Effects of chlorsulfuron and cadmium on metabolites of maize seedlings

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Abstract

The impact of persisting herbicide residues on succeeding crops is of great concern to farmers because even the presence of very low concentrations can inhibit growth of crop and cause crop reduction. Furthermore, wastewater irrigation can lead to cadmium accumulation in soils. Thus, the co-occurrence of low amounts of herbicide residues and cadmium within agricultural fields are difficult to avoid. How the combination of these two pollutants affect plant metabolites remains to be elucidated and thus warrants investigation. Maize seeds were planted in soil that had been sprayed with chlorsulfuron and Cd, then we studied the effects of exposure to the herbicide chlorsulfuron (0.001, 0.003, 0.005, 0.008, and 0.010 mg kg⁻¹) and cadmium (as 5.0 mg kg⁻¹ CdCl₂) on maize seedlings by utilizing nuclear magnetic resonance (NMR) after 21 d. Principle component analysis of ¹H NMR spectra clearly discriminated between control and treatment groups. Compared with chlorsulfuron-only treatments, treatments using both contaminants showed higher content of phenolic acids, aspartic acid, choline, β-galactose, and α-glucose in the seedlings. Contrary to previous reports, we found larger pools of branched-chain amino acids in seedlings exposed to chlorsulfuron and CdCl₂. These findings indicate that CdCl₂ did not aggravate the effects of chlorsulfuron on maize seedlings metabolites. CdCl₂ elicited significant changes in plant metabolism at a concentration that did not impair plant growth. Moreover, chlorsulfuron did not inhibit branched chain amino acid synthesis.

Keywords: branched-chain amino acid, joint effect, metabolomics, nuclear magnetic resonance, Zea mays.

Introduction

Chlorsulfuron (2-chloro-N-[(4-methoxy-6-methyl-1,3,5-triazine-2-yl)aminocarbonyl]-benzene-sulfonamide) is a selective, systemic post-emergence herbicide. It was the first sulfonylurea herbicide approved in the United States (Zhou et al. 2020), and globally, it was one of the most commonly used herbicides of its type for removing broadleaf weeds and other weedy annuals in wheat and barley fields. According to existing reports, the primary target of chlorsulfuron and other sulfonylurea herbicides is acetolacetate synthase (ALS), the enzyme specific to branched-chain amino acid biosynthesis (Fan 2003). In addition, chlorsulfuron inhibits spermidine accumulation in mitotic tissues of root tips more effectively than other specific inhibitors of spermidine synthesis. Because of its high efficacy as a weed killer, chlorsulfuron is applied to wheat fields at very low concentrations. However, several field studies indicate a long residual time, with a half-life of 4 to 10 weeks, depending on soil properties (Shan 1998). Moreover, the previous study reported that chlorsulfuron persisted for 3 - 5 years in alkaline soils (Hollaway et al. 2006). The other study also reported that it would theoretically take 271.8 d for chlorsulfuron to degrade to 0.2 µg kg⁻¹ (below this amount is considered safe for rice roots) (Zhang et al. 2015). Chlorsulfuron has been used

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Abbreviations: ALS - acetolacetate synthase; CAT - catalase; GST - glutathione S - transferase; MDA - malondialdehyde; NMR - nuclear magnetic resonance; SOD - superoxide dismutase; POD - peroxidase; TSP - sodium-3-trimethylsilylpropionate.

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for several years in China and the soil in northern China is alkaline, so chlorsulfuron degrades slowly there. Thus, it is likely that application of chlorsulfuron produces residual effects on terrestrial plant reproduction.

The heavy metal cadmium has long been known for its detrimental physicochemical properties that can result in environmental damage. Consequently, the environmental discharge limit of Cd is far lower than for most heavy metals. Cadmium is non-biodegradable and its accumulation in crops poses a threat to human health. In plants, Cd binds to apoplastic and symplastic target sites and disrupts the active components affecting fundamental processes such as cell division, mineral nutrition, and saccharide metabolism (Ahmad et al. 2009, Moussa and El-Gamal 2010, Deng et al. 2020, Li et al. 2020).

