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THE AMELIORATIVE EFFECT OF PROTEIN HYDROLYSATE ON THE IMAZAMOX-DAMAGED YOUNG WHEAT PLANTS

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Abstract

The plant biostimulants (PBs) are a wide range of microbial and/or organic compounds applied to crops to improve the physiological processes such as nutrition efficiency, plant development and abiotic stress tolerance. Imazamox is a herbicide characterised with a wide spectrum of weed control, low application rates and low mammalian toxicity, but also with a high soil persistence. Therefore, the residual amounts of imazamox may negatively affect subsequent sensitive crops in the crop rotation. In the current study we investigated the effect of a single and combined treatment with imazamox and a plant biostimulant (protein hydrolisate) on the antioxidative defense system and the detoxification metabolism of wheat young plants. The result showed that the seed imbibition with 10 μM imazamox inhibits the growth of the young wheat plants. A slight improvement was found due to the additional treatment with protein hydrolysate of the wheat plants damaged by imazamox herbicide. According to the results, this improving effect on the growth does not ameliorate the plant detoxification metabolism such as glutathione S-transferases or antioxidative defense. However, the improving effect is low and insufficient to restore the plant growth and functioning and its effects on wheat production are not studied yet.

Key words: imazamox, wheat, biostimulant, protein hydrolysate

INTRODUCTION

A plant biostimulant (PB) is any substance or microorganism applied to plants aiming to improve the nutrition efficiency, abiotic stress tolerance and/or crop quality, regardless of its nutrients content, by stimulating the plant physiological processes (du Jardin., 2015; Colla et al., 2017). The biostimulants have gained sustainable interest in recent years, because they stimulate the plant development and reduce fertilizers consumption (Kunicki et al., 2010). They benefit plants via biological interactions in the cultivated ecosystem and are often included in the agricultural management practices to reduce the input of chemicals and restore the natural equilibrium in agro-ecosystems (Woo and Pepe, 2018). Moreover, these products do not lead to the accumulation of harmful ingredients and residues in soil or crops. The plant-derived protein hydrolysates (PHs) are an important group of PBs based on a mixture of peptides and amino acids. They are produced mainly by enzymatic and/or chemical hydrolysis of proteins from an animal- or plant-derived materials. Many studies have reported the benefits of PHs applications on growth, yield, product quality and tolerance to abiotic stress factors on crops (Colla et al., 2017)

Weeds are interrupting crop plants, leading to high yield losses that necessitate weed control practices crucial in ensuring sufficient food production for the rapidly increasing human population. The herbicides and the integrated weed management are the most widely used weed management tools for farmers and plant growers. The new technologies using herbicide-tolerant crops in combination with selective herbicides give an

additional superiority and positively affect the environment (Hussain et al., 2021). However, the herbicides can affect target or non-target plants within or near the target area during the treatment by a herbicide drift, amiss application or overdose and after the treatment and harvest as residual amounts (Sea et al., 2012).

Imazamox is a part of the imidazolinone herbicide family (IMI), which mode of action is blocking the activity of the enzyme acetohydroxy acid synthase (AHAS, EC 2.2.1.6), also known as an acetolactate synthase (ALS) (Tan et al., 2005). The AHAS enzyme is a key enzyme in the biosynthetic pathway of branched chain amino acids valine, leucine and isoleucine. Therefore the IMI herbicide inhibits the protein turnover in plants, leading to the death of IMI-susceptible species. Imazamox is widely used in many herbicide tolerant crops as it provides effective control against annual and perennial, parasitic, grasses and broadleaf weeds (Hess et al., 2010). It also possesses a high crop selectivity and might be applied either as a pre- or post-emergence weed management tool (Hess et al., 2010). In addition, imazamox possesses a favorable environmental profile (low application rate and toxicity) (Shaner and Singh, 1997), making it preferable to many farmers and this further contributes to its widespread use in agriculture.

