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ORIGINAL ARTICLE

Molybdenum mediated mitigation of oxidative stress in *Triticum durum* (HI 8737) seedlings caused due to aluminium

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Abstract India is one of the largest producers of wheat, however, its productivity is adversely affected by aluminium (Al) disposed as industrial waste into soil. Molybdenum (Mo) is a trace element essential for plant growth. Metal effects induce oxidative stress which can be reduced due to strong antioxidative defense system. The present study investigates the effect of Al and Mo in terms of antioxidative parameters either alone or in combination under acidic condition (pH 5.0) using seedlings of *Triticum durum* var. HI 8737. Al supply using 25–200 µM $AlCl_3$ significantly reduced fresh tissue weight and superoxide dismutase (SOD) activity at all the concentrations in root and shoot both, but the catalase (CAT) and ascorbate peroxidase (APX) activities increased upto 50 µM Al and then decreased. Al toxicity resulted in enhanced malondialdehyde (MDA) and H_2O_2 content, being more substantial at higher concentration and exerting strong correlation. The results demonstrate the tolerance capacity of the used wheat cultivar upto 50 µM Al under acidic conditions. Mo supply (0.25–5 µM) as ammonium molybdateincreased fresh tissue weight of root, antioxidative enzymes, but reduced MDA and H_2O_2 content in both root and shoot. With binary treatment Mo ameliorated the effect of Al stress at least partly, as concentration of MDA and H_2O_2 were reduced and CAT and APX activities were enhanced for Al–Mo combination. A significant increase for nitric oxide (NO) content along with increased activity of nitrate reductase (NR), an Mo-enzyme, in root, suggests a key role of Mo on Al stress.

Keywords Aluminium · Molybdenum · Wheat seedling · Antioxidants · Nitric oxide

Introduction

India ranks first for highest net cropped area followed by US and China and ranks second in the world in farm outputs (Majumdar et al., 2017). Higher area coverage is reported from Madhya Pradesh for wheat production in recent years. However, anthropogenic activities in the vicinity of Malwa zone and use of synthetic fertilizers have led to increase of metal concentration in the soil, thereby reducing natural fertility of soil. Availability of aluminium (Al) is high in acid soils (Aggarwal et al., 2015a) whereas molybdenum (Mo) availability decreases with increase in soil acidity (Kaiser et al., 2005). Al even in small concentrations adversely affects plant growth and productivity whereas, Mo is an essential micro nutrient. Plants exhibit resistance against abiotic stresses caused due to metal toxicity in soil, which induces superoxide dismutase (SOD) to scavenge superoxide radical ($O_2^{\cdot-}$) that further reduces to H_2O_2. Enzymes, such as, catalase (CAT) decompose the H_2O_2 into water and oxygen. While, ascorbate peroxidase (APX) and guaiacol peroxidase (Gu-POX) catalyze H_2O_2 dependent oxidation of ascorbate and guaiacol, respectively (Karuppanapandian et al., 2011). In addition, malondialdehyde (MDA), the product of lipid peroxidation caused by reactive oxygen species is related to injuries in plants and the increased H_2O_2 under toxic environment is a stress marker in plants.

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Al limits crop production over 40% of the world's arable irrigated land and adversely affects the nutrient uptake and metabolic processes. Under acidic condition, Al inhibits the root growth by disrupting root cell expansion and elongation (Kochian et al., 2005). With prolonged exposures, Al interacts with the root cell nuclei, causing disruption of cell division (Silva et al., 2000; Zheng & Yang, 2005). In wheat, disruption of cell membrane, observed as increased lipid peroxidation under Al toxicity has been reported (Dong et al., 2002, Xu et al. 2012, Aggarwal et al., 2015b, Liu et al., 2018). Moreover, under Al toxicity, increase in H_2O_2 content and variation in antioxidative enzyme activities have been reported, which adversely affected the wheat growth (Xu et al. 2012; Aggarwal et al., 2015b; Liu et al., 2018). In both monocots and eudicots, the exclusion of Al through the efflux of organic acid anions from root apices has been demonstrated as a main mechanism to overcome Al stress. The organic acid efflux is associated with callose accumulation, restriction to Al induced lipid peroxidation, lower accumulation of Al in root tips and better root regrowth under Al stress (Garcia-Oliveira et al., 2016).

Mo is a trace element and is vital for plant growth. Toxic effects of Mo in plants are rare, while deficiency of Mo is a widespread agricultural problem, especially in acidic soils (Marschner, 1991). Mo is reported to enhance antioxidant enzymes, such as, SOD, CAT, POX simultaneously reducing lipid peroxidation under low temperature (Sun et al., 2006), under varying nitrogen sources (Imran et al., 2020) and induce nitrate reductase (NR) activity (Abd El-Samad et al., 2005; Yaneva et al., 1996) and nitric oxide (NO) content (Wu et al., 2017) under varying condition. Ameliorating effect of Mo on Al stress has not been reported yet. Our current investigation analyses effects of Al and Mo and their interaction to study the ameliorating effects of Mo based on antioxidative parameters, NR activity and NO content using static hydroponic culture at pH 5 and temperature of 20 °C during raising the seedlings of *Triticum durum* var. HI 8737.