Soil pollution is rarely caused by a single contaminant and toxic effects on an individual organism are not easily attributable to a single chemical (Olsvik and Søfteland 2020). Such is the case in agricultural fields in which both pesticide and heavy metal residues may affect non-target plants. Because heavy metals are likely to co-occur with sulfonylurea herbicides in contaminated soils, their combined effects on non-target plants are a matter of interest to researchers. Cadmium, the most toxic heavy metal, and chlorsulfuron, an effective herbicide commonly used in agriculture, have been shown to negatively affect plants with sulfonylurea herbicides in contaminated soils, their combined effects on non-target plants are a matter of interest to researchers. Cadmium, the most toxic heavy metal, and chlorsulfuron, an effective herbicide commonly applied to wheat fields, were investigated in this study. We tested their effects on content of metabolites in maize seedlings.

According to our previous study (Zhao et al. 2018), chlorsulfuron inhibited shoot growth more severely after 21 d, with inhibitory rate 28 % after exposure to chlorsulfuron inhibited shoot growth more severely after 21 d, with inhibitory rate 28 % after exposure to 0.001 mg kg⁻¹ chlorsulfuron in test soil. Chlorsulfuron also significantly (P < 0.05) impaired root growth. Even at the very low concentration of 0.001 mg kg⁻¹, chlorsulfuron reduced root length by 50 %. Cadmium at 5.0 mg kg⁻¹ had no significant effect on shoot elongation, but it did enhance root growth (P < 0.05). We also found that chlorsulfuron negatively affected the chlorophyll content, photochemical efficiency of photosystem II in the dark-adapted state, the maximum efficiency of photosystem II, photochemical quenching coefficient, and steady-state fluorescence decline ratio in the leaves of maize seedlings. However, cadmium did not produce noticeable changes on these parameters.

Nuclear magnetic resonance (NMR) spectroscopy and multivariate data analysis have been used to detect differences between samples and identify biomarkers in diverse fields (Gall et al. 2001, Charlton et al. 2002, Azam et al. 2020, Masclllani et al. 2020). We used NMR in order to clarify the changes of metabolites in maize seedlings when chlorsulfuron and cadmium are added in alkaline soil.

Materials and methods

Plants and treatments: Maize (Zea mays L.) seeds were obtained from Shanxi Qiangsheng Seed Company (Taiyuan, Shanxi, China). Soils were collected from the 0 - 20 cm surface layer of an uncontaminated field at the Shanxi Experimental Station. Soil properties were as follows: pH = 8.08 (in 1 mol dm⁻³ KCl); organic matter = 1.21 %; cation exchange capacity = 25.4 cmol kg⁻¹; and texture (% by volume) = 24.7 % clay, 32.9 % silt, and 42.4 % sand. Samples of soil were dried at room temperature, gently crumbled, and then passed through a 2-mm mesh sieve; the samples were stored for subsequent analysis. The soil used in this paper meets the GB 15618-2018 soil environmental quality standard, all elements meet the environmental background value, and do not contain chlorsulfuron. The herbicide chlorsulfuron was a commercial formulation of 25 % wettable powder (Jiangsu Institute of Ecomines Company, Jiangsu, China). CdCl₂ was from Chengdu XiYa Chemical Technology Company (Chengdu, China) and methanol-d₄ for NMR, 99.8 atom % D from Sigma-Aldrich (St. Louis, USA).

All experiments were performed in controlled laboratory conditions, at a constant temperature of 25 ± 2 °C, a relative humidity between 40 and 60 %, a 12-h photoperiod, and an irradiance of 87.5 µmol m⁻² s⁻¹ on soil surfaces. The moisture of soil was 60 % of the water holding capacity during the experiment. Depending on the treatment conditions, a single application of herbicide and/or CdCl₂ was added to soils before maize seeds were sown. According to previous studies (Zeng and Zhu 2005, Chen et al. 2013), cadmium accumulates up to 5.0 mg kg⁻¹ in sewage irrigation areas in China and content of cadmium 5.0 mg kg⁻¹ was selected in experiments. According to Shan et al. (2001), residue of chlorsulfuron found in field was 0.005 - 0.013 mg kg⁻¹(soil) after spraying at 15 and 30 g ha⁻¹. The five concentrations of chlorsulfuron (0.001, 0.003, 0.005, 0.008, or 0.01 mg kg⁻¹) were used in this study. Six single-treatments received CdCl₂ at 5 mg kg⁻¹ or chlorsulfuron at 0.001, 0.003, 0.005, 0.008, or 0.01 mg kg⁻¹. Six combined-treatment conditions received both CdCl₂ and chlorsulfuron at the same concentrations. The control was grown at the same type of soil but without added chlorsulfuron or Cd.

