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SEED IMBIBITION BY IMAZAMOX INDUCES ACTIVATION OF GLUTATHIONE S-TRANSFERASES (GSTS) IN WHEAT SEEDLINGS

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Abstract

Imidazolinone herbicides are widely used in agriculture. In some cases, they may be very persistent in soils, generating risks for the next crop in crop rotation. In several plant species, glutathione S-transferases (GSTs) take part in the detoxification of imazamox, but there exist no reports about their activity in wheat. Our study evaluated the effects of the imbibition of wheat seeds in imazamox containing solutions (10, 25 and 50 μM) on seedling growth, protein content as well as on the activity of GSTs. Growth of the young wheat seedlings was significantly inhibited after imbibition of the seeds in imazamox containing solutions. The protein content of both roots and shoots was lower compared to controls. This was partially due to a lower acetohydroxy acid synthase (AHAS) activity, leading to slowed protein turnover. The GSTs activity, determined using two different substrates 1-Chloro-2,4-dinitrobenzene (CDNB) and p-nitrophenyl acetate (pNPA), was enhanced, indicating their possible participation in cellular defence mechanisms against imazamox. The obtained new information about GSTs will be further verified in our subsequent studies.

Key words: imazamox, wheat, glutathione S-transferases (GSTs), acetohydroxy acid synthase (AHAS)

INTRODUCTION

Imazamox is an herbicide of the imidazolinone group whose mechanism of action is blocking the acetohydroxy acid synthase activity (AHAS, EC 2.2.1.6). AHAS is a crucial enzyme in the biosynthesis of branched chain amino acids and thus, the IMI herbicides inhibit the protein turnover, leading to the death of IMI-susceptible (IMI-S) plants (Tan et al., 2005). The imidazolinone herbicides are characterized by high effectiveness at low doses and a broad spectrum of weed control, but also by a high persistence in soils (Loux and Reese, 1993). Most studies report a half-life for imidazolinone herbicides between 10 and 100 days, but some authors also report periods longer than 600 days (Gherke et al., 2021). The half-life of imazamox varies between 17 and 92 days depending on soil type, initial concentration, temperature and soil moisture (Vischetti et al., 2002). In the case of crop rotation, the residual amounts of imazamox may affect subsequent sensitive crops (Liu et al., 2016). Adverse effects due to residues of imazamox in soils have been reported in sorghum, corn, rice and millet (Cobucci et al., 1998), but not in wheat. It has been reported that wheat was not very sensitive and was not significantly negatively affected by imazamox residues (Pannacci et al., 2006; Suzer and Boyuk, 2010).

In some imidazolinone resistant (IMI-R) crops, it has been reported that in addition to target site resistance, some non-target mechanisms, like fortified detoxification, are involved in imidazolinone resistance (Dominguez-Mendez et al., 2017). Garcia-Garijo et al. (2014) reported activation of GSTs in imazamox-treated IMI-R seedlings of common bean and vetch. In a previous study, we found that the GSTs contribute to the resistance in IMI-R sunflower genotypes (Balabanova et al., 2018; Balabanova et al., 2020). Manabe

(2007) found enhanced expression of some detoxification genes, including GSTs, in response to imazapyr application on A. thaliana. Recently, Arda et al. (2020) reported that imazamox accumulation leads to changes of the GST gene expression in IMI-S and IMI-R sunflower hybrids. Concerning the effect of imazamox on GSTs activity in IMI-S wheat, no reports were found, which motivates the current study.

The mentioned reports suggest that the GSTs enzyme family may be involved in the plant cellular defence against imazamox, however, the available database is still limited to prove or reject this statement. Our study aimed to establish whether and if yes - to what extent GSTs enzymes are triggered by imazamox in IMI-S wheat seedlings.

MATERIALS AND METHODS

Wheat (*Triticum aestivum* L.) seeds (cv. Karina; IMI-S) were soaked for 4 h in water containing different concentrations of the commercial product Pulsar 40® (imazamox) (10, 25 and 50 μM). The applied imazamox concentrations were chosen after preliminary study using as a criteria observed degrees of chronic toxicity in wheat seedlings. Subsequently, the seeds were sown in plastic cups (10 seeds / 200 ml cup) filled with perlite and irrigated with ½ Hoagland solution. Each variant was presented in triplet (3 cups). The growth and biochemical analyses were done 10 days after the treatment (DAT). The experiment was repeated twice with identical results. The data on one representative experiment are presented and discussed here.

