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RESEARCH ARTICLE

Reactive oxygen species, antioxidant enzyme activity, and gene expression patterns in a pair of nearly isogenic lines of nicosulfuron-exposed waxy maize (*Zea mays* L.)

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Abstract

Nicosulfuron is a post-emergence herbicide used for weed control in maize fields (*Zea mays* L.). Here, the pair of nearly isogenic inbred lines SN509-R (nicosulfuron resistant) and SN509-S (nicosulfuron sensitive) was used to study the effect of nicosulfuron on growth, oxidative stress, and the activity and gene expression of antioxidant enzymes in waxy maize seedlings. Nicosulfuron treatment was applied at the five-leaf stage and water treatment was used as control. After nicosulfuron treatment, the death of SN509-S might be associated with increased oxidative stress. Compared with SN509-R, higher $O_2^{\cdot-}$ and H_2O_2 accumulations were observed in SN509-S, which can severely damage lipids and proteins, thus reducing membrane stability. The effects were exacerbated with extended exposure time. Both $O_2^{\cdot-}$ and H_2O_2 detoxification is regulated by enzymes. After nicosulfuron treatment, superoxide dismutase (SOD), catalase, ascorbate peroxidase (APX), monodehydroascorbate reductase (MDHAR), dehydroascorbate reductase (DHAR), glutathione reductase (GR), and glutathione-S-transferase (GST) of SN509-S were significantly lower than those of SN509-R. Compared to SN509-R, ascorbate content (AA), glutathione (GSH) content, GSH to glutathione disulfide ratios, and AA to dehydroascorbate ratios significantly declined with increasing exposure time in SN509-S. Compared to SN509-S, nicosulfuron treatment increased the transcript levels of most of the *APX* genes except for *APX1*, and in contrast to *Gst1*, upregulated the transcription of *sod9*, *MDHAR*, *DHAR*, and *GR* genes in SN509-R. These results suggest that on a transcription level and in accordance with their responses, detoxifying enzymes play a vital role in the $O_2^{\cdot-}$ and H_2O_2 detoxification of maize seedlings under nicosulfuron exposure.

Keywords Waxy maize · Antioxidant enzyme · Gene expression · Oxidative stress · Nicosulfuron

Abbreviations

AA	Ascorbate
APX	Ascorbate peroxidase
CAT	Catalase
DAT	Days after nicosulfuron treatment
DHAR	Dehydroascorbate reductase
DHA	Dehydroascorbate
GST	Glutathione-S-transferase
GR	Glutathione reductase
GSH	Glutathione
GSSG	Glutathione disulfide
MDHAR	Monodehydroascorbate reductase
NILs	Nearly isogenic lines
ROS	Reactive oxygen species

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Introduction

Nicosulfuron [2-[(4,6-dimethoxypyrimidin-2-ylcarbamoyl)sulfamoyl]-*N*,*N*-dimethylnicotinamide] is an effective, broad-spectrum, inner-absorption conducting

sulfonylurea herbicide, which is mainly used for the control of annual weeds and broadleaf weed species during maize production (Stall and Bewick 1992; Williams and Harvey 2000; Wang et al. 2016). Nicosulfuron residue in both soil and rivers is degraded via hydrolysis and microbial activity; therefore, the toxicity of nicosulfuron has a minor effect on the next crop (Wang et al. 2011). The effectiveness of nicosulfuron compensates for the paucity of post-emergence herbicides in corn, and it has also been important for the development of the herbicide industry in China.

The different nicosulfuron susceptibilities of weeds and crops explain its ability to effectively control the growth of weeds. Nicosulfuron kills weeds selectively, while causing minimal harm to crops (Dobbels and Kapusta 1993; Beckie and Tardif 2012; Yu and Powles 2014). Nicosulfuron is a sulfonylurea herbicide that inhibits the activity of acetolactate synthase (ALS), reduces the synthesis of branched chain amino acids, and kills weeds (Rey-Caballero et al. 2016). In contrast, nicosulfuron can be degraded into inactive substances and thus loses its activity when it is combined with glucose in maize (Pataky et al. 2008; Meyer et al. 2010).

The resulting effects of corn exposure to nicosulfuron have been widely reported. A large number of studies indicated severe injury of maize seedlings after nicosulfuron treatment between 7 and 14 days. The severity of the resulting injury decreased in the following manner: common corn < waxy corn < sweet corn (O'Sullivan et al. 2002). Both sweet corn inbred and hybrid lines exhibit different resistance to nicosulfuron, and while some are extremely resistant, others are completely susceptible. Several studies have demonstrated that nicosulfuron induced significant injuries in some sweet corn hybrids, including Delmonte 2038, Merit, and Silver Extra Sweet, following nicosulfuron application at 25 g hm^{-2} or 50 g hm^{-2} (O'Sullivan et al. 1995; O'Sullivan and Bouw 1998). Different corn varieties basically suffer the same injury after nicosulfuron treatment: during the early stage of development, the leaves show local chlorosis and etiolation, which is accompanied by the purple leaf phenomenon. With further plant growth, new leaves show cauda equina curl. Consequently, plant growth is inhibited, which results in dwarfing plants, and secondary stems may be produced. With further aggravating phytotoxicity, sensitive maize lines cease to grow, stalks become purple, the leaves become yellowish, and plants eventually die. Several studies indicated that nicosulfuron can reduce the chlorophyll content and photosynthesis, which consequently inhibits plant growth (Hussain et al. 2010; Li et al. 2018; Tan et al. 2012; Yang et al. 2017). Herbicide application damages the photosynthetic apparatuses within both photosystem I (PSI) and photosystem II (PSII). This in turn reduces the maximum photochemical efficiency of photosystem II (F_v/F_m), the photochemical quenching coefficient (q_P), and the quantum yield of PSII electron transport (n_{PSII}) (Yuan et al. 2014; Hu et al. 2014). Therefore, it is vitally important to improve the nicosulfuron resistance of maize and to understand the resistance and detoxification mechanisms of maize.