Seed germination and seedling growth tests were performed following the procedures in ISO11269-2 (2013), with the following modifications. Plastic vessels were filled with soil of 155 g (dry mass equivalent). The diameters of each test vessel were 9 cm (top) and 6.5 cm (bottom) and the height was 6.5 cm; the bottom of each vessel had a drainage hole. Soil moisture was checked by weighing several randomly selected pots daily. Ten uniform and undressed seeds were planted in each pot filled with approximately 40 g of soil. After the emergence of 50 % of the seedlings in the control (uncontaminated soil) condition, emergence rates were determined for all conditions. After two weeks, the remaining plants were harvested for analyses of growth, lipid peroxidases, enzyme activity and metabolites.

Chlorophyll content, lipid peroxidation, and enzyme activities: The content of chlorophyll and malondialdehyde was detected according to the method described in our previous study (Zhao et al. 2018). The activities of superoxide dismutase (SOD), peroxidase (POD), catalase
Table 1. Metabolites from extracts of maize seedlings identified in the 600 MHz ^1H spectrum. ^1H chemical shift is referenced to a 1 mM sodium-3-trimethylsilylpropionate (TSP) internal standard. Abbreviations are reported in parentheses. Chemical shifts are referenced to TSP signal (δ=0.000). s - singlet, d - doublet, dd - double doublets, t - triplet, q - quartet, m - multiplet, - unknown.

| Classification | No. | Metabolite | Assignment | H δ | Multiplicity | Coupling constant J [Hz] |
|----------------|-----|------------|------------|-----|--------------|--------------------------|
| Amino acids    | 1   | isoleucine (Ile) | δ-CH$_3$ | 0.950 | t | 7.5 |
|                |     |            | γ-CH$_3$ | 1.030 | d | 7.2 |
|                |     |            | γ-CH   | 1.250 | m |     |
|                |     |            | Γ'-CH  | 1.450 | m |     |
|                | 2   | leucine (Leu) | δ-CH$_3$ | 0.980 | d | 6.0 |
|                |     |            | Δ'-CH$_3$ | 0.970 | d | 6.0 |
|                | 3   | valine (Val) | Γ'-CH$_3$ | 1.000 | d | 7.2 |
|                |     |            | γ-CH   | 1.060 | d | 6.6 |
|                | 4   | threonine (Thr) | γ-CH$_3$ | 1.340 | d | 6.6 |
|                |     |            | β-CH   | 4.250 | m |     |
|                | 5   | alanine (Ala) | R-CH   | 3.770 | q |     |
|                |     |            | β-CH$_3$ | 1.480 | d | 7.2 |
|                | 6   | arginine (Arg) | β-CH$_2$ | 1.650 | m |     |
|                |     |            | γ-CH$_2$ | 1.920 | m |     |
|                | 7   | proline (Pro) | γ-CH$_2$ | 2.010 | m |     |
|                |     |            | β-CH   | 2.360 | m |     |
|                |     |            | B'-CH  | 2.080 | m |     |
|                | 8   | glutamine (Gln) | β-CH$_2$ | 2.150 | m |     |
|                |     |            | γ-CH$_2$ | 2.450 | m |     |
|                | 9   | γ-aminobutyrate (GABA) | R-CH$_2$ | 2.320 | t | 7.2 |
|                |     |            | γ-CH$_2$ | 3.010 | t | 7.8 |
|                | 10  | aspartate (Asp) | R-CH   | 3.950 | dd | 8.1, 4.2 |
|                |     |            | β-CH   | 2.680 | dd | 17.7, 9.6 |
|                |     |            | B'-CH  | 2.790 | dd | 12.0, 4.2 |
|                | 11  | asparagine (Asn) | R-CH   | 3.950 | dd | 8.1, 4.2 |
|                |     |            | β-CH   | 2.830 | dd | 17.4, 8.4 |
|                |     |            | B'-CH  | 2.950 | dd | 17.4, 3.9 |
|                | 12  | tyrosine (Tyr) | C2,6H ring | 7.170 | s |     |
|                |     |            | C3,5H 510 ring | 6.890 | d | 9.0 |
|                | 13  | glycine (Gly) | 1-CH$_2$ | 3.570 | s |     |
|                | 14  | trimethylamine | CH$_3$ | 2.900 | s |     |
| Saccharides    | 15  | sucrose (Suc) | CH-1 (Glc) | 5.420 | d | 3.6 |
|                |     |            | CH$_2$-1’ (Fru) | 3.650 | s |     |
|                |     |            | CH-3’ | 4.170 | d | 9.0 |
|                | 16  | β-glucose (β-Glc) | CH-1 | 4.590 | d | 7.8 |
|                | 17  | α-glucose (α-Glc) | CH-1 | 5.190 | d | 3.6 |
|                | 18  | α-galactose (α-Gal) | C1H | 5.270 | d | 3.6 |
|                | 19  | β-galactose (β-Gal) | C1H | 4.600 | d | 3.6 |
| Organic acids  | 20  | succinic acid (SA) | αβ-CH$_2$ | 2.481 | s |     |
|                | 21  | acetic acid (AA) | α-CH$_3$ | 1.910 | s |     |
|                | 22  | lactic acid (LA) | β-CH$_3$ | 1.390 | d | 6.6 |
|                | 23  | malic acid (MA) | α-CH | 4.300 | dd | 8.4, 3.9 |
|                |     |            | β-CH   | 2.730 | dd | 15.9, 3.9 |
|                | 24  | citric acid (CA) | α γ-CH | 2.540 | d | 17.4 |
|                |     |            | α'γ'-CH | 2.700 | d | 16.8 |
|                | 25  | pyruvic acid (PA) | β-CH$_3$ | 2.350 | s |     |
|                | 26  | fumaric acid (FA) | αβ-CH=CH | 6.530 | s |     |
|                | 27  | formic acid | HCOOH | 8.460 | s |     |
|                | 28  | p-hydroxybenzoic acid | - | 6.930 | d | 9.0 |
|                |     |            | - | 7.930 | d | 8.4 |
miscellaneous compounds 29 choline N(CH₃)₃⁺ 3.210 s
30 choline chloride O-(dihydrogen phosphate) N-CH₃ 3.280 s
31 adenosine H-7 8.213 s
32 phenolic acids H-7' 7.610 d 16.2
-OC₄H₃ 3.800 s

