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Letter to Editor

Glufosinate Phytotoxicity to Maize under Salt Stress Conditions

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Abstract

In this report we describe the responses of two maize varieties (Koka and Limko) to combined action of glufosinate and salinity. Glufosinate (phosphinothricin) is a non-selective herbicide that binds to the active site of glutamine synthetase (GS) and irreversibly inhibits this enzyme. Maize seedlings were grown in hydroponic cultures in complete nutrient solution under the following conditions: 16h photoperiod (220 $\mu mol \cdot m^{-2} \cdot s^{-1}$) at 26/20°C day/night, 65-70% relative humidity. The growth experiment (determination of dry matter production) was 2 × 2 factorial arrangement with two levels of NaCl in nutrient solution, 0 and 60 $mmol \cdot dm^{-3}$ NaCl and four levels of glufosinate in nutrient 0, 0.010, 0.025, 0.050 $mmol \cdot dm^{-3}$. Salt stress significantly decreased dry weight of both maize varieties. Glufosinate also caused reduction in growth of maize seedlings and the amount of inhibition was dependent on herbicide concentration and part of plant. Combined action of glufosinate and NaCl caused marked reduction in plant growth but in some cases (roots of Koka and shoots of Limko), this negative effect was mainly induced by NaCl. Biochemical analyses (determination of ammonium and nitrate concentration, content of water-soluble protein) were carried out at two levels of each treatment (0 and 0.050 $mmol \cdot dm^{-3}$ glufosinate, 0 and 60 $mmol \cdot dm^{-3}$ NaCl). Plants treated by glufosinate accumulated 3-4-fold more ammonium than control plants and also contained more nitrate than control. The combined action of glufosinate and NaCl resulted in a significant decrease in ammonium content in maize leaves compared to sole herbicide treatment, whereas in roots ammonium concentration remained at the same level. Under combined actions of herbicide and NaCl alterations in concentration of water-soluble protein and nitrate content were relatively small.

Keywords: glufosinate, salinity, ammonium, nitrate, maize growth

Introduction

Pesticides are increasingly used in modern crop management, and herbicides represent about 60% of the chemical additives used in agriculture [1]. Modern herbicides are non-toxic, readily degradable in the environment and used in low doses. But there is a risk of herbicide contamination in soil and injury to some crops in particular conditions. Such factors as light intensity, temperature, relative humidity, and nutrient level (including salt) stress may affect the efficacy and selectivity of herbicides [2-4]. Most studies are conducted under optimal conditions, hence limited data are available on relationships between herbicide efficacy and environmental stresses, particularly soil salinity. Salinization of land is a progressively increasing phenomenon, hence recognizing the interactions between salinity and herbicides is essential for more efficient application of herbicides and plants protection. In this report, we describe the response of two maize varieties to combined action of glufosinate and salinity. Glufosinate (phosphinothricin) is a

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non-selective herbicide that binds to the active site of glutamine synthetase (GS) and irreversibly inhibits this enzyme [5, 6]. GS plays a crucial role in plant nitrogen metabolism, it assimilates ammonium from photorespiration and nitrate reduction. Application of glufosinate causes glutamine deficiency and induces ammonium accumulation in plant tissues [5, 6]. Therefore, some researchers maintain that ammonium concentration in plant tissues may serve as an indicator of plant damage and the glufosinate tolerance index [7, 8].

Experimental Procedures

All experiments were conducted on two maize varieties (Koka and Limko; seeds were obtained from the "Nasiona Kobierzyc" Company) grown in hydroponic culture under the following conditions: 16h photoperiod (220 $\mu mol \cdot m^{-2} \cdot s^{-1}$) at 26/20°C day/night, 65-70% relative humidity. The composition of the nutrient solution was (in $mmol \cdot dm^{-3}$): 3 $Ca(NO_3)_2$, 2KNO_3, 1$MgSO_4$, 1KH_2PO_4, 0.2Fe-EDTA and microelements, pH 6.5±0.1. The experimental design consisted of the following treatments:

I control - complete nutrient solution,
II glufosinate treatment - complete nutrient solution plus herbicide at three concentrations: 0.010, 0.025, 0.050 $mmol \cdot dm^{-3}$,
III salt treatment – control plus 60 $mmol \cdot dm^{-3}$ NaCl and,
IV combined action of glufosinate and salt – control plus glufosinate and NaCl.

The experiment was arranged in a randomized complete block design and was repeated six times. After 7 days of cultivation plants were harvested, roots and shoots were separated and dried at 70ºC for 48 h and weighed for dry weight (DW) determination. Plant constituents such as ammonium, nitrate and water-soluble protein were extracted from fresh plant material (second leaf and roots). Protein was determined according to the Lowry method [9]. Nitrate and ammonium determinations were made, respectively, according to Cataldo et al. [10] and Weathernburn [11].