Oxygenic photosynthetic organisms such as maize, photosystem I (PSI), photosystem II (PSII), and mitochondrial-mediated electron transport are major sites for the production of reactive oxygen species (ROS, e.g., $O_2^{\cdot-}$, H_2O_2, and $^1O_2$) under both natural and stress conditions (He and Häder 2010; Prasad et al. 2016). As a consequence, the presence of toxic factors, such as nicosulfuron, can enhance the production of ROS, using the same electron transport systems and thus accelerating the chance of physiological and biochemical damage (Sheeba et al. 2011). The antioxidant system plays an important role for scavenging ROS in plants and for preventing herbicide stress-related injuries in plant cells (Sun et al. 2017). Under herbicide stress, the redox balance in the plant is broken, which leads to a significant increase in ROS. Excessive $O_2^{\cdot-}$ in plants induces the generation of superoxide dismutase (SOD), which specifically removes superoxide anion radicals (Foyer et al. 1994; Baek and Skinner 2003; Gill and Tuteja 2010). SOD scavenges $O_2^{\cdot-}$ by catalyzing its dismutation, where one $O_2^{\cdot-}$ molecule is decreased to H_2O_2 and another is oxidized to O_2 (Table 1). Part of H_2O_2 can be catalyzed to form H_2O and O_2 via catalase (CAT). However, overly abundant H_2O_2 can be removed via the ascorbate-glutathione cycle (AA-GSH). The ascorbate-glutathione metabolic pathway, also named the Foyer-Halliwell-Asada pathway, is an important antioxidant pathway for higher plants during the process of resisting biotic and abiotic stresses, and it is also an important pathway for the metabolism of toxic substances in higher plants (Li et al. 2015). In this pathway, ascorbate peroxidase (APX) catalyzes hydrogen peroxide and produces a monodehydroascorbate radical (MDHA) and water. Then, MDHA is converted to ascorbate (AA) in a reaction that is catalyzed via monodehydroascorbate reductase (MDHAR). Furthermore, MDHA can be used to generate dehydroascorbate (DHA) by disproportionation, and subsequently, it can be reduced via dehydroascorbate reductase (DHAR) to the AA. Then, the glutathione disulfide (GSSG) is converted to glutathione (GSH) in a reaction catalyzed via glutathione reductase (GR; Fig. 1, Table 1) (Zhang and Kirkham 1996; Kaur et al. 2015; Sytykiewicz 2016). At the physiological and biochemical levels, a variety of abiotic and biotic stresses (chilling, drought, heavy metals, salt, and aphid infection) have been reported to lead to changes in the ascorbate-glutathione pathway enzyme activity (Anjum et al. 2014; Gill and Tuteja 2010). Until now, no data has been published on the effects of nicosulfuron on the expression of genes that encode isozymes in higher plants that are involved in the AsA-GSH cycle.

Apparently, studying the gene expression involved in antioxidant enzyme activities can further enhance our understanding of the molecular adaptation and detoxification

Table 1 Major ROS scavenging antioxidant enzymes

Enzymatic antioxidants	Enzyme code	Reactions catalyzed
Superoxide dismutase (SOD)	EC 1.15.1.1	$O_2^{\cdot -} + O_2^{\cdot -} + 2H^+ \rightarrow 2H_2O_2 + O_2$
Catalase (CAT)	EC 1.11.1.6	$H_2O_2 \rightarrow H_2O + ½O_2$
Ascorbate peroxidase (APX)	EC 1.11.1.11	$H_2O_2 + AA \rightarrow 2H_2O + DHA$
Monodehydroascorbate reductase (MDHAR)	EC 1.6.5.4	$MDHA + NADPH \rightarrow AA + NADP^+$
Dehydroascorbate reductase (DHAR)	EC 1.8.5.1	$DHA + 2GSH \rightarrow AA + GSSG$
Glutathione reductase (GR)	EC 1.6.4.2	$GSSG + NADPH \rightarrow 2GSH + NADP^+$

mechanisms of plants to various biotic and abiotic stresses. In fact, ROS that are overproduced in plant cells can regulate the expression of various genes related to antioxidant and other stresses. For example, overexpression of *Cu/Zn SOD* in transgenic tobacco plants caused an increased resistance to drought, chilling, and oxidative stress (Zhang et al. 2017). Upregulation in the transcript abundance of antioxidative enzyme genes was also observed in response to drought, salt, and stress (Li et al. 2017; He et al. 2017; Diaz-Vivancos et al. 2016). Sytykiewicz (2016) showed that *GR1* and *GR2* genes were significantly upregulated by aphid infestation of maize leaves. In response to a variety of abiotic stresses, the generation of ROS, increased antioxidant enzyme activity, and elevated transcript level of antioxidant enzymes has been extensively reported. In contrast, the regulatory mechanism of the family of antioxidant system genes in maize after spraying with nicosulfuron has not been reported. Therefore, we selected the pair of nicosulfuron-resistant and nicosulfuron-sensitive nearly isogenic lines (NILs) of maize SN509-R and SN509-S, to minimize effects caused by differences in the genetic background. Here, we aimed to (1) investigate the accumulation of both $O_2^{\cdot -}$ and H_2O_2 and the antioxidant enzyme activity of maize seedlings in the presence of nicosulfuron and (2) investigate changes in the expressions of gene for antioxidant enzymes in maize seedlings in response to nicosulfuron exposure.

Materials and methods

Experimental design

The experiment was designed at the southern farm of the Shenyang Agricultural University (41° 49′ N, 123° 34′ E), which is an area within the north temperate zone, with a monsoon-affected semi-humid continental climate. The fundamental nutrient content of the tested soil was 26.79 g kg^{-1} of organic matter, 2.45 g kg^{-1} of total nitrogen, 110.75 mg kg^{-1} of alkaline hydrolyzed nitrogen, 11.09 mg kg^{-1} of available phosphorous, and 105.74 mg kg^{-1} of available potassium. The pair of NILs SN509-R and SN509-S was developed by the Institute of Specialty Corn at the Shenyang Agricultural University. The nicosulfuron-resistant inbred line SN509-R developed normally after nicosulfuron exposure. In contrast,

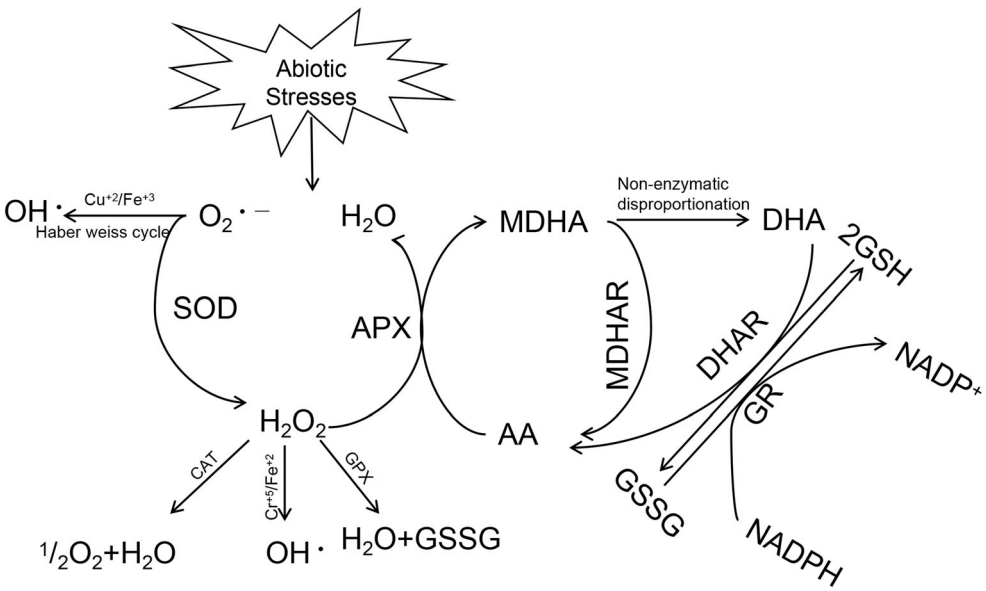

Fig. 1 ROS and antioxidant defense mechanism

nicosulfuron can inhibit the growth of the nicosulfuron-susceptible inbred line SN509-S, which ultimately causes plant death (Fig. S1). A split-plot experimental design was used with nicosulfuron treatment defining main plots and inbred lines within subplots. The row length of one plot was 6 m, and the row width was 0.6 m. We set up eight rows per plot and each plot had an area of 28.8 m^2.