Unassigned resonances 33 -
34 - 7.330 d 9.0
35 - 7.178 d 8.4
36 - 7.140 d 9.0
37 - 7.104 d 8.4
38 - 7.551 d 12.0
39 - 6.720 d 5.4

(CAT), and glutathione-S-transferase (GST) were also described in detail in the previous published article (Zhao et al. 2018).

NMR spectroscopy: The whole plants were ground to a powder in liquid nitrogen, and 200 mg of the powder was extracted using 6 cm³ of CH₃OH and water (1:1) in a 10-cm³ centrifuge tube. The extracts were vortexed, sonicated for 60 min, and centrifuged at 2,012 g for 20 min. Supernatant (4 cm³) was transferred to a 25 cm³ round flask and evaporated to dryness at 45 °C using a rotary evaporator. The dried extract was dissolved in 0.4 cm³ deuterated methanol-d₄ and 0.4 cm³ buffer (pH 6.0) containing 0.05 % (m/v) 2,2,3,3-D₄-3-(trimethylsilyl) propionic acid sodium (TMSP-2,2,3,3-D₄). Then the solution was centrifuged at 6,540 g for 15 min. Supernatant (0.6 cm³) was transferred to a NMR tube and analyzed by NMR.

The 1H NMR spectra were automatically reduced to ASCII files using MestReNova (v. 6.1.1, Mestrelab Research, Santiago de Compostela, Spain). Spectral intensities were sorted into bins of equal width (0.04) corresponding to the region of δH9.28 - 0.64. The regions of δH4.96 - 4.80 (water) and δH3.34 - 3.28 (methanol) were excluded from the analysis. Although the differences between spectra could be clearly visualized, we further examined the data using multivariate analysis for a more objective comparison. Principal component analysis (PCA) is one of the most widely used techniques in multivariate analysis. The purpose of PCA is to describe the variance in a set of multivariate data in terms of underlying orthogonal variables (principal components). The original variables (metabolite concentrations) can be expressed as linear combinations of the principal components (Sumner et al. 2003, Brereton 2018). The PCA and partial least squares discriminant analysis (PLS-DA) were performed with SIMCA-P software (v. 11.0, Umetrics, Umea, Sweden), using the Pareto scaling method for PCA and the unit variance method for PLS-DA.