The protein extraction was performed according to Schröder and Götzberger (1997). The protein concentrations were measured according to Bradford (1976).

Acetohydroxyacid synthase (AHAS, EC 2.2.1.6) enzyme activity was measured according to Ray (1984), with some modifications given elsewhere (Balabanova et al., 2020).

Glutathione-S-transferase (GST, EC 2.5.1.18) activity assays were carried out following the method of Jakoby and Habig (1981).

The statistical analysis was performed using one-way ANOVA (for $P < 0.05$). The Duncan test for the mean comparison was performed based on ANOVA results for a 95% confidence level to test for significant differences among treatments. In the figures, different letters (a, b, c) express significant differences.

RESULTS AND DISCUSSION

Winter wheat is often sown after IMI-R crops such as soybean, sunflower and maize. Therefore, the consequences of imazamox residues for wheat growth and functioning are of high interest. Although some authors reported considerable damages and yield reductions to rotational crops (mentioned above) due to insufficient imazamox decay, the reports for its carryover in wheat suggest relatively low susceptibility. Pannacci (2006) reported that wheat was not very sensitive to imazamox residues. Suzer and Boyuk (2010) demonstrated that stand establishment and seed yield of wheat, barley, and maize were not significantly affected by imazamox residues in either year. The wheat seedlings in our study were exposed to chronic phytotoxic concentrations of imazamox and therefore their growth was retarded. The inhibition of the plant fresh weight was 34, 47 and 59 % for 10, 25 and 50 μM imazamox, respectively (Fig. 1B). Similar inhibitions were observed for the length of both leaves and roots. The damaging effect of imazamox on wheat seedlings was accompanied by a reduced protein content (Fig. 1C), caused by blocking the AHAS enzyme activity (Fig. 1D).

