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Synergistic application of melatonin and silicon alleviates chromium stress in *Brassica napus* through regulation of antioxidative defense system and ethylene metabolism

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ABSTRACT

Melatonin (MT) reacts with oxygenated compounds to form 2-hydroxymelatonin (2-HMT). The potential role of 2-HMT and silicon (Si) in moderation of chromium (Cr) stress in *Brassica napus* was evaluated in this study. *B. napus* seedlings have reduced growth and phytochemical attributes when grown in Cr-contaminated pots. Application of 2-HMT and Si, alone or as combined treatment, minimized Cr-stress in *B. napus* seedlings. Supplementation of 2-HMT and Si improved stomatal conductance (Gs), transpiration rate (Tr) and intercellular CO_2 concentration (Ci) in *B. napus* seedlings subjected in Cr-toxic soil. Combined application of 2-HMT and Si prominently enhanced antioxidantive enzymes activity i.e., SOD (superoxide-dismutase), APX (ascorbate-peroxidase) and CAT (catalase) enzyme in *B. napus* seedlings, compared to seedlings treated with MT-only. Moreover, 2-HMT and Si also reduced MDA (malondialdehyde) content, H_2O_2 (hydrogen peroxide) content and EL (electrolyte leakage) in *B. napus* seedlings of Cr-amended soil. Co-supplementation of 2-HMT and Si significantly enhanced ethylene level in *B. napus* seedlings grown in Cr-contaminated soil, as compared to rest of treated seedlings. Increased ethylene level activates antioxidantive defence system. Therefore, it is suggested that combined treatment of 2-HMT and Si can be useful to eliminate abiotic stresses in other crops.

1. Introduction

Heavy metal contamination is most solemn pollution due to modern anthropogenic activities. Many of the heavy metals have polluted the environment (Briffa et al. 2020). Chromium exists in two forms: trivalent (III) and hexavalent (VI). Hexavalent chromium is more toxic as compared to trivalent Cr. Many researchers have revealed, elevated hexavalent chromium (Cr^{6+}) in soil has reduced growth and physiological properties of several crops (Balali-Mood et al. 2021). Excessive accumulation of Cr disturbs photosynthetic rate in plants along with nutrient accumulation. This excessive Cr enters in human body through food chain and causes various diseases such as bronchitis, tuberculosis, dermatitis and also results in cancer (Lopez-Bucio et al. 2022).

Melatonin (N-acetyl-5-methoxytryptamine) is a decisive compound involved in regulation of morphophysiological, physiochemical and molecular process in all plant tissues (Arnao and Hernandez-Ruiz, 2019). This unique low molecular weight organic compound has ROS scavenging properties, thus can remediate harmful heavy metals in plants (Zeng et al. 2022). Melatonin (MT) protects plants from deleterious impacts of heavy metals, this illustrates MT role as powerful antioxidant (Hoque et al. 2021). Chen et al. (2017) reported, exogenous supplementation of MT reduced toxic salinity stress in *Arabidopsis* plants. Foliar application of melatonin also induced Cd-sequestration in *Nicotiana tabacum*, thereby reduced Cd-induced reduction in growth and

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photo-inhibition, in MT treated seedlings (Wang et al. 2019). 2-Hydroxymelatonin is predominant over melatonin in plants. This is proved by the fact that ratio of 2-HMT to MT in plants is 368:1 (Byeon et al. 2015). Lee et al. (2019) reported that 2-HMT protected tomato, cucumber and tobacco against combined stress of drought and cold. Additionally, as per our previous research findings 2-HMT mitigated Cd-stress in *C. sativus* by incrementation in activity of polyamine synthetic enzymes and scavenging action of antioxidantive enzymes (Shah et al. 2020). Lee and Back (2016) also reported that 2-HMT modulate the physiological role of transcription factors, and there by alleviated the collective effect of cold and drought in rice plants.

Silicon is a crucial plant nutrient and is most prevalent element after oxygen (Tripathi et al. 2020). This beneficial mineral plays vital part in growth, photosynthesis, chlorophyll stabilization and upregulation of crucial enzymes (Verma et al. 2019). Silicon is crucial for various molecular processes, which are involved in scrutiny of metabolomics in plants. Si as an essential element, produces many distinguish character for growth and developments in plants (Souri et al. 2021). Si mitigates stress caused by accumulation of toxic metals and various other abiotic factors (Bhat et al. 2019). Si triggered some internal as well as external processes that mitigate toxic effects of heavy metals. As far as external mechanisms are concerned, they include Si role in amelioration of heavy metal through absorption and activity of heavy metal (Zhao et al. 2022). Internal mechanisms include compartmentalization of silicon with metal ion, activation of antioxidantive enzymes and changing cell wall architecture and composition (Khan et al. 2022). The effective contribution of Si in alleviation of stresses is due to accretion of polysialic acid. With incrementation of these acids, tolerance in plants to abiotic stresses is increased (Emamverdian et al. 2018). Bao et al. (2021) reported that Si in combination with MT reduced As and Cd translocation in rice grown in polluted soil.

Ethylene in plants contributes to physiological activities e.g photosynthesis and respiration (Chandwani and Amaresan 2022). Ethylene biosynthesis, occurred in almost all cells of plant. It is not limited to specific tissue or organ (Chen et al., 2022). Certain type of internal or external stimuli are involved in ethylene biosynthetic pathway, and require extensive research to understand mechanism laying behind this process (Muller 2021). Ethylene biosynthesis is mediated in abiotic stresses, and regulated through feedback mechanism involving several signaling agents such as NO (nitric oxide), hydrogen sulphide (H_2S), and others (Husain et al., 2020). Ethylene activates the plant's antioxidative defence mechanism, that reduced oxidative stress, enhanced photosynthetic efficiency, and recovered plant growth (Sharma et al., 2019). Chilling, salt, drought, heat, floods, heavy metals, and photo-oxidative stress all stimulate ethylene production (Khan et al., 2020). In grapevine, exogenous MT induced salt tolerance through promoting ethylene synthesis (Xu et al., 2019). Plants generate ethylene against heat stress, which emphasis its role in combating abiotic stresses (Poor et al., 2021). Ethylene is also identified as a key positive modulator of salt-stress resistance (Riyazuddin et al., 2020).

Brassica napus (Czern) L. is from Brassicaceae family, its common name is "Indian-mustard" (Rai *et al.*, 2022). It is a common amphidiploid species resulting from a hybrid of *Brassica rapa* with *Brassica nigra* (Aslan, 2022), commonly grown in India, Canada, Australia, China, and Russia (Nanjundan et al. 2022). Efforts were made to increase their commercial and agriculture related qualities such as oil extract, seed size, fractured pods, and disease resistance (Srivastava et al. 2022).

On basis of above facts, this novel research was conducted to examine potential of 2-HMT and Si in alleviation of Cr stress in *B. napus* seedlings. Furthermore, the study was intended to explore effect of 2-HMT and Si on growth and physiochemical features of *B. napus* seedlings grown in Cr-stressed conditions.