The data for all parameters were statistically analyzed using variance analysis, and differences among mean values of treatments were compared by the least significant difference test (LSD, $p<0.05$).

Results and Discussion

The results presented in Fig. 1. show that mild salt stress (60 $mmol \cdot dm^{-3}$ NaCl) significantly inhibited growth of both maize varieties. It is worth noting, that there were differences in susceptibility to salt stress between different organs of examined maize varieties. Roots of Limko were more tolerant to salinity than roots of Koka, whereas Koka shoots grew better in saline medium than shoots of Limko. But when considering salt sensitivity on the basis of the reduction of total dry mass both maize varieties are similarly salt-sensitive. Fortmeier and Schubert [12] classified maize (*Zea mays* L.) as a salt-sensitive crop, although there are differences in this sensitivity dependent on the variety. Examined maize varieties were also sensitive to glufosinate. Herbicide caused reduction in their growth and the amount of inhibition was dependent on herbicide concentration. Besides, reduction in growth of shoots and roots was not the same. Shoots of Limko were relatively tolerant to glufosinate, whereas roots were very sensitive. The highest concentration of glufosinate (0.050 $mmol \cdot dm^{-3}$) caused solely 12% reduction in dry mass of shoots, whereas root growth was reduced by 65% in comparison to control plants. The lowest herbicide concentration (0.010 $mmol \cdot dm^{-3}$) allowed 29% inhibition in growth of roots and did not inhibit shoot growth. Response of the Koka variety was different than Limko. 0.010 $mmol \cdot dm^{-3}$ glufosinate caused 19% reduction in shoot dry weight and did not fall significantly in root growth. The highest herbicide concentration (0.050 $mmol \cdot dm^{-3}$) caused marked decrease in dry weight of shoots (52% reduction) and roots (40% reduction compared to control). Obtained results show that the range of glufosinate concentrations that is toxic and well tolerated by maize seedlings is not very wide. Pornprom et al. [7] had tested 15 hybrid corn varieties and stated that there are varietal differences in tolerance to glufosinate. Further investigations conducted on three maize varieties (two refered as "tolerant" and one as "susceptible")

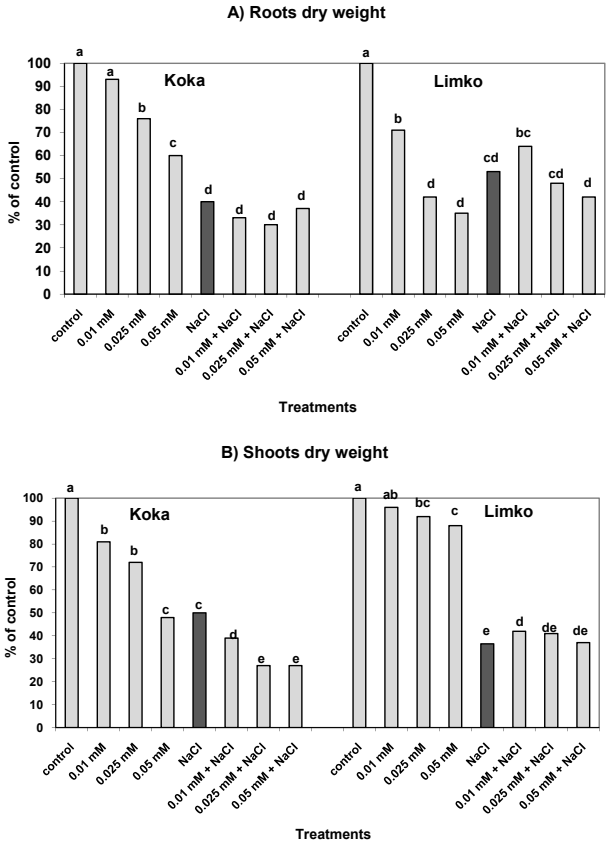

Fig. 1. The influence of glufosinate (at different concentrations: 0.01, 0.025 and 0.05 mM), 60 mM NaCl and combined action of glufosinate and NaCl on dry weight of Koka and Limko roots (A) and shoots (B). Values marked with the same letter do not differ significantly (5%).

Table 1. Influence of glufosinate (0.05 mmol·dm^{-3}), NaCl (60 mmol·dm^{-3}) and combination of glufosinate and NaCl on nitrogen compounds in roots and leaves of 7-day-old maize seedlings. Values in columns marked with the same letter do not differ significantly ($p<0.05$).

