



Oxidative enzymes activity during abiotic and biotic stresses in *Zea mays* leaves and roots exposed to Cu, methyl jasmonate and *Trigonotylus caelestialium*

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Abstract The activities of antioxidative enzymes, i.e. superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), and guaiacol peroxidase (GPX), in the leaves and roots of *Zea mays* L. plants exposed to abiotic (methyl jasmonate, MJ, or/and copper, Cu) and biotic (*Trigonotylus caelestialium*) factors were examined. The contribution of MJ as a signal molecule in the defense mechanism against abiotic and biotic stresses was studied. All plants were cultivated hydroponically and divided into three groups: not treated by abiotic factors (control), treated by MJ only (MJ) and by MJ and Cu (MJ + Cu) and in each group half of the plants were exposed to *T. caelestialium* attack. The enzymatic activities of SOD, CAT, APX, and GPX in the leaves were higher in the insect-treated than non-insect-treated control plants, but lower in both MJ + Cu- or MJ- and insect-treated plants. In the roots, the enzyme activities were elevated in all insect-treated plants with the highest rise in MJ + Cu, in comparison with the MJ-treated plants. The results showed that MJ and MJ + Cu were efficient in reducing the activity of the antioxidative enzymes in the leaves under the insect influence by elevating enzyme activity in the roots.

Keywords Catalase · Insect · Peroxidase · Maize · Methyl jasmonate · Superoxide dismutase

Abbreviations

APX	Ascorbate peroxidase
CAT	Catalase
MJ	Methyl jasmonate
GPX	Guaiacol peroxidase
ROS	Reactive oxygen species
SOD	Superoxide dismutase

Both abiotic (e.g. Cu excess) and biotic (e.g. insect attack) stresses trigger cross-talk among hormones, including jasmonic acid signaling pathway (Paudel et al. 2013) and generate reactive oxygen species (ROS), which can be directly toxic towards insects (Wasternack and House 2013). Therefore, the activities of enzymes, used as markers of oxidative stress, are crucial, i.e. superoxide dismutase (SOD) degrading O_2^- to H_2O_2 and catalase (CAT), ascorbate peroxidase (APX), and guaiacol peroxidases (GPX) converting H_2O_2 to H_2O .

Plants attacked by herbivorous insects induce volatile organic compounds, such as methyl jasmonate (MJ) to regulate the attack by attracting other organisms that destroy damaging insects (Turlings et al. 2012), or to inform other plants about a coming danger to prepare for fighting (Kim and Felton 2013). The defense signaling against chewing insects is mediated by the biosynthesis, transport, and perception of molecules of jasmonates pathway and the following interaction of these molecules with other plant hormones and messengers (Boughton et al. 2006; Tian et al. 2014).

Maize (*Z. mays* L.) is a crucial agricultural crop in the world after wheat and rice. Moreover, it is used not only as a food, but also as a pharmaceutical and industrial product. Also it is worth studying insect infestations by the Holarctic plant bug *T. caelestialium* (Kirkaldy)

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(Heteroptera: Miridae, Stenodemini) as one of the possible biotic stresses to maize limiting its productivity. Moreover, there is a limited number of papers studying *T. caelestialium* on plants used in agriculture (Ito 2004) and exposed to exogenous jasmonates (Koramutla et al. 2014).

The information about the exact regulation of ROS by MJ in the insect attack is still patchy. The aim of this study was to determine the activities of the antioxidative enzymes, SOD, CAT, APX, and GPX, in the leaves and roots of *Z. mays* L. plants exposed to abiotic factors, Cu and MJ (in concentrations chosen on the grounds of the previously published data; Hanaka et al. 2015, 2016) and a biotic factor, *T. caelestialium*.

Trigonotylus caelestialium insects were collected from grasslands near Lublin, Poland. The insects were reared in a plastic cage in an insect-rearing room at 25 ± 1 °C under a 16 h photoperiod and maintained on grass and maize leaves, which were exchanged with new ones every 3 days. The sucking insects at the 4th larval stage were taken to the experiment with plants.