2. Material and Methods

This experiment was carried out in the wire house of Department of Botany, University of Education, Lahore, Pakistan. Healthy garden soil was obtained for experiment designed in pots. The collected garden soil contained nitrogen (N) 1.79 g kg^{-1}, zinc (Zn) 27.43 g kg^{-1}, organic content 3.98 g kg^{-1}, nickel (Ni) 0.16 mg kg^{-1}, chromium (Cr) 0.001 mg $kg^{-1,}$ potassium (K) 2.27 g $kg^{-1}$and pH 7.5. Certified and pathogen free seeds were acquired from Punjab Seed Centre, Lahore, Punjab, Pakistan. Seeds of *B. napus* were sterilized with sodium hypochlorite, followed by rinsing with distilled H_2O. For Cr toxification, potassium dichromate ($K_2Cr_2O_7$) was used during experimentation. Sodium silicate (Na_2SiO_3) was used for application of Si (1.7 mM) in soil. The pots in which there was no toxification of Cr as well as no growth regulator (either 2-HMT or Si) was applied, was termed as uncontaminated control (C). In case when only Cr was added in potted soil and there was no addition of any growth regulator, the treatment was named as contaminated control (Cr-stress). 2-Hydroxymelatonin was obtained from sigma Aldrich. As per our previous study, concentration used for 2-HMT was 100 μM (Shah et al. 2020). Also, in a pilot experiment, highest germination rate (95%) was observed in *B. napus* seedlings treated with 100 μM 2-HMT. So, in the current research, 100 μM 2-HMT was used in potted soil through seed priming approach. Similarly, Si treatment was also applied using seed priming approach.

2.1. Determination of Growth

Plants of *B. napus* were harvested after 30 days. Growth characters (root and shoot length and their respective weights) were recorded.

2.2. Estimation of Chlorophyll Content

Arnon method (1949) was used to determine chl *a*, chl *b* and total chl content from acetone extracts.

2.3. Determination of Stomatal Conductance (Gs), Net Photosynthetic Rate (Pn) and Transpiration Rate (Tr)

Gs, Pn and Tr of *B. napus* leaves was determined using LCi-SD (ADC Bioscientific Ltd. Hoddesdon, UK) portable photosynthesis system.

2.4. Determination of Antioxidantive Enzymes Activities

For preparation of enzyme extract, 250 gm plant leaves were subjected to homogenous mixture of potassium phosphate buffer (3 ml of 100 μM) containing EDTA (1mM) and polyvinyl polypyrrolidone. The obtained homogenous mixture was subjected to centrifugation at 12,000 rpm for ten minutes at 4°C. The extract was collected and used for further proceedings.

The activity of SOD was quantified as per method determined by Nishikimi and Rao (1972). SOD activity. SOD is regarded to inhibit chemical induced nitroblue tetrazolium (NBT) reduction. 1 unit of enzyme was regarded as amount of enzyme used to inhibit 50% reduction of NBT.

For measurement of APX activity, Nakano and Ascada (1981) method was employed. For this, ascorbate oxidation was measured in the presence of hydrogen peroxide at 250 nm. The activity of APX was measured in terms of oxidized ascorbate.

Catalase activity was recorded with the help of reaction mixture made by mixing phosphate buffer (50 mM/L) and hydrogen peroxide (150 mM/L) for 2 minutes, for measurement of hydrogen peroxide decomposition (Chance and Maehly, 1955).

2.5. Determination of Malondialdehyde content

Du et al. (1992) protocol was followed to determine malondialdehyde content. MDA content was estimated using modified thiobarbituric acid method. In liquid nitrogen and ethanol (80%), 0.4 g leaf samples were homogenised, then centrifuged in microcentrifugation tubes at

6000 rpm (5 min). Subsequently, 0.7 mL supernatant assorted with TBA (thiobarbituric acid 0.65%), TCA (trichloroacetic acid 20%) and BHT (butylated hydroxytoluene 0.01%). Another set of 0.7 mL was nixed with TCA (20%, 0.7 mL) and BHT (0.01%). Following incubation of micro-centrifuges tubes at 95°C and cooling, these tubes are then centrifuged at 6000 rpm for 5 minutes. UV-Vis spectrophotometer was used for measurement of absorbance at 440nm, 532 nm and 600 nm to estimate MDA content with 157 mM cm^{-1} extinction coefficient.

2.6. Determination of Hydrogen peroxide Content

At the 50% blooming stage, the amount of H_2O_2 produced was estimated using spectrophotometer and expressed as μmol g^{-1} fresh weight (FW). The H_2O_2 concentration (molar extinction coefficient 0.28 μM cm^{-1}) was determined using Jana and Choudhuri's (1981) technique, and density of yellow color in supernatant was determined at 410 nm.

2.7. Determination of Electrolyte leakage (EL)

Ten fresh leaf discs were taken, sluiced in deionized water. Samples were inserted in tubes having 5 mL of deionized water, after that these tubes were incubated at 10 °C for 24 hrs. EC_1 (initial electrical conductivity) of samples was checked using GRYF 158 conductometer (GRYF HB, Ltd., Czech Republic). Following that, samples were incubated at 95°C for 20 min, then cooled to 25 °C and EC_2 (final EC) was assessed. EL was calculated from EL (%) = $(EC_1/EC_2)\times100$ (Yang et al. 1996).

2.8. Determination of Ascorbate Content

Plant sample was minced in liquid nitrogen, 0.5-1 g powder was normalized with 600 μL of 6% ice cold TCA. Centrifuge samples for 15 min (16000 x g) at 4 °C (Gautier et al. 2010). To quantify ascorbate, 200 μL of water (distilled) was added to 200 μL of extract. Then, 10 % TCA (200 μL), 44% phosphoric acid (200 μL), 4% 2, 2'-dipyridyl (200 μL) and 3% $FeCl_3$ (100 μL) were added to solution. Diluted K-Na phosphate buffer (pH 7.4) was taken as control. After incubating all samples for 60 minutes, their absorbance was measured on a spectrophotometer at 524 nm. Concentration of ascorbate was calculated using a molar absorption coefficient, $\varepsilon = 8.7\ mM^{-1}\ cm^{-1}$ (Alen'kina & Nikitina, 2020).

2.9. Determination of Protein content

Protein contents were estimated using bovine serum albumin as a baseline. Leaf samples were grinded in liquid nitrogen and stored at -20°C for protein and enzyme activity evaluation. 0.5 g of material and 4 mL of buffer were used for extraction. 0.1 M Sodium-phosphate buffer (pH 6.4), 0.1 mM phenyl-methyl-sulfonyl fluoride), and 0.2% TWEEN were used. Prepared samples were then centrifuged at 10,000 rpm and 8°C for 10 min, after homogenization. After vortexing, 1 mL of supernatant was separated with 5% poly-vinyl-pyrrolidone. Centrifuged again for 10 min at 10,000 rpm. The absorbance at 550 nm was employed to analyse soluble proteins in the supernatant (Hodzic et al. 2021).

2.10. Determination of chromium content

Plant samples were uprooted, washed with water and subsequently oven dried for 2 days. $HClO_4$ and HNO_3 were used for digestion of samples. Chromium concentration was estimated using atomic absorption spectrophotometer AA-7000.