The half-life of the herbicide imazamox in the soil varies between 17.1 and 92.4 days, depending on the amounts sprayed (initial concentration), temperature, soil moisture, soil type and microbiological activity (Vischetti et al., 2002). Imidazolinone degradation in soil occurs mainly through biodegradation. The herbicide biodegradation rate depends on environmental conditions and the bioavailability of the herbicide in the soil. The bioavailability of the herbicide is affected by soil conditions, where with a higher bioavailability in soil with less clay texture, higher pH, higher soil moisture and a moderate organic content (Gehrke et al., 2021). However, their residual amounts may have on subsequent sensitive crops in the crop rotation (Liu et al., 2016). In a greenhouse biological test, it was found that sensitive species such as sugar beet and spinach could be damaged after imazamox, thus requiring additional time between replanting the affected areas (Pannacci et al., 2006). Piao et al. (2002) also found that imazamox applied in high doses (50-100 g ha-1) was dangerous for species such as cabbage and potatoes. García-Garijo et al. (2012, 2013) found that the herbicide imazamox rapidly accumulates in the next crop rotation, such as beans and vetch, leading to inhibition of the AHAS enzyme mainly in growing tissues. Adverse effects due to the after-effects of the same herbicide have been reported in tomatoes (Umiljendic et al., 2016), pumpkin and sweet corn (O'Sullivan et al., 1998). Ball (2003) reported that imazamox applied to IMI-wheat caused damage to barley and oilseed rape 1 year after the treatment with the herbicide at low humidity and pH. There is also evidence of impairment as a result of the soil effects of imazamox on non-target crops such as wheat, barley and rapeseed (Yadav and Bhullar, 2014).

The possibility of restoration of the herbicide-damaged plants depends on the degree of structural and functional disorders. Some studies have shown that the herbicide phytotoxicity can be overcome (to some extent or completely) by applying biostimulants, foliar fertilizers, growth regulators, herbicide antidotes and others (Jablonkai, 2013). Soltani (2015) demonstrated a significant increase in the oat and winter wheat yields as a result of the addition of a biostimulant after a vegetative treatment of crops with the herbicides glyphosate, toprameson and atrazine. In our previous study, we found that due to the combined application of the herbicide imazamox and a biostimulant containing protein hydrolysates in IMI sunflower, the negative effect of the herbicide is significantly reduced (Balabanova et al., 2016).

The cited literature indicates that the plant biostimulants can positively affect the

herbicide-damaged plants, but their mechanisms of action on the physiological and biochemical processes in plants are not fully understood. There is also limited information on the effect of this type of biostimulant on the imidazoline-sensitive crops. This motivates us to study the biological response of wheat plants to (1) the excess of imazamox at seed bed level as well as (2) the subsequent foliar treatment with a protein hydrolysate (PH).]

MATERIALS AND METHODS

Experimental design. Wheat seeds (*Triticum aestivum* L., cv. Karina) were soaked for 6 hours in 200 lm, 10 µM imazamox (Pulsar 40®) or with tap water. After that, the seeds were sown in plastic cups, filled by perlite, enriched by ½ Hoagland solution (15 seeds per cup, cup volume 200 ml) and left for germination. Twelve-day-old wheat plants were separated in three groups, namely: (1) Non treated by imazamox plants (control), (2) Imazamox-treated plants as well as (3) Imazamox-treated plants receiving subsequent foliar treatment by a PH. Water solution of protein hydrolysate Terra-Sorb® Foliar (1%) was sprayed on the wheat shoots in a dose of approximately 2 ml per cap.

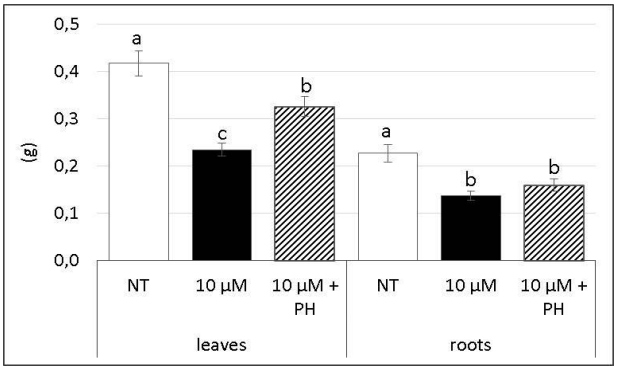

Figure 1: Dry weight of young wheat plants exposed to herbicide imazamox and/or protein hydrolysate (PH). The values represent the mean of three biological replicates. Different letters (a, b, c) express significant differences ($P < 0.05$).

Three repetitions (pots) were made for each treatment. Five days after the foliar treatment the dry weight of both roots and shoots of wheat plants were measured (Fig. 1). Samples for the biochemical analyses were taken, fixed in liquid nitrogen and stored in – 86 C° until measurements.