Material and method

Plant growth and treatment

Triticum durum var. HI 8737 was collected from Directorate of Wheat Research, Indian Agriculture Research Institute, Indore, India. The seeds were surface sterilized with 0.1% $HgCl_2$ for 2 min, washed thoroughly and rinsed with milli Q water. Seeds were germinated in petri dish lined with moistened filter paper for 3 days at 25 ± 1 °C and transferred to static hydroponic culture containing 50 ml solution of $AlCl_3$ and $(NH_4)_6Mo_7O_{24}.4H_2O$ at pH 5 and temperature of 20 °C for 4 days. The hydroponic cultures of seedlings were grown in a growth chamber under the conditions of 90 mW cm^{-2} light intensity, 55% relative humidity and 12 h day/night cycle. The treatments involved were:

Single treatment: To study the effect of Al and Mo, salt concentrations usedwere 0, 25, 50, 100 and 200 µM of $AlCl_3$ and 0.00, 0.25, 0.50, 1.00, 2.00, 5.00 µM of $(NH_4)_6Mo_7O_{24}.4H_2O$.

Binary treatment: To analyse interactive effects Al_{50} (T_1), $Mo_{0.5}$ (T_2), Mo_2 (T_3), Al_{50} $Mo_{0.5}$ (T_4) and $Al_{50}Mo_2$ (T_5) were used.

Root and shoot of treated seedlings grown for 4 days in static hydroponic system were analyzed for various antioxidative parameters, like superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), guiacol peroxidase (Gu-POX), lipid peroxidation (MDA), H_2O_2 content. With binary treatment, nitrate reductase (NR) activity and nitric oxide (NO) content were also measured. Average values with ± S.D. of three replicate experiments are presented.

Analytical procedures

Root and shoot material were homogenized with 50 mM phosphate buffer (pH 7.0) containing 1 mM EDTA using pestle and mortar at 4 °C. The homogenate was centrifuged at 12,000 g for 20 min at 4 °C using Remi Cooling centrifuge "C-24". The supernatant was used for different enzyme assays and protein estimation. The protein content was determined by method of Lowry et al (1951). The SOD activity was assayed by the method of Beauchamp and Fridovich (1971) based upon the ability to inhibit the photochemical reduction of Nitrobluetetrazolium (NBT) by measuring absorbance at 560 nm using the spectrophotometer "Schimadzu" UV-1800. The activity was

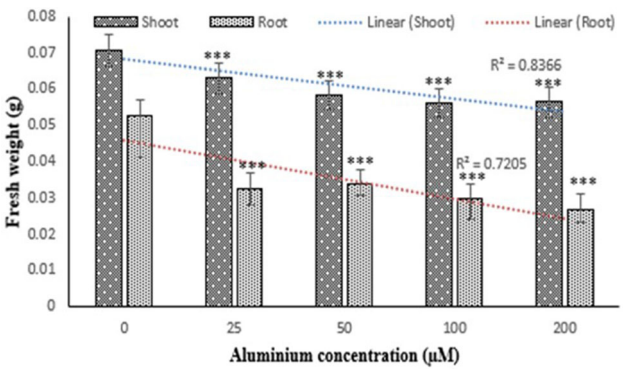

Fig. 1 Effects of Al application on fresh tissue weight in the *T. durum* var. HI8737. Data represent the means ± SD of three independent experiments (n = 3), $*P < 0.05$ $**P < 0.01$, $***P < 0.001$ using *t* test

