ROLE OF GLUTATHIONE IN THE CHEMICAL STRESS-TOLERANCE OF PLANTS

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Abstract
In the research presented we tested the effects of the plant fertilizer ammonium sulphate and the chloroacetanilide herbicide acetochlor on the germination of maize and wheat seeds and the growth of the seedlings by studying the dose-response and time-course effects of the treatments. Possible antagonistic effects of the herbicide safener MG-191 and the L-cysteine precursor OTC to phytotoxicity caused by the fertilizer and the herbicide were also investigated. Our results show that low concentrations of ammonium sulphate and acetochlor (<100 mM and <100 μM, respectively) stimulate the plant growth, while higher concentrations (>100 mM and >100 μM) are inhibitory.

Keywords: glutathione, chemical stress-tolerance, herbicide, safener, OTC

Introduction

Glutathione (GSH, γ-L-glutamyl-L-cysteinylglycine) is the major non-protein thiol in plant cells. In some legume species it is replaced by homoglutathione (γ-L-glutamyl-L-cysteinyl-β-alanine). GSH plays many roles in plants, for example, in cell differentiation, growth and development, cell apoptosis and senescence, resistance against pathogens, enzymatic regulation, antioxidant, detoxification of xenobiotics, signal pathways, maintenance of cell homeostasis, and also in the storage and transport of reduced sulphur (Srivalli and Khanna-Chopra, 2008). The biosynthesis of GSH, among other factors, is influenced by environmental stress. Biotic (e.g. pathogen attack) and abiotic stress (e.g. drought, high temperature, salinity, and toxic chemicals) lead to the formation of reactive oxygen species (ROS) that cause oxidative stress in plant tissues. GSH reacts with ROS thereby reducing their concentration and antagonizing the stress effects.

Detoxification transformations of foreign organic compounds (pollutants or molecules synthesized by other organisms) in plants are determined by a highly complex set of biochemical and chemical reactions. Directions and rates of these reactions depend on the plant species, its developmental stage and fitness, as well as on a number of environmental (chemical, physical, and biological) factors. The reactions typically involve an oxidation step (Phase I, catalysed by cytochrome P-450 enzymes), a conjugation reaction with endogenous sugars or thiols (Phase II) (Behringer et al., 2011), followed by the elimination of the conjugate from the cytosol into the cell vacuole (Phase III, carried out by multidrug resistance-associated proteins) (Riechers et al., 2010). Conjugates in the vacuole are further processed in different reactions (Riechers et al., 2010); (Behringer et al., 2011) and the products may be built in the cell wall (Phase IV).

GSH provides protection to plants under stress in two ways: 1) under oxidative conditions it can be converted to glutathione disulfide (GSSG) (Noctor et al., 1998); (Srivalli and Khanna-Chopra, 2008) thereby acting as a reducing agent, and 2) under chemical stress conditions caused by different electrophilic xenobiotics (e.g., chemical carcinogens, environmental pollutants, antitumor agents, and several herbicides) GSH together with the enzyme glutathione-S-transferases (GST, EC 2.5.1.18) acts as protectant *via* converting the xenobiotic molecule to a non-toxic conjugate derivative in a Phase II type reaction (Hayes et al., 2005).

In addition, GSH functions as the most important storage of reduced sulfur in plants, and as a precursor of phytochelatins, which bind (and thereby detoxify) heavy metals (Noctor et al., 1998).

Acetochlor and other chloroacetanilide herbicides are used to control mono- and dicotyledonous weeds in maize. They are known to be detoxified by GSH conjugation catalyzed by GST.

Ammonium sulphate is a fertilizer that supplies nitrogen and sulphur to plants. At higher doses it damages the crop plants because of the toxicity of ammonium (Bittsánszky et al., 2015).

Safeners (also called herbicide antidotes) are chemicals used to protect crop plants against the toxic effects of herbicides by inducing herbicide tolerance in plants without affecting the herbicide sensitivity of the target weed species (Riechers et al., 2010); (Behringer et al., 2011). The biochemical mode of action of safeners involves the induction of Phases I, II, III, and IV. MG-191 is highly effective to protect maize plants against chloroacetanilide herbicide damage.

Objectives

The objectives of our research were to determine the effects of the plant fertilizer ammonium sulphate, the chloroacetanilide herbicide acetochlor (Figure 1), the herbicide safener MG-191 (Figure 1), the plant activator acibenzolar-methyl (Figure 1), and the antioxidant L-2-oxothiazolidine-4-carboxylate (OTC, Figure 1) on the germination and growth of maize (*Zea mays*) and wheat (*Triticum aestivum*) plants. Of these compounds acetochlor is used to control mono- and dicotyledonous weeds in maize, ammonium sulphate is applied as a mineral fertilizer that supplies nitrogen and sulphur to plant tissues, and MG-191 is highly effective safener that protects maize plants against chloroacetanilide herbicide damage. In plant cells OTC is known to be converted to L-cysteine that is rapidly built into GSH, thereby increasing its content.