Translocation factor was determined by dividing Cr content in *B. napus* shoot to that of root (Mattina et al. 2003). Chromium tolerance index was calculated with the help of following formula;

$$CTI = \frac{DWTP}{DWNP} \times 100$$

Where DWTP= dry weight of 2-HMT and Si treated *B. napus* seedlings, DWNP = dry weight of non-treated plants and CTI= Chromium tolerance index

2.11. Determination of Proline Content

Plant samples (0.2 g) were grinded, mixed with 3 mL of 3% sulfosalicylic acid (w/v) and then centrifuged for 20 min at 3000×g. Subsequently, supernatant was mixed with HCl (3 mL) and 2.5% acid ninhydrin (1.5 mL), vortexed and then kept in boiling water bath for 1 h. After cooling in ice bath, extraction was done with 5 mL toluene, waited for layer formation, captivating toluene in upper layer. Estimation of absorbance value was done at 520 nm (Yan et al. 2021a).

2.12. Determination of Ethylene Content

Leaf samples (0.1 g) were collected, transferred to glass tube (50 ml) and sealed with a rubber septum for 24h. After that, gas chromatography (GC-2010; Shimadzu, Tokyo, Japan) was used to measure ethylene content (Naing et al., 2022; Xu et al., 2021).

2.13. Statistical Analysis

One-way ANOVA was applied on the data composed of 5 replicates, and proposed to Duncan's Multiple Range Test ($P\leq 0.05$). DSAASTAT software was used to evaluate mean values and significant differences at 5% were highlighted using lower-case letters.

3. Results

3.1. Effect of 2-hydroxymelatonin and silicon on growth and photosynthetic pigmentation

Table 1 indicates that Cr stress reduced growth parameters like root length (RL), shoot length (SL), shoot fresh weight (SFW), root fresh weight (RFW), shoot dry weight (SDW) and root dry weight (RDW)) of *B. napus* seedlings in control as well as in Cr-contaminated soil. On the other hand, supplementation of 2-HMT and Si enhanced the aforementioned growth attributes in *B. napus* seedlings. In normal soil, 2-HMT enhanced RL (17.4%), SL (17.03%), SFW (23%), RFW (39.02%), SDW (71.79%) and RDW (34.58%) in *B. napus* seedlings as compared to C-treatment. Co-application of 2-HMT and Si also significantly enhanced growth characteristics in *B. napus* prone to Cr-toxified soil, compared with seedlings grown in Cr-only treatment.

3.2. Effect of 2-hydroxymelatonin and silicon on photosynthetic pigmentation and proline content

Chromium toxicity significantly reduced Chl content in *B. napus* seedlings, compared with C-treatment. However, application of 2-HMT and Si elevated Chl *a*, Chl *b* and total Chl content in *B. napus* seedling grown in all the treated pots. In case of Cr-polluted soil, 2-HMT and Si combine treatment enhanced total Chl content by 40.38% as compared to Cr-only treated *B. napus* seedlings. Same trend was observed for proline determination in *B. napus* seedlings. Co-treatment of 2-HMT and Si significantly incremented proline content by 71.81%, as compared with normal condition (Table 3).

3.3. Effect of 2-hydroxymelatonin and silicon on net photosynthetic rate (Pn), transpiration rate (Tr) and stomatal conductance (Gs)

Fig. 1 indicates that 2-HMT and Si augmented Gs, Pn and Tr in *B. napus* seedlings encountering C-treatment and Cr-polluted soil. 2-

Table 1
Effect of 2-HMT and Si on growth parameters of *B. napus* grown in Cr-contaminated soil.

Treatments	Traits					
	Root length (cm)	Shoot length (cm)	Root FW (g plant^{-1})	Shoot FW (g plant^{-1})	Shoot DW (g plant^{-1})	Root DW (g plant^{-1})
C	7.89b ± 0.07	21.08bc ± 1.06	0.82bc ± 0.04	6.78bc ± 0.38	0.78c ± 0.03	0.042ab ± 0.003
Cr	4.23d ± 0.46	12.87d ± 0.98	0.45d ± 0.08	3.76d ± 0.21	0.45d ± 0.02	0.021bc ± 0.002
Si	8.47ab ± 0.75	21.09bc ± 1.09	1.03ab ± 0.03	6.91b ± 0.57	1.21ab ± 0.06	0.045ab ± 0.001
2-HMT	9.27a ± 0.56	24.67a ± 1.37	1.14a ± 0.02	8.34a ± 0.67	1.34a ± 0.07	0.061a ± 0.006
Si + Cr	5.89cd ± 0.24	17.98c ± 1.87	0.68c ± 0.05	4.65c ± 0.48	0.97bc ± 0.08	0.023bc ± 0.004
2-HMT + Cr	6.72c ± 0.65	20.87bc ± 1.08	0.73bc ± 0.07	6.19bc ± 0.45	1.08b ± 0.09	0.031b ± 0.009
2-HMT + Si + Cr	7.08bc ± 0.27	21.98b ± 1.54	0.92b ± 0.06	7.29ab ± 0.56	1.19ab ± 0.04	0.041ab ± 0.008

Values demonstrate means ± SD (n=5). C, control; Cr, chromium; 2-HMT, 2-hydroxymelatonin; Si, silicon. Values are means ± SD of five replicates. Significant differences at 5% are represented by lower-case letters.

HMT treatment in normal soil significantly increased all forementioned parameters in *B. napus* seedlings. Co-application of 2-HMT and Si significantly increased these physiological attributes as compared to *B. napus* seedlings subjected to Cr-only treatment. For seedlings in Cr-contaminated soil, co-application of 2-HMT and Si increased Gs, Pn and Tr in *B. napus* by 16.36%, 12.34% and 8.33%, respectively, in comparison with 2-HMT treatment.

3.4. Effect of 2-hydroxymelatonin and silicon on activity of antioxidantive enzymes

Chromium stress enhanced activity of APX by 32.83%, as compared to *B. napus* seedlings given normal conditions. 2-HMT and Si application enhanced APX activity in *B. napus* seedlings in control and Cr-contaminated soil. Combined supplementation of 2-HMT and Si significantly enhanced APX activity, compared to Cr-only treated seedlings. In *B. napus* seedlings of Cr-contaminated pots, 2-HMT and Si combined treatment enhanced APX activity by 43.56%, compared with 2-HMT. A similar trend was observed in which 2-HMT + Si significantly incremented SOD and CAT enzyme activity as compared to Cr-only treatment (Fig. 2).

3.5. Effect of 2-hydroxymelatonin and silicon on malondialdehyde and hydrogen peroxide content

Chromium stress enhanced MDA content (> 1-fold) in *B. napus* seedlings prevailing in Cr-contaminated soil, compared to C-treatment. 2-HMT and Si, when applied alone or combinedly, impeded MDA content of *B. napus* seedlings in control condition and Cr-polluted soil. This interactive application of 2-HMT and Si reduced MDA content by 80.5% as compared to 2-HMT treated *B. napus* seedlings subjected to Cr-toxificated soil (Fig. 3).

3.6. Effect of 2-hydroxymelatonin and silicon on electrolyte leakage

Electrolyte leakage (EL) is a crucial for determination of membrane integrity in plants proned to stressed conditions. During the current study, it was noted that highest EL value was observed in *B. napus* seedlings proned to Cr-only treatment. Application of 2-HMT, alone in synergistic application with Si, significantly decreased EL in *B. napus* seedlings in comparison to Cr-only treatment. Co-treatment of 2-HMT and Si reduced EL by 41.17% as compared to 2-HMT treatment in seedlings potted in Cr-contaminated soil (Fig. 3).