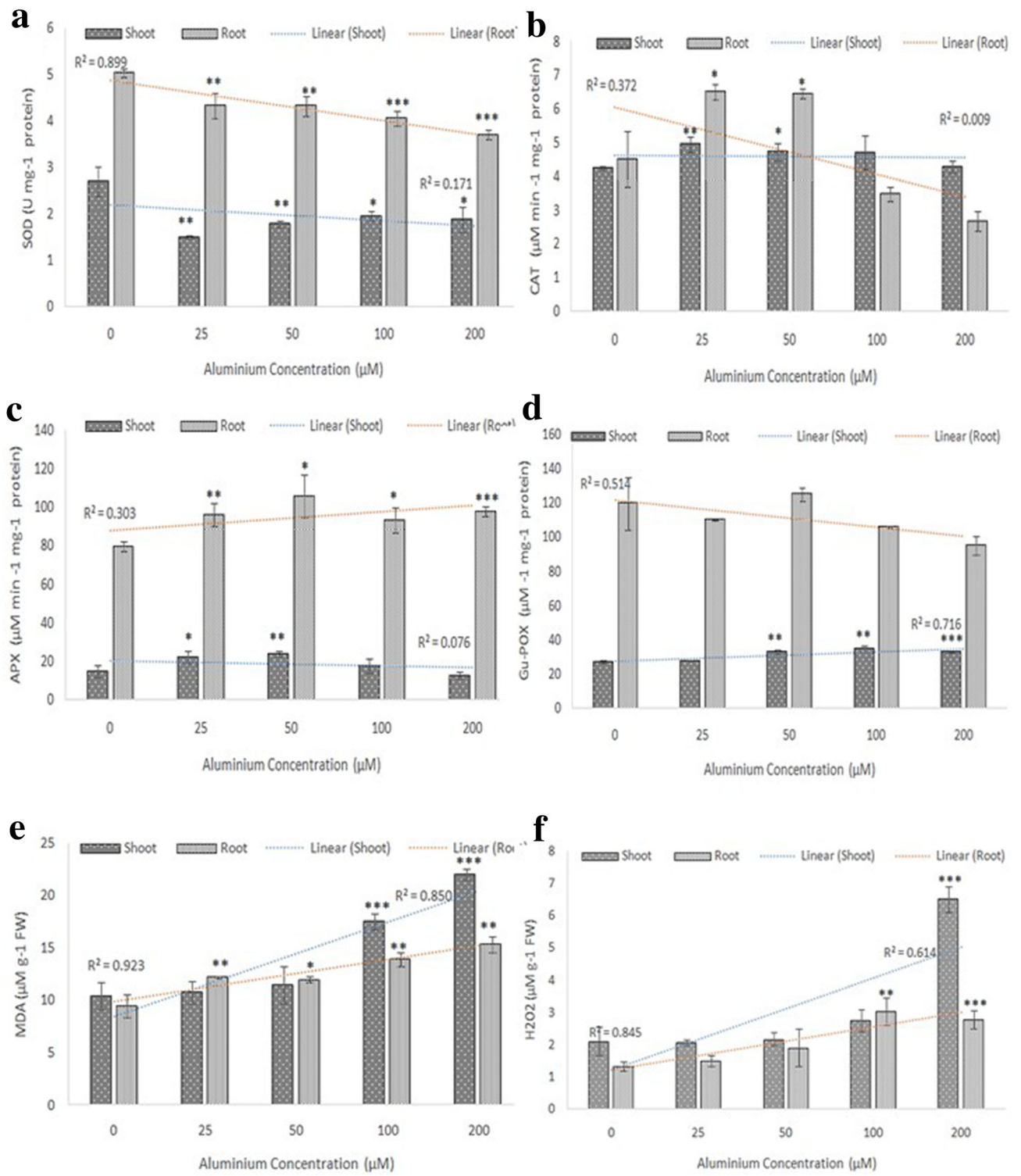

Fig. 2 Effects of Al application on the activities of SOD, CAT, APX, Gu-Pox, MDA and H_2O_2 in the *T.durum* var. HI8737. Data represent the means ± SD of three independent experiments (n = 3), $*P < 0.05$, $**P < 0.01$, $***P < 0.001$ using *t* test

expressed as Units/mg protein and one unit was defined as the amount of enzyme required to cause 50% inhibition in the rate of photoreduction of NBT. The CAT activity was determined by monitoring the decrease in absorbance at 240 nm for 3 min using the substrate H_2O_2 and calculated with ε = 39.4 mM^{-1} cm^{-1} (Aebi, 1984). The APX activity was determined by monitoring the rate of H_2O_2 dependent oxidation of ascorbic acid during assay by following the

decrease of absorbance at 290 nm for 3 min (Nakano & Asada, 1981). Extinction coefficient ($\varepsilon = 2.89\ mM^{-1}$ - cm^{-1}) was used to calculate the enzyme activity. The Gu-POX activity was determined by monitoring the increase of absorbance at 475 nm for 2 min (Putter, 1974) andthe activity was calculated from extinction coefficient ($\varepsilon = 26.6\ mM^{-1}\ cm^{-1}$).

For the determination of MDA and H_2O_2 content, fresh root and shoot tissue were extracted in ice cold TCA (0.1% w/v). The MDA content determinationwas based on thiobarbituric acid (TBA) reaction. The specific absorbance was measured at 532 nm and non-specific absorbance at 600 nm (Hodges et al., 1999). The level of lipid peroxidation was expressed as μM MDA g^{-1} fresh weight. As described by Velikova et al. (2000), the H_2O_2 level was measured by using 1 M potassium iodide and absorbance was recorded at 390 nm. The H_2O_2 content was determined by using extinction coefficient ($\varepsilon = 0.28\ mM^{-1}\ cm^{-1}$) and expressed as μM $H_2O_2\ g^{-1}$ fresh weight.

In vivo NR activity was assayed by estimating the amount of nitrite produced according to the method of Shrivastava (1975) and the activity was expressed as nmoles NO_2^- formed $h^{-1}\ g^{-1}$ fresh weight. The NO content was measured by using Griess reagent as per procedure of Zhou et al. (2005) and content was expressed as nmoles of nitrite g^{-1} fresh weight.

Statistical analysis

Data presented are average values of 3 independent experiments with ± S.D. Difference between means obtained was tested by student's t test at level of significance $*p < 0.05$, $**p < 0.01$, $***p < 0.001$for Al/Mo treatment and also for combination treatment (T_4 and T_5) with respect to T_1. For combination treatments $\#p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ denote significant difference between T_2 and T_4 and $^{+}p < 0.05$, $^{++}p < 0.01$, $^{+++}p < 0.001$ between T_3 and T_5. For correlation analysis XY scatter charts were prepared and correlation coefficient, R^2 were calculated using Excel 2013.

