suppresses Arsenic Uptake in *Brassica napus* L. *Front. Plant Sci*7: 468-468. <https://doi.org/10.3389/fpls.2016.00468>

Farouk S, Al-Amri SM (2019) Exogenous melatonin-mediated modulation of arsenic tolerance with improved accretion of secondary metabolite production, activating antioxidant capacity and improved chloroplast ultrastructure in rosemary herb. *Ecotoxicol. Environ. Saf* 180: 333-347. [https://doi: 10.1016/j.ecoenv.2019.05.021](https://doi.org/10.1016/j.ecoenv.2019.05.021)

Gill RA, Zang L, Ali B, Farooq MA, Cui P, Yang S, Ali S, Zhou W (2015) Chromium-induced physio-chemical and ultrastructural changes in four cultivars of *Brassica napus* L. *Chemosphere* 120: 154-164. [https://doi: 10.1016/j.chemosphere.2014.06.029](https://doi.org/10.1016/j.chemosphere.2014.06.029)

Goussi R, Manaa A, Derbali W, Cantamessa S, Abdelly C, Barbato R (2018) Comparative analysis of salt stress, duration and intensity, on the chloroplast ultrastructure and photosynthetic apparatus in *Thellungiella salsuginea*. *J. Photochem. Photobiol. B, Biol*183: 275-287. [https://doi: 10.1016/j.jphotobiol.2018.04.047](https://doi.org/10.1016/j.jphotobiol.2018.04.047)

Hanelt D (2018) Photosynthesis assessed by chlorophyll fluorescence. In: *Bioassays*. Elsevier, pp 169-198.

Hasan M, Ahammed GJ, Yin L, Shi K, Xia X, Zhou Y, Yu J, Zhou J (2015) Melatonin mitigates cadmium phytotoxicity through modulation of phytochelatin biosynthesis, vacuolar sequestration, and antioxidant potential in *Solanum lycopersicum* L. *Front. Plant Sci* 6: 601. <https://doi.org/10.3389/fpls.2015.00601>

He S, Chen Z, Zhang X, Zhao Y, Huang D, Zhao J, Zhu T, Hu M, Zeng L (2010) Measurement of atmospheric hydrogen peroxide and organic peroxides in Beijing before and during the 2008 Olympic Games: Chemical and physical factors influencing their concentrations. *J. Geophys. Res. Atmos* 115(D17). <https://doi.org/10.1029/2009JD013544>

Jahan MS, Shu S, Wang Y, Chen Z, He M, Tao M, Sun J, Guo S (2019) Melatonin alleviates heat-induced damage of tomato seedlings by balancing redox homeostasis and modulating polyamine and nitric oxide biosynthesis. *BMC Plant Biol* 19(1): 1-16. <https://doi.org/10.1186/s12870-019-1992-7>

- Jiang W-D, Feng L, Liu Y, Jiang J, Hu K, Li S-H, Zhou X-Q (2010) Lipid peroxidation, protein oxidant and antioxidant status of muscle, intestine and hepatopancreas for juvenile Jian carp (*Cyprinus carpio* var. Jian) fed graded levels of myo-inositol. *Food Chem* 120(3): 692-697.
<https://doi.org/10.1016/j.foodchem.2009.10.062>
- Lambrev PH, Miloslavina Y, Jahns P, Holzwarth AR (2012) On the relationship between non-photochemical quenching and photoprotection of photosystem II. *BBA – Bioenergetics* 1817(5): 760-769.
<https://doi.org/10.1016/j.bbabi.2012.02.002>
- Li X, Brestic M, Tan DX, Zivcak M, Zhu X, Liu S, Song F, Reiter RJ, Liu F (2018) Melatonin alleviates low PS I-limited carbon assimilation under elevated CO₂ and enhances the cold tolerance of offspring in chlorophyll b-deficient mutant wheat. *J. Pineal Res* 64(1). <https://doi.org/10.1111/jpi.12453>. Epub 2017
- Lohani N, Jain D, Singh MB, Bhalla PL (2020) Engineering Multiple Abiotic Stress Tolerance in Canola, *Brassica napus*. *Front. Plant Sci* 11. <https://doi.org/10.3389/fpls.2020.00003>
- Maxwell K, Johnson GN (2000) Chlorophyll fluorescence—a practical guide. *J. Exp. Bot.* 51(345): 659-668.
<https://doi.org/10.1093/jexbot/51.345.659>
- Meng JF, Xu TF, Wang ZZ, Fang YL, Xi ZM, Zhang ZW (2014) The ameliorative effects of exogenous melatonin on grape cuttings under water-deficient stress: antioxidant metabolites, leaf anatomy, and chloroplast morphology. *J. Pineal Res* 57(2): 200-212. <https://doi.org/10.1111/jpi.12159>
- Ni J, Wang Q, Shah FA, Liu W, Wang D, Huang S, Fu S, Wu L (2018) Exogenous melatonin confers cadmium tolerance by counterbalancing the hydrogen peroxide homeostasis in wheat seedlings. *Molecules* 23(4): 799. <https://doi.org/10.3390/molecules23040799>
- Niyogi KK, Truong TB (2013) Evolution of flexible non-photochemical quenching mechanisms that regulate light harvesting in oxygenic photosynthesis. *Curr. Opin. Plant Biol.* 16(3): 307-314. <https://doi.org/10.1016/j.pbi.2013.03.011>
- Oukarroum A, Bussotti F, Goltsev V, Kalaji HM (2015) Correlation between reactive oxygen species production and photochemistry of photosystems I and II in *Lemna gibba* L. plants under salt stress. *Environ. Exp. Bot* 109: 80-88. <https://doi.org/10.1016/j.envexpbot.2014.08.005>
- Ruban, A.V., M.P. Johnson and C.D. Duffy. 2012. The photoprotective molecular switch in the photosystem II antenna. *BBA – Bioenergetics*, 1817: 167-181.
<https://doi.org/10.1016/j.bbabi.2011.04.007>
- Sipka G, Müller P, Brettel K, Magyar M, Kovács L, Zhu Q, Xiao Y, Han G, Lambrev PH, Shen JR (2019) Redox transients of P680 associated with the incremental chlorophyll-a fluorescence yield rises elicited by a series of saturating flashes in diuron-treated photosystem II core complex of *Thermosynechococcus vulcanus*. *Physiol. Plant* 166(1): 22-32. <https://doi.org/10.1111/ppl.12945>