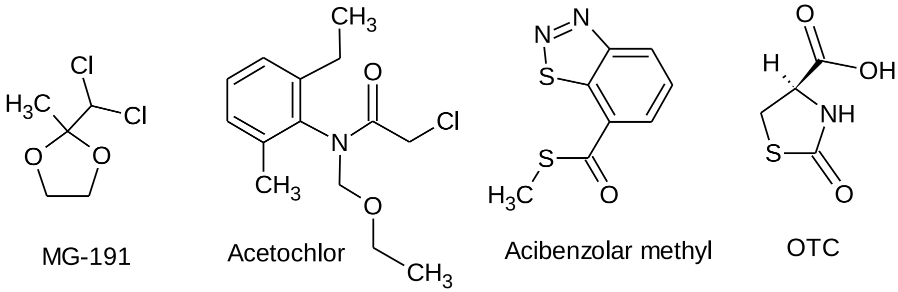

Figure 1. Chemical structures of compounds used in the study

Material and methods

Seed germination and seedling growth tests

Wheat (ARIESAN variety) and maize (MV 3122 hybrid) seeds were sterilized with 20x diluted hypochlorite solution then washed six times with distilled water. The sterilized seeds were germinated under controlled conditions on water for two-three days, followed by a treatment period of ten days. Germination tests were carried out in a plant growth chamber (Sanyo MLR-351, Versatile Environmental Test Chamber) at 22˚C, 70% relative humidity and 12 h illumination period/day with 2500 lx.

Treatment of seeds

Seeds were treated with different concentrations of ammonium sulphate and acetochlor to determine the effects of the chemicals on the germination of the seeds and the growth of the seedlings. The concentrations applied are listed in Table 1.

Table 1. The used treatments

Concentration of stock solution	Concentrations	Ammonium sulphate solution [mL]	Distilled water [mL]
900 mM ammonium sulphate solution	Control	-	100
	100 mM	11.1	75
	300 mM	33.5	50
	900 mM	100	-
Concentration of stock solution	Concentrations	Acetochlor solution [µL]	Distilled water [mL]
50 mM acetochlor	Control	-	100
	0.1 mM	200	100

solution	0.3 mM	600	100
	0.9 mM	1800	100

Results and discussion

Results of the treatments are summarized on Figures 2-5. The effect of ammonium sulphate on the maize seedling development can be observed on Figure 2. The toxicity of ammonium sulphate was observed for the used concentrations (100 mM-900 mM), inhibiting both the shoot and root growth. In case of the ammonium sulphate treatment the root development of maize was inhibited with 96.1%, whereas the shoot development of maize with 89%.

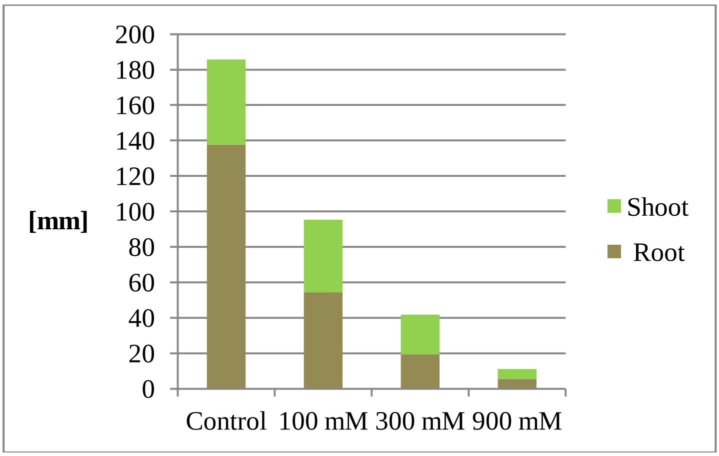

Figure 2. Effects of ammonium sulphate on the shoot and root growth of maize seedlings

The acetochlor treatment of the maize seedlings resulted in the inhibition of shoot and root development (Figure 3.) In case of acetochlor treatment the root development of maize was inhibited with 77% and the shoot development with 51%.

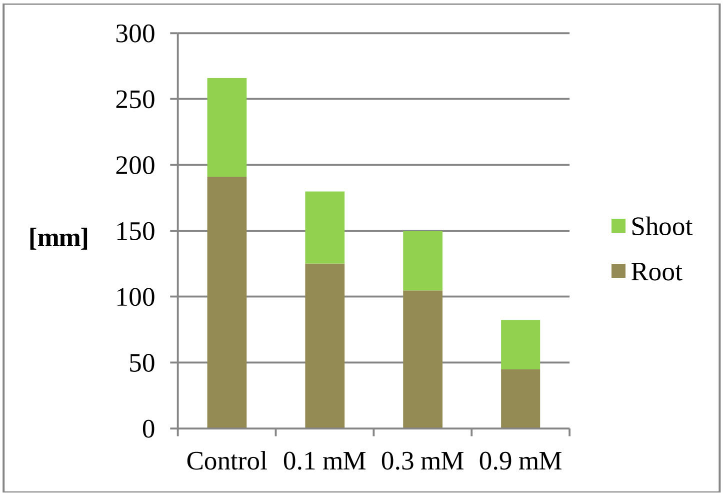

Figure 3. Effects of acetochlor on the shoot and root growth of maize seedlings

The ammonium sulphate treatment in used concentrations (100 mM-900 mM) resulted in the inhibition of wheat seedlings development (Figure 4.) In case of ammonium sulphate treatment of wheat seedlings the root development was inhibited with 74%, whereas the shoot development of wheat with 95.7%.