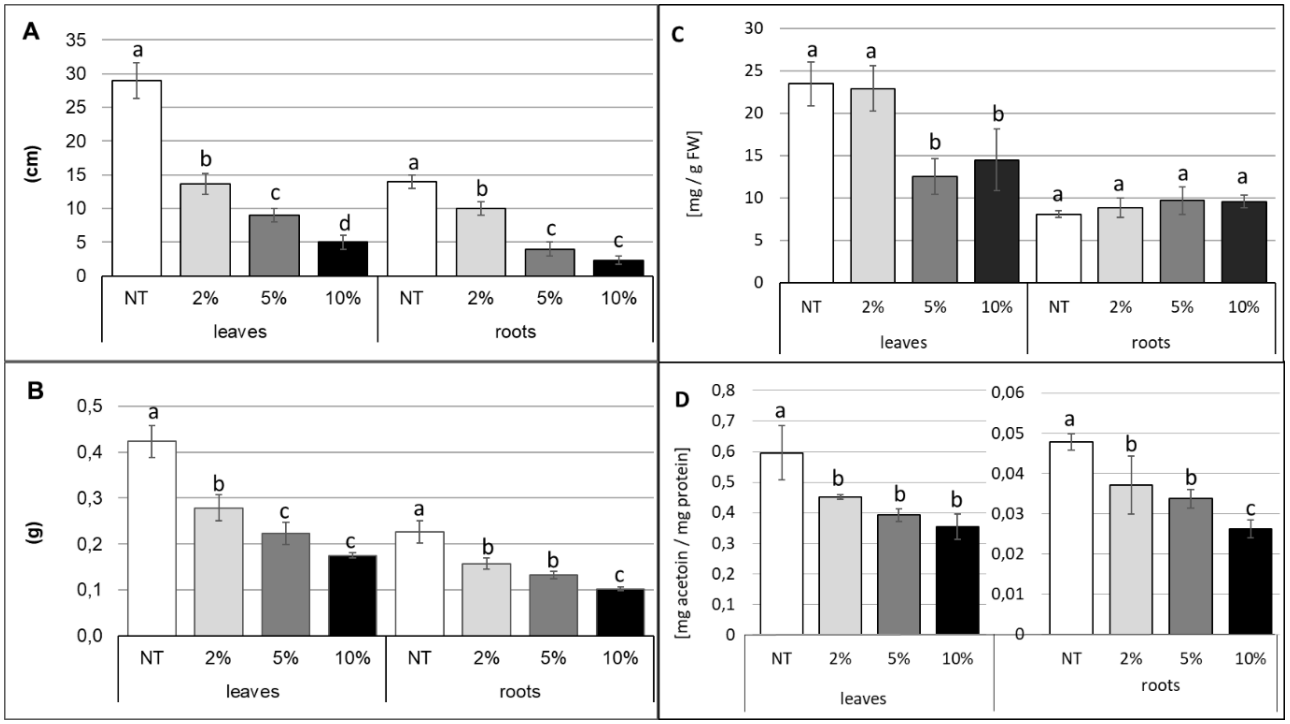

Figure 1: Growth parameters (A - plant height; B - fresh weight); C - protein content and D – acetohydroxyacid synthase (AHAS) enzyme activity of young wheat seedlings after seed imbibition with different doses of the herbicide imazamox. The values represent the mean and SD of three biological replicates. Different letters (a, b, c) express significant differences ($P < 0.05$).

The detoxification mechanisms aim to defend the plant cell from the damaging effect caused by xenobiotic compounds such as pesticides. However, the best-investigated group of plant enzymes implicated in herbicide detoxification is undoubtedly the GSTs, where CDNB is a common substrate (Riechers et al., 2010). In our experiment, its activity increases due to imazamox imbibition of the seeds in both leaves and wheat roots (Table 1). The maximal activity is observed in leaves of wheat grown from seeds that were imbibed with 10 µM imazamox, which is 41 % and 54% higher than the values found for the non-treated control for CDNB and pNPA substrates, respectively. The activity is also significantly increased in the roots. These results undeniably indicate the activation of cellular detoxification mechanisms. The results of both used substrates overlap, which further confirms the activation of GSTs in IMI-S wheat due to residual amounts of imazamox. These results correspond to those of Arda et al. (2020), who reported that the GSTs gene expression increased in all imazamox treated groups of IMI-S sunflowers. This activation of detoxification mechanisms in IMI-S species is not sufficient to prevent the harmful effects of imazamox caused on the protein metabolism, which inevitably leads to the observed growth retardation of the seedlings. In addition, the detoxification rate of imazamox by GSTs in IMI-S wheat is significant but not as high compared to IMI-R cultivars. The results correspond to those obtained by Rojano-Delgado (2014) who reported that IMI-S wheat cultivars are metabolizing the imazamox more slowly than resistant cultivars.

Table 1: Activity of glutathione S-transferases, substrate CDNB and pNPA, in young wheat seedlings after seed imbibition with different doses of the herbicide imazamox. The values represent the mean and SD of three biological replicates. Different letters (a, b) express significant differences between treatments at each time point ($P < 0.05$).

substrates	*treatment*	*GSTs enzyme activity*	
		leaves	***roots***
CDNB *[mU/mg protein]*	*NT*	104,9 ± 7,5 (b)	29,3 ± 1,7 (c)
	10 μM	177,8 ± 25,6 (a)	34,9 ± 4,5 (bc)
	25 μM	149,5 ± 20,6 (a)	45,5 ± 6,9 (b)
	50 μM	142,8 ± 38,8 (a)	63,5 ± 11,2 (a)
pNPA *[mU/mg protein]*	*NT*	11,74 ± 1,3 (b)	not detected
	10 μM	33,48 ± 7,4 (a)	not detected
	25 μM	26,79 ± 7 (a)	not detected
	50 μM	26,22 ± 5,9 (a)	not detected

CONCLUSION

The imbibition of wheat seeds by the herbicide imazamox had an inhibiting effect on the growth of wheat seedlings. The activation of the xenobiotic detoxification mechanism through the enhanced activities of GSTs in IMI-S wheat is noticeable. It demonstrates that GSTs are taking part in the cellular defence mechanisms against imazamox in wheat seedlings. According to our knowledge this is the first report showing the involvement of GSTs in imazamox detoxification in wheat. Nevertheless, the activation of GSTs is not sufficient to compensate for the detrimental effects of imazamox on the protein metabolism and plant functioning.

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