Zea mays L. cv. Reduta plants were grown hydroponically on the Hoagland nutrient solution (3 plants per pot). The plants were cultivated in a growth chamber at 25 °C (day)/18 °C (night) under a 16 h photoperiod and photosynthetic photon flux density of $130 \mu\text{mol m}^{-2} \text{s}^{-1}$. 10-day-old plants were divided into three groups: cultivated without abiotic stress (control), treated by MJ for 24 h (MJ) and treated by MJ for 24 h and then transferred to a fresh Hoagland nutrient solution with addition of Cu^{2+} for 5 h (MJ + Cu). MJ at 10 μM and Cu at 50 μM (in the form of $\text{CuSO}_4 \times 5 \text{H}_2\text{O}$) were used and applied straight to the Hoagland solution. In each group, half of the 13-day plants were additionally exposed to insect attack, then covered with a plastic cage enabling gas exchange for the following 7 days. After that period, all insects were adult. 20-day-old leaves and roots were cut and frozen in liquid nitrogen for analyses of SOD, CAT, APX, and GPX.

Proteins concentration were determined according to Bradford (1976). Activities of SOD, CAT, APX, and GPX were measured following Verma and Dubey (2003), Aebi (1984), Nakano and Asada (1987), and Milosevic and Slusarenko (1996), respectively.

All data were analyzed using one-way ANOVA and Fisher's post hoc test with significance determined at $p < 0.05$ on Statistica ver. 6 software (SoftStat, Inc., USA). The mean values (three to five separate experiments with at least three replicates each) \pm SE were presented.

The total protein concentration in the leaves was from 1.99 to 3.29 times higher than in the roots, with the highest difference detected for the MJ + Cu- and MJ-treated plants in the presence of insects (Table 1). In the leaves, all agents applied to the plants, compared with the control, significantly elevated the protein concentration, and the most

pronounced rise was detected for both the MJ- and insect-treated plants in the following order (and statistical significance): MJ + insect (a) > MJ – insect (b) = MJ + Cu + insect (b) > MJ + Cu – insect (c) = control + insect (c) > control – insect (d) (Table 1). In the roots, the MJ + Cu- or MJ-treated plants without the presence of insects achieved the highest protein concentration and the exact order was as follows (with statistical significance): MJ + insect (a) = MJ – insect (a) = MJ + Cu – insect (a) > control – insect (b) > control + insect (c) = MJ + Cu + insect (c) (Table 1).

The higher activities of SOD and CAT in the leaves than in the roots were accompanied by higher activities of APX and GPX in the roots than in the leaves (Fig. 1a–h). Both in the leaves and roots, higher activity of the enzymes was detected in the control plants with *T. caelestialium*, in comparison with the control plants not sucked by the insect (Fig. 1a–h).

In the leaves, the most pronounced enzyme activity was detected without insect sucking after MJ + Cu or MJ supplementation, but it declined after the insect treatments (Fig. 1a, c, e, g). In the leaves of the insect-treated plants, after MJ + Cu or MJ addition, the enzyme activity was the same or lower than that in the insect-treated control (Fig. 1a, c, e, g).

In the roots of the *T. caelestialium*-stimulated maize plants, the enzyme activities were significantly higher than in all corresponding treatments without the insect infestation (Fig. 1b, d, f, h). The most pronounced activities were detected in the MJ + Cu- and MJ-treated plants after the insect attack with the highest activity observed in the first of the cases mentioned (Fig. 1b, d, f, h).

The insects themselves caused enhanced stress measured as elevated activity of antioxidant enzymes in all root treatments and in the leaves of control plants (Fig. 1a–h). Contrary to this statement, the insects reduced stress interpreted as reduced activity of enzymes in the leaves of the MJ + Cu- or MJ-treated plants in comparison with the non-insect-treated plants (Fig. 1a–h).

The concentration of total proteins, which take part in protection against stress conditions, was significantly higher in the leaves of *Z. mays* than in the roots. Such a result suggests that leaves can be better protected than the roots. Moreover, in our research, exogenous MJ under insect attack caused production and significant accumulation of proteins in the leaves, but to a lesser extent also in the roots. Maize plants tested in the experiment represent diversified activity of antioxidative enzymes between the leaves and the roots, which is in accordance with our previous results (Strubińska and Hanaka 2011; Hanaka et al. 2016). *Zea mays* “enzymatic kit” seems to be well-balanced because the activity of two enzymes, SOD and CAT, is higher in the leaves, but activity of both

Table 1 Protein concentration in *Zea mays* leaves and roots exposed to abiotic, i.e. MJ (10 μ M applied for 24 h) or both MJ (10 μ M applied for 24 h) and Cu (50 μ M applied for 5 h), and biotic (*T. caelestialium*) factors

Treatment	Protein concentration [% of control without insect]	
	Leaves	Roots
MJ + Cu without insect	114 c	128 a
MJ without insect	128 b	119 a
Control with insect	113 c	86 c
MJ + Cu with insect	124 b	84 c
MJ with insect	145 a	102 a

