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RESEARCH ARTICLE

β-Pinene moderates Cr(VI) phytotoxicity by quenching reactive oxygen species and altering antioxidant machinery in maize

Priyanka Mahajan[1] · Harminder Pal Singh[2] · Shalinder Kaur[1] · Daizy R. Batish[1] · Ravinder Kumar Kohli[1,3]

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Abstract

We examined the possible role of monoterpene β-pinene in providing protection against Cr(VI) toxicity in maize (*Zea mays*). Treatment with β-pinene (10 μM) significantly alleviated Cr(VI) accumulation and recuperated Cr(VI) caused decline in root and coleoptile growth in maize. β-Pinene addition caused a decline in Cr(VI)-induced accumulation of superoxide anion, hydroxyl ion, hydrogen peroxide and confirmed by in-situ detection of ROS using histochemical localization. It suggested that the β-pinene quenches/neutralizes enhanced ROS generated under Cr(VI) exposure. β-Pinene also reduced Cr(VI)-induced electrolyte leakage, thereby suggesting its role in membrane stabilization. Further, β-pinene regulated the activity of scavenging enzymes, thereby suggesting a role in modulating Cr(VI)-induced oxidative damage. In conclusion, our results suggest that the addition of β-pinene has a protective role against Cr(VI) stress and provides resistance to maize against Cr(VI) toxicity.

Keywords Monoterpenes · Hexavalent chromium · Oxidative damage · Free radicals · Stress amelioration

Introduction

Chromium (Cr), a naturally occurring element, is one of the major contaminants in the environment (Vimercati et al. 2017). Cr exists in various valence states ranging from −2 to +6; however, its trivalent (+3) and hexavalent (+6) states are the most common and stable forms (Mondal et al. 2017; Shahid et al. 2017; Stambulska et al. 2018). Cr speciation and availability in the soil depends greatly on pH, redox potential, organic matter, and microbes in the soil (Shahid et al. 2017). In soil, Cr(III) gets oxidized into Cr(VI) by oxidized manganese (MnO_2), whereas Cr(VI) is reduced into Cr(III) by Fe^{2+}, Sr^{2+}, sulfides, Vanadium and organic materials like fulvic acid (Singh et al. 2013; Shahid et al. 2017). Among various ionic forms, Cr(VI) is the most reactive species due to its rapid mobility, higher solubility, and easy uptake by the plant membrane system (Rodriguez et al. 2007; Shahid et al. 2017). Cr(VI) enters the natural environment from petroleum refineries, mines, steel works, paints and pigments production, fungicide development, improper sanitary landfills and electroplating, leather tanning, wood processing, and pulpwood and dyeing industries (Singh et al. 2013). Excess of Cr(VI) inhibits seed germination, disrupts cell membrane integrity, induces microscopic changes in mitochondria and chloroplasts leading to chlorosis, stunted growth, and reduced productivity in plants (Shanker et al. 2009; Singh et al. 2013; Medda and Mondal 2017; Shahid et al. 2017; Stambulska et al. 2018). In addition, it results in the significant accumulation of free radicals, thereby inducing the oxidative stress, and consequently leading to cellular oxidative damage (Shanker et al. 2009). Earlier, attempts have been made to achieve mitigation of Cr through the process of phytoremediation, mycorrhizal associations, and application of chelators (Singh et al. 2013; Agarwal et al. 2014; Shahid et al. 2017). In the past, active molecules such as silicon (Zeng et al. 2011), phosphorus (Sayantan and Shardendu 2013), glutathione (GSH) (Zeng et al. 2012), and brassinosteroids (Choudhary et al. 2011) have been evaluated for providing resistance against Cr-stress. However, the complete eradication of Cr from the environment is not possible, but plant defense mechanism can be regulated through the exogenous application of novel

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✉ Harminder Pal Singh
hpsingh_01@yahoo.com

[1] Department of Botany, Panjab University, Chandigarh 160014, India

[2] Department of Environment Studies, Panjab University, Chandigarh 160014, India

[3] Central University of Punjab, Mansa Road, Bathinda 151001, India

compounds/methods that could ameliorate the toxic effects of Cr, provide cellular homeostasis, and avoid Cr-induced stress in plants.