- Tang Y, Lin L, Xie Y, Liu J, Sun G, Li H, Liao Ma, Wang Z, Liang D, Xia H (2018) Melatonin affects the growth and cadmium accumulation of *Malachium aquaticum* and *Galinsoga parviflora*. *Int. J. Phytoremediation* 20(4): 295-300. [https://doi: 10.1080/15226514.2017.1374341](https://doi.org/10.1080/15226514.2017.1374341)
- Ulhassan Z, Gill RA, Huang H, Ali S, Mwamba TM, Ali B, Huang Q, Hamid Y, Khan AR, Wang J (2019) Selenium mitigates the chromium toxicity in *Brassicca napus* L. by ameliorating nutrients uptake, amino acids metabolism and antioxidant defense system. *Plant Physiol. Biochem* 145: 142-152. <https://doi.org/10.1016/j.plaphy.2019.10.035>
- Wang P, Sun X, Li C, Wei Z, Liang D, Ma F (2013) Long-term exogenous application of melatonin delays drought-induced leaf senescence in apple. *J. Pineal Res*54(3): 292-302. [https://doi: 10.1111/jpi.12017](https://doi.org/10.1111/jpi.12017)
- Yang S, He M, Zhi Y, Chang SX, Gu B, Liu X, Xu J (2019) An integrated analysis on source-exposure risk of heavy metals in agricultural soils near intense electronic waste recycling activities. *Environ. Int*133: 105239. [https://doi: 10.1016/j.envint.2019.105239](https://doi.org/10.1016/j.envint.2019.105239)
- Zhang N, Zhao B, Zhang HJ, Weeda S, Yang C, Yang ZC, Ren S, Guo YD (2013) Melatonin promotes water-stress tolerance, lateral root formation, and seed germination in cucumber *Cucumis sativus* L. *J. Pineal Res*54(1): 15-23. [https://doi: 10.1111/j.1600-079X.2012.01015.x](https://doi.org/10.1111/j.1600-079X.2012.01015.x)
- Zhang X (1992) The measurement and mechanism of lipid peroxidation and SOD, POD and CAT activities in biological system. *Research methodology of crop physiology*. Agriculture Press, Beijing: 208-211.
- Zhao D, Wang R, Meng J, Li Z, Wu Y, Tao J (2017) Ameliorative effects of melatonin on dark-induced leaf senescence in gardenia (*Gardenia jasminoides* Ellis): leaf morphology, anatomy, physiology and transcriptome. *Scientific reports* 7(1): 1-19. <https://doi.org/10.1038/s41598-017-10799-9>
- Zhao D, Yu Y, Shen Y, Liu Q, Zhao Z, Sharma R, Reiter RJ (2019) Melatonin Synthesis and Function: Evolutionary History in Animals and Plants. *Front. Endocrinol* 10: 249-249. <https://doi.org/10.3389/fendo.2019.00249>
- Zheng F, Xu B, Xiao P, Zhang X, Manlike A, Jin Y-X, Li C, Feng X, An S (2020) Estimation of chlorophyll content in mountain steppe using in situ hyperspectral measurements. *Spectrosc Lett*: 1-12. <https://doi.org/10.1080/00387010.2019.1711131>