Protein extraction - according to Schröder and Götzberger (1997). The proteins were precipitated by the addition of ammonium sulfate in two steps of 40 and 80% saturation, respectively. The protein concentrations were measured spectrophotometrically according to the method described by Bradford (1976) using the Biorad Bradford Protein Assay and a bovine serum albumin standard curve.

Biochemical analyses

Protein extraction was done according to Schröder and Götzberger (1997).

Acetohydroxyacid synthase (AHAS, EC 2.2.1.6) activity was measured according to Ray (1984) with some modifications.

Glutathione-S-transferase (GST, EC 2.5.1.18) activity assays were carried out following the method of Jakoby and Habig (1981) with model substrates: 1-chloro-2,4-dinitrobenzene (CDNB), fluorodifen and p-nitrophenyl acetate (pNPA).

Glutathione reductase (GR, EC 1.8.1.7) activity was determined according to Zhang and Kirkham (1996).

Lipid peroxidation in shoots and roots was estimated by determining the concentration of thiobarbituric acid-reactive compounds (TBA-rm) spectrophotometrically according to Dhindsa et al. (1981).

Imazamox determination. Imazamox was extracted first with acetone and second with petroleum ether/dichloromethane mixture (1/1). After extraction 20 g of $NaSO_4$ were added, then 30 min incubation at room temperature. The solvents were evaporated to dryness on a rotary evaporator at 40°C bath temperature and the residues were dissolved in methanol/water mixture. The imazamox residues were analyzed by liquid chromatography LC-MS/MS tandem

quadrupole mass spectrometer Acquity XevoTQ UPLS/MS/MS, from Waters (Waters, USA) as was explained elsewhere (Balabanova et al., 2016).

RESULTS

The herbicide imazamox is inhibiting the growth of the young wheat plants by reducing the leaf dry mass with 46.5 % for a leaf and 39.2 % for the root weight. The additional application of PH has a slight ameliorative effect on the tested plants with 39 % and 15,8 % for the leaf and root mass, respectively, compared to the plants treated only with 10 μM imazamox.

The treatment of the IMI-S wheat plants with the herbicide imazamox leads to a significant inhibition of the AHAS enzyme activity, which is normal since the imidazolinone herbicides' mechanism inhibits the activity of AHAS. The protein content is not affected by the imazamox treatment. This could be because of the early measurement of the protein, where the inhibited AHAS enzyme could not yet have affected the protein turnover, while the enzyme inhibition is an immediate effect (Fig. 2). The addition of the plant biostimulant containing amino acids to the wheat plants imbibed with 10 μM imazamox does not considerably change the AHAS activity and the protein content.

Along with the biochemical measurements, we also analysed the imazamox content in shoots and roots of the wheat plants treated with imazamox (Table 1). The results show no difference between the samples with added PH and the one treated only with imazamox. In the leaves might be seen that the imazamox is slight but not significantly lower from the variant that received additional treatment with a biostimulant.

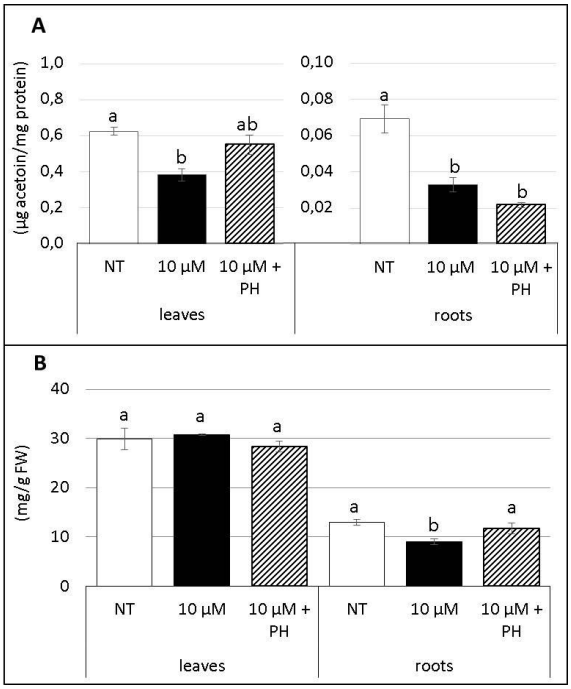

Figure 2: **A** - acetohydroxyacid synthase (AHAS) enzyme activity and **B** – protein content in young wheat plants, exposed to herbicide imazamox and/or protein hydrolysate (PH). The values represent the mean of three biological replicates. Different letters (a, b, c) express significant differences ($P < 0.05$).