3.7. Effect of 2-hydroxymelatonin and silicon on protein and ascorbic acid content

Chromium stress reduced protein content in *B. napus* seedlings prone to Cr-contaminated pots. 2-HMT and Si enhanced protein content in both non-polluted and polluted soil. In Cr-polluted soil, combined treatment of 2-HMT and Si enhanced protein level by 20.44% in comparison with 2-HMT only treated *B. napus* seedlings grown in normal soil.

Chromium stress reduced ascorbic acid (ASA) content by 32.58% in *B. napus* in comparison with C-treatment. 2-HMT and Si escalated ASA content in control and contaminated potted soil. Combined treatment of 2-HMT and Si enhanced ASA content by 10.06 % in *B. napus* seedlings of Cr-contaminated pots, in comparison with 2-HMT only treatment. 2-HMT, alone or its combined treatment with Si, expressively incremented ASA content in *B. napus* seedlings in comparison with Cr-only treatment (Fig. 4).

3.8. Effect of 2-hydroxymelatonin and silicon on chromium content

As far as uptake of Cr is concerned, 2-HMT and Si reduced Cr uptake in *B. napus* seedlings. 2-HMT and Si significantly reduced Cr content in *B. napus* seedlings as compared to Cr-treatment. Apart from this, highest values of metal tolerance index were observed when 2-HMT and Si were applied synergistically in *B. napus* seedlings of Cr-amended soil (Table 2).

3.9. Effect of 2-hydroxymelatonin and Si on ethylene level

Fig 5 reveals the role of 2-HMT and Si in regulation of ethylene level in *B. napus* seedlings. Chromium treatment enhanced ethylene level (69.5%) as compared to *B. napus* seedlings grown in normal soil. Although 2-HMT and Si incremented ethylene level in treated seedlings as equated to control. However, co-supplementation of 2-HMT and Si significantly enhanced ethylene level as compared to C and Cr-only treatment.

3.10. Pearson's relationship

The Pearson's relationship was performed to checked the correlation of a different studied parameters of *B. napus* under varied level of Cr given in Fig. 6. The results of Pearson's relationship of *B. napus* indicated that the concentration of Cr in plant is significantly positively linked MDA, H_2O_2, and EL while negatively relationship with the SOD, pro, ASA, Pro, APX, CAT, DPPH and P. rate, transpiration rate, stomatal conductance and ethylene in plants.

3.11. Principal component analysis

The loading plots of principal component analysis (PCA) to evaluate the effects of various levels of Cr treatments on plant physiochemical attributes of B. napus are given in Fig. 7. The result show that 94.3 contribution of the total variance in the dataset. The 2nd group of variables which contribute the 28.5 PC2 is positively linked with H_2O_2, MDA and EL level in plant While, a significant negative correlation of PC2 parameters was found with the parameters aligned plant enzymatic antioxidants (SOD, CAT, APX), non enzymatic (ASA, DPPH, proline, protein) and gas exchange attributes (photosynthetic rate, transpiration rate, and stomatal conductance) and ethylene in plants.

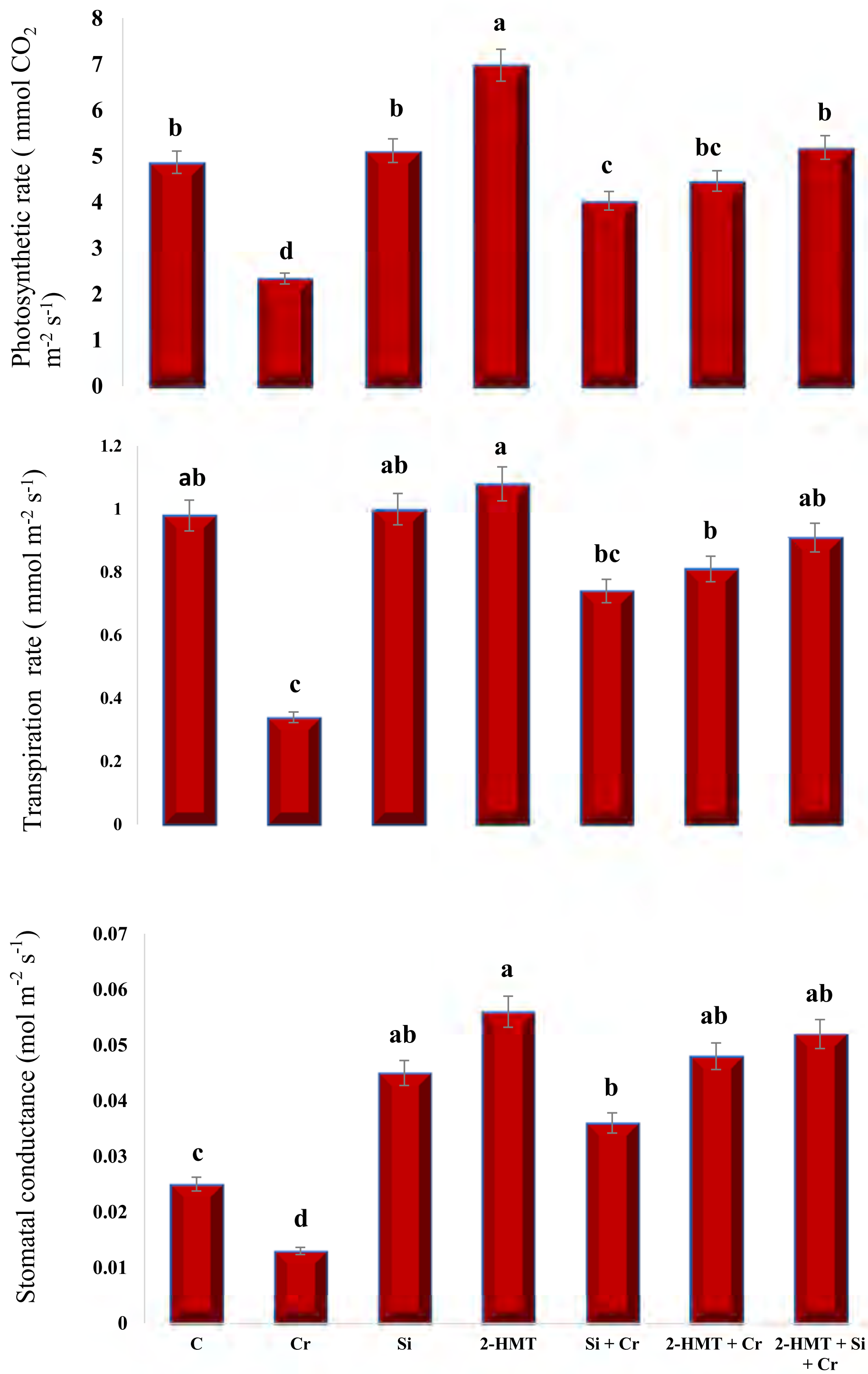

Fig. 1. Effect of 2-HMT and Si on photosynthetic rate, transpiration rate and stomatal conductance on *B. napus* grown in Cr-contaminated soil. C, control; Cr, chromium; 2-HMT, 2-hydroxymelatonin; Si, silicon. Values are means ± SD of five replicates.