Results

Effect of Al on antioxidative parameters

Supply of 25–200 μM $AlCl_3$ significantly reduced fresh weight in both shoot ($R^2 = 0.836$) and root ($R^2 = 0.720$) tissues being higher in former, in dose dependent manner at all the concentrations (Fig. 1). **Al** supply significantly reduced SOD activity in shoot as well as root with the level being higher in later (Fig. 2a). Further, observed correlation ($R^2 = 0.899$) is very strong for root tissue. The CAT activity in both root and shoot increased significantly up to 50 μM Al, but thereafter decreased (Fig. 2b). In root the activity was 1.4 folds higher than shoot and Al effect was more substantial, while in shoot marginal changes were observed (Fig. 2b). The APX activity also increased up to 50 μM Al and then decreased in both root and shoot with higher level being found in former (Fig. 2c). Exogenous application of Al decreased Gu-POX activity insignificantly in root ($R^2 = 0.515$), but increased for shoot with strong correlation ($R^2 = 0.717$) and being significant at and above 50 μM concentration (Fig. 2d). The level of MDA and H_2O_2 were higher for shoot as compared to root (Fig. 2e, f). In roots the MDA content increased significantly at all the concentrations of Al with very strong correlation ($R^2 = 0.850$) (Fig. 2e). In shoot significant increase was observed at 100 and 200 μM Al only and correlation was very strong ($R^2 = 0.924$). The H_2O_2 content increased with Al concentration in root ($R^2 = 0.845$) being significant at 100 and 200 μM Al and being respectively, 2.33 and 2.1 folds higher (Fig. 2f). In shoot significant increase was observed at 200 μM Al (3.09 folds) only with lesser degree of correlation ($R^2 = 0.614$).

Effect of Mo on antioxidative parameters

The treatment of wheat seedlings with 0.25–5 μM Mo, significantly iincreased the fresh weight of root ($R^2 = 0.614$) at 2 and 5 μM, whereas insignificant changes resulted for shoot (Fig. 3). Mo supply significantly increased the SOD activity in root at and above 0.5 μM exerting strong correlation ($R^2 = 0.768$). Mo effect was less substantial in shoot and had lower activity than root (Fig. 4a). The CAT activity was significantly increased at 2

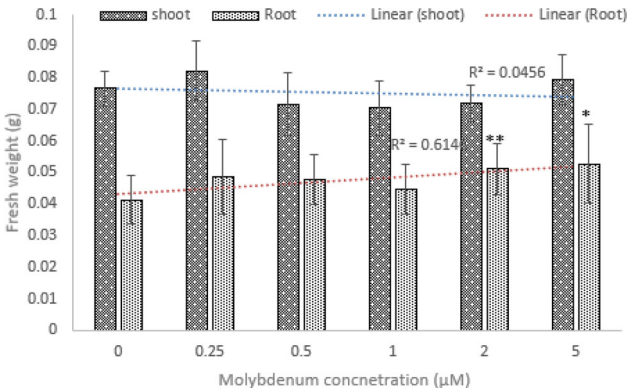

Fig. 3 Effects of Mo application on fresh tissue weight in the *T. durum* var. HI8737. Data represent the means ± SD of three independent experiments (n = 3), $*P < 0.05$, $**P < 0.01$, $***P < 0.001$ using t.test