Different letters indicate significant differences between the treatments. Values are the means of three–five separate experiments \pm SE (three replicates each)

peroxidases, APX and GPX, is higher in the roots. In the plant reaction to adverse conditions, a broad range of proteins is involved, thus besides detoxifying enzymes, e.g. SOD, CAT, APX, and GPX, also genes encoding pathogenesis-related proteins are induced by MJ (Faurie et al. 2009). At the early stage of insect attack, it seems that MJ treatment causes rapid increase of O_2^- and H_2O_2 and subsequently elevated activities of SOD, CAT, and peroxidases detoxify ROS molecules (Boughton et al. 2006; Koramutla et al. 2014; Farooq et al. 2016). In our experiment as well as in the papers of Botelho-Júnior et al. (2008), Ye et al. (2013), and Rigsby et al. (2016), MJ application elevates activity of peroxidases. It is known that peroxidases participate in lignification, as a response to the pathogen infection and wounding (Rigsby et al. 2016). Moreover, in the response to herbivore attack, concentrations of detoxifying enzymes in plants could be elevated in a MJ dose-dependent manner (Boughton et al. 2006). We proved that the roots react statistically more spectacularly than the leaves because in all measurements with the insects, the activity of enzymes increased. It means that the information about a stimulus acting in one part of plant body (an insect sucking the leaf) is quickly moved to the distant parts (the roots), e.g. by MJ. Thus, the defense system works systemically and helps the plant to survive in a changing environment.

Cellular redox changes appear in response to the mechanical damage after insect feeding (Paudel et al. 2013). In our experiment, the insect attack elicited response in the plant organism by elevating antioxidant enzyme activities in all root treatments and in the leaves of control plants, which was connected with increased O_2^- and H_2O_2 accumulation in plant tissues (Wasternack and Hause 2013). In the roots, independent of the presence of insects, MJ addition reduced enzyme activities in comparison with MJ + Cu supplementation (except for CAT activity). Furthermore, during metal treatment, the level of jasmonates is reduced, which suggests a defensive role in mitigation of excess ROS (Dar et al. 2015). The present results have shown the effect of Cu on defense against

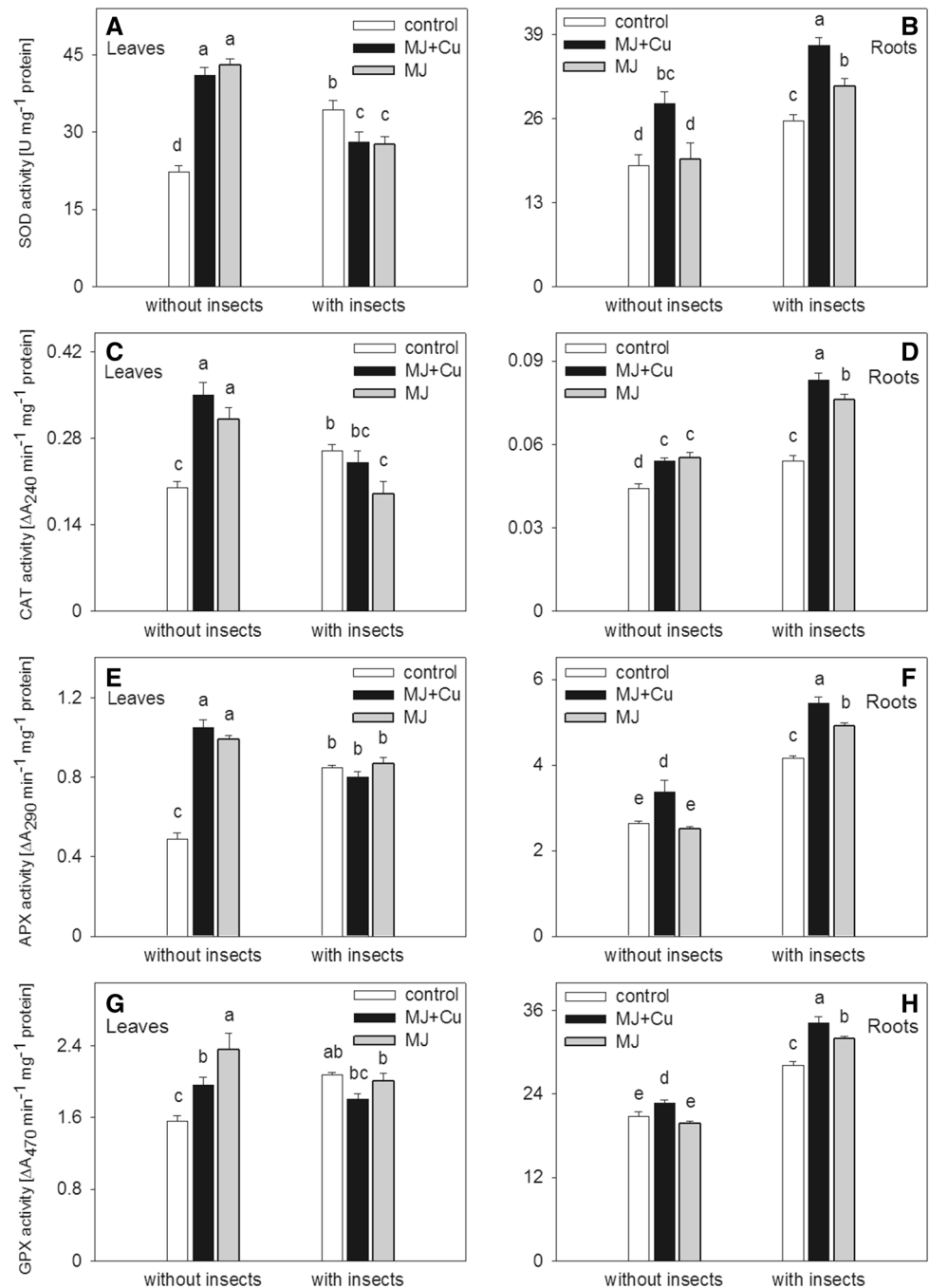
insects by reduction of the stress level in the leaves and simultaneously enhancement thereof in the roots.