4. Discussions

Chromium enrichment in soil reduces germination of seeds, which reduced overall yield of vital agronomic crops (Singh et al. 2020). Higher Cr level reduces growth and physiological attributes in treated seedlings which ultimately results in reduction in yield (Hassan et al. 2022; Qadir et al. 2020). Various studies have revealed, increase in concentration of Cr reduces germination rate and finally growth of plants (Srivastava et al. 2021; Naveed et al. 2021). This decrease in growth is due to fact that higher Cr level results in reduced nutrient uptake by plants (Ahmad et al. 2020a, b). Increase in Cr concentration lowered uptake of vital mineral nutrients like calcium, magnesium, phosphorous and iron (Sharma et al. 2020). A study by Hoque et al. (2021) reported that melatonin treatment recovers tomato seedling from oxidative stress through incrementation in Mg, N, Mg and Mn. Melatonin is a powerful antioxidant and regulates development of roots, stem, leaves, nutrient, water translocation and metabolomics in plants (Ayyaz et al. 2022). MT had ameliorating capability against abiotic

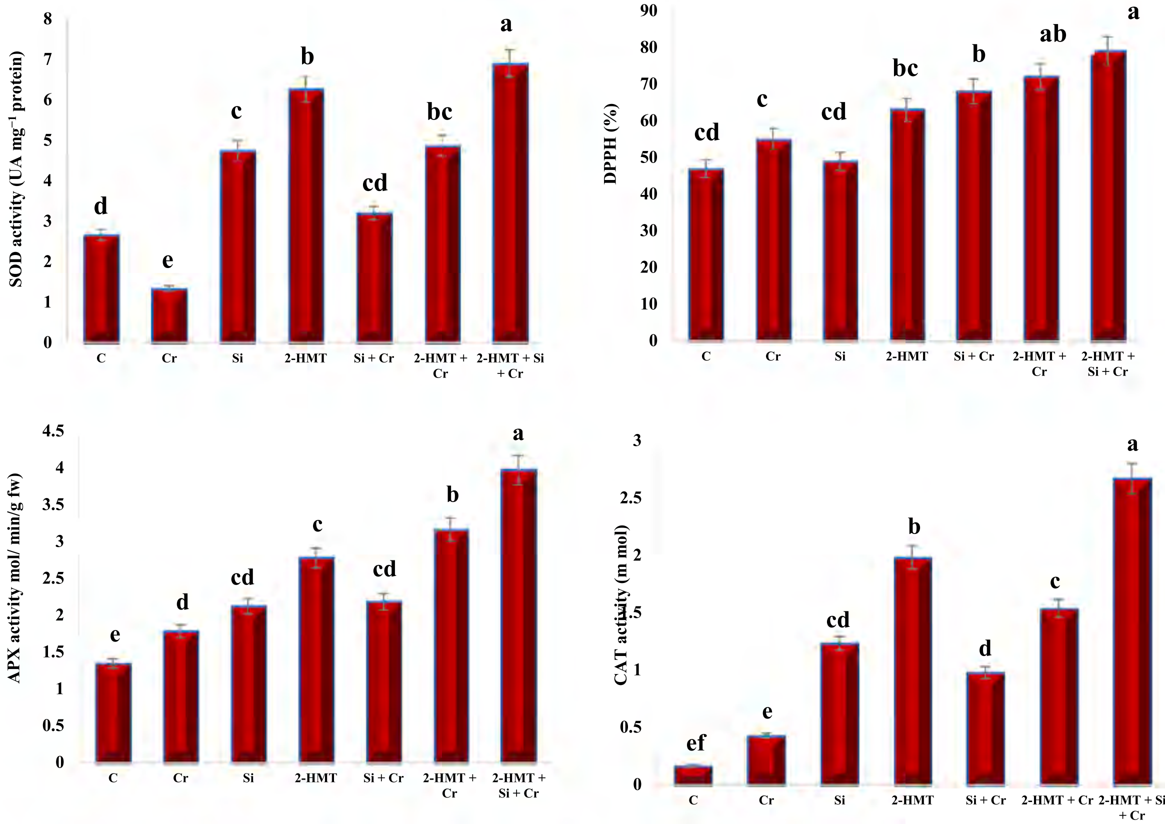

Fig. 2. Effect of 2-HMT and Si on SOD, DPPH, APX and CAT activity on *B. napus* grown in Cr-contaminated soil. C, control; Cr, chromium; 2-HMT, 2-hydroxymelatonin; Si, silicon. Values are means ± SD of five replicates.

stresses, owing MT vital phytohormone (Giraldo Acosta et al. 2022). MT treatment progresses growth of strawberry under Cd stressed conditions (Wu et al. 2021). Ayyaz et al. (2020) reported, MT treatment incremented growth of canola grown under Cr stress. This study also proved that 2-HMT improved growth in *B. napus* seedlings potted in control and Cr-polluted soil.

During the current research, Cr stress reduced growth, photosynthetic and transpiration rate in *B. napus* seedlings (Fig. 1). This decrease in physiological and biochemical properties of *B. napus* might be owed to increased accumulation of MDA and H_2O_2 content. This finding is similar to Sharma et al. (2020), which emphasised that Cr toxicity reduced plant growth due to reduction in cell division, cell elongation, cell wall biosynthesis and decrease in water content. Our data also revealed that Cr-treatment decreased chlorophyll content in *B. napus* seedlings potted in Cr-contaminated soil. This might be due to escalated activity of chlorophyllase enzyme, imbalance in nutritional homeostasis, disruption in thylakoid content and disruption of pigment structure due to increase in oxidative-stress (Sachdev et al. 2021). ROS resulted in damage of macromolecular cellular structures viz. proteins, carbohydrates, lipids, nucleic acids (Hasan et al. 2015). Current research also revealed that higher Cr toxicity in *B. napus* seedlings enhanced MDA and H_2O_2 content, which resulted in cytotoxic effects on cellular structures.

Melatonin plays a phyto-protective role in different plants and assists in alleviation of abiotic stress like temperature (high/slow), water logging, drought stress, heavy metal and nutritional deficit conditions by regulating antioxidantive and non-antioxidantive enzymes (Pardo-Hernández et al. 2020). MT supplementation plays significant role in chlorophyll structure stabilization and leads to enhanced photosynthetic rate in Cucumber seedlings, thereby protected plant from damaging effects of Cr stress (Rajora et al. 2022). Another assumption is that MT application resulted in incremented efficiency of photosystem-II in light/dark (Zhang et al. 2014). Exogenous MT averts deprivation of photosynthetic process and increase photosynthetic efficiency in *B. napus* (Ayyaz et al. 2020). As per our previous findings, 2-HMT decreased harmful footprints of Cd on *Cucumis sativus* through changes in polyamine content and improvement of antioxidantive enzymes activity (Shah et al. 2020). This current research also revealed that 2-HMT reduced Cr-stress in *B. napus* seedlings subjected to Cr-polluted soil through enhancement of antioxidantive enzymes.

Current research stated that Cr stress reduced chlorophyll and carotenoid content as equated with control. This reduced Chl content resulted in reduction of photosynthetic rate and ultimately crop productivity (Ahmad et al. 2022). Melatonin treatment reduced Chl inhibition and alleviated salt stress in a concentration dependant manner (Yan et al. 2021b). As of previous work on maize plants (Bashir et al. 2021), apart from melatonin induced alteration in pigment composition, MT treatment also altered antioxidantive enzymes activity. In a work on rice seedlings proned to salt stress conditions, this pleotropic phytohormone increased antioxidantive enzymes activity (Yan et al. 2021a).