Pesticide treatments

From 2015 to 2016, an experiment was conducted in an incubator maintained at 27 °C with a relative humidity of 85%. Uniformly sized seeds of two inbred lines were sown after rinsing with a small volume of distilled water in culture dishes. After 7 days, uniformly sized buds (0.01 m) were transferred to new culture dishes via filter paper. Nicosulfuron concentrations of 0 (control), 8, 40, 80, 120, 160, 240, 320, and 400 mg kg^{-1} were applied to each of the cultures to screen for the nicosulfuron resistances of SN509-R and SN509-S under a wide range of herbicide concentrations. Fifty buds of each line were evenly spread into the culture dishes, with three replicates. After 2 days, buds were transferred to the field, and the survival rate was investigated at the five-leaf stage. This initial screening test revealed that for a nicosulfuron concentration of 80 mg kg^{-1}, SN509-R plants were able to attain normal growth via self-defense mechanisms, while SN509-S plants either wilted or died (Table 2). In 2016, an experiment was conducted in the field. Maize seedlings were treated with nicosulfuron at the five-leaf stage using a laboratory pot sprayer, equipped with a nozzle. The nicosulfuron concentration was maintained at 80 mg kg^{-1} to investigate both physiological parameters and antioxidant enzyme activities of maize seedlings. Due to the fact of seedlings of SN509-S either died or wilted after 15 days after nicosulfuron treatment (DAT), data were determined every 2 days after 0 DAT.

H_2O_2 assay, $O_2^{\cdot-}$ production rate, and lipid peroxidation

A method described by Jena and Choudhuri (1981) was used to determine the content of H_2O_2. A reaction mixture containing 1 ml of 0.1% titanium sulfate in 20% H_2SO_4 (*v*/*v*) and 1 ml of supernatant was centrifuged at 8000×*g* for 15 min at 25 °C. The absorbance of the yellow color of the reaction solution was recorded at 415 nm. The content of H_2O_2 was calculated via a standard curve with known concentrations, and the extinction coefficient (= 0.28 μmol^{-1} cm^{-1}) was used to calculate the content of H_2O_2, which was expressed in micromoles per gram (FW). The $O_2^{\cdot-}$ production rate was assayed according to the method described by Jiang and Zhang (2002) with some modifications. 0.1-g seedling samples of waxy maize seedling leaves were homogenized with 1 ml of 50 mM Tris-HCl (pH 7.5). The homogenate was then centrifuged at 10,000×*g* at 4 °C for 10 min. The decrease in absorbance caused by the reduction of 2,3-Bis-(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide (XTT sodium salt) was measured at 470 nm, and the extinction coefficient (= 2.16×10^4 M^{-1} cm^{-1}) was used to calculate the $O_2^{\cdot-}$ production rate. The $O_2^{\cdot-}$ production rate was expressed in nanomoles per minute per gram (FW).

After 0 DAT, the seedling leaves were sampled, placed in liquid nitrogen, and then stored at − 80 °C until further analyses. The thiobarbituric acid method was used to determine the malondialdehyde (MDA) content of samples (Heath and Packer 1968). MDA was extracted by grinding 0.5 g of leaf samples in 5 ml of phosphate buffer (0.05 mM, pH 7.8). The homogenate was centrifuged at 4500×*g* for 10 min and the supernatant was collected to measure MDA. The 2 ml of extract liquid and 3 ml of 0.5% thiobarbituric acid (TBA) containing 5% trichloroacetic acid (TCA) were boiled for 10 min, and the solution was then cooled to room temperature. This mixture was centrifuged at 4500×*g* for 10 min. The

Table 2 Effect of nicosulfuron on germination rate of waxy maize seed of SN509-R and SN509-S. Asterisk indicates differences under different days after herbicide treatment at $P < 0.05$, according to the least significant difference (LSD) test. *CK* control

Spaying concentration [mg kg–^{1}]	Germination percentage				Survival rate after germination			
	SN509-R	Than CK [%]	SN509-S	Than CK [%]	SN509-R	Than CK	SN509-S	Than CK [%]
0	91.3	–	91.5	–	100	–	100	–
8	90.6	− 0.8	8.8	− 90.4*	100	0.0	0.0	− 100.0*
40	89.2	− 2.4	2.5	− 97.3*	100	0.0	0.0	− 100.0*
80	88.5	− 3.2	–		99.1	− 0.9	–	
120	79.2	− 13.3*	–		94.2	− 5.8*	–	
160	66.4	− 27.3*	–		82.9	− 17.1*	–	
240	18.8	− 79.5*	–		14.7	− 85.3*	–	
320	7.2	−92.1*	–		1.8	− 98.2*	–	
400	3.8	− 95.8*	–		0.4	− 99.6*	–	

absorbance of the supernatant was recorded at 450, 532, and 600 nm with a *UNICO™ UV-2000* spectrophotometer (*UV-2000*, *UNICO*, USA). MDA was expressed as nanomoles per gram (FW), and the extinction coefficient (155 mM^{-1} cm^{-1}) was used to calculate lipid peroxidation.

Enzyme assay

After nicosulfuron treatment, at 0 DAT, the seedling leaves were sampled, frozen in liquid nitrogen, and then stored at − 80 °C until subsequent analysis. Enzyme activities were measured spectrophotometrically, and absorbances were recorded with a *UNICO™ UV-2000* spectrophotometer (*UV-2000*, *UNICO*, USA). Enzymes were extracted by grinding 0.5 g of leaf samples in 5 ml of phosphate buffer (pH 7.5) containing 1 mM EDTA, 1% PVP, 1 mM DTT, and 1 mM PMSF. The homogenate was centrifuged at 15,000×*g* at 4 °C for 30 min, and the supernatant was collected to measure the enzyme activity.