Figures

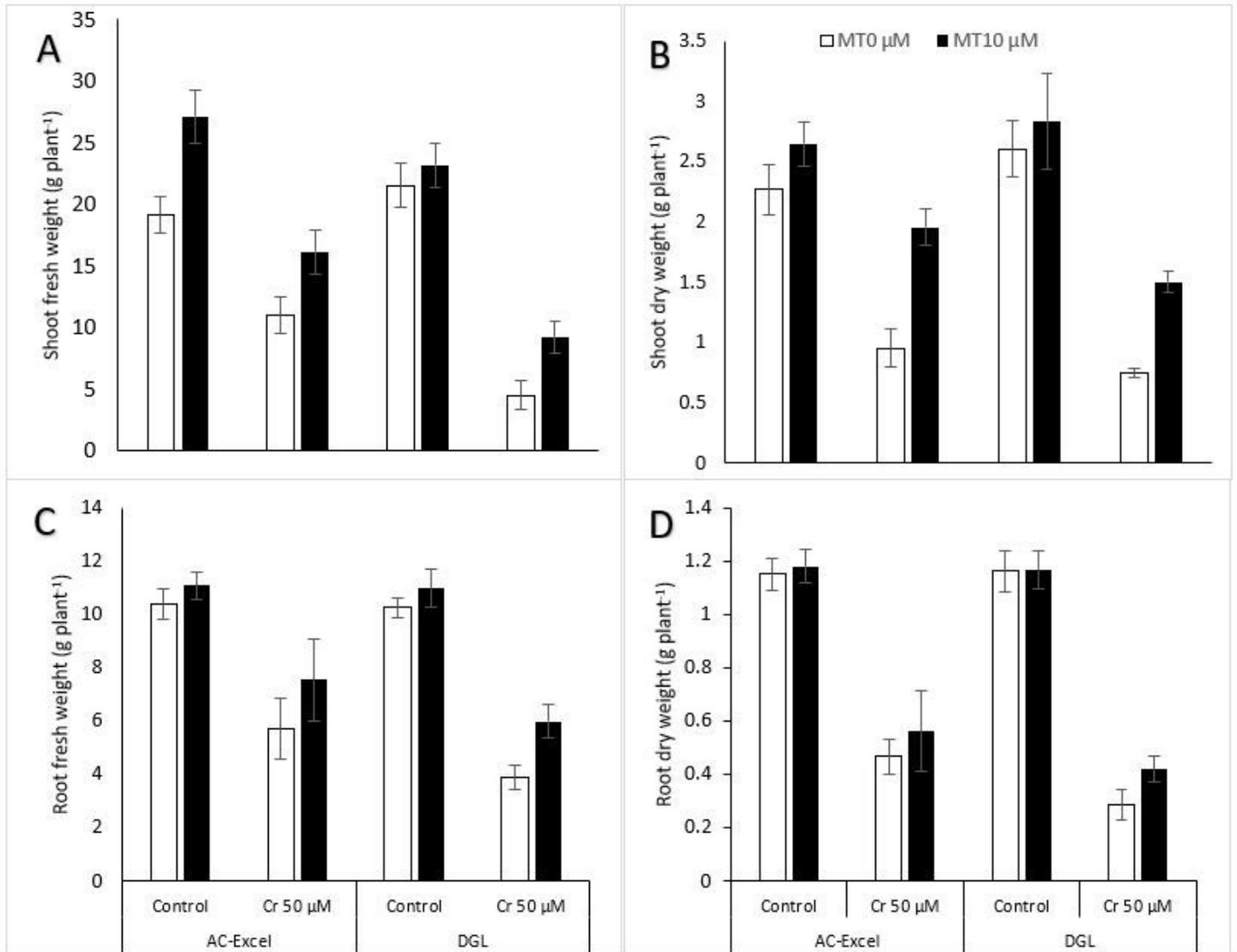


Figure 1

(A) Shoot fresh weight (B) shoot dry weight (C) Root fresh weight (D) Root dry weight of *Brassica napus* L. cultivars under melatonin and chromium stress.

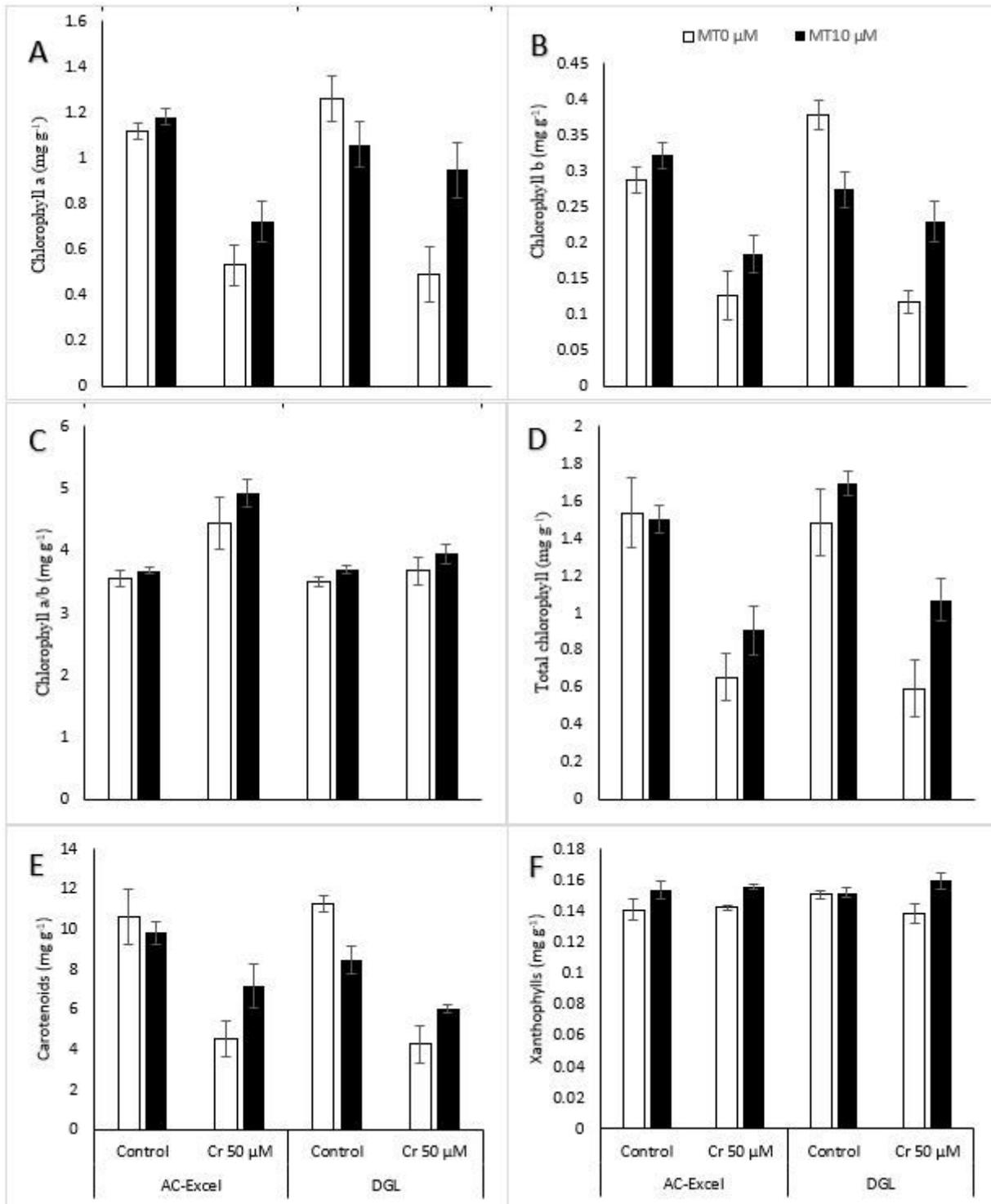


Figure 2

(A) Chlorophyll a (B) Chlorophyll b (C) Chlorophyll a/b (D) Total chlorophyll (E) Carotenoids (F) Xanthophyll's of *Brassica napus* L. cultivars under melatonin and chromium stress.