Plant volatile organic compounds, particularly isoprenes and monoterpenes, are abundantly present in the natural environment and perform a multitude of ecological functions (Dudareva et al. 2006; Sharkey et al. 2007). These provide protection against high temperature and oxidative stress by stabilizing membranes (Peñuelas et al. 2005) and scavenging free radicals (Loreto et al. 2004). Recently, pinenes, the primary volatiles in pine trees, have been found to mediate inter-plant communication and act as defense signals (Farquharson 2017; Riedlmeier et al. 2017). However, studies investigating the potential of monoterpenoids in providing tolerance against heavy metal toxicity are largely lacking. We hypothesized that β-pinene, one of the most abundant monoterepenes released into natural environment from forests (Geron et al. 2000), could scavenge free radicals and regulate Cr-induced oxidative stress. In the present study, we examined the effect of β-pinene supplementation on generation and metabolism of ROS in maize during 48–144 h of Cr(VI) exposure.

Materials and methods

Materials

Maize seeds (var. Aruna 5) procured locally from the market were surface-sterilized prior to use. Cr(VI) was supplied as potassium dichromate (Mol. wt. = 294.18 g; 99.9%; Merck, India). β-Pinene (99% pure) was obtained from Sigma-Aldrich. The other chemicals used in the present study were of analytical reagent grade.

Experimental design

Maize seeds (pre-hydrated for 14 h in distilled water) were germinated at 25 °C on mesh at the top of glass vessels containing distilled water. Four-day old seedlings were exposed to different treatments. In all, there were four treatments: water alone (control), 10 μM β-pinene, 250 μM Cr, 250 μM Cr + 10 μM β-pinene. The concentration of Cr(VI) that has been used is based upon the earlier studies by Mahajan et al. (2013). β-pinene concentration selected in the present study is non-toxic for the plant growth (Chowhan et al. 2011). The experiment was conducted under controlled conditions at 30 °C day and 25 °C night temperature, 76% RH, and 16 h of daylight with 240 μmol photons $m^{-2}s^{-1}$photosynthetic flux density. As the toxic effect of Cr(VI) was more pronounced in roots than in coleoptile, roots were excised at 48, 96, and 144 h after treatment and stored at − 80 °C prior to use.

Cr content

Root samples oven-dried at 70 °C were powdered and digested in 3:1:: HNO_3:$HClO_4$. Amount of Cr was measured in digested material using Atomic Absorption Spectrophotometer (Analytik Jena, Germany) against a reference of Cr, and a parallel reagent blank was also run.

Determination of ROS

To estimate the regulatory effect of β-pinene in Cr-affected maize roots, the alterations in the content of various stress markers: $^{\bullet}OH$ (hydroxyl) and $O_2^{-\bullet}$ (superoxide) radical, H_2O_2 (hydrogen peroxide), conjugated dienes (CD), and malondialdehyde (MDA) were assessed. Membrane peroxidation was determined in terms of MDA at 532 nm (corrected at 600 nm) as per Heath and Packer (1968). $O_2^{-\bullet}$ generation was measured at 480 nm by the acceptor method using epinephrine (Misra and Fridovich 1972). The content of $^{\bullet}OH$ was measured at 532 nm (corrected at 600 nm) using $\varepsilon = 155\ mM^{-1}\ cm^{-1}$ according to the method of Halliwell et al. (1987). H_2O_2 was measured at 390 nm by the method of Velikova et al. (2000). CD content was measured at 234 nm as per Singh et al. (2007).

Membrane disintegration

Membrane disruption was estimated by measuring relative electrolyte leakage (REL), according to Singh et al. (2007).

Qualitative determination of ROS

The membrane disintegration was localized using 0.025% of Evans blue (prepared in 100 μM $CaCl_2$), as per Yamamoto et al. (2001). The staining with Schiff's reagent was performed to histochemically detect in vivo membrane peroxidation (Pompella et al. 1987). H_2O_2 accumulation was visualized using 0.3 mg/ml of diaminobenzidine, accoridng to Thordal et al. (1997). In all three cases, stained roots were washed and photographed using Stereo Zoom Microscope fitted with Nikon imaging system.

