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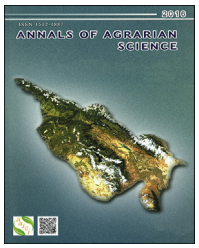

Effect of soil herbicides on the antioxidant system of maize vegetative organs during ontogenesis

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ABSTRACT

The impact of soil herbicides Harnes, Frontier and Merlin on the activity of enzymes superoxid dismutase (SOD, EC 1.15.1.1), catalase (CAT, EC 1.11.1.6), and benzidine peroxidase (POD, EC 1.11.1.7) in maize (*Zea mays* L.; cultivar Kadr 267 MV) roots and leaves was studied in the field experiment. It was established that the adaptation of maize plants to the herbicides treatment was accompanied by significant activation of antioxidant enzymes both in roots (39%, 57%, and 67% above control level) and leaves (50%, 64%, and 77% above control), respectively during different vegetation stages (shoots emergence; 3–5 leaves phase; florescence). The herbicides-induced changes of enzymes activity high correlated with ontogenetic dynamics of control plants activity: r = 0.98 for SOD; r = 0.96 for POD; r = 0.98 for CAT.

Introduction

The wide use of herbicides in the crops based on selectivity which eliminates the possibility of herbicides impact to their sites of action in the cells of crop plants [1]. However, recent research suggested the significant influence of herbicides treatment on the ontogenesis of different agricultural plants. For example, reducing leaves length and surface area, and seed production decline in maize [2], and increase of sorghum pollen sterility [3], and the violation of chlorophyll fluorescence in wheat leaves [4] were caused by herbicides action. It was shown in our previous work [5] that increase in 1000 seeds weight, reduction of protein and glutathione content, and antioxidant enzymes activity changes in maize mature seeds should be attributed to the aftereffect of herbicides Harnes, Stellar, Mayster and Proponit. For today, the state of the antioxidant enzyme system in maize vegetative organs under the influence of herbicides has been studied in fragments [6,7]. The objective of this study was to investigate whether the soil herbicides cause the activation of maize antioxidant enzymes during early stage of ontogenesis only, or the herbicides treatment has a prolonged effect on the defense system of plants.

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Objectives and methods

The test objects were roots and leaves of maize (*Zea mays* L.; cultivar Kadr 267 MV) plants from field experimental plots of Institute of Steppe Zone Agriculture (Dnipropetrovsk, Ukraine). The plots soil was the ordinary black soil with the humus content 3.8–4.2%, and with the neutral reaction of the soil solution (pH = 6.75–7.0). Herbicide Harnes was introduced into the soil at a dose 2.5 L/ha; Frontier – at a dose 1.5 L/ha; Merlin – at a dose 0.13 kg/ha, and any herbicide was introduced on control plots.

The roots and leaves of 7–10 plants were selected during different stages of plant vegetation (I – shoots emergence; II – phase of 3–5 leaves; III – florescence). Supernatants for determination of enzymes activity were obtained by centrifugation (12,000 g for 20 min at 4 °C) of plants' crude extracts. The detection of enzymatic activity was made by spectrophotometric registration of optical density of reactive mixtures. SOD activity estimation was based on method of Ranieri et al. [8]. Benzidine-POD activity determined by Gregory [9] method. The activity of CAT was measured in according to Goth [10]. All determinations were performed in three replicates. Significance of differences was estimated using Student's t-test ($P < 0.05$).

Results and analysis

The activity of SOD both in maize roots and leaves exceeds control level significantly ($P < 0.05$) during all ontogenetic phases which were examined, as shown in Fig. 1.

Increase in SOD activity under herbicide Harnes action amounted to 39%, 49%, and 61% above control in maize roots, and 50%, 64%, 74% in leaves, respectively during shoots emergence, phase of 3–5 leaves, and florescence. The action of herbicide Frontier caused the similar increase in SOD activity both in maize roots and leaves. The highest SOD activation was initiated by herbicide Merlin in maize roots (41%, 57%, and 67% above control) and leaves (54%, 66%, and 76% above control), respectively during I, II, and III ontogenetic stages.

In our study, benzidine-POD activity grew significantly ($P < 0.05$) under the action of all herbicides in comparison with control both in maize roots and leaves during developmental stages, as shown in Fig. 2.

Herbicide Harnes influence resulted in reliable ($P < 0.05$) POD activation in roots (37%, 51%, and 59% above control) and in leaves (49%, 64%, and 74% above control), respectively during shoots emergence, 3–5 leaves phase, and florescence. The similar enchancing POD activity both in roots and leaves was observed under Frontier impact. The highest level of enzyme activation was caused by Merlin in maize leaves (66% and 77% above control) during II and III phases of ontogenesis.

The results proved that activity of CAT both in maize roots and leaves increased under all herbicides impact in comparison with the control plants during stage of shoots emergence, phase of 3–5 leaves, and florescence, as shown in Fig. 3.

An exceeding control level of CAT activity under the impact of Harnes has reached 38%, 54%, and 62% in roots and 50%, 64%, and 66% in leaves, respectively at I, II and III exemined phases of maize growth. The influence of herbicide Frontier caused the similar increase in enzyme activity in both maize organs. The highest CAT activity was observed under the Merlin impact in roots (57% and 67% above control) and in leaves (65% and 77% above control), respectively at phases of 3–5 leaves and florescence of maize plants.

The herbicides Harnes and Frontier belong to chloroacetanilides which are able to delay the plant cells division; herbicide Merlin can blocking the synthesis of carotenoids. In our study, the prevail enchancing each enzyme activity was observed under the impact of Merlin both in maize roots and leaves. This effect should be attributed to the specificity of Merlin mode of action with the ability to reactivation in the soil. All herbicides are xenobiotics regardless of mode of action, and their penetration into plant cells causes the disturbance of the redox balance, and involves reactive oxygen species (ROS) accumulation [11]. Maintaining an optimal level of free radical processes in plant cells is provided by enzyme superoxid dismutase whose functioning under stresses leads to the excessive accumulation of hydrogen peroxide [12]. In turn, the enzymes catalase and peroxidase ensure the preservation of a safe level of toxic H_2O_2 in the plant cells [13]. Peroxidases are responsible for the scavenging of H_2O_2 by the oxidation of different substrats [12], but catalases don't require the reductants to eliminate H_2O_2.

Our results showed that herbicides action was accompanied by the significant enchansing both CAT and POD activities in maize roots and leaves. It well known that the presence of a few enzymes with the same catalytic function expands the plant organism adaptable possibilities which are very important to sustain the vital functions under stress [14]. Consequently, the adaptation of the maize plants to soil herbicides impact was assosiated with the induction both of catalase and peroxidase pathway of H_2O_2 scavenging.

In our study, high correlation coefficients of herbicides-induced activity of SOD ($r = 0.98$), POD ($r = 0.96$), and CAT ($r = 0.98$) were revealed with enzymes activity in control maize plants during respective ontogenetic phases. These data indicate the coordinated changes of maize roots and leaves antioxidant system under herbicides treatment. This conclusion is consistent with the statement of Jaleel et al. [15] that the antioxidant enzymes activities in plants are upregulated in response to several abiotic stresses. In addition, the results obtained confirm the prolonged effect of the soil herbicides impact on the activity of the defense antioxidant system of maize plants.

Conclusion

Soil herbicides Harnes, Frontier and Merlin caused the significant increase in activity of key antioxidant enzymes both in maize roots and leaves during phases of shoots emergence, 3–5 leaves, and florescence of plants. The changes of SOD, POD, and CAT activities have a coordinated character in both maize organs regardless of the mode of herbicide action. The prolonged action of soil herbicides on the maize