In a study on wheat, Cr stress increase MDA content in comparison with control condition, result in oxidative burst (Lei et al. 2021). Sun et al. (2023), exposed that melatonin reduced oxidative damage in wheat seedlings by reducing MDA content.

Silicon (Si) is beneficial for plant's growth. Si is abundantly found in soil and can be easily absorbed by plants (Kim et al. 2014). It is a vital element for plant tolerance against many of abiotic stresses mainly heavy-metal stress. Various studies have established that Si supplementation mitigate abiotic stresses especially excessive level of metals and induced plant tolerance to metal stress (Huang et al. 2012; Farooq et al. 2013; Adrees et al. 2015).

Si application improved morpho-physiological characters as well as nutrient absorption in plants. Especially, in extreme and stressful

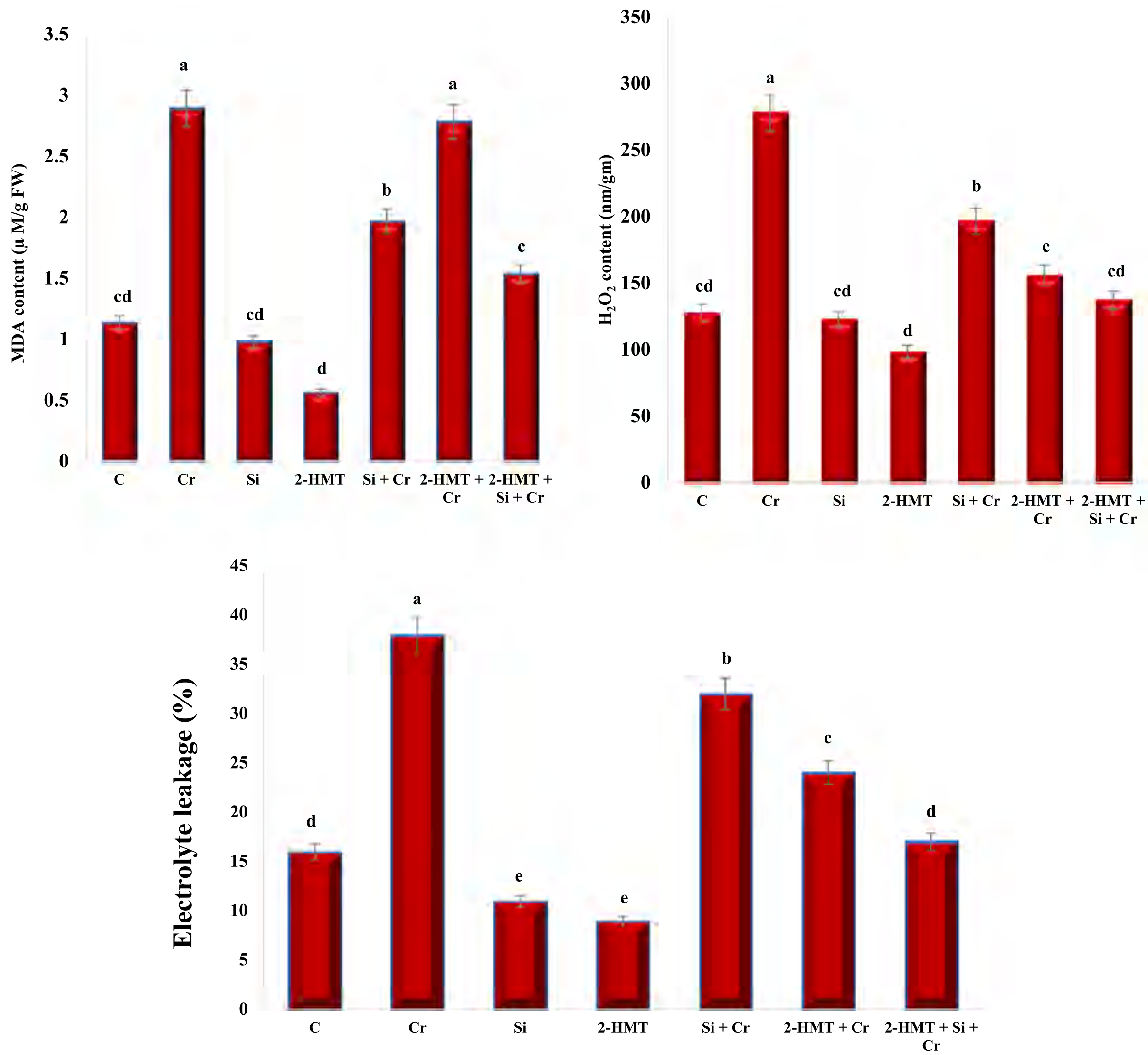

Fig. 3. Effect of 2-HMT and Si on MDA, H_2O_2 and EL on *B. napus* grown in Cr-contaminated soil. C, control; Cr, chromium; 2-HMT, 2-hydroxymelatonin; Si, silicon. Values are means ± SD of five replicates.

conditions e.g drought, excessive metals and salt, Si treatment will be more effective in improving plant growth (Meena et al. 2014)

Exogenous Si application mitigated arsenate stress and improved wheat growth through modulating nitrogen metabolism (Sil et al. 2020). Si addition reduced Cr level in aboveground parts of rice and reversed its negative impacts on protein content through detoxifying excessive Cr (Nazmul Huda et al. 2017). Similarly, Si application reversed negative impacts of Ni toxicity on morpho-physiological characters of cotton plant (Khaliq et al. 2016). According to an other study, Si addition had non-significant effect on Cr content in roots but significantly reduced Cr content in aboveground parts of plant as compared to those plants not given Si treatment (Nazmul Huda et al. 2017).

Si supplementation activated plants' antioxidative defense to encounter oxidative stress. It comprises both enzymatic and non-enzymatic antioxidants. In cucumber, Mn stress caused lipid peroxidation. Si application increased antioxidant level and minimized lipid-peroxidation resulted by Mn-toxicity (Shi et al. 2005). Similarly, in Solanum nigrum, Si supplementation reduced H2O2 and electrolyte leakage induced under Cd toxicity (Liu et al. 2013). Si application prominently reduced malondialdehyde, hydrogen peroxide and electrolyte leakage, ultimately the oxidative stress due to Cd (Hussain et al. 2015), Zn (Anwaar et al. 2015), and Pb stress (Bhatti et al. 2013).

Ethylene activates the plant's antioxidative defence mechanism, resulting reduction in oxidative stress (Sharma et al., 2019). In heat stressed rice plants, ethylene treatment enhanced Pn, Gs, and Ci compared to control (Gautam et al., 2022). Ethylene production was greater in most of plants given abiotic stress (Chandwani and Amaresan 2022). Abiotic stressors stimulated plant ethylene biosynthesis. For example, Cr(VI) increased the expression of ethylene signalling and biosynthetic genes. Furthermore, during Cr(VI) stress, ethylene production inhibited ROS generation by decreasing expression of enzymatic antioxidantive enzyme-related genes (Wakeel et al., 2019). In the study of Husain et al., (2022) under Cr(VI) stress, ethylene reduced oxidative stress indicators, resulting in lower ROS levels in both black and mung beans. Ethylene reduced oxidative damage and growth inhibition caused by CuO nanoparticles in *Arabidopsis thaliana* leaves (Azhar et al., 2020). Under ABA-inhibited conditions, high salt or dehydration stimulates expression of ERF (ethylene responsive factor) genes (Debbama et al., 2019). Our research indicated that co-treatment of 2-HMT and Si enhanced ethylene level in *B. napus* seedlings grown in Cr-contaminated pots. Thus, revealed that ethylene has a role in defence against Cr induced toxicity in *B. napus* plants.