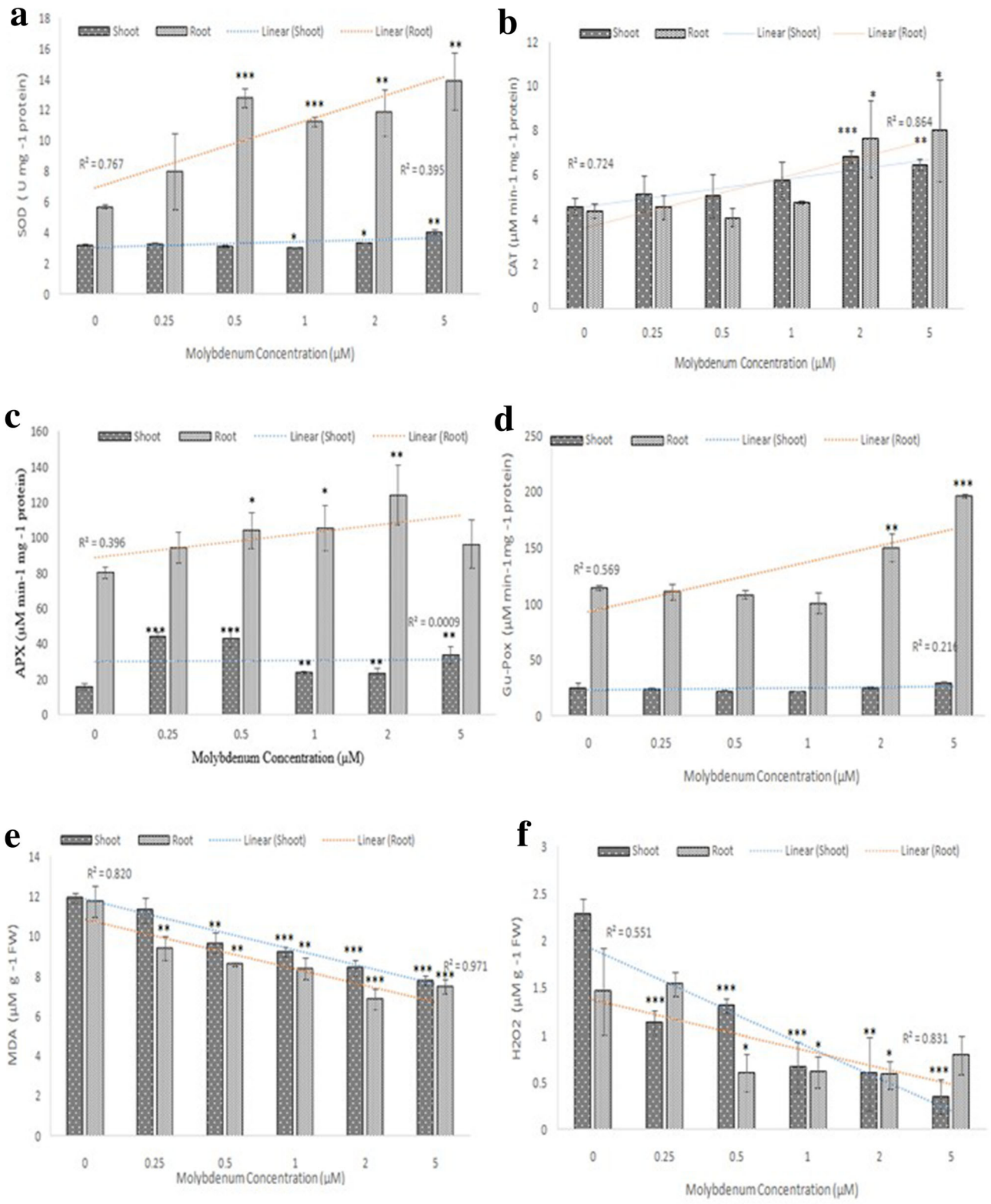

Fig. 4 Effects of Mo application on the activities of SOD, CAT, APX, Gu-POX, MDA and H_2O_2 in the T. *durum* var. HI8737. Data represent the means ± SD of three independent experiments (n = 3), $*P < 0.05$, $**P < 0.01$, $***P < 0.001$ using t.test

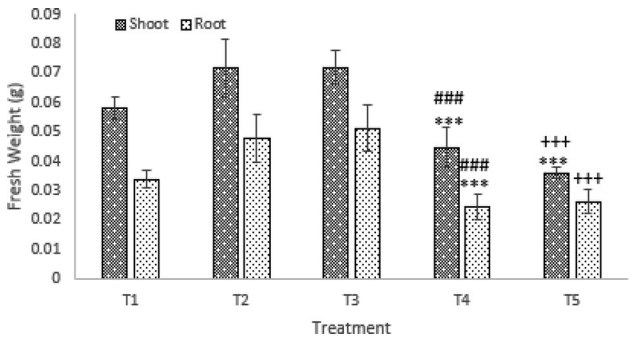

Fig. 5 Effects of binary treatment i.e., T_1 (Al_{50}), T_2 ($Mo_{0.5}$), T_3 (Mo_2), T_4 ($Al_{50}Mo_{0.5}$) and T_5 ($Al_{50}Mo_2$) on fresh tissue weight in the *T. durum* var. HI8737. Data represent the means ± SD of three independent experiments (n = 3). using t.test where, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ for T_4 and T_5 with respect to T_1 (Al_{50}), $^{\#}P < 0.\ 05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ for T_4 with respect to T_2 ($Mo_{0.5}$) and $^{+}P < 0.\ 05$, $^{+}P < 0.01$, $^{+++}P < 0.001$ for T_5 with respect to T_3 (Mo_2)

and 5 µM Mo in root as well as in shoot (Fig. 4b). The observed correlation was stronger ($R^2 = 0.864$) with root than shoot ($R^2 = 0.724$). Significant increase in APX activity of root resulted at 0.5–2 µM Mo and of shoot at all the concentrations, though the activity was lower than root (Fig. 4c). The Gu-POX activity increased significantly in root at 2 and 5 µM with $R^2 = 0.570$, while it remained unaffected in shoot (Fig. 4d). MDA and H_2O_2 content were observed to be high in shoot as compared to root (Fig. 4e, f). Significant decrease in MDA content was observed at all the concentrations of Mo in root with very strong correlation ($R^2 = 0.820$) whereas in shoot MDA content reduced at and above 0.5 µM with perfect correlation ($R^2 = 0.971$). The H_2O_2 content decreased significantly at and above 0.5 µM Mo in root ($R^2 = 0.551$) and at all the concentrations in shoot with a very strong correlation ($R^2 = 0.831$).

Effect of Al–Mo interaction on antioxidative parameters

The interactive effects of Al at 50 µM (T_1) and Mo 0.5 µM (T_2) and 2 µM (T_3) were studied in combination $Al_{50}Mo_{0.5}$ (T_4) and $Al_{50}Mo_2$ (T_5). Higher level of fresh tissue weight was maintained in T_2 and T_3 treatments as compared to T_1. Reduction in fresh weight of shoot as well as root was observed in binary treatments (T_4 and T_5) as compared to Al alone as well as respective Mo controls (Fig. 5). The SOD activity in both root as well as shoot was comparable for T_1, T_4 and T_5 treatments, but T_4 and T_5 had significantly lower level as compared to T_2 and T_3 respectively (Fig. 6a). The CAT activity significantly increased in root and shoot with treatment T_5 as compared to T_1 and in relation to T_2 and T_3 differential effect resulted showing significant increase for root in T_4 but significant decrease for shoot in T_5 (Fig. 6b). For APX activity in shoot significant increase resulted in T_4 and T_5 treatments as compared to T_1 and incomparison to Mo treatment the observed activity was significantly lower in T_4 whereas significantly higher in T_5 (Fig. 6c). No significant changes were observed in roots of T_4 and T_5 as compared respectively, to T_2 and T_3. MDA content in T_4 and T_5 root was significantly high as compared to T_2 and T_3 respectively but significant decrease in MDA in shoot of T_4 was observed (Fig. 6d). Remarkable decrease in H_2O_2 content resulted for T_5 being significant in shoot only (Fig. 6e). For T_4 treatment H_2O_2 content was signifcantly higher in both root and shoot with respect to T_2,whereas remarkable decrease was observed for T_5 being significant in shoot only (Fig. 6e).