Table 1: Imazamox content [mg/kg] in young wheat plants, exposed to herbicide imazamox and/or protein hydrolysate. The values represent the mean of three biological replicates. Different letters (a, b) express significant differences between treatments within each column ($P < 0.05$).

Treatment	Roots	Leaves
Non-treated plants	not detected	not detected
10 μM imazamox	0,11 ± 0,02 (a)	0,087 ± 0,04 (a)
10 μM imazamox + PH	0,10 ± 0,01 (a)	0,080 ± 0,01 (a)

The response of the antioxidative defense system of the wheat plants is shown in Fig. 3. The application of 10 μM imazamox significantly increases the activity of GPOD more than 5 times in leaves and 46% in roots of the wheat plants. GR activity is also significantly higher in imazamox treatment, which is well seen by the leave samples of

imazamox + PH – 49% and the root samples treated with imazamox – 150%. The level of lipid peroxidation (Fig. 3B) is not significantly changed by the imazamox treatment for both leaves and roots. The rate of MDA is not significantly changed by adding PH and the values of variants treated with imazamox and both imazamox + PH are similar.

The xenobiotic detoxification system of the plant cells consists of a few enzyme families, including GSTs, that dispose of the foreign compounds. The GSTs are taking part in the second phase of the detoxifying metabolism by conjugating with the metabolite glutathione (Dixon et al., 2002), and for this reason, their activity gives us important information about its functioning. From our results (Table 2), the GSTs activity measured with the substrate CDNB and pNPA is significantly increased in leaves with 56% and 106%, respectively, and the substrate fluorodifen in roots with 25%. The additional application of PH does not significantly affect the GSTs activity as the only exception is the activity with substrate pNPA, where the additional treatment with PH increases the GSTs activity by 54% compared to the activity in leaves treated with imazamox only.

DISCUSSION

The plant biostimulants are a new tool of the plant growers for a higher and good quality yield. They, therefore, are often included in the agricultural management practices aiming to reduce the input of chemicals and restoring the natural equilibrium in agro-ecosystems (Woo and Pepe, 2018). They function by stimulating the plant nutrition processes or improving the plant rhizosphere, leading to a higher tolerance to abiotic stress and ameliorating quality traits and yield (Woo and Pepe, 2018). The commercial formulations of PBs may contain various bioactive natural substances: (1) humic and fulvic acids, (2) animal and vegetal protein hydrolysates, (3) macroalgae seaweeds extracts, and (4) silicon, as well as beneficial microorganisms (Rouphael and Colla, 2020).