Antioxidant enzymes assay

Frozen root material was crushed in 100 mM K-PO_4^{3-} buffer (pH 7.0) and then centrifuged at 15,000×*g* at 4 °C rotor temperature for 30 min. All the operations were carried under ice-cold conditions. The supernatant thus obtained was used for the assay of antioxidant enzymes spectrophotometrically at 25 °C. Protein content was estimated according to the method of Bradford (1976). SOD (Superoxide dismutase) was assayed by observing the inhibition in photoreduction of nitroblue tetrazolium, as per the method of Beauchamp and Fridovich (1971). APX (Ascorbate peroxidase) was assayed by

following the oxidation of ascorbate at 290 nm (Nakano and Asada 1981). Catalases (CAT) activity was determined as H_2O_2 disappearance rate at 240 nm, according to the method of Cakmak and Marschner (1992). Assay of GR (Glutathione reductase) was performed at 340 nm by measuring the rate of oxidation of NADPH as described by Foyer and Halliwell (1976). Activity of GPX (Glutathione peroxidase)was measured by monitoring guaiacol polymerization at 470 nm (Egley et al. 1983). All enzyme activities were expressed in terms of unit milligram^{-1} protein (Singh et al. 2007).

Statistical analyses

For each treatment, there were five vessels (beakers), each representing independent replication. These were arranged in completely randomized design (CRD). All the biochemical estimations included at least five independent (tissue) replications. The significance among treatment means was determined by applying Tukey's test at $P \leq 0.05$.

Results

Cr(VI) exposure caused 30% and 41% decline in coleoptile and root length of maize over the control, when measured at 144 h after the exposure (Fig. 1a). However, β-pinene supplementation recuperated these effects by enhancing the coleoptile and root length by ~16% and 36%, respectively, over Cr(VI) treatment (Fig. 1a). Accumulation of Cr(VI) was greater in roots than in coleoptile, and it corresponded with the observed reduction in root or coleoptile length (Fig. 1b). Cr(VI)-treated roots and coleoptile accumulated 61 and 27 folds greater Cr over that in the control, respectively, when measured after 144 h of exposure (Fig. 1b). In contrast, the Cr(VI) accumulation was lesser (by 65% in roots and 27% in coleoptile) upon β-pinene supplementation (Fig. 1b).

Cr(VI)-exposure enhanced $O_2^{\bullet-}$ by 69–164% in maize roots over the control during 48–144 h of treatment (Table 1). However, upon the addition of β-pinene, $O_2^{\bullet-}$ generation was reduced by 32–46% (Table 1). Cr(VI) increased the amount of $OH^{\bullet}$ by ~37–87% over the control. However, β-pinene supply declined $OH^{\bullet}$ content by ~23–31%, when measured within 48–144 h of treatment (Table 1). Likewise, H_2O_2 content spiked to ~178% after 144 h of Cr(VI) treatment; however, it declined by ~56% in treatments supplemented with β-pinene (Table 2). CD content declined by ~42%, 56%, and 71% in maize roots over the control; however, it was recuperated by ~16% at 48 h, 28% at 96 h, and 55% at 144 h on β-pinene supply (Table 2). Cr(VI)-exposure enhanced MDA content in maize roots by 17–63% during 48–144 h of treatment, over the control. However, supplementation of β-pinene decreased MDA by ~22–51%, over Cr(VI) treatment (Table 3). Parallel to LP, membrane disintegration measured in terms of REL was enhanced by ~64–147% during 48–144 h of Cr(VI)-treatment, whereas β-pinene supplementation caused a reduction in REL by 24–43% over Cr(VI) treatment (Table 3). The modulating effect of β-pinene on ROS generation ($O_2^{\bullet-}$ and H_2O_2) and membrane peroxidation (MDA and REL) under Cr(VI)-induced stress was evident from histochemical analyses wherein roots from Cr(VI) + β-pinene treatments stained lesser over Cr(VI) only (Fig. 2).