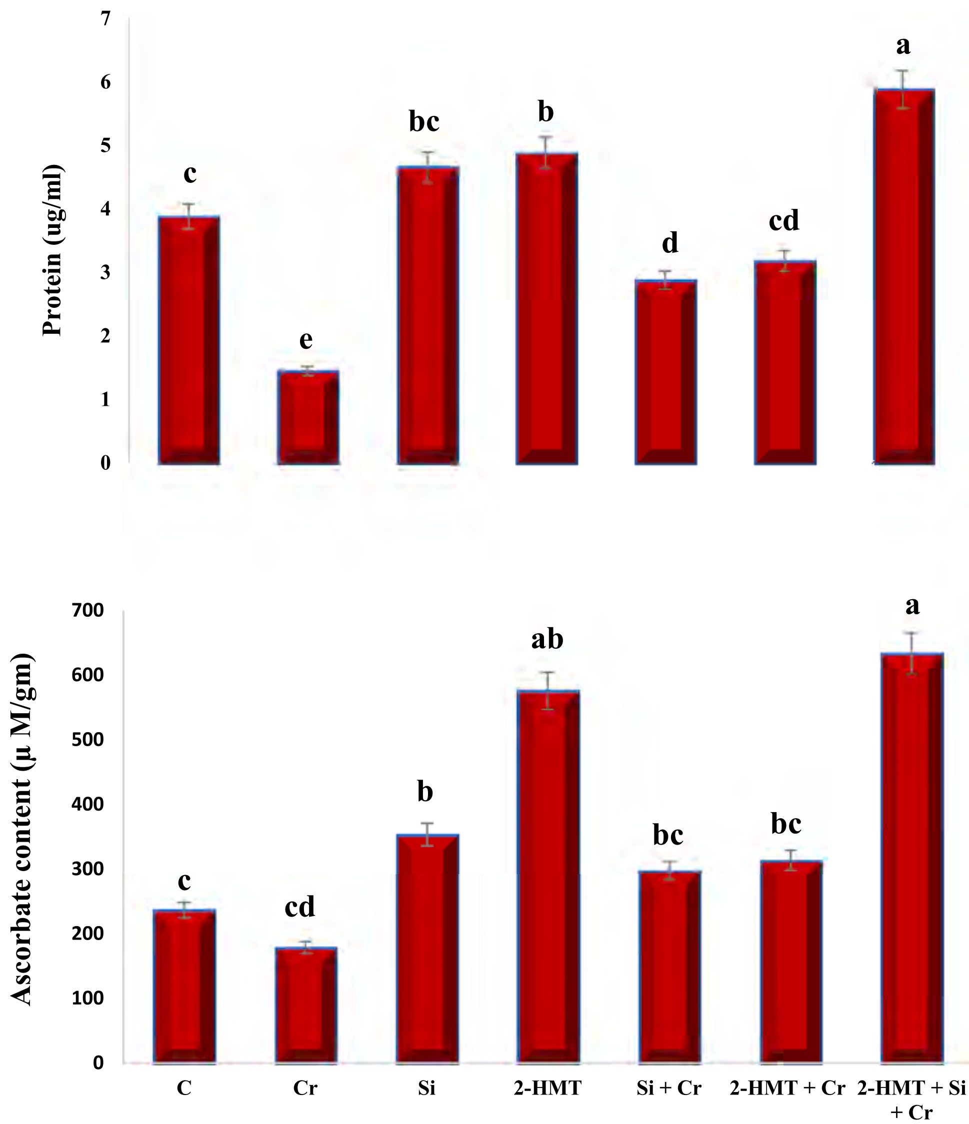

Fig. 4. Effect of 2-HMT and Si on protein and ASC content on *B. napus* grown in Cr-contaminated soil. C, control; Cr, chromium; 2-HMT, 2-hydroxymelatonin; Si, silicon. Values are means ± SD of five replicates.

Table 2
Effect of 2-HMT and Si on Cr content of *B. napus* grown in Cr-contaminated soil.

Treatments	Chromium Content			
	Root (mg kg^{-1})	Shoot (mg kg^{-1})	TF	MTI
C	ND	ND	ND	-
Cr	7.81a ± 0.45	6.54a ± 0.18	0.83a ± 0.05	57.69b ± 4.89
Si	ND	ND	ND	-
2-HMT	ND	ND	ND	-
Si + Cr	5.67ab ± 0.36	4.05ab ± 0.54	0.71ab ± 0.03	124.35ab ± 6.13
2-HMT + Cr	4.78b ± 0.24	3.54b ± 0.32	0.74ab ± 0.02	138.46ab ± 5.28
2-HMT + Si + Cr	3.67bc ± 0.48	1.79c ± 0.24	0.48b ± 0.07	152.56a ± 8.94

Values demonstrate means ± SD (n=5). C, control; Cr, chromium; 2-HMT, 2-hydroxymelatonin; Si, silicon. Values are means ± SD of five replicates. Significant differences at 5% are represented by lower-case letters.

Table 3
Effect of 2-HMT and Si on Chl a, Chl b, total Chl and proline content of *B. napus* grown in Cr-contaminated soil.

	Chl a (mg g^{-1} FW)	Chl b (mg g^{-1} FW)	Total Chl content (mg g^{-1} FW)	Proline content (µg/ g FW)
C	1.02b ± 0.03	0.54b ± 0.04	1.56b ± 0.07	110d ± 5.76
Cr	0.76d ± 0.06	0.38c ± 0.03	1.04d ± 0.06	45e ± 3.48
Si	1.23ab ± 0.09	0.67ab ± 0.07	1.90ab ± 0.02	156b ± 6.89
2-HMT	1.78a ± 0.07	0.69a ± 0.02	2.47a ± 0.07	179ab ± 7.29
Si + Cr	0.83c ± 0.02	0.39bc ± 0.03	1.22c ± 0.09	126c ± 4.98
2-HMT + Cr	0.91bc ± 0.04	0.41bc ± 0.06	1.32bc ± 0.05	148bc ± 5.28
2-HMT + Si + Cr	0.98bc ± 0.03	0.48c ± 0.08	1.46bc ± 0.04	189a ± 6.18

Values demonstrate means ± SD (n=5). C, control; Cr, chromium; 2-HMT, 2-hydroxymelatonin; Si, silicon. Values are means ± SD of five replicates. Significant differences at 5% are represented by lower-case letters.