Effect of Al–Mo interaction on NR activity and NO content

NR activity of root tissue was higher in T_2 and T_3 as compared to T_1, while T_4 and T_5 treatments increased it synergistically and significantly (Fig. 7a). The effect was insignificant for shoot tissue. Higher level of NO content was found in root as well as shoot with T_2, T_3, T_4 and T_5 treatments as compared to T_1 and was more substantial in T_2than $T_3$and in T_4 than T_5 (Fig. 7b). With T_4 treatment root NRA was significantly increased in relation to T_2, but shoot activity was reduced and the NO content was significantly increased in shoot with T_5 as compared to T_3 (Fig. 7a, b).

Discussion

Effect of aluminium on antioxidative parameters

Aluminium is one of the most abundant element in the soil. When the soil pH reduces below 5, Al forms soluble complexes that can be potentially phytotoxic. Root inhibition is considered as a primary symptom of Al toxicity. Under acidic condition, Al inhibits the root growth by disrupting root cell expansion and elongation (Kochian et al., 2005). In our study, decreased growth measured as fresh weight of shoot and root was observed in *T. durum* var. HD8737 with Al supply (Fig. 1a) showing adverse effect of Al on growth of HI 8737. One of the mechanisms of Al resistance in plants is suppression of reactive oxygen produced as a result of oxidative damage caused by Al. Further, Al tolerance in wheat occur due to increase in antioxidative enzymes (Aggarwal et al., 2015b; Dong et al., 2002; Liu et al., 2018). In the present study, Al effects analyzed for antioxidative enzymes in wheat seedlings under acidic conditions indicated that root tissue maintains higher activities than shoot (Fig. 1a–d). Thus root tissue