CAT, APX, and GR activities were upregulated, whereas SOD and GPX activities declined on Cr(VI) exposure (Fig. 3). However, β-pinene addition regulated the activity of these enzymes to certain extent. SOD activity declined by ~43%, 53%, and 72% in Cr(VI)-treatments over the control, after 48 h, 96 h, and 144 h, respectively. However, upon addition of β-pinene, the reduction in SOD activity ranged from 21 to 44%, thus showing an improvement by 66–98% over the Cr(VI)-alone treatments after 48–144-h treatment (Fig. 3). Cr(VI) spiked activity of CAT to 1.6–1.7 times of that in control. β-pinene addition further increased CAT activity to 2.1 times at 48 h, 2.5 times at 96 h, and 3.2 times at 144 h treatment, respectively. Likewise, APX activity was increased upon Cr(VI)-treatment, and was 1.74–2.34 times of the control after 48–144 h of treatment. It was further enhanced with addition of β-pinene by 2.54, 3.16, and 4.65 times of the control (Fig. 3). Activity of GR also enhanced upon Cr(VI) treatment and increased to 2.17, 2.44, and 2.71 times to that of the control after 48, 96, and 144 h of treatment. However, the increase was less pronounced (by 22–34% of

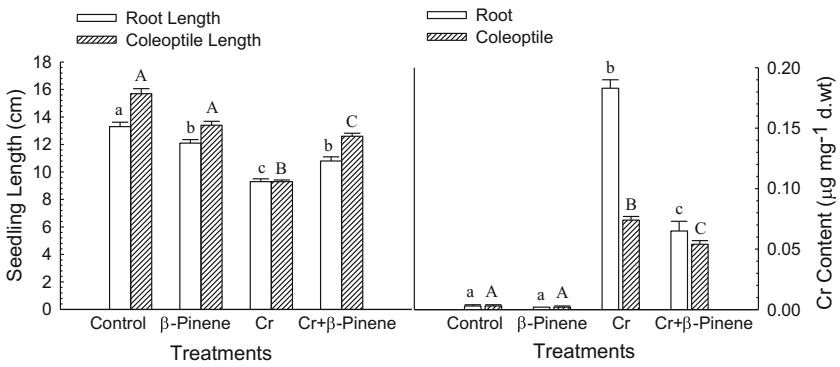

Fig. 1 Effect of β-pinene (10 µM) supplementation on **a** Cr(VI)-induced changes in root and coleoptile length, and **b** Cr accumulation in root and coleoptile of maize, determined 144 h after treatment. Different alphabets (lower case for roots and upper case for coleoptile) represent significant difference among treatments at $P \leq 0.05$, according to Tukey's test

Table 1 Effect of β-pinene (10 μM) on the Cr (250 μM)-induced increase in $O_2^{-\bullet}$ (μM g^{-1} fwt) and $OH^{\bullet}$ (nmol g^{-1} fwt) content in roots of maize, determined 48, 96, and 144 h after Cr(VI) exposure

Treatment	$O_2^{-\bullet}$ content (μM g^{-1} fwt)			$OH^{\bullet}$ content (nmol g^{-1} fwt)		
	48 h	96 h	144 h	48 h	96 h	144 h
Control	1.3 ± 0.04aA	1.3 ± 0.06aA	1.4 ± 0.04aA	27.5 ± 0.78aA	29.9 ± 0.22aB	31.0 ± 0.37aB
β-Pinene (10 μM)	1.7 ± 0.06bA	1.8 ± 0.04bA	2.1 ± 0.06bB	31.4 ± 0.43bA	34.0 ± 0.22bB	35.7 ± 0.22bC
	(+ 30.8)[1]	(+ 38.5)	(+ 50.0)	(+ 14.2)	(+ 13.7)	(+ 15.2)
Cr(VI) (250 μM)	2.2 ± 0.02cA	2.8 ± 0.07cB	3.7 ± 0.04cC	37.8 ± 0.57cA	46.0 ± 0.57cB	58.1 ± 0.37cC
	(+ 69.2)	(+ 115.4)	(+ 164.3)	(+ 37.4)	(+ 53.8)	(+ 87.4)
Cr(VI) + β-pinene	1.5 ± 0.06bA	1.7 ± 0.04bA	2.0 ± 0.04bB	29.0 ± 0.37abA	34.2 ± 0.37bB	40.2 ± 0.43dC
	(+ 15.4)	(+ 26.3)	(+ 42.8)	(+ 5.4)	(+ 14.4)	(+ 29.7)
	[− 31.8][2]	[− 39.3]	[− 46.0]	[− 23.3]	[− 25.6]	[− 30.8]