	NH_4^+ concentration [nmol(NH_4^+)×g^{-1} FW]		Protein content [mg×g^{-1} FW]		NO_3- concentration [mmol(NO_3^-)×g^{-1} FW]	
	Roots	Leaves	Roots	Leaves	Roots	Leaves
Koka						
Control	120 b	120 b	3400 c	8800 b	22 b	16 a
Glufosinate	450 a	400 a	5240 a	10000 a	28 a	18 a
NaCl	60 c	60 d	3700 b	7600 c	16 c	8 b
Glufosinate + NaCl	450 a	83 c	3400 c	8000 c	18 bc	14 a
$LSD_{0.05}$	50	23	238	695	6	5
Limko						
Control	110 b	140 bc	4150 bc	7400 ab	23 ab	14 b
Glufosinate	420 a	420 a	4950 a	7600 a	25 a	22 a
NaCl	70 b	100 c	4400 b	6800 bc	15 c	8 c
Glufosinate + NaCl	410 a	160 b	3800 c	6200 c	20 b	12 b
$LSD_{0.05}$	41	55	365	703	4	3

showed that a clear varietal difference in herbicide tolerance took place only at low doses of glufosinate. With increasing glufosinate doses all corn varieties responded similarly [7].

As mentioned above, the growth-response of Koka and Limko varieties to increasing concentrations of glufosinate was dependent on plant part. The amount of growth inhibition was different in shoots and roots, with the exception of Koka seedlings grown under 0.025 mmol·dm^{-3} glufosinate. In this case reduction in dry weight of shoots and roots was similar and it amounted to approximately 30% compared to control. Combined action of NaCl and herbicide resulted in significant alterations in growth of maize compared to control plants. In Koka variety, reduction in root growth was caused mainly by NaCl while in shoots the cooperative action of NaCl and glufosinate was synergistic viz. negative effect was greater than under each stress factor applied separately. Whereas in Limko roots the combined action of NaCl and glufosinate was antagonistic – observed response appears to be less than expected. Reduction in dry weight of Limko shoot was caused primarily by NaCl. However, the presence of herbicide at concentrations of 0.010 and 0.025 mmol·dm^{-3} improved growth of Limko shoots in comparison to plants grown in medium with sole NaCl.

Similar relations – a slight increase in shoot dry matter accumulation under combined action of NaCl and a low dose of herbicide – were observed in other maize varieties grown under NaCl plus rimsulfuron (inhibiting biosynthesis of branched amino acids) [3]. Biochemical analysis indicated that under glufosinate treatment (0.050 mmol·dm^{-3}) roots and shoots of both maize varieties accumulated high amounts of ammonium. Leaves accumulated approximately 3-fold more ammonium than control plants, whereas roots accumulated nearly 4-fold more compared to control. Regarding ammonium accumulation in plant tissues as an indicator of glufosinate tolerance it might be stated that Limko and Koka are similarly sensitive to this herbicide. Some reports indicated that glufosinate-susceptible cells/varieties accumulate more ammonia than tolerant ones [7, 8, 13]. Our results show that under glufosinate treatment maize seedlings also accumulated more nitrate than control plants, but a statistically significant increase was observed in leaves of Limko and roots of Koka. This might indicate that under glufosinate treatment nitrate assimilation is disturbed.

NaCl stress caused a marked decrease in nitrate and ammonium concentrations in roots and shoots of both maize varieties. It is well documented that salt stress decreases the uptake of nutrients such as NO_3^- and, consequently, nitrate content in plant tissues is very low [14-16]. It is interesting that under salt stress, plants accumulated less ammonium that control plants and under combined action of NaCl and herbicide ammonium concentration in leaves was significantly lower compared to plants grown in the presence of herbicide alone. In roots, the concentration of ammonium remained at the same level as under glufosinate alone. It is possible that under NaCl stress ammonium is utilized to synthesize nitrogen-containing compounds required for osmotic adjustment. It may be that under these conditions glutamate dehydrogenase (GDH) operates effectively. GDH plays an important role in stress resistance when the GS-GOGAT cycle is affected and ammonium

accumulates in plant tissues [17, 18]. Under the combined action of NaCl and herbicide, concentrations of nitrate were similar to that in control plants. Alterations (decrease or increase) in nitrate and ammonium concentrations in plant tissues did not affect accumulation of water-soluble protein. Under herbicide treatment leaves and roots of Koka contained significantly more water-soluble protein than control plants. A similar effect occurred in the roots of Limko and did not appear under salt stress and salt stress plus glufosinate.

Conclusions

Obtained results show that the growth response of examined maize varieties to sole glufosinate was dependent on the part of the plant. Combined action of glufosinate and NaCl caused marked reduction in plant growth but in some cases (roots of Koka and shoots Limko) this negative effect was mainly induced by NaCl. The presence of glufosinate in rooting medium resulted in a 3-4-fold increase in ammonium concentration in roots and leaves of both maize varieties, whereas combined action of glufosinate and NaCl resulted in a significant decrease in ammonium accumulation in maize leaves. Under the combined actions of herbicide and NaCl, alterations in the concentration of water-soluble protein and nitrate content were relatively small. Finally, it can be concluded that there are not clear relationships between phytotoxicity of glufosinate and salt stress.

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