Exogenous application of MJ reduced feeding damage caused by insects through minimizing the number and size of feeding scars (Fedderwitz et al. 2016), or reducing the growth rate of insect population (Koramutla et al. 2014), but not through deterring the insects from attacking the plant (Fedderwitz et al. 2016). Furthermore, MJ stimulated defense genes expression and increased density of glandular trichomes on leaves, which negatively influenced the herbivore population (Boughton et al. 2006; Horgan et al. 2009; Tian et al. 2014). Moreover, MJ applied exogenously induced reactions similar to the natural wounding, which proves that MJ can be a substantial molecule in stimulating plant resistance in the natural environment (Thaler et al. 2012). On the other hand, endogenous MJ production is stimulated by the damage signals for which the molecular pattern seems to be similar to the insect attack (Wasternack and Hause 2013).

It should be also mentioned that MJ pathway do not play its role in the isolation from other defense systems. For instance, MJ treatment may result in the enhancement of trypsin protease inhibitor levels (Ye et al. 2013) or production of enzymes involved in the SA and JA biosynthesis (i.e. lipoxygenase, allene oxide synthase) (Botelho-Júnior et al. 2008). Moreover, most scientists confirm the JA–SA antagonistic interaction (Zhang et al. 2017). But in *Z. mays* plants attacked by caterpillars, no antagonistic crosstalk between the JA and SA pathways was stated (Rostás et al. 2013). On the other hand, it was proved that the elevated levels of SA in Cu treated maize plants were directly responsible for JA induction (Engelberth et al. 2011). It means that in *Zea* plants relationship between the JA and SA pathways in a specific stress can be confusing and should not be generalized.

In conclusion, our results have shown that, compared with the control, the MJ treatment did not change the antioxidative enzyme activities in the plant roots not subjected to the insect attack (except for the elevation of CAT activity), whereas in the same conditions, MJ + Cu

Fig. 1 Activities of SOD (a), CAT (b), APX (c), and GPX (d) in *Zea mays* leaves and roots exposed to abiotic, i.e. MJ (10 μ M applied for 24 h) or both MJ (10 μ M applied for 24 h) and Cu (50 μ M applied for 5 h), and biotic (*T. caelestialium*) factors. Different letters indicate significant differences between the treatments. Values are the means of three–five separate experiments \pm SE (three replicates each)



elevated the enzyme activities. Moreover, both MJ and Cu were potent molecules in modulating enzyme activity in the presence of the sucking insect because they prepared the roots for reception of the stress, in consequence limiting the stress in the leaves. This implies involvement of the MJ-dependent signaling pathway in the plant defense against the insect attack under the experimental conditions used. Thus, MJ has a potential in agricultural research and application.

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Compliance with ethical standards

Conflict of interest The authors declare there is no conflict of interest.

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