Superoxide dismutase (SOD, EC 1.15.1.1) activity

The method described by Giannopolitis and Ries (1977) was used to measure the SOD activity. Three-milliliter reaction mixture contained 100 mM phosphate buffer (pH 7.8), 3.0 mM EDTA, 200 mM methionine, 2.25 mM nitroblue tetrazolium chloride (NBT), 60 μM riboflavin, and 1.5 M sodium carbonate. The photoreduction of NBT was measured by recording the absorbance at 560 nm. The enzyme activity was calculated as 50% inhibition expressed in units per gram (FW). One unit of SOD activity was defined as the enzyme activity required to inhibit the photoreduction of NBT to purple formazan by 50%.

Catalase (CAT, EC 1.11.1.6) activity

Catalase activity levels were assayed according to a method described by Aebi (1984). A decrease in the absorbance due to the disappearance of H_2O_2 was measured at 240 nm (= 0.036 mM^{-1} cm^{-1}). The catalyzed reaction system consisted of 100 mM phosphate buffer (pH 7.0), 10 mM H_2O_2, and enzyme extract. The enzyme activity was expressed in micromoles (H_2O_2 oxidized) per minute per gram (FW).

Ascorbate peroxidase (APX, EC 1.11.1.11) activity

The reaction mixture contained 50 mM phosphate buffer (pH 7.0), 0.3 mM ascorbate, 0.1 mM EDTA, 0.06 mM H_2O_2, and enzyme extract. The decrease in the absorbance caused by the reduction of ascorbate concentration was measured at 290 nm, and the extinction coefficient (2.8 mM^{-1} cm^{-1}) was used to calculate enzyme activity. The enzyme activity of APX was expressed in micromoles (product) per minute per gram (FW) according to Nakano and Asada (1981).

Monodehydroascorbate reductase (MDHAR, EC 1.6.5.4) activity

Monodehydroascorbate reductase activity was measured according to the method described by Hossain et al. (1984). A decrease in the absorbance was measured at 340 nm, and the extinction coefficient (6.2 mM^{-1} cm^{-1}) was used to calculate the enzyme activity. The enzyme activity of MDHAR was expressed in nanomoles per minute per gram (FW).

Dehydroascorbate reductase (DHAR, EC 1.8.5.1) activity

A method initially described by Nakano and Asada (1981) was used to measure the DHAR activity. The catalyzed reaction system consisted of 2.5 mM GSH, 0.1 mM EDTA, 0.2 mM DAsA, 50 mM phosphate buffer (pH 7.0), and enzyme extract. An increase in the absorbance caused by the formation of AA was recorded at 265 nm (= 14 mM^{-1} cm^{-1}) up to 2 min, and it was expressed in nanomoles per minute per gram (FW).

Glutathione reductase (GR, EC 1.6.4.2) activity

A method initially described by Cakmak et al. (1993) was used to measure GR activity. The reaction mixture contained 1 mM EDTA, 0.2 mM NADPH, 100 mM phosphate buffer (pH 7.8), 1 mM GSSG, and enzyme extract. Changes in the absorbance of the reaction solution described below at 340 nm (= 6.2 mM^{-1} cm^{-1}) caused by NADPH oxidation were recorded for 3 min. The enzyme activity of GR was expressed in nanomoles per minute per gram (FW).

Glutathione-S-transferase (GST, EC 2.5.1.18) activity

GST activity levels were assayed according to a method initially described by Habig et al. (1974). Changes in the absorbance of the reaction solution described above at 340 nm (= 9.6 mM^{-1} cm^{-1}) caused by the formation of conjugate production were recorded for 5 min. The enzyme activity was calculated and expressed in nanomoles per minute per gram (FW).

GSH, GSSG, AA, and DHA contents

GSH and GSSG contents were measured according to a method described by Queval and Noctor (2007) with some modifications. 0.1 g of leaf sample was homogenized in 5% sulfosalicylic acid and centrifuged at

10,000×*g* for 10 min. The supernatant was collected to measure GSH and GSSG contents. The reaction mixture contained 120 mM phosphate buffer (pH 8.0), 0.6 mM 5,5′-dithiobis (2-nitro-benzoic acid) DTNB, 6 mM EDTA, and 0.1 mM NADPH. The reaction was initiated by adding 5 μl GR (0.12 unit μl^{-1}). Absorbance was recorded at 412 nm, and GSH and GSSG content was expressed in micromoles per gram (FW). AA and DHA contents were measured according to a method initially described by Gossett et al. (1994). The increase in the absorbance caused by the formation of red chelate was determined at 265 nm. AA and DHA content was expressed in nanomoles per gram (FW).

RNA isolation and real-time RT-PCR

Total RNA of maize leaves (control and treatment of NILs) was isolated via the RNA pure Plant Kit (Kangwei Company, China). One microgram of total RNA was used to synthesize the cDNA using the HiScript II Q RT Super Mix reagent (Vazyme, China). Primers were designed using the Primer Premier 3 software (bioinfo.ut.ee/primer3-0.4.0/primer3/) according to gene sequences in the GenBank Database. The PCR efficiency of all primers for gene expression analysis was examined (Table 3). Only primers with higher amplification efficiency (> 90%) were used for this experiment. Maize cDNA sequences of SOD, APX, MDHAR, DHAR, GR, and GST were searched against GenBank, www.maizegdb.org and www.ncbi.nlm.nih.gov/ (Table 4). PCR amplification was performed with the HiScript II Q RT Super Mix reagent (Vazyme, China) on a real-time PCR detection system according to the manufacturer's instructions (CFX Connect Optics Module, Singapore). The $2^{-\Delta\Delta ct}$ method was used to calculate the relative transcript levels, and the *gapdh* gene was used as the housekeeping gene. Three biological repeats were performed for each analysis. The expression of each transcript was normalized against the amount of *gapdh* control transcript in each sample. Values are means ± standard error (SE) of three biological repeats.

Data analysis

Microsoft Excel and SigmaPlot 12.5 were used for data processing and mapping, and each reported data point represents the mean ± standard error (SE) of three replicates combined in the three experimental repeats. SPSS version 12.0 (SPSS Inc., Chicago, IL, USA) was used to conduct an analysis of variance (ANOVA), and mean values were compared via least significant difference (LSD) test at a $P < 0.05$ significance threshold indicated significant difference.

Results

$O_2^{\cdot-}$ production rate, H_2O_2 content, and lipid peroxidation

In SN509-R, the $O_2^{\cdot-}$ production rate first increased and then decreased with extended exposure time, while the $O_2^{\cdot-}$ production rate increased throughout in SN409-S (Fig. 2a). The average $O_2^{\cdot-}$ production rate of SN509-R was 8.93% higher than that of the control (NR). Compared to the control (NS), the $O_2^{\cdot-}$ production rate of SN509-S increased by 0.26, 18.43, 12.94, and 119.73% at 1, 3, 5, and 7 DAT, respectively. After nicosulfuron treatment, the average $O_2^{\cdot-}$ production rate of SN509-S was 14.18% higher than that of SN509-R. Our study showed that the accumulation of ROS in SN509-S was increased significantly, and the redox balance in the plant was severely damaged.