Data presented as mean ± standard error

Different alphabets (upper case in a row and lower case in a column) represent significant difference at $P \leq 0.05$ applying post hoc Tukey's test

[1] Figures within parenthesis represent percent increase (+) over the control

[2] Figures within square brackets represent percent decrease (−) over the Cr alone treatments

the control) upon addition of β-pinene to Cr(VI) (Fig. 3). In contrast, activity of GPX declined by 24–54.5% during 48–144 h of Cr(VI) treatment. The decline was less (6.6–26.7% during 48–144 h) when β-pinene was supplemented, showing an improvement of GPX activity by 23–61% upon Cr(VI)-alone treatments (Fig. 3).

Discussion

The study documented that Cr(VI) inhibited root and coleoptile growth, increased ROS accumulation, and caused oxidative stress in maize, and it paralleled greater accumulation of Cr(VI). These findings are corroborated by several earlier reports that Cr(VI) is toxic and retards plant growth vis disruption of oxidative metabolism (Singh et al. 2013; Shahid et al. 2017 and references therein). However, β-pinene supply ameliorated Cr(VI)-toxicity as depicted by better root and coleoptile growth, less Cr accumulation, and decreased ROS generation and modulation of scavenging enzymes in maize roots. The decreased levels of ROS ($O_2^{-\bullet}$ and $OH^{\bullet}$) on β-pinene supplementation indicate that β-pinene scavenges ROS and thus provides protection against Cr-stress. However, not much is known about the protective role of monoterpenes, including β-pinene. Nevertheless, monoterpenes act as stress regulators and have protective role. For example, thymol protects rice

Table 2 Effect of β-pinene (10 μM) on the Cr (250 μM)-induced increase in H_2O_2 (nmol g^{-1} fwt) and conjugated diene (CD; μ mol g^{-1} fwt) content in roots of maize, determined 48, 96, and 144 h after Cr(VI) exposure

Treatment	H_2O_2 content (nmol g^{-1} fwt)			CD content (μ mol g^{-1} fwt)		
	48 h	96 h	144 h	48 h	96 h	144 h
Control	8.3 ± 0.24aA	8.1 ± 0.24aA	8.8 ± 0.63aA	6.4 ± 0.04aA	6.6 ± 0.08aA	7.0 ± 0.02aB
β-Pinene (10 μM)	8.6 ± 0.41aA	9.5 ± 0.24aAB	10.7 ± 0.41aB	5.2 ± 0.04bA	4.9 ± 0.05bB	4.5 ± 0.02bC
	(+ 3.6)[1]	(+ 17.3)	(+ 21.6)	(− 18.8)	(− 25.8)	(− 35.7)
Cr(VI) (250 μM)	15.2 ± 0.24bA	17.9 ± 0.41bA	24.5 ± 1.04bB	3.7 ± 0.04cA	2.9 ± 0.06cB	2.0 ± 0.01cC
	(+ 83.1)	(+ 121.0)	(+ 178.4)	(− 42.2)	(− 56.1)	(− 71.4)
Cr(VI) + β-pinene	8.8 ± 0.24aA	8.6 ± 0.41aA	10.9 ± 0.24aB	4.3 ± 0.04dA	3.7 ± 0.02 dB	3.1 ± 0.03dC
	(+ 6.0)	(+ 6.2)	(+ 23.9)	(− 32.8)	(− 43.9)	(− 55.7)
	[− 42.1][2]	[− 52.0]	[− 55.5]	[+ 16.2]	[+ 27.6]	[+ 55.0]

Data presented as mean ± standard error

Different alphabets (upper case in a row and lower case in a column) represent significant difference at $P \leq 0.05$ applying post hoc Tukey's test

[1] Figures within parenthesis represent percent increase (+) or percent decrease (−) over the control

[2] Figures within square brackets represent percent decrease (−) or percent increase (+) over the Cr alone treatments

Table 3 Effect of β-pinene (10 μM) on the Cr (250 μM)-induced increase in MDA content (nmol g^{-1} fwt) and REL (%) in roots of maize, determined 48, 96, and 144 h after Cr(VI) exposure