Results

After exposure to the chlorsulfuron, the inhibition on shoot and root length was 28 - 87 % (Fig. 1A). However, the inhibitions on shoot and root length were 47 - 90 % after exposure to chlorsulfuron and cadmium.

Chlorsulfuron reduced fresh mass by 55.8 - 93 % (Fig. 1A); the combined pollutants also led to a significant decrease in fresh mass (63 - 93 %, Fig. 1A). However, Cd alone did not have a significant effect on the fresh mass (P > 0.05).

Both chlorsulfuron alone and in combination with Cd caused significant decrease in the amount of chlorophyll (26.78 - 83.93 %, Fig. 1B). The content of MDA was the highest (approximately 1.5-times in comparison with control) after exposure to 0.005 mg kg⁻¹ chlorsulfuron. As the concentration of chlorsulfuron continued to increase, the content of MDA in seedling showed a downward trend. Only at the concentrations of 0.003 and 0.005 mg kg⁻¹, the content of MDA was significantly different from the control (P < 0.05), and the other treatments had no significant effects. The content of MDA showed no significant change after exposure to chlorsulfuron and cadmium (P > 0.05). Chlorsulfuron caused a significant increase in SOD, POD, CAT, and GST activities. Cd alone had no significant effect on SOD, POD, CAT, and GST (Fig. 1C, D). Meanwhile, there was no significant difference between the effect of chlorsulfuron and Cd combination and chlorsulfuron single pollution on SOD and POD. But, there were some significant differences between chlorsulfuron and Cd pollution and chlorsulfuron single pollution on GST and CAT.

Table 1 lists 39 identified metabolites, including 14 amino acids, 9 organic acids, 5 sugars, 4 miscellaneous
EFFECTS OF CHLORSULFURON AND CADMIUM ON MAIZE SEEDLINGS

Fig. 1. Effects of chlorsulfuron, Cd, and the mixture of chlorsulfuron and Cd on fresh mass, shoot length and root length (A), on chlorophyll $a$+$b$ and malondialdehyde (MDA) content (B), superoxide dismutase (SOD) and peroxidase (POD) activities (C), and glutathione S-transferase (GST) and catalase (CAT) activities (D) of maize seedlings grown for 14 d in soil. Means ± SEs, $n = 4$, ** and * indicate significant difference at $P < 0.01$ and $P < 0.05$ between the treated and control plants, respectively. One unit of SOD is defined as amount of enzyme necessary for 50% inhibition of photochemical reduction of nitroblue tetrazolium; one unit of POD is defined as 0.01 increase of absorbance per min; one unit of GST is defined as amount of enzyme that conjugated 1nmol dm$^{-3}$ of dinitrobenzene with reduced glutathione per min; one unit of CAT is defined as 0.01 decrease of absorbance per min.
compounds, and 7 unknown compounds with their characteristic chemical shifts and coupling constants.

As shown in Fig. 2A, the 2-D score plot produced by PCA showed distinct separation between NMR data from control and chlorsulfuron-only treatment groups. Principle component 1 (PC1) and principle component 2 (PC2) accounted for 30 and 19 % of the variation, respectively. Similarly, Fig. 2B shows the distinct separation between NMR data from the treatment group exposed to CdCl₂ alone and the groups exposed to CdCl₂ and chlorsulfuron. Principle component 1 (PC1) and principle component 2 (PC2) accounted for 31 and 15 % of the variation, respectively.

To better understand the variables (i.e., metabolites) contributing to the classification components, the spectral data were further subjected to PLS-DA (partial least squares discriminant analysis) and OPLS-DA (orthogonal partial least squares discriminant analysis). Figs. 3 - 4 and 1 - 3 Suppl. show PCA score plots, validation models, and corresponding coefficient plots for the control treatment and 0.001, 0.003, 0.005, 0.008, and 0.01 mg kg⁻¹ chlorsulfuron treatments. The random left values of $R^2$ and $Q^2$ in Figs. 3B, 4B, 1B Suppl., 2B Suppl., and 3B Suppl. that were lower than the right values indicated that these models were of reasonable quality. The loading plots of the NMR data (Figs. 3C, 4C, 1C Suppl., 2C Suppl., and 3C Suppl.), which were obtained from comparisons of control seedlings and those exposed to chlorsulfuron, indicate that the herbicide had marked effects on plant metabolites. Chlorsulfuron treatments increased the content of glutamine, the primary product of ammonia (re) assimilation. This increase in glutamine was accompanied by markedly increased pools of two other major amino acids, such as alanine and tyrosine.