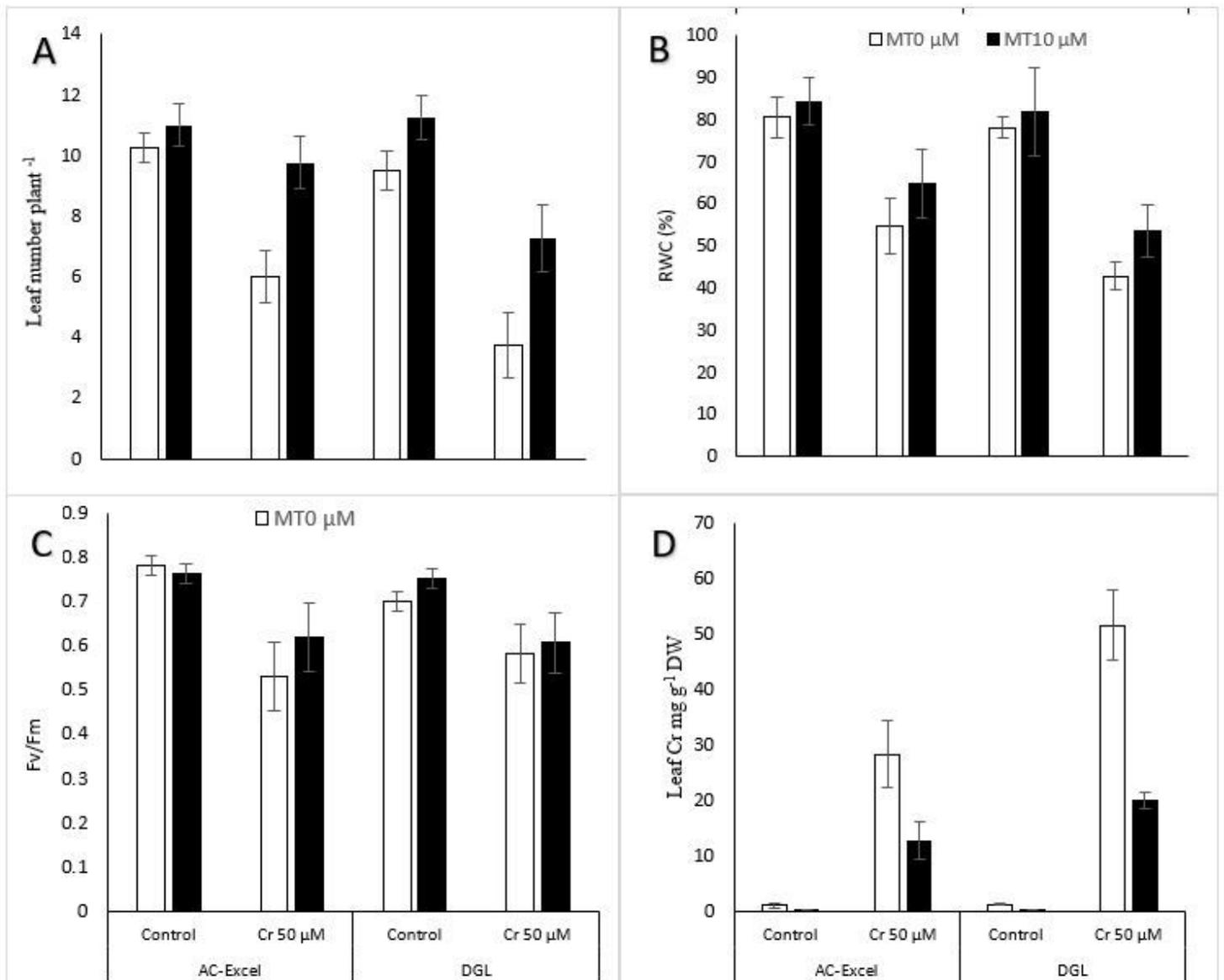


Figure 3

(A) Leaf number (B) Relative water content (C) Quantum yield (Fv/Fm) (D) Leaf chromium content of *Brassica napus* L. cultivars under melatonin and chromium stress.