Treatment	MDA content (nmol g^{-1} fwt)			REL (%)		
	48 h	96 h	144 h	48 h	96 h	144 h
Control	28.8 ± 0.78abA	31.2 ± 1.20aA	31.8 ± 0.94aA	22.8 ± 0.79aA	23.8 ± 0.59aA	24.7 ± 0.80aA
β-Pinene (10 μM)	31.0 ± 0.37bcA	37.9 ± 0.22bB	39.6 ± 0.43bC	28.1 ± 0.63abA	29.8 ± 1.37bAB	32.4 ± 0.24bB
	(+ 7.6)[1]	(+ 21.5)	(+ 4.5)	(+ 23.2)	(+ 25.2)	(+ 31.2)
Cr(VI) (250 μM)	33.8 ± 1.08cA	46.9 ± 0.86cB	51.8 ± 0.22cC	37.4 ± 1.90cA	49.1 ± 1.23cB	60.9 ± 1.03cC
	(+ 17.4)	(+ 50.3)	(+ 62.9)	(+ 64.0)	(+ 106.3)	(+ 146.6)
Cr(VI) + β-pinene	26.5 ± 0.37aAB	29.0 ± 0.94aA	25.4 ± 0.57 dB	28.5 ± 1.05bA	32.8 ± 1.70bA	34.8 ± 1.71bA
	(− 8.0)	(− 7.1)	(−2 0.1)	(+ 25.0)	(+ 37.8)	(+ 40.9)
	[− 21.6][2]	[− 38.2]	[− 51.0]	[− 23.8]	[− 33.2]	[− 42.8]

Data presented as mean ± standard error

Different alphabets (upper case in a row and lower case in a column) represent significant difference at $P \leq 0.05$ applying post hoc Tukey's test

[1] Figures within parenthesis represent percent increase (+) or percent decrease (−) over the control

[2] Figures within square brackets represent percent decrease (−) over the Cr alone treatments

roots from oxidative damage by reducing ROS accumulation and endogenous nitric oxide levels (Wang et al. 2017). In an earlier study, β-pinene has been found to protect emerging crop seedlings from Cr(VI) stress by modulating protein and oxidoreductase enzymatic pathways (Mahajan et al. 2016). Greater Cr accumulation creates imbalance in the oxidative metabolism, which leads to excessive generation of free radicals ($O_2^{-\bullet}$ and $OH^{\bullet}$) and other ROS (H_2O_2), as observed in the present study. Enhanced MDA levels and greater REL upon Cr(VI) exposure suggests Cr(VI)-mediated membrane damage; Lipid peroxidation involves peroxidation of polyunsaturated fatty acids forming MDA and enhancing free radical eruption due to which the membranes become leaky as evidenced from high REL (Heath and Packer 1968). However, recuperation of MDA levels and reduced REL on supplementation of β-pinene indicates the role of latter in membrane protection. It was in agreement with decrease in ROS generation and membrane damage, upon addition of β-pinene compared to Cr-alone treatment. Volatile organic compounds (VOCs), including monoterpenes, are involved in mediating the defense mechanism in plants under abiotic stress (Holopainen and Gershenzon 2010). Isoprenes and monoterpenes act as antioxidants and neutralize ROS (Affek and Yakir 2002) and protect against oxidative damage (Vickers et al. 2009). These stabilize and protect the membranes by disrupting lipid peroxidation under stress, thereby suggesting their role in plant protection under adverse conditions (Loreto and Velikova 2001). It paralleled the present findings that roots from β-pinene supplied treatments retained membrane integrity and accumulated less MDA, H_2O_2, and $O_2^{-\bullet}$, thereby suggesting scavenging of free radicals by β-pinene.