The content of arginine, asparagine, proline, and glutamine increased with the increasing dose of chlorsulfuron. The content of proline, another messenger involved in plant response to stress, also increased in maize seedlings exposed to chlorsulfuron. Chlorsulfuron decreased content of malic and citric acids, which are important in the tricarboxylic acid cycle.

As shown in Fig. 4 Suppl., OPLS-DA analysis of the NMR data revealed a pronounced separation between data from maize seedlings grown in control and CdCl₂ treatment. The values of $R^2$ and $Q^2$ indicated that these models were of reasonable quality (Fig. 4B Suppl.). The NMR data in the loading plot obtained by comparing control and 5.0 mg kg⁻¹ CdCl₂ (Fig. 4C Suppl.) show that maize seedlings grown under the CdCl₂ treatments had lower content of sucrose, malic acid, asparagine, alanine, isoleucine, and valine; however, they had higher content of phenolic acids, glycine, β-galactose, and α-glucose. The results show that addition of 5.0 mg kg⁻¹ CdCl₂ to soil markedly inhibited biosynthesis of branched-chained amino acids, such as isoleucine and valine. The decrease in malic acid observed from this analysis for CdCl₂-exposed seedlings also indicates that Cd affected the tricarboxylic acid cycle.

There were significant differences ($P < 0.05$) between the only chlorsulfuron exposed groups and chlorsulfuron (0.001 and 0.003 mg kg⁻¹) and Cd exposed groups (Fig. 5 Suppl. and 6 Suppl.). The values of $R^2$ and $Q^2$ indicated that these models were of reasonable quality (Fig. 5B Suppl., 6B Suppl.). At 0.005, 0.008, and 0.010 mg kg⁻¹ chlorsulfuron, there were no significant differences between chlorsulfuron-exposed groups and chlorsulfuron and Cd exposed groups. For these, PLS-DA validation models were not used.

Loading plots (Fig. 5C Suppl., 6C Suppl.) show that phenolic acids, which are synthesized through shikimate-independent pathways, increased in maize seedlings exposed to both CdCl₂ and chlorsulfuron, as did sucrose, aspartate, choline, β-galactose, and α-glucose. In contrast, the branched-chain amino acids, which include valine and isoleucine, decreased in maize seedlings exposed to CdCl₂ and chlorsulfuron. These results indicate differences in content of metabolites between maize seedlings exposed to CdCl₂ and chlorsulfuron, and those

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Fig. 2. Results of principle component analysis of nuclear magnetic resonance data. A - Comparison of data from maize seedlings grown in control (CK) and chlorsulfuron-exposed conditions. B - Comparison of data from maize seedlings grown in soil treated with cadmium chloride (Cd) alone or in addition to chlorsulfuron. PC1 - Principle component 1, PC2 - Principle component 2.
exposed to chlorsulfuron alone, at low concentrations of chlorsulfuron.

**Discussion**

In our present study, we investigated shoot length, root...
length, and fresh mass of maize seedlings after exposure to chlorsulfuron and Cd. Chlorsulfuron was the main factor inhibiting the growth of maize seedlings, especially the growth of their roots. This finding suggested that small amounts of chlorsulfuron residue in the soil affected maize seedlings by inhibiting root growth, leading to plant growth retardation at later developmental stages. Moreover, we also found that low-concentration of chlorsulfuron and Cd did not increase MDA. These results indicated that chlorsulfuron was the main factor causing the change of

![Comparison of control and chlorsulfuron-exposed (0.003 mg kg$^{-1}$) maize seedlings. A - PCA score plot, $R^2 = 0.991, Q^2 = 0.961$. $R^2$ - the predictable variables of the model, $Q^2$ - the predictive degree of the model. B - OPLS-DA loading plot. OSC is orthogonal signal correction. The numbers at peaks represent the corresponding metabolites, which can be found in Table 1.](image)

Fig. 4. Comparison of control and chlorsulfuron-exposed (0.003 mg kg$^{-1}$) maize seedlings. A - PCA score plot, $B$ - Validate mode, $R^2 = 0.991, Q^2 = 0.961$. $R^2$ - the predictable variables of the model, $Q^2$ - the predictive degree of the model. C - OPLS-DA loading plot. OSC is orthogonal signal correction. The numbers at peaks represent the corresponding metabolites, which can be found in Table 1.
growth, fresh mass, and activity of antioxidant enzymes in maize seedlings. In agreement to information from previous reports (Piccioni et al. 2009, Shuib et al. 2011, López-Gresa et al. 2012, Wang et al. 2012a,b, Zhi et al. 2012, D’Abrosca et al. 2013, Mi et al. 2013, Pereira et al. 2014) 39 metabolites were identified from maize seedlings under chlorsulfuron or/and Cd stress.