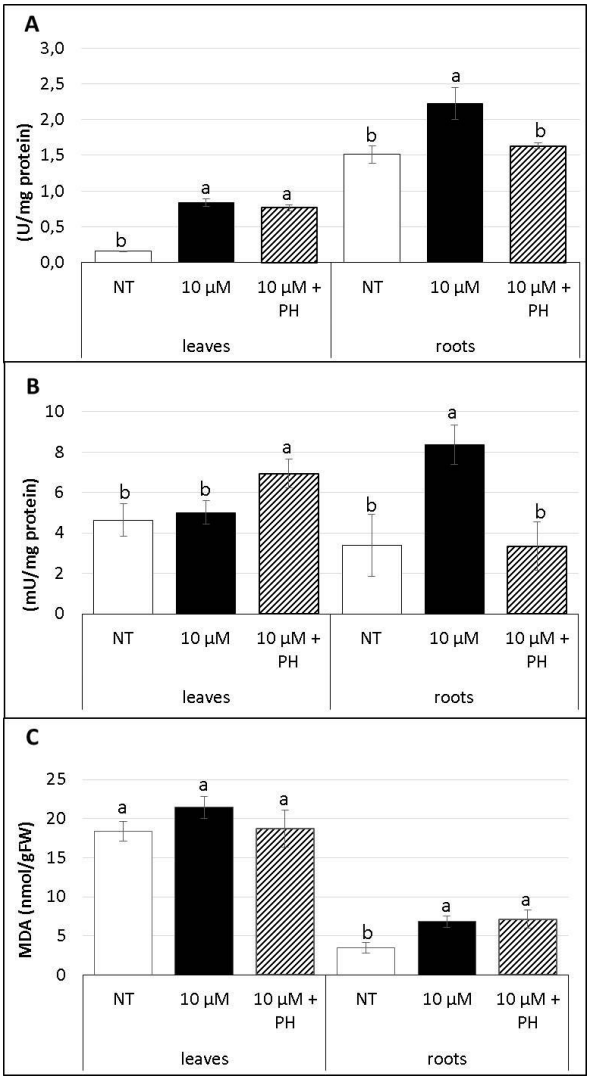

Figure 3: **A** - Guajacol peroxidase (GPOD) enzyme activity, **B** – glutathione reductase (GR) enzyme activity and **C** - lipid peroxidation in young wheat plants, exposed different doses of herbicide imazamox and/or protein hydrolysate (PH). The values represent the mean of three biological replicates. Different letters (a, b, c) express significant differences ($P < 0.05$).

Table 2: Activity of glutathione S-transferases, substrate CDNB, fluorodifen and pNPA, in young wheat plants, exposed different doses of herbicide imazamox and/or protein hydrolysate (PH). The values represent the mean of three biological replicates. Different letters (a, b) express significant differences ($P < 0.05$).

Substrate	Treatment	Leaves	Roots
CDNB [mU/mg protein]	Non-treated plants	131,7 ± 19,4 (b)	38,5 ± 2 (a)
	10 μM imazamox	204,9 ± 24,5 (a)	41,3 ± 12,2 (a)
	10 μM imazamox + PH	179,5 ± 11 (ab)	31,2 ± 10 (a)
Fluorodifen [μU/g protein]	Non-treated plants	76,8 ± 8,8 (a)	23,9 ± 3,1 (b)
	10 μM imazamox	71,7 ± 3,3 (b)	49,7 ± 14,3 (a)
	10 μM imazamox + PH	55,5 ± 10,8 (b)	51,9 ± 3,6 (a)
pNPA [mU/mg protein]	Non-treated plants	14,9 ± 1,1 (c)	Not detected
	10 μM imazamox	30,7 ± 0,1 (b)	Not detected
	10 μM imazamox + PH	47,2 ± 8,2 (a)	Not detected

The protein hydrolysates are obtained by chemical or biological hydrolysis. Sources of protein for hydrolysis can be plants, animals and/or microbial products. It has been reported that the protein hydrolysates might stimulate carbon and nitrogen metabolism in plants by the increase in gene expression and the activity of key enzymes of nitrogen assimilation and the Citric acid cycle such as nitrate and nitrite reductase, glutamine synthetase, glutamate synthase, aminotransferase and citrate synthase (Calvo et al., 2014; Nardi et al., 2016). The protein hydrolysates have been shown to activate the synthesis of various antioxidants (carotenoids, polyphenols, flavonoids, etc.), which increases their resistance to stress factors (Prado et al., 2007). There is reports for the ameliorative effect on plants injured by the herbicide application (Jablonkai, 2013; Soltani et al., 2015).

This study investigated the effect of the protein hydrolysate on the wheat plants injured by imazamox. Our results showed that the imbibition of seed in 10 μM imazamox inhibits the growth of the young wheat plants. This inhibition is also apparent after an additional PH application, but is less pronounced, which shows a slight ameliorative effect of the biostimulant on imazamox treated wheat plants. Our results show that, this improving effect on growth is not due to the amelioration of the plant detoxification metabolism. The imazamox residues in the plant tissues are not affected by the treatment with the plant biostimulant and the activity of GSTs does not rise markedly. The antioxidative defense system also does not show the emphasized activation that is facilitated by the amino acid addition. This leads us to suppose that the weak ameliorative effect of the protein hydrolysate might be due to its effect on the photosynthetic performance and/or mineral nutrition by their direct or indirect implementation in the photosynthetic compartments, enzymes or generally in the protein turnover. In our previous research on the imazamox effect on IMI sunflower, we found that it has a negative effect on both the light-light dependent photosynthetic redox reactions and the leaf gas exchange processes, which was much less pronounced after the combined application of imazamox and amino acid extract (Balabanova et al., 2016). The improving effect of the protein hydrolysate on wheat may be due to a similar effect, but this needs to be further studied in detail.

CONCLUSION

In summary, we found a slight improvement effect due to the additional treatment with protein hydrolysate of the wheat plants damaged by imazamox herbicides. This

ameliorative effect is not the result of the accelerated xenobiotic detoxification mechanisms or the antioxidative defense. However, the improving effect is low and insufficient to restore the plant growth and functioning and its effect on the wheat production is not yet studied.

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