As regards the uptake of Cr in plants, there is no specific mechanism. Nevertheless, the entry of Cr in the plant occurs via plasma membranes possibly through carriers of essential elements such as Fe, S, and P (da Conceição Gomes et al. 2017). Cr(VI) uptake is an active process occurring via sulphate or phosphate transporter (Kim et al. 2006; López-Bucio et al. 2014; Shahid et al. 2017). Presence of phosphates and sulphates

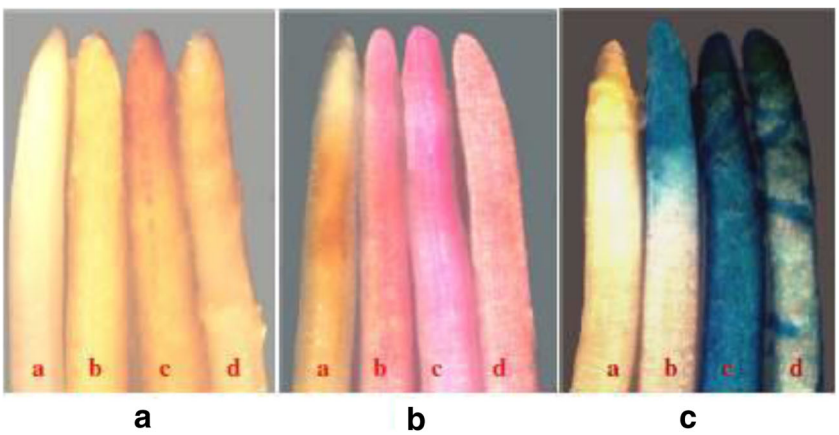

Fig. 2 In situ localization of (**a**) H_2O_2 and (**b**) MDA content, and (**c**) disruption in membrane integrity in roots of maize, observed after 144 h of treatment. In each figure, from left to right: (a) control, (b) 10 μM β-pinene, (c) 250 μM Cr(VI), and (d) 250 μM Cr(VI) + 10 μM β-pinene

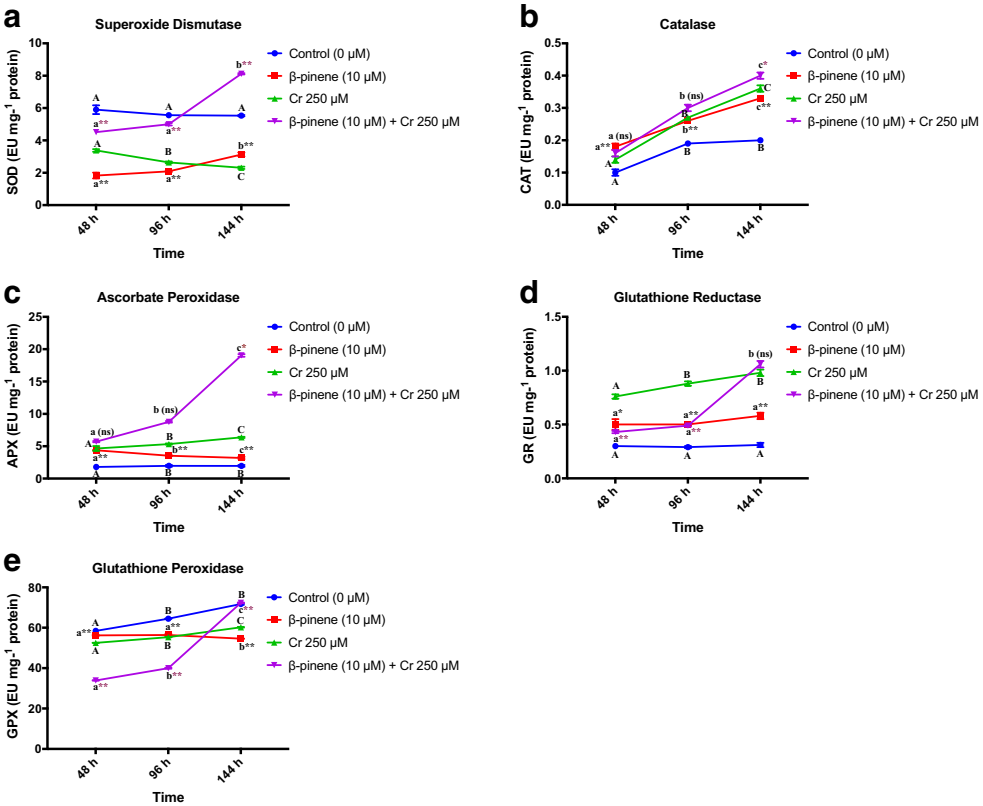

Fig. 3 Effect of β-pinene (10 µM) supplementation on the Cr(VI) (250 µM)-induced alterations in the activities (EU mg^{-1} protein) of **a** SOD, **b** CAT, **c** APX, **d** GR, and **e** GPX in roots of maize, determined at 48, 96, and 144 h after treatment. Data presented as mean ± SE. Different alphabets (lower case at a particular time period, upper case within a particular treatment at different time periods) represent significant difference at $P \leq 0.05$, according to Tukey's test. * and ** represent significance difference between Cr(VI) and Cr(VI)) + β-pinene treatments at $P \leq 0.05$ and $P \leq 0.01$, respectively