After 1 DAT, nicosulfuron increased the H_2O_2 content by different amounts in the two inbred lines (Fig. 2c). Compared to the control (NR), nicosulfuron increased the H_2O_2 content of SN509-R at 1, 3, 5, and 7 DAT by up to 17.61, 22.01, 21.65, and 15.39%, respectively. Compared to the control (NS), nicosulfuron significantly enhanced the H_2O_2 content of SN509-S at 1, 3, 5, and 7 DAT by up to 28.10, 27.30, 44.98, and 65.07%, respectively. This suggested that H_2O_2 accumulation in SN509-S was increased throughout with the exposure time.

A large amount of ROS production will result in cell membrane lipid peroxidation. After 5 DAT, the MDA content of SN509-R reached a maximal value, followed by a decrease, while the MDA content of SN509-S increased with exposure time (Fig. 2b). Compared to control, MDA content of SN509-S significantly increased by 49.42, 119.38, 209.47, and 220.94% at 1, 3, 5, and 7 DAT, respectively; the MDA content of SN509-R increased by an average of 79.33% from 1 DAT to 7 DAT. Additionally, the MDA content of SN509-S was significantly higher than that of SN509-R at 3, 5, and 7 DAT, with increases of 53.33, 40.64, and 191.17%, respectively. The accumulation of MDA indicated that the resistant inbred line SN509-R has a greater capacity to adapt to herbicide stress.

Nicosulfuron induced changes in antioxidant enzymes activities

The accumulation of ROS induces the production of SOD (Fig. 3a). After herbicide treatment, the SOD enzyme activity of SN509-R was first increased and then decreased each day following treatments. However, the SOD enzyme activity of SN509-S was much lower than that of control. After 1 DAT, nicosulfuron decreased CAT activity by different amounts in the two inbred lines, while the CAT enzyme activity of SN509-S was much lower than that of SN509-R. The average

Table 3 List of primers used for the real-time RT-PCR

Sequence ID	Forward primer	Reverse primer
GRMZM2G058522	CCTGCGAGCAAGGAGCAT	TCTGCTCCAGCTGTCACATTTC
GRMZM2G137839	TGATGCCACTAAGGGTTCT	ATCACTCCAGGATAGGGTCT
GRMZM2G140667	CTCAGGCAGGTTTTCTCCAC	GGATCAGAGAGGAGGGCTTT
GRMZM2G004211	CCAGATCTGCGAATAAACACAA	AAATACATGTGCACAGAACTGAAA
GRMZM2G084881	TACTCCCGATCATTCGACCT	GGCAATGACCTTGTTCTCGT
GRMZM2G134708	TCAAGGAGCAGAATCCAACA	GCCCTATGTAACCACCTCCA
GRMZM2G320307	CAGCTCTGTGTATGCCGTTG	ATCGATGTCCCTCGTCTTTG
GRMZM5G828229	GTGCAAAGAAGGTGGTGGTT	TTCTTAGCAAGCGAGGGTGT
GRMZM2G035502	TCCCAACTCCATCTCTGGTC	CGCCCTGGGAATTAACATAG
GRMZM5G826194	CGAGGAAAAATGGATTGGTG	TGTTCCATCGCTTGGATCTT
GRMZM5G855672	CAATGTCCATGCCTACACCA	CAGGTAGCACCAAAGCACAA
GRMZM2G005710	CATCAAGACTAAGCCCACCAA	TAGAAACATGGCCACCACAA
GRMZM2G172322	CGGTGCAATAGTGGTTGATG	CCTATTGGTGGTTGGGAGAA
GRMZM5G806449	CGATATTGCGGTTAAATGTG	AAGTTCGTCTTTGGCTTGGA
GRMZM2G116273	CGGTGACTTGTACCTCTTCGAATC	ATCCACCATTGCTGCCTCC

CAT enzyme activity of SN509-R was 32.3% higher than that of SN509-S (Fig. 3b).

In the AA-GSH cycle, The APX enzyme activity of the inbred lines showed different responses to nicosulfuron over time (Fig. 3c). The APX enzyme activity of SN509-S first increased and then reduced each day following treatments, while the APX enzyme activity of SN509-R increased significantly. The APX activity was compared between both inbred lines: compared to SN509-S, the nicosulfuron treatment significantly increased the APX enzyme activity of SN509-R at 1, 3, 5, and 7 DAT by up to 59.49, 14.26, 57.31, and 52.67%, respectively. After herbicide treatment, the MDHAR enzyme activity of SN509-R increased with increasing exposure time, while the MDHAR enzyme activity of SN509-S decreased with increasing exposure time. Compared to the control, the average MDHAR enzyme activity of SN509-R increased by 71.46% and the average MDHAR enzyme activity of SN509-S decreased by 23.33%. In addition, nicosulfuron treatment induced significant increases in DHAR enzyme activity in both inbred lines at each sampling time. The DHAR enzyme activity of SN509-R was much higher than that of SN509-S. After the spraying, the GR activity of SN509-R increased significantly, while the GR activity of SN509-S did not significantly changed (Fig. 4).

GST plays an important role in maintaining the plant redox balance. The most important biological function of GST is to regulate the binding of electron affinity substances to GSH, thus preventing continuous bursts of oxidation (Dixon et al. 2010). Following herbicide treatment, nicosulfuron caused an increase of GST activity in SN509-R, while GST activity of SN509-S increased only slightly. The average GST enzyme activity of SN509-R was 54.89% higher than that of SN509-S (Fig. 4d).

GSH, GSSG, AA, and DHA contents

After nicosulfuron treatment, GSH increased in leaves of SN509-R at 1 and 5 DAT. In contrast, GSSG of SN509-R significantly increased at each sampling time point. The GSH/GSSG value of SN509-R decreased with increased exposure time. In contrast, nicosulfuron had little effect on the GSH/GSSG of SN509-S (Fig. 5). Compared to SN509-S, nicosulfuron significantly increased both AA and DHA contents in SN509-R. AA/DHA of SN509-R remained at a high level at 7 DAT (Fig. 6).

Nicosulfuron induced changes in gene expression

The expression levels of antioxidant enzymes in leaves of maize and their control group were measured 12 h after exposure. The obtained results showed that, compared to the control, the *Cu/Zn sod9* gene of SN509-R was significantly upregulated, while the *Cu/Zn sod9* gene of the sensitive inbred line SN509-S was remarkably downregulated (Fig. 7a).