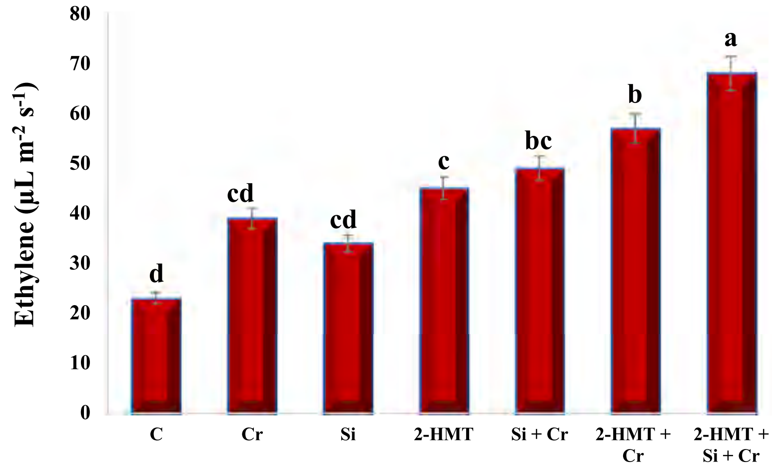

Fig. 5. Effect of 2-HMT and Si on ethylene content on *B. napus* grown in Cr-contaminated soil. C, control; Cr, chromium; 2-HMT, 2-hydroxymelatonin; Si, silicon. Values are means ± SD of five replicates.

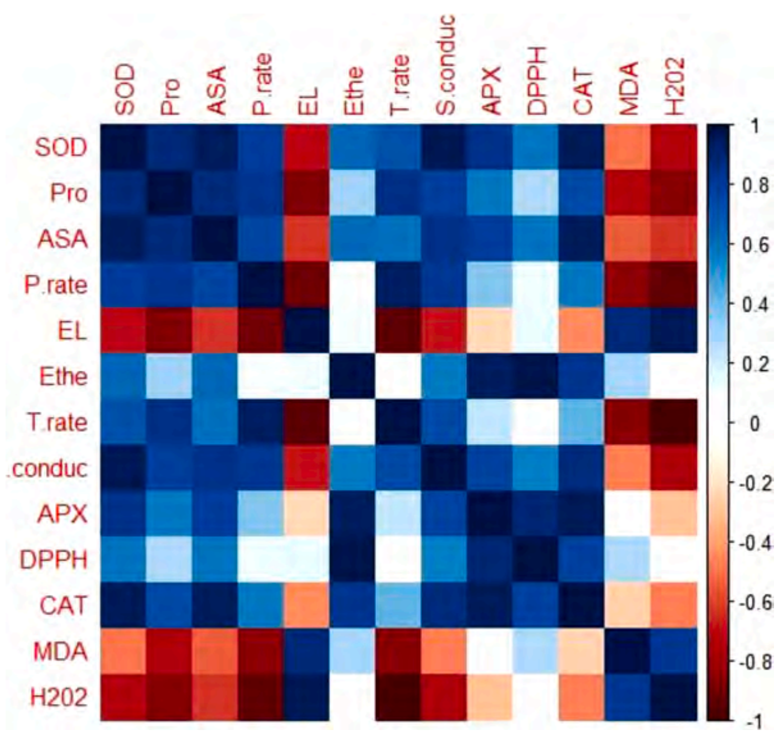

Fig. 6. Pearson correlation among the various variables of *Brassica napus* grown under the influence of silicon and 2-HMT and Cr stress. The abbreviations of the various variables, includes catalase (CAT), SOD (superoxidase dismutase), peroxidase (POD), ascorbate peroxidase (CAT), Ascorbate (ASA), proline (pro), photosynthetic rate (P. rate), transportation rate (T. rate), stomatal conductance (S. conduc), (DPPH), Ethelyene (Ethe), Malondialdehyde (MDA), Electrolyte leakage (EL), Hydrogen peroxidase (H_2O_2).

5. Conclusion

Chromium stress declined growth and physiological properties of *B. napus* seedlings. As seed priming is cost-effective technique to alleviate abiotic stresses in plants, this proved as a better approach in mitigation of Cr-stress in *B. napus* seedlings. A very novel approach was used during this study i.e., 2-HMT and Si application was carried out to reduce toxic effects of Cr on *B. napus* seedlings. Chromium stress enhanced MDA, H_2O_2 and EL in *B. napus* seedlings encountering Cr-contaminated pots. Contrarily, Application of 2-HMT and Si reversed the toxic effects of Cr through incrementation in activity of antioxidantive enzymes and lowering level of MDA, H_2O_2 content and EL. Additionally, 2-HMT and Si enhanced ethylene level in *B. napus* seedlings treated with Cr-contaminated conditions, thereby reversing the toxic effect of Cr-toxicity. This study promises to explore more melatonin metabolites that can be used in improvement of growth in plants facing stressed conditions.

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Authors Contribution

Mansour K. Gatasheh; Analysis Experimentation Research Design Funding, Anis Ali Shah; supervision, research design, Sajid Ali; statistical analysis Validation, Musarrat Ramzan; Research Design Statistical analysis, Sumera Javad; Analysis and Validation, Research Design, Laiba Waseem; Experimentation Analysis, Hafeez Noor; Review Writing and Drafting, Shakil Ahmed; Research Design, Abdul Wahid; Review,

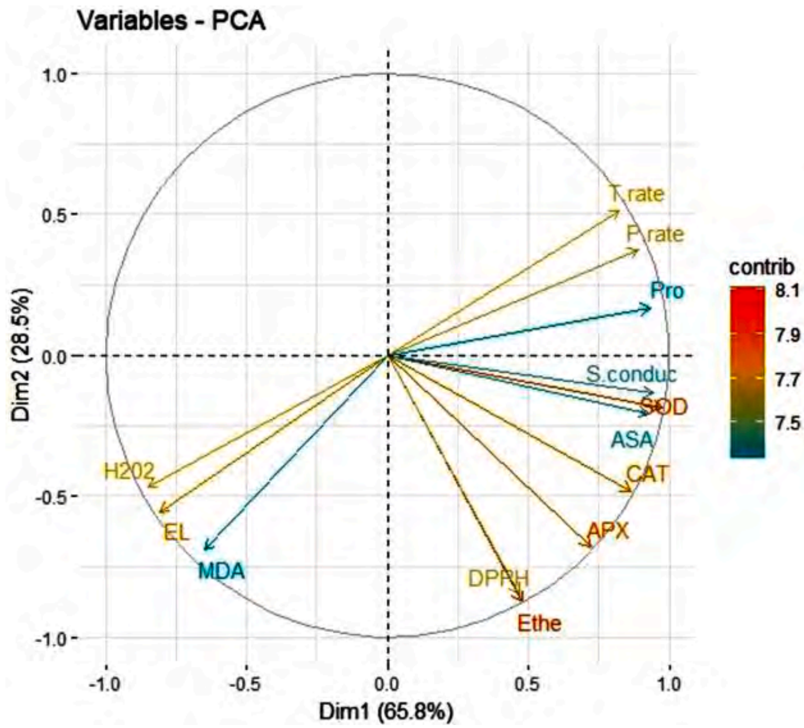

Fig. 7. Principal component analysis of *Brassica napus* grown under varied level Cr stress. The abbreviations of the various variables, includes catalase (CAT), SOD (superoxidase dismutase), peroxidase (POD), ascorbate peroxidase (CAT), Ascorbate (ASA), proline (pro), photosynthetic rate (P. rate), transportation rate (T. rate), stomatal conductance (S. conduc), (DPPH), Ethelyene (Ethe), Malondialdehyde (MDA), Electrolyte leakage (EL), Hydrogen peroxidase (H_2O_2).

Writing and Drafting.

Declaration of Competing Interest

All the authors declare no conflict of interest.

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