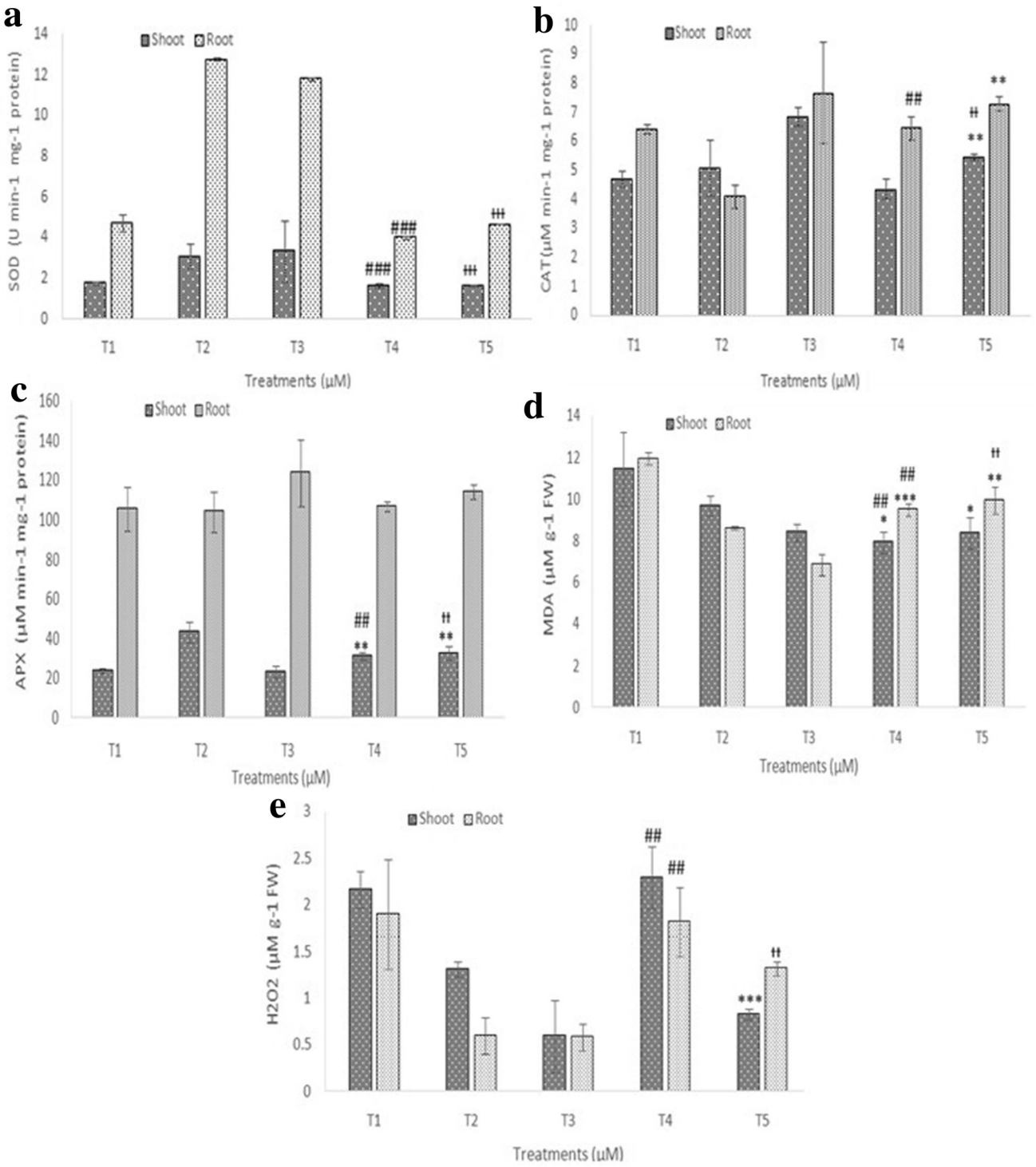

Fig. 6 Effects of binary treatment i.e., T_1 (Al_{50}), T_2 ($Mo_{0.5}$), T_3 (Mo_2), T_4 ($Al_{50}Mo_{0.5}$) and T_5 ($Al_{50}Mo_2$) on the SOD, CAT, APX, MDA and H_2O_2 concentration in the *T. durum* var. HI8737. Data represent the means ± SD of three independent experiments (n = 3). using t test where, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ for T_4 and T_5 with respect to T_1 (Al_{50}), $^{\#}P < 0.\ 05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ for T_4 with respect to T_2 ($Mo_{0.5}$) and $^{+}P < 0.\ 05$, $^{+}P < 0.01$, $^{+++}P < 0.001$ for T_5 with respect to T_3 (Mo_2)

seems to be the target site for Al stress in this wheat variety. Gradual decrease in SOD activity with Al treatment being significant (Fig. 2a) suggest concentration dependent increase of O_2^- radical, but with reduced scavenging activity at higher concentration and the observed stronger correlation in root. Other enzymes, like CAT and

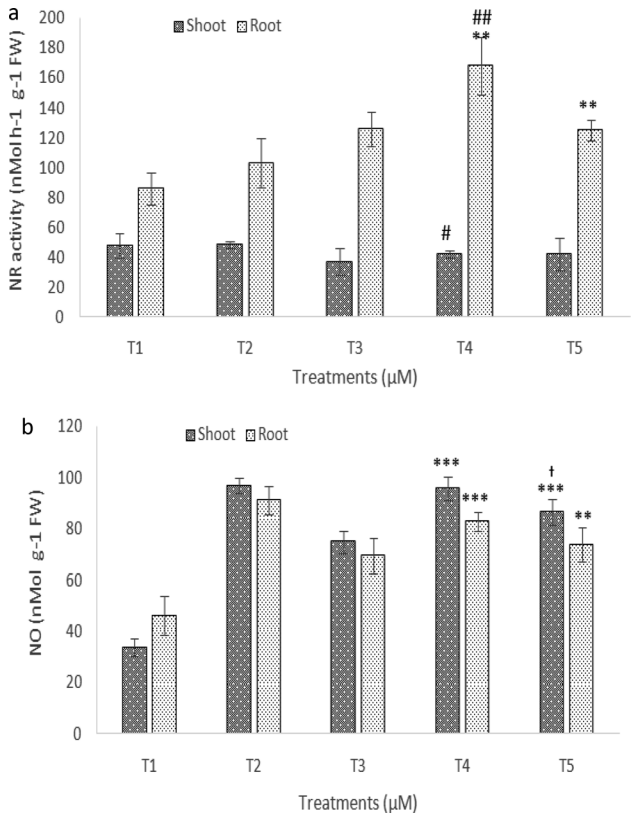

Fig. 7 Effects of binary treatment i.e., T_1 (Al_{50}), T_2 ($Mo_{0.5}$), T_3 (Mo_2), T_4 ($Al_{50}Mo_{0.5}$) and T_5 ($Al_{50}Mo_2$) on the NR activity and NO concentration in the *T. durum* var HI8737. Data represent the means ± SD of three independent experiments (n = 3) using t test where, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ for T_4 and T_5 with respect to T_1 (Al_{50}), $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ for T_4 with respect to T_2 ($Mo_{0.5}$) and $^{+}P < 0.05$, $^{+}P < 0.01$, $^{+++}P < 0.001$ for T_5 with respect to T_3 (Mo_2)

APX increased upto 50 µM Al and then decreased (Fig. 2b, c) and also more substantial increase in MDA content above 50 µM Al (Fig. 2e) suggest a tolerance capacity of the cultivar upto 50 µM Al under acidic conditions. Further Gu-POX activity in shoot increased significantly at and above 50 µM Al exerting strong correlation ($R^2 = 0.717$) (Fig. 2d) and the H_2O_2 content increased above 50 µM Al (Fig. 2f). Here it is worth to discuss antioxidative parameters analyzed in Al tolerant and sensitive varieties of wheat. Thus Liu et al. (2018) showed that upon Al application the activities of CAT and APX were higher while that of SOD and POD were lower in roots of the Al-tolerant (jian-865) genotype as compared to the Al-sensitive (yangmai-5) genotype of *Triticum aestivum*. These alterations occurred concomitantly with the inhibition of root growth and increased lipid peroxidation. In a study conducted by Xu et al. (2011) Al increases accumulation of H_2O_2 and $O_2^{\cdot-}$ leading to more predominant lipid peroxidation, programmed cell death and inhibition of root elongation in *Triticum astevium* genotype Yangmai-5 (Al-sensitive) than in Jian-864 (Al-tolerant). Moustaka et al. (2016) reported higher lipid peroxidation (MDA) in Al sensitive *Triticum astevium* cultivar (Dio) as compared to Al tolerant (YecoraE).