Comparing the results of the metabolites in the control and chlorsulfuron-treated groups, we found that different concentrations of chlorsulfuron induced an increase in asparagine, arginine, and proline. The content of asparagine is significantly increased in wheat grains under severe disease (Navrotskyi et al. 2018). Moreover, the upregulation of glutamine, asparagine and malonic acid was found in recovered-tolerant genotype of rice, suggesting a role in the regulation of panicle branching and spikelet formation for survival (Ma et al. 2021). Arginine, an important amino acid in plants, not only serves as a nitrogen reserve and in nitrogen recycling, but also as a precursor of polyamines and nitric oxide, important messengers in almost all physiological and biochemical processes. Therefore, asparagine and arginine accumulation in the treatment group can be used as one of the signs of stress in maize seedlings. CdCl₂ induced the decrease of sucrose, mafic acid, asparagine, alanine, isoleucine, and valine.

The previous studies reported that the primary target of chlorsulfuron and other sulphonyleurea herbicides is acetylacetate synthase, the enzyme significantly involved in branched-chain amino acids biosynthesis (Zhou et al. 2007, Orcaray et al. 2010, 2011). But, there was no significant decrease of branched-chain amino acids under chlorsulfuron stress in maize. However, CdCl₂ inhibited the biosynthesis of branched-chain amino acids.

Moreover, the content of sucrose increased in maize seedlings exposed to chlorsulfuron and CdCl₂, it is maybe associated with the increase in energy metabolism. The increase in sucrose content in roots also suggests that it was transported from the leaves to the roots at a higher rate than it was utilized. Under stress, the sugar gradient required for long-distance transport is abolished; thus, phloem transport is inhibited, suggesting that the saccharide accumulation in the leaves of treated plants reflected a reduction in sink strength (Zabalza et al. 2013).

Conclusions

This study revealed that chlorsulfuron is a main limiting factor in growth of maize seedlings, and the addition of Cd aggravated or ameliorated the inhibitory effect of chlorsulfuron. Moreover, there were synergism between chlorsulfuron and cadmium on activity of some antioxidant enzymes. Variation of metabolites in maize seedlings under chlorsulfuron or/and CdCl₂ suggested that chlorsulfuron was the main factor, and CdCl₂ did not aggravate the effects of chlorsulfuron on maize seedlings metabolites. The present work also found that CdCl₂ elicited significant changes in plant metabolism at a concentration that did not impair plant growth. Furthermore, the results also suggest that the changes of metabolites in maize seedlings could be a good indicator for the early toxic effects from environmental pollutants.

References

Ahmad, I., Naeem, M., Khan, N., Samiullah, A.: Effects of cadmium stress upon activities of antioxidative enzymes, photosynthetic rate, and production of phytochelatins in leaves and chloroplasts of wheat cultivars differing in yield potential. - Photosynthetic 47: 146-151, 2009.

Azam, A.A., Ismail, I.S., Vidyasaran, S.: ‘H NMR-based metabolomics of Clinacanthus nutans leaves extracts in correlation with their anti-neuroinflammation towards LPS-induced BV2 cells. - Rec. natu. Prod. 14: 231-247, 2020.

Breteron, R.G.: Classification and Supervised Pattern Recognition in Chemometrics. - John Wiley & Sons, New Jersey, 2018.

Chen, Z.F., Zhao, Y., Guo, T.Z., Wang, S.F., Tian, Q.: Impacts of sewage irrigation on heavy metal distribution and chemical fractions in anable soils: a case study about sewage-irrigated farmlands of the Fenggangjian River in Tongzhou district of Beijing, China. - Scientia geogr. sin. 33: 1021-1027, 2013 [In Chinese].

D’Abrosca, B., Scognamiglio, M., Fiumano, V.: Plant bioassay to assess the effects of allelochemicals on the metabolome of the target species Aegilops geniculata by an NMR-based approach. - Physicochemistry 93: 27-40, 2013.