Nicosulfuron significantly affected the expression level of enzyme genes in the AA-GSH cycle. After herbicide treatment, compared to control, *APX2* and *APX3* transcripts of SN509-R were significantly increased. While the *APX2* transcript of SN509-S was not altered by nicosulfuron stress, the *APX3* transcript was significantly reduced in SN509-S (Fig. 7b–d). Nicosulfuron significantly affected the expression of *MDHAR* genes (Fig. 8a–d). After nicosulfuron spraying, *MDHAR1*, *MDHAR2*, *MDHAR3*, and *MDHAR4* transcripts of SN509-R were significantly higher than those of control at 12 h treatment, showing increases of 156.05, 181.23, 91.37, and 193.56%, respectively. Nicosulfuron treatment significantly reduced the transcript levels of *MDHAR1* and

Table 4 Superoxide dismutase, ascorbate peroxidase, monodehydroascorbate reductase, dehydroascorbate reductase, glutathione reductase, and glutathione-S-transferase genes of *Zea mays*

Name	Locus name	Location	Chromosome	Transcript length (bp)	Translation length (aa)
SOD9	GRMZM2G058522	131494602–131498241	9	1083	152
APX1	GRMZM2G137839	43682818–43686019	1	1181	250
APX2	GRMZM2G140667	225337094–225339718	2	1028	250
APX3	GRMZM2G004211	101526024–101527964	10	1086	290
MDHAR1	GRMZM2G084881	170386767–170391447	2	1590	435
MDHAR2	GRMZM2G134708	55307251–55311577	4	1659	433
MDHAR3	GRMZM2G320307	206752168–206757174	5	1918	478
MDHAR4	GRMZM5G828229	81348038–81352528	10	1790	499
DHAR1	GRMZM2G035502	35975667–35978706	6	1150	214
DHAR2	GRMZM5G826194	117483743–117495154	6	1128	262
DHAR3	GRMZM5G855672	137153528–137156513	8	1086	214
DHAR4	GRMZM2G005710	137157191–137159710	8	1260	181
GR1	GRMZM2G172322	13177263–13183612	1	2074	550
GR2	GRMZM5G806449	221145010–221153973	5	1871	495
GST1	GRMZM2G116273	178412108–178414563	8	929	214

MDHAR3 in SN509-S, whereas nicosulfuron stress did not alter *MDHAR2* and *MDHAR4* transcripts of SN509-S. In SN509-R, the transcript levels of *DHARs* also increased significantly after nicosulfuron treatment (Fig. 8e, f; Fig. 9a, b). In contrast, the expressions of *DHAR1*, *DHAR2*, *DHAR3*, and *DHAR4* in SN509-S were lower than those of the control at 12 h, showing reductions of 18.12, 36.51, 31.19, and 57.59%, respectively. *GR1* and *GR2* of SN509-R showed similar responses to nicosulfuron with strong transcript inductions (Fig. 9c, d). Changes in *GRs* expression levels were not significant in SN509-S, although slight decreases were found in *GR1*and *GR2*. Twelve hours after spraying and compared to the control, the *Gst1* gene in SN509-R was not significantly changed; however, the *Gst1* gene in SN509-S was significantly upregulated (Fig. 9e).

Discussion

The damaging effect of nicosulfuron in plants caused an accumulation of $O_2^{\cdot-}$ and H_2O_2 that has been suggested to be associated with a reduction of electron transport activity as reported in earlier studies (Wang et al. Photosynthetica Wang et al. Accepted). Due to inhibited electron transport activity, an excess of electrons is delivered to O_2, which results in the generation of $O_2^{\cdot-}$. This $O_2^{\cdot-}$ induces the production of SOD enzymes, which results in a dismutation of $O_2^{\cdot-}$ to H_2O_2 (Prasad et al. 2016; Hassan and Alla 2005). In this study, a significant increase in the accumulation of $O_2^{\cdot-}$ in SN509-S was only observed at 7 DAT. Due to the low activities of SOD and CAT, the $O_2^{\cdot-}$ in SN509-S cannot be removed and eventually converted into a large number of H_2O_2 molecules. It has been suggested that nicosulfuron, as a toxic factor, creates oxidative stress by increasing ROS levels in plants, eventually inactivating SOD and CAT in plants. This is supported by previous results reported by Alla and Hassan (2007) showing that isoproturon significantly accelerated the production of H_2O_2 in maize seedlings. Compared to the unstable $O_2^{\cdot-}$, H_2O_2 is more stable and easier to transport between cells. Under abiotic stress, high concentrations of H_2O_2 will produce hydroxyl radicals, thus exacerbating the toxicity of ROS (Basu et al. 2017; Gill and Tuteja 2010). Furthermore, excessive accumulation of H_2O_2 will lead to membrane lipid peroxidation. Numerous studies have demonstrated that membrane lipid peroxidation leads to the accumulation of MDA and herbicide treatment also causes the accumulation of MDA (He et al. 2017; Yu et al. 2015). Here, the MDA content of SN509-S increased with increasing exposure time, while the content of SN509-R was significantly lower than that of SN509-S. These findings suggest that oxidative stress in the leaves of SN509-S via nicosulfuron increased with exposure time. An effective defense mechanism in the leaves of SN509-R could protect plants from nicosulfuron damage.

H_2O_2 can be effectively detoxified in chloroplasts and other organelles; here, APX is a key enzyme of the AA-GSH pathway, which has been reported to be involved in the transformation of H_2O_2 into H_2O that utilizing AA as electron donor (Gill and Tuteja 2010). In the present study, after nicosulfuron treatment, APX activity of SN509-R was higher than that of SN509-S. Under nicosulfuron stress, the low level of APX enzyme activity