Effect of molybdenum on antioxidative parameters

Molybdenum is an essential micronutrient required for nitrogen, carbon and sulphur metabolism. Deficiency of Mo is a widespread agricultural problem, especially in acidic soils (Marschner, 1991). However, Mo deficiency or excess in soil has resulted in production of light weight immature seeds, poor in vigor and germination potential (Chatterjee & Nautiyal, 2001). Mo supply has been reported to induce wheat growth measured as dry weight (Imran et al., 2020; Wang et al., 1999; Wu et al., 2014) and also different root growth parameters (Imran et al., 2020). While high dosage of Mo have negative effect on wheat growth (Buekers et al., 2010). In our study significant increase in root weight was observed (Fig. 3), thus suggesting a beneficial role of Mo in growth. Also, Mo application can potentially overcome low temperature stress by increasing antioxidant enzyme activity, such as, SOD, CAT and POX (Sun et al., 2006). Mo effects in wheat seedlings of the present study has shown increase in SOD activity of both root and shoot with higher level and strong correlation in root (Fig. 4a) indicating a role for Mo in scavenging oxidative stress induced generation of $O_2^{\cdot-}$ radical. Increased CAT and APX activity (Fig. 4b, c) while reduced MDA and H_2O_2 content (Fig. 4e, f) suggest that application of Mo has the potential to elevate antioxidative parameters thereby overcoming stress.

Effect of Al–Mo interaction on antioxidative parameters

Exogenous application of ammonium molybdate induces resistance against various stress via enhancing antioxidant defense mechanism (Nie et al., 2015; Sun et al., 2006; Wu et al., 2014) and by inducing nitric oxide (Wu et al., 2017). Al toxicity under acidic soil condition is prominent phenomenon and none of the reports regarding alleviating effect of Mo on Al stress has been found. The interactive effects of Al and Mo were investigated in this study to evaluate it. In this Al–Mo interactive effects have shown, lower fresh tissue weight as compared to single Al treatment (Fig. 5a), indicating the ineffectiveness of Mo to improve fresh tissue weight reduction caused by Al stress. The binary treatments (T_4 and T_5) have SOD activities comparable to Al treatment (T_1) alone (Fig. 6a) indicating that Mo do not exert any protective effect against Al stress induced $O_2^{\cdot-}$ radical generation. Although Mo supply has

increased SOD activity (Fig. 4a) suggesting a role for Mo in scavenging oxidative stress induced generation of $O_2^{\cdot-}$ radical. However, Mo supplementation effectively reduced Al induced lipid peroxidation, as MDA content is significantly reduced in root as well as shoot of T_4 and T_5 treatments as compared to T_1 (Fig. 6d). Al stress effects for CAT and APX activities and H_2O_2 content partially overcome by higher concentration of Mo in shoot, as these parameters were significantly altered in T_5 treatment (Fig. 6b, c, e). Thus the study reports for the first time that Mo has potential to alleviate Al stress to some extent.

Effect of Al–Mo interaction on NR activity and NO content

Mo requirement in plants is as Mo cofactor for enzymes, nitrogenase and nitrate reductase, involved in nitrogen metabolism. Nitrate reductase catalyzes the first and rate-limiting step of nitrate assimilation in fungi, algae and higher plants that often limits growth and productivity (Campbell, 2001). Increased activity of NR by Mo application has been found in wheat leaves (Wu et al., 2017) and under low temperature and acidic condition Mo enhances NR activity (Wang et al., 1999; Yaneva et al., 1996). In addition to nitrate assimilatory function, NR has been reported to be involved in the synthesis of NO (Yamasaki & Sakihama, 1999). NO is a bioactive signaling molecule exerting both the beneficial and harmful effects in plant cells and is involved in different stresses (Magdalena & Jolanta, 2007). Studies on wheat using Al-induced oxidative stress suggests that exogenous supply of NO affects plant growth by arresting oxidative stress in root and reducing respiratory dysfunction resulting from Al stress (He et al., 2006, 2007). Zhang et al. (2008) suggested that NO boosted Al tolerance in wheat seedlings by not only reducing membrane lipid peroxidation damage and H_2O_2 but also increased SOD, CAT, APX activities and proline content. Higher NR activity of root tissue and NO content in root as well as shoot in T_2, T_3, T_4 and T_5 treatments (Fig. 7a, b) suggest a role of Mo to overcome Al stress via NO signaling partially.

Conclusion

The influence of Al, Mo and their interaction were analyzed under acidic condition. Antioxidative enzyme activity was more prominent in root as compared to shoots in single as well as binary experiments. Activity of SOD reduced with increase in the Al concentration while CAT and APX significantly reduced after 50 µM. Parameters such as, MDA and H_2O_2 were more substantially increased above 50 µM Al, suggesting tolerance of *T. durum* var. HI 8737 upto 50 µM Al. Significant increase of SOD, CAT, APX was observed with application of Mo, but MDA and H_2O_2 content decreased. With combination of Al–Mo treatment, the effect of Al stress is mitigated by Mo at least partly, as concentration of MDA and H_2O_2 were reduced and CAT and APX activities were enhanced for binary treatments. Further, increased NRA and NO content for combined Al–Mo treatment suggested NO mediated effect of Mo in alleviation of Al toxicity. Thus supplementation of Mo can be favorable in increasing plant resistance under Al toxicity in acid soil condition.

Declarations

Conflict of interest The authors declare that they have no conflict of interests.

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