negatively correlates to chromate ion uptake in maize and *Brassica juncea* (Schiavon et al. 2008; López-Bucio et al. 2014). Schiavon et al. (2008) observed greater accumulation of Cr in *Brassica juncea* under sulfur-deprived conditions and vice-versa, suggesting that sulfate and chromate compete for uptake in plants (Schiavon et al. 2008). Kaszycki et al. (2005) observed increased accumulation of Cr in giant duckweed uponexogenous application of high sulfate levels (100 mM), thereby suggesting opening of new transport pathways possibly due to damage to plasma membrane. Though we did not make any such observation in the present study, yet the observed protective effect of β-pinene on membrane integrity correlates positively with less toxicity and accumulation of Cr(VI) in β-pinene-supplied treatments.

To prevent ROS-mediated damage, cells have an efficient mechanism comprising of antioxidant enzymes and non-enzymatic antioxidants to scavenge excessive ROS (del Río 2015). In our study, β-pinene addition was found to regulate the activity of antioxidant enzymes. SODs modulate both O_2 and H_2O_2 and act as the first line of defense (Alscher et al. 2002). However, in our study, SOD activity declined upon Cr-treatment, despite the increased $O_2^{-\bullet}$ generation. It is in agreement with earlier findings that Cr(VI) exposure declined SOD activity in pea (Dixit et al. 2002). Reduction in SOD activity indicates inactivation/degradation of SOD protein due to excessive ROS generation (Casano et al. 1997). $O_2^{-\bullet}$ if not dismutated by SOD, induce oxidative stress in cells. It was evident from greater damage to membrane integrity and enhanced lipid peroxidation. Nevertheless, β-pinene increased/recuperated SOD activity, thereby suggesting its role in protecting SOD protein. In our study, the major H_2O_2 metabolizing enzymes, CAT and APX, were increased significantly and paralleled the observed H_2O_2 accumulation under Cr(VI) stress.

The antioxidant behavior of isoprenes and monoterpenes has been attributed to the presence of conjugated double bond that may arbitrate electron and energy transfer, enabling them to scavenge free radicals (Vickers et al. 2009). Monoterpenes being lipophilic can permeate through cell membrane (Chowhan et al. 2013; Pham et al. 2015) and manipulate defense genes and certain transcription factors in plants (Godard et al. 2008). Monoterpenes act as signaling molecules and trigger a signal similar to a mild stress, and thereby may incite the expression of certain stress-linked genes (Lee and Seo 2014). Kriegs et al. (2010) opined that discontinuous or short-term exposure to bioactive monoterpenes may lead to reversible and constructive response and strengthens plant fitness (Kriegs et al. 2010). Of late, α- and β-pinene have been demonstrated to act as air-borne signals to propagate defense signaling in plants and induce Systemic Acquired Resistance (Riedlmeier et al. 2017). Recently, thymol, another monoterpene, has been reported to confer resistance towards Cd-induced injury in rice roots through modulation of endogenous NO level (Wang et al. 2017). However, we did not explore such a mechanism in the present study.

Conclusions

In summary, our results show that the addition of β-pinene has a protective role against Cr(VI) stress and provides resistance to maize against Cr(VI) toxicity. β-Pinene quenches/neutralizes enhanced ROS generated under Cr(VI) exposure. However, the exact biochemical mechanism/mode underlying the antioxidant role of β-pinene is still evasive. Nevertheless, the findings of the present study provide some insights that monoterpenes may be exogenously used to combat/partially ameliorate metal-induced stress in plants. To unravel the physiological and molecular mechanisms underlying such an action, future studies are required.

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