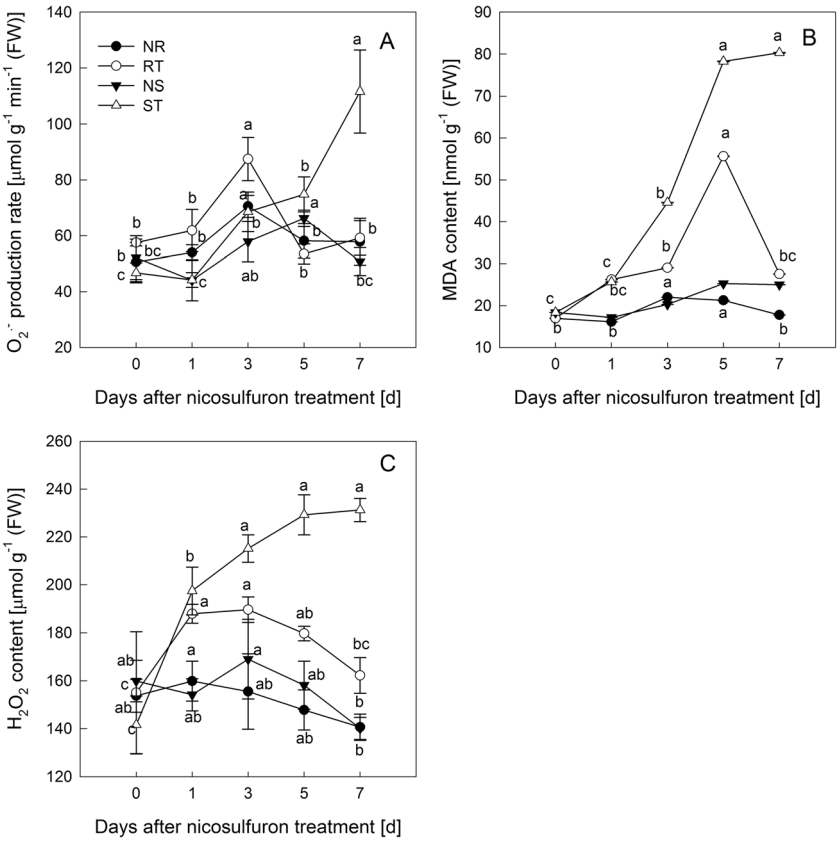

Fig. 2 **a**–**c** Effects of nicosulfuron on the $O_2^{\cdot-}$ production rate, H_2O_2 content, and lipid peroxidation in leaves of maize seedlings. NR, water treatment in SN509-R; RT, nicosulfuron 80 mg kg^{-1} treatment in SN509-R; NS, water treatment in SN509-S; ST, nicosulfuron 80 mg kg^{-1} treatment in SN509-S. Vertical bars represent the SE ($n = 3$). Small letters indicate differences between values obtained on different days after nicosulfuron treatment ($P < 0.05$) according to a least significant difference (LSD) test

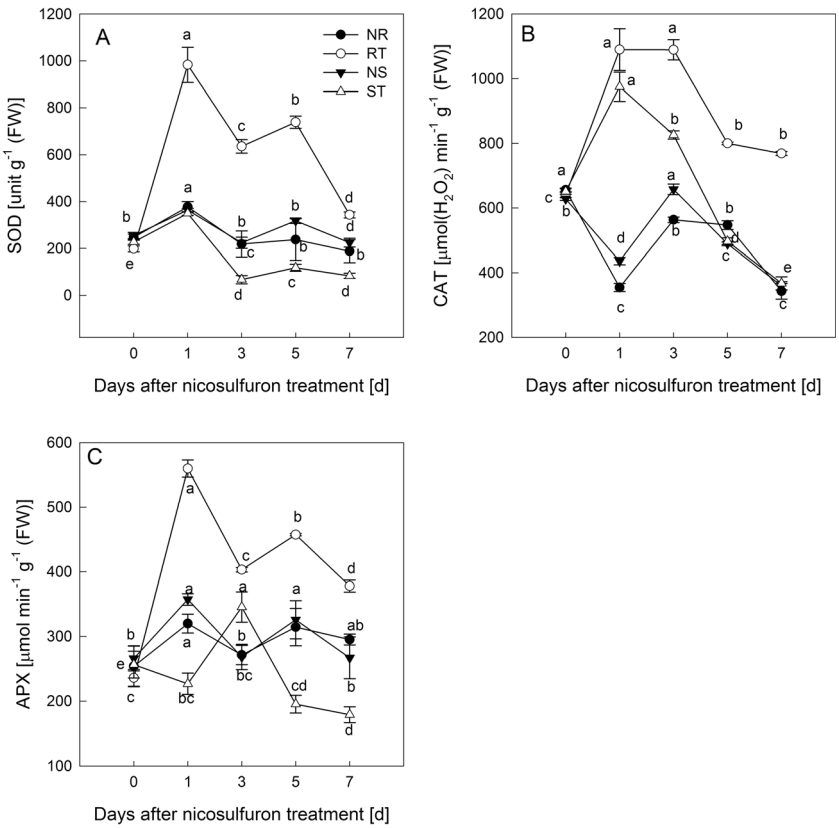

Fig. 3 **a**–**c** Effects of nicosulfuron on superoxide dismutase (SOD), catalase (CAT), and ascorbate peroxidase (APX) in leaves of SN509-R and SN509-S. NR, water treatment in SN509-R; RT, nicosulfuron 80 mg kg^{-1} treatment in SN509-R; NS, water treatment in SN509-S; ST, nicosulfuron 80 mg kg^{-1} treatment in SN509-S. Vertical bars represent the SE ($n = 3$). Small letters indicate differences in values obtained on different days after nicosulfuron treatment ($P < 0.05$) according to a least significant difference (LSD) test

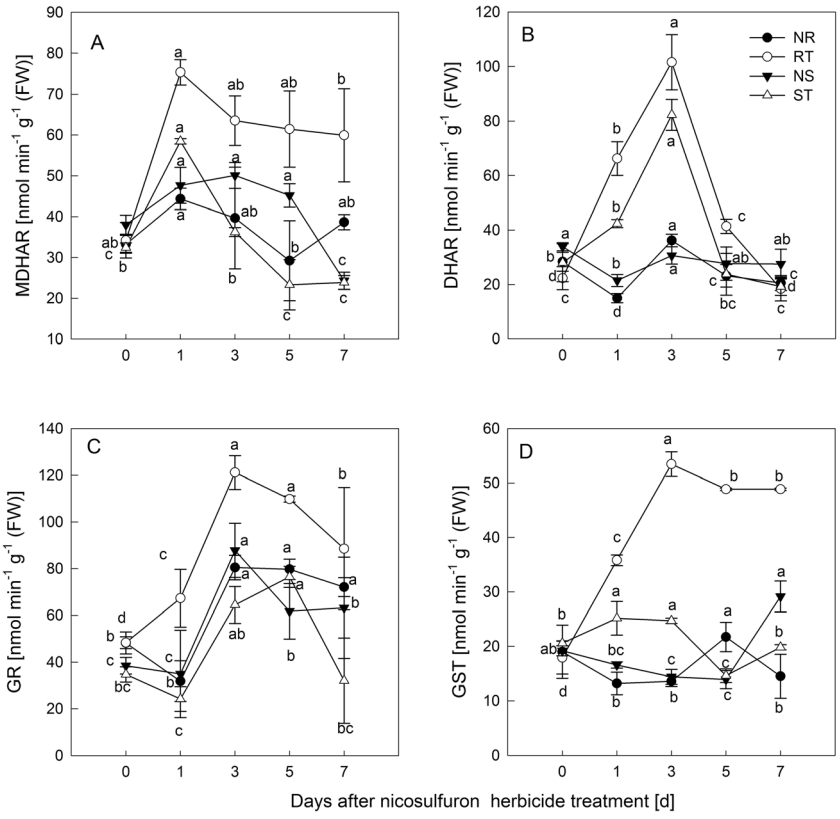

Fig. 4 **a**–**d** Effects of nicosulfuron on monodehydroascorbate reductase (MDHAR), dehydroascorbate reductase (DHAR), and glutathione reductase (GR) in leaves of SN509-R and SN509-S. NR, water treatment in SN509-R; RT, nicosulfuron 80 mg kg^{-1} treatment in SN509-R; NS, water treatment in SN509-S; ST, nicosulfuron 80 mg kg^{-1} treatment in SN509-S. Vertical bars represent the SE ($n = 3$). Small letters indicate differences in values obtained on different days after nicosulfuron treatment ($P < 0.05$) according to a least significant difference (LSD) test

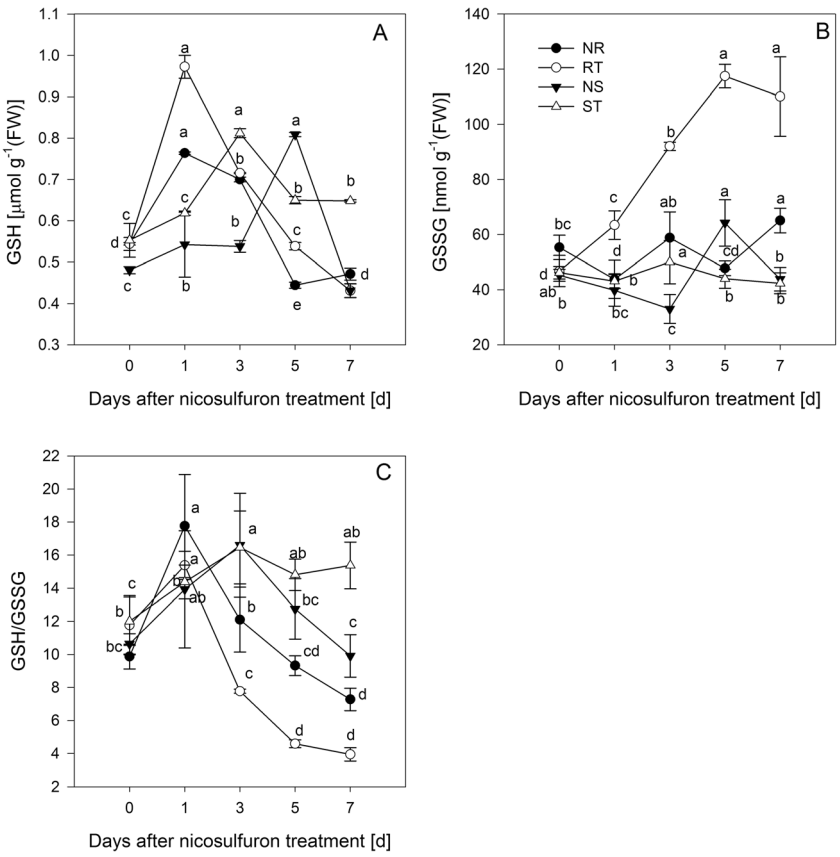

Fig. 5 **a**–**c** Effects of nicosulfuron on ascorbate (AA), dehydroascorbate content (DHA), and AA/DHA ratios in leaves of SN509-R and SN509-S. NR, water treatment in SN509-R; RT, nicosulfuron 80 mg kg^{-1} treatment in SN509-R; NS, water treatment in SN509-S; ST, nicosulfuron 80 mg kg^{-1} treatment in SN509-S. Vertical bars represent the SE ($n = 3$). Small letters indicate differences in values obtained on different days after nicosulfuron treatment ($P < 0.05$) according to a least significant difference (LSD) test

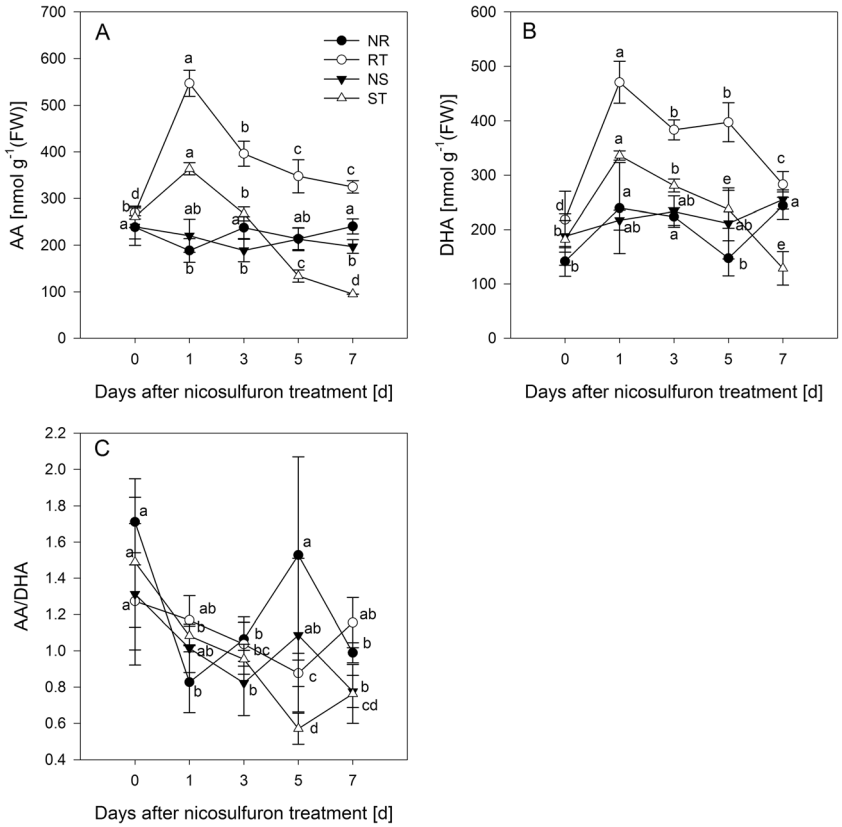

Fig. 6 **a**–**c** Effects of nicosulfuron on reduced glutathione (GSH), and oxidized glutathione content (GSSG), and GSH/GSSG ratios in leaves of SN509-R and SN509-S. SN509-R-CK, water treatment as control; SN509-R, nicosulfuron 80 mg kg^{-1} treatment; SN509-S-CK, water treatment as control; SN509-S, nicosulfuron 80 mg kg^{-1} treatment. Vertical bars represent the SE (n = 3). *Small letters* indicate differences in values obtained on different days after nicosulfuron treatment ($P < 0.05$) according to a least significant difference (LSD) test