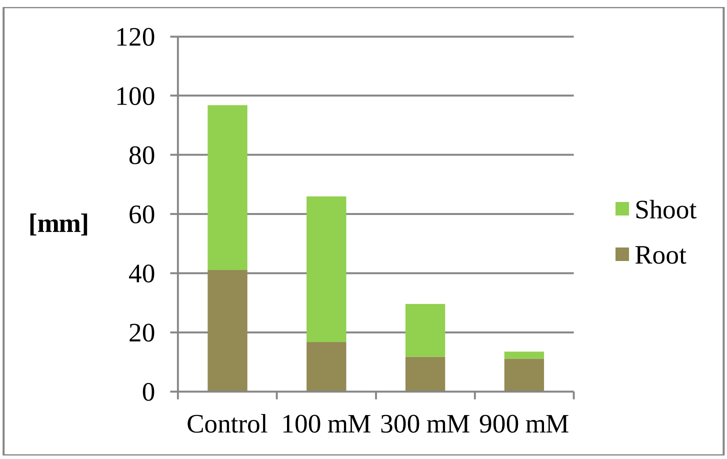

Figure 4. Effects of ammonium sulphate on the shoot and root growth of wheat seedlings

The phytotoxicity of acetochlor can be observed in Figure 5, the used concentrations (0.1 mM-0.9 mM) inhibited the development of root and shoot of wheat seedlings. The acetochlor treatment inhibited the root of wheat development with 90%, whereas the shoot development was inhibited with 85%.

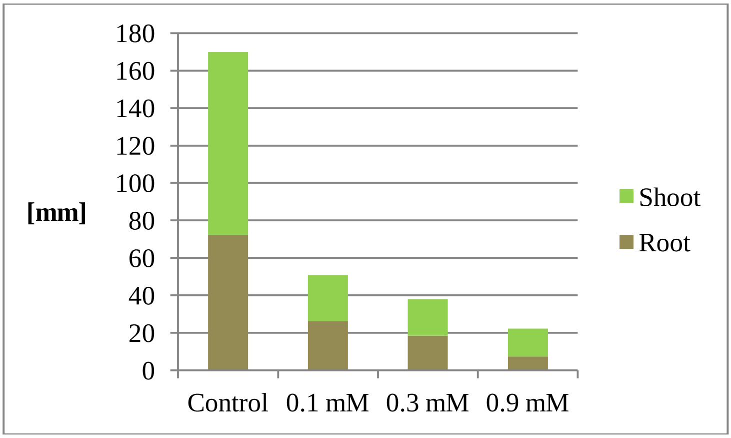

Figure 5. Effects of acetochlor on the shoot and root growth of wheat seedlings

Acetochlor was found to be moderately toxic to maize and wheat: it significantly inhibited the growth of maize seedlings at concentrations higher than 0.1 mM. On the other hand, ammonium sulphate stimulated the growth of maize and wheat seedlings at 100-times higher concentrations (10 mM) and became significantly phytotoxic only above 100 mM. All seeds germinated 100% with the exception of the highest concentration of ammonium sulphate (900 mM): at this dose germination levels of maize and wheat seeds were 80% and 40%, respectively. At this moment we have preliminary data on the effects of a combined treatment: they show that MG-191 (0.1 mM) increased the growth of maize seedlings and antagonized the toxic effects of 300 mM ammonium sulphate, but the effects did not reach the $p<0.05$ level of significance. Further research is necessary to investigate this phenomenon.

Conclusions

At different concentrations acetochlor and ammonium sulphate are toxic to maize and wheat plants. In order to protect the crop plants against these phytotoxic effects, chemicals may be applied that can increase the level of the endogenous thiol GSH. Further studies are necessary to determine the optimum timing and concentrations of these treatments.

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GLUTATION SZEREPE NÖVÉNYEK KÉMIAI STRESSZTŰRŐ KÉPESSÉGÉBEN

Összefoglaló: Jelen kutatásban vizsgáltuk az ammónium-szulfát műtrágya és az acetoklór herbicid (klóroacetanilid) hatását a kukorica és a búza magok csírázására. A kísérletek során a kezelések okozta dózis-válaszokat és az idő hatását vizsgáltuk a csírázás folyamatára. Az MG-191 herbicid antidótum és az OTC, mint L-cisztein prekurzor lehetséges antagonista hatásait is próbáltuk meghatározni a műtrágya és a herbicid okozta fitotoxicitás ellen. Eredményeink alapján elmondható, hogy az alacsony ammónium-szulfát és acetoklór koncentráció (<100 mM, illetve <100 μM) serkentette a növényi növekedést, míg a magasabb koncentrációk (>100 mM és >100 μM) gátló hatást fejtettek ki.

Kulcsszavak: glutation, kémiai stressztűrés, herbicid, herbicid antidótum, OTC