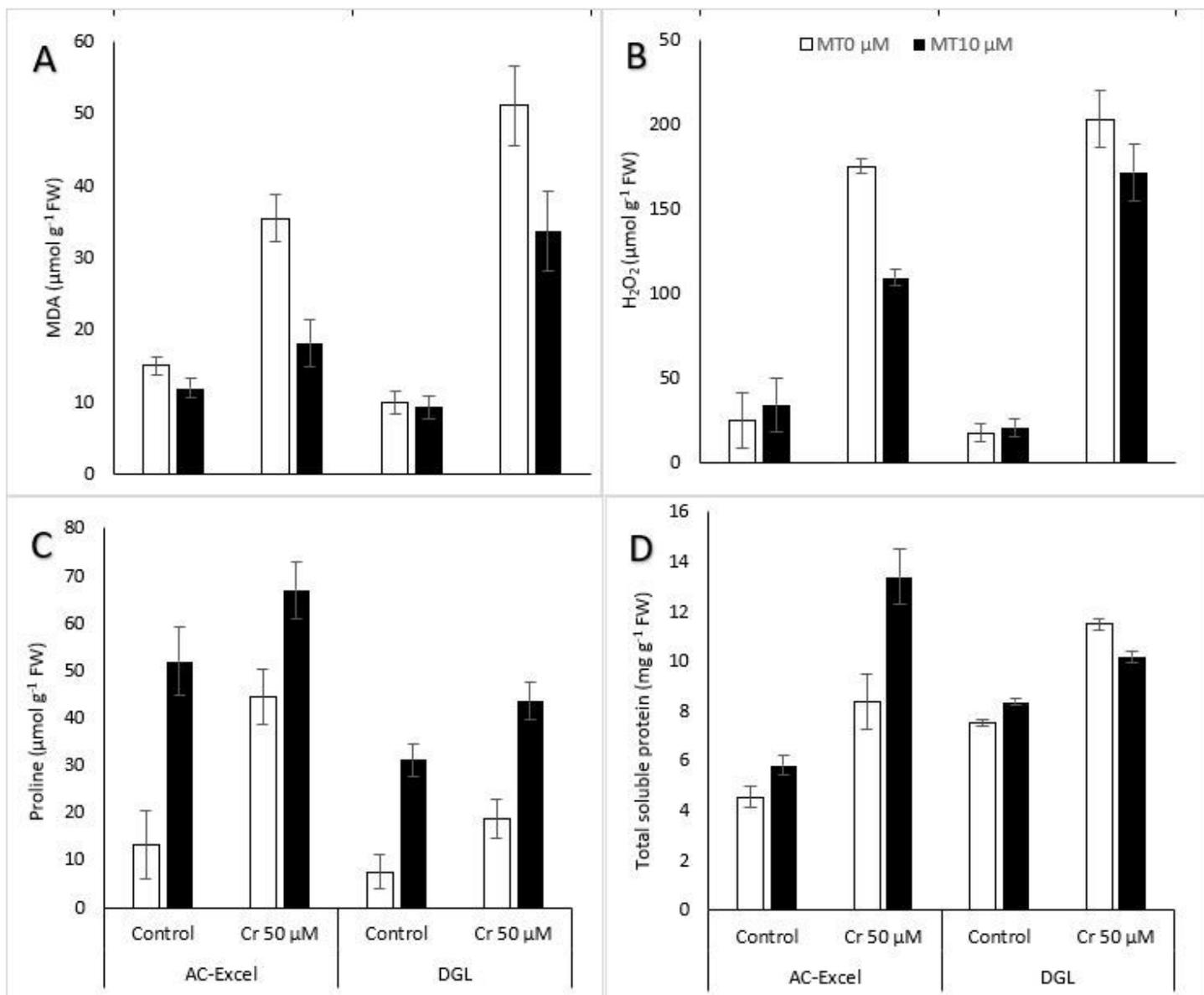


Figure 4

(A) Malonaldehyde (MDA) content (B) Hydrogen peroxide H₂O₂ content (C) Proline estimation (D) Total soluble protein contents of *Brassica napus* L. cultivars under melatonin and chromium stress.

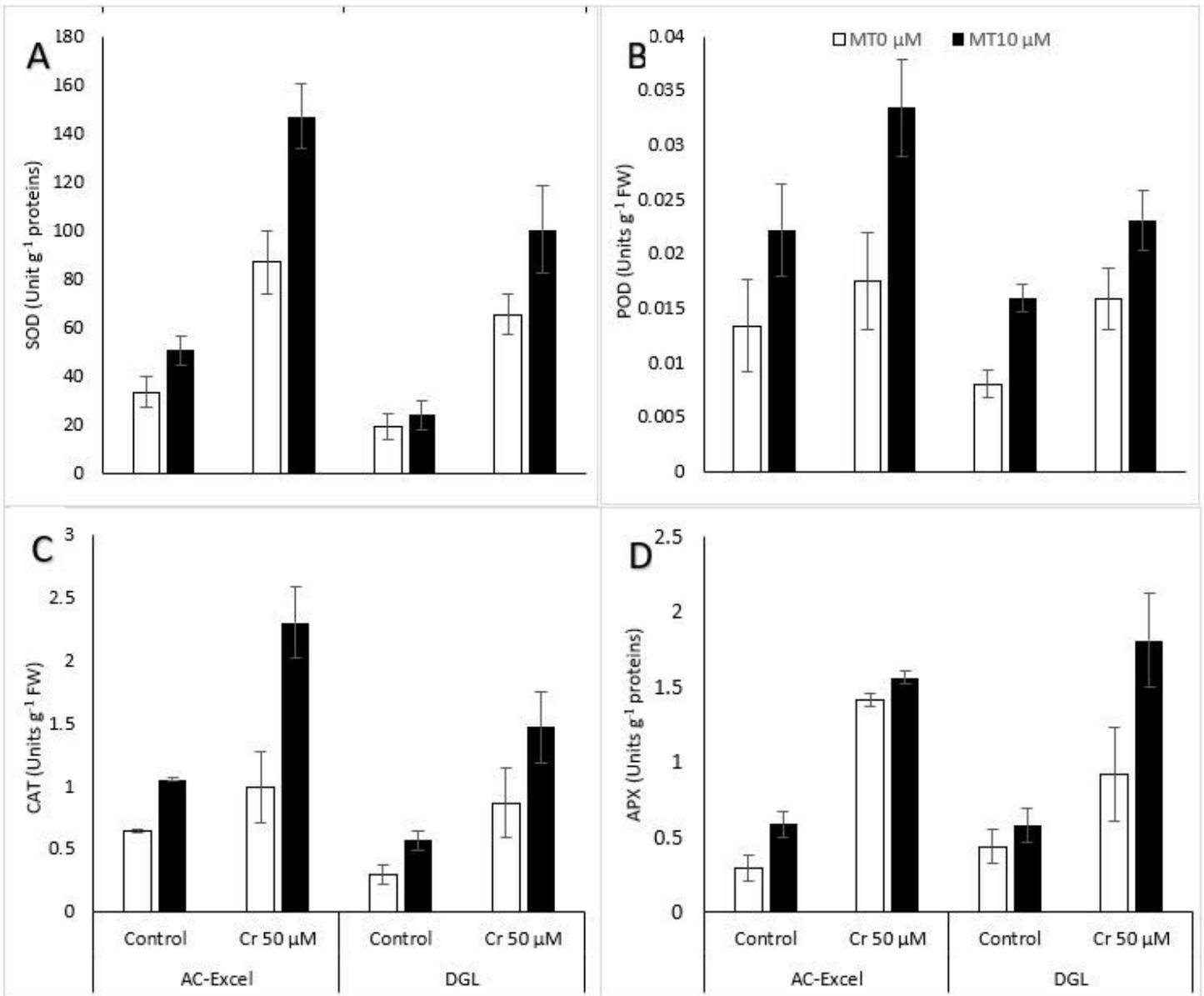


Figure 5

(A) Superoxide dismutase (SOD) (B) Peroxidase (POD) (C) Catalase (CAT) (D) Ascorbate peroxidase (APX) of Brassica napus L. cultivars under melatonin and chromium stress.

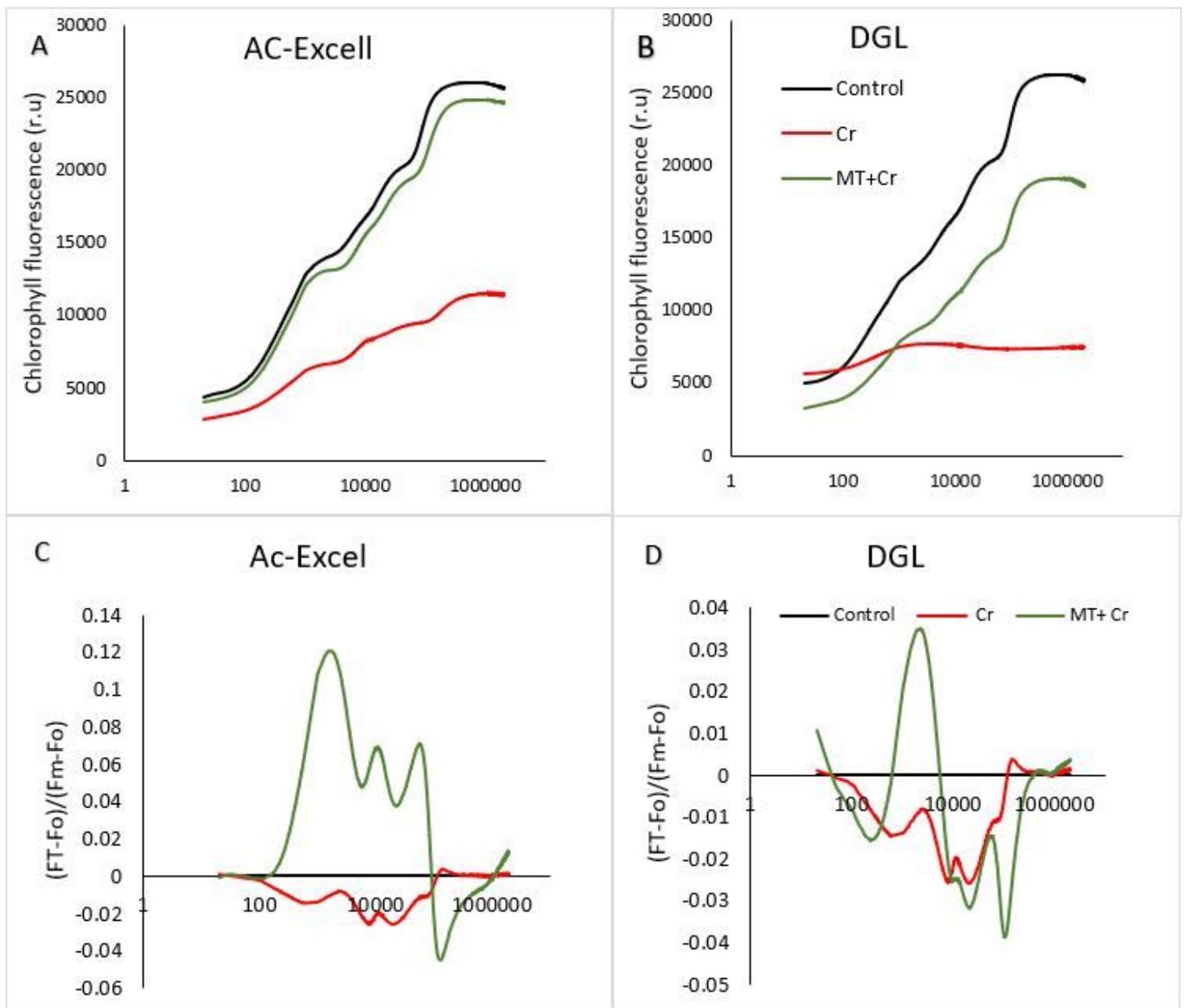


Figure 6

(A) Chlorophyll fluorescence curves of Ac-Excel (B) Chlorophyll fluorescence curves of DGL (C) OP band of Ac-Excel (D) OP band of DGL *Brassica napus* L. cultivars under melatonin and chromium stress.

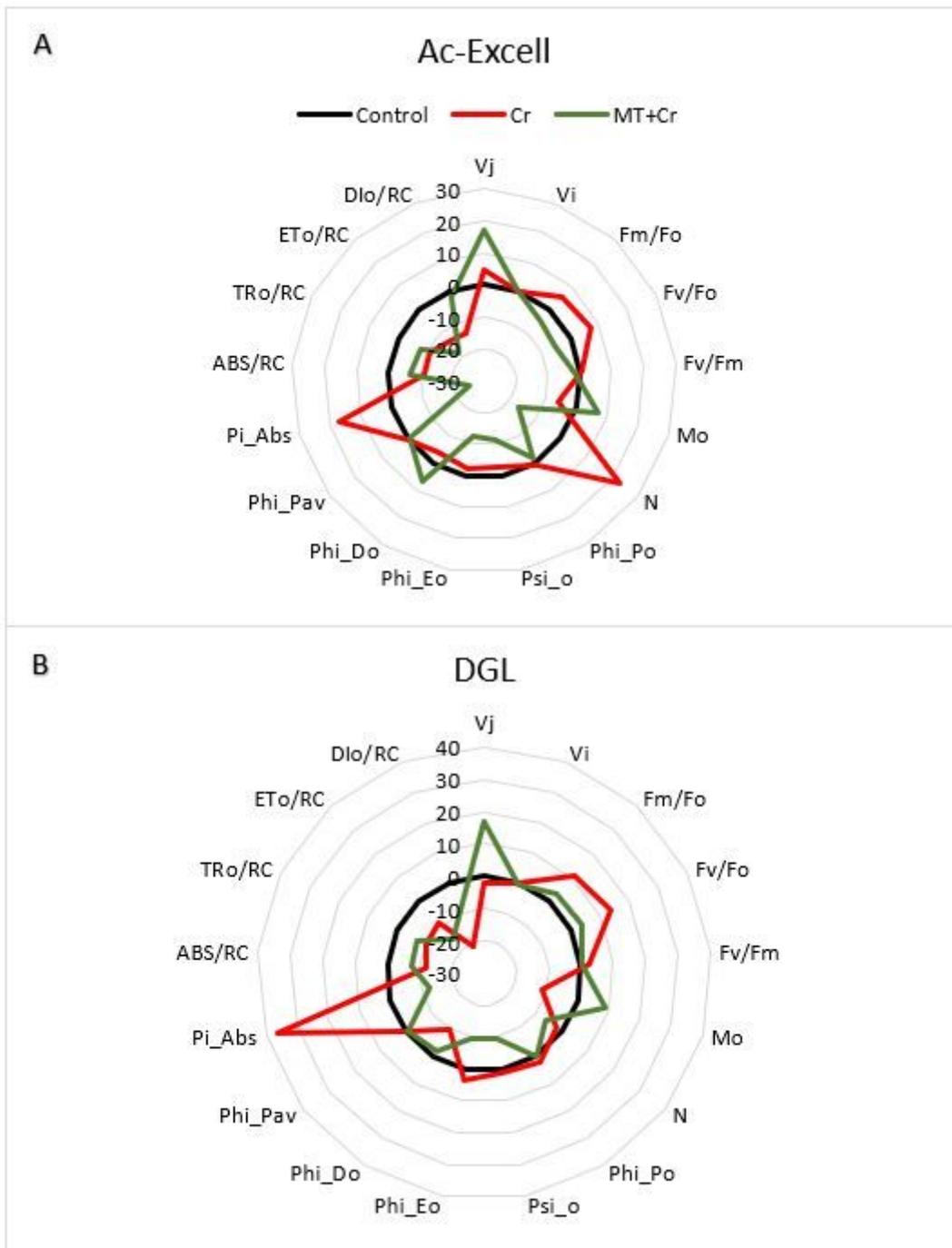


Figure 7

Radar plot showing different photosynthetic attributes of *Brassica napus* L. cultivars (A) Ac-Excel (B) DGL under melatonin and chromium stress.

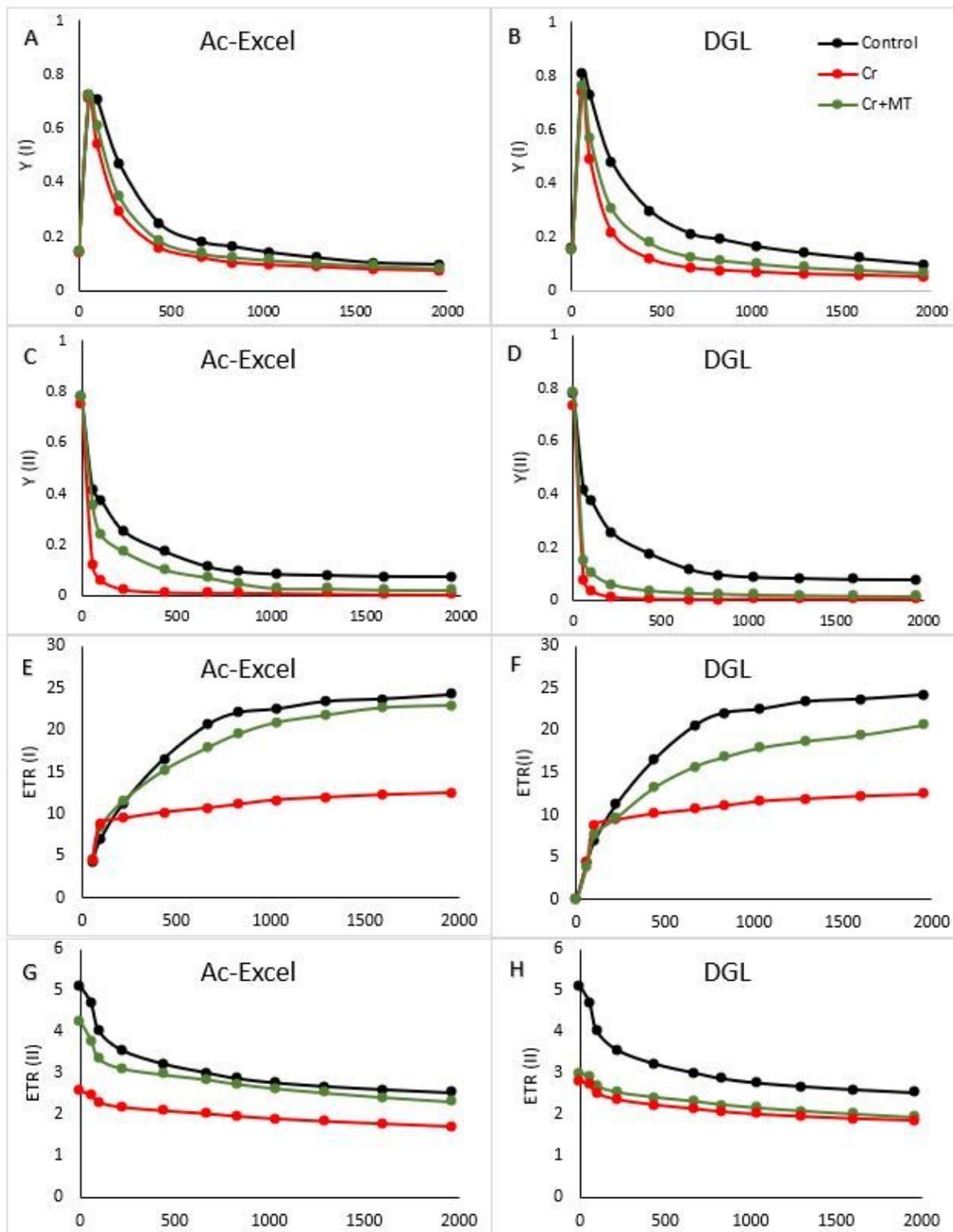


Figure 8

(A, B) Quantum yield of PSI Y(I), (C, D) Quantum yield of PSII Y(II), (E, F) Electron transport rate of PSI ETR(I), (G, H) Electron transport rate of PSII ETR(II) from light curve analysis by Dual-PAM of two Brassica napus cultivars (Ac-Excel and DGL) under melatonin and chromium stress.

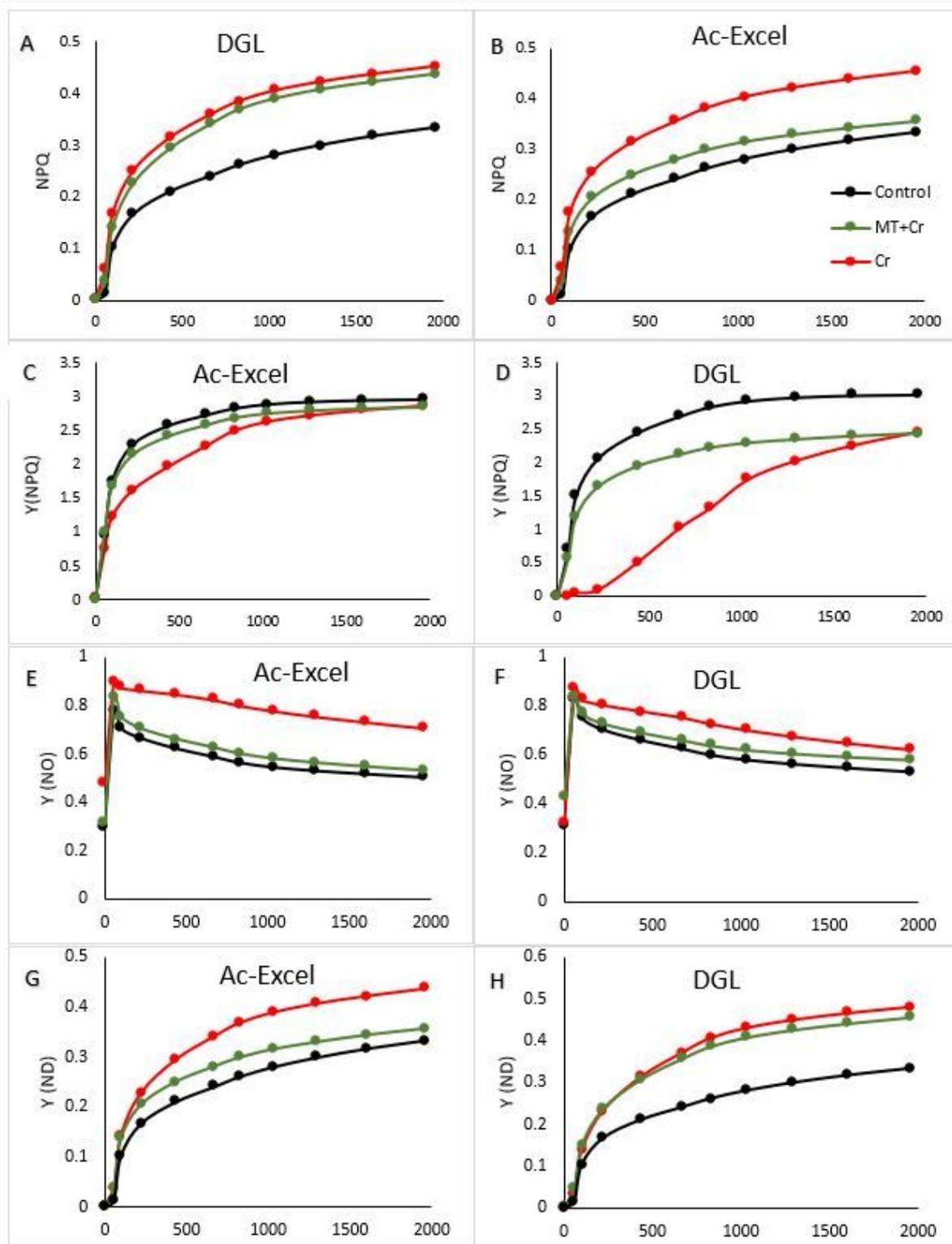


Figure 9

(A, B) Non photochemical quenching (NPQ), (C, D) Photoprotective heat dissipation Y(NPQ), (E, F) Non photoprotective heat dissipation Y(NO), (G, H) Donor side limitation of PSI Y(ND) from light curve analysis by Dual-PAM of two *Brassica napus* cultivars under melatonin and chromium stress.