Deng, J., Fu, D., Hu, W.: Physiological responses and accumulation ability of Microcystis aeruginosa to zinc and cadmium: implications for bioremediation of heavy metal pollution. - Bioresour. Technol. 303: 122963, 2020.

Fan, Z.: Study on enzymatic inhibition of acetylacetate synthase from maize (Zea mays L.) by chlorsulfuron and tribenuron-methyl. - Scientia agr. sin. 36: 173-178, 2003.

Gall, G.L., Puaud, M., Colquhoun, I.J.: Discrimination between orange juice and pulp wash by 1H nuclear magnetic resonance spectroscopy: identification of marker compounds. - J. Agr. Food Chem. 49: 580-588, 2001.

Hollaway, K.L., Kookana, R.S., Noy, D.M., Smith, J.G., Wilhelm, N.: Persistence and leaching of sulfonylurea herbicides over a 4-year period in the highly alkaline soils of south-eastern Australia. - Aust. J. exp. Agr. 46: 1069-1076, 2006.

Li, G., Li, Q., Wang, L.: Cadmium tolerance and detoxification in Ma, N.L., Su, D.L., Wan, A.: Monitoring of chemical components with 1H NMR-based metabolomics. - Acta pharm. sin. 48: 1692-1697, 2013. [In Chinese].

201
Chinese
Moussa, H.R., El-Gamal, S.M.: Effect of salicylic acid pretreatment on cadmium toxicity in wheat. - Biol. Plant. 54: 315-320, 2010.

Navrottsky, S., Baenziger, P.S., Regassa, T.: Variation in asparagine concentration in Nebraska wheat. - Cereal Chem. 95: 264-273, 2018.

Olsvik, P.A., Softeiland, L.: Mixture toxicity of chlorpyrifos-methyl, pirimiphos-methyl, and nonylphenol in Atlantic salmon (Salmo salar) hepatocytes. - Toxicol. Rep. 7: 547-558, 2020.

Orcaray, L., Igal, M., Marino, D., Zabalza, A., Royuela, M.: The possible role of quinate in the mode of action of glyphosate and acetolactate synthase inhibitors. - Pest. Manage. Sci. 66: 262-269, 2010.

Orcaray, L., Igal, M., Zabalza, A., Royuela, M.: Role of exogenously supplied ferulic and p-coumaric acids in mimicking the mode of action of acetolactate synthase inhibiting herbicides. - J. Agr. Food Chem. 59: 10162-10168, 2011.

Pereira, S.I., Figueiredo, P.I., Barros, A.S.: Changes in the metabolome of lettuce leaves due to exposure to mancozeb pesticide. - Food Chem. 154: 291-298, 2014.

Piccioni, F., Capitani, D., Zolla, L.: NMR metabolic profiling of transgenic maize with the Cry1Ab gene. - J. Agr. Food Chem. 57: 6041-6049, 2009.

Shan, Z.: Reviews on the environmental behavior of chlorsulfuron and its safety to plants. - Pestic. Sci. Admin. 3: 12-14, 1998.

Shan, Z.J., Wang, L.S., Cai, D.J.: Residue of chlorsulfuron in soil and its effects on following crop. - Rural Eco-Environ. 17: 35-38, 2001.

Shuib, N.H., Shaari, K., Khatib, A.: Discrimination of young and mature leaves of Melicope ptelefolia using 1H NMR and multivariate data analysis. - Food Chem. 126: 640-645, 2011.

Sumner, L.W., Mendes, P., Dixona, R.A.: Plant metabolomics: large-scale phytochemistry in the functional genomics era. - Phytochemistry 62: 817-831, 2003.

Wang, X., Wang, L., Yu, P.: NMR spectroscopy based metabolomic analysis of Thellungiella salsuginea under drought stress. - Acta ecol. sin. 32: 4737-4744, 2012a.

Wang, X., Li, Z., Xue, S.: Quality control over different processed products of polygalae radix based on plant metabolomics. - Chin. traditional herbal drugs 43: 1727-1737, 2012b.

Zhao, L.J., Xie, J.F., Zhang, H., Wang, Z.T., Jiang, H.J., Gao, S.L.: Enzymatic activity and chlorophyll fluorescence imaging of maize seedlings (Zea mays L.) after exposure to low doses of chlorsulfuron and cadmium. - J. Integr. Agr. 17: 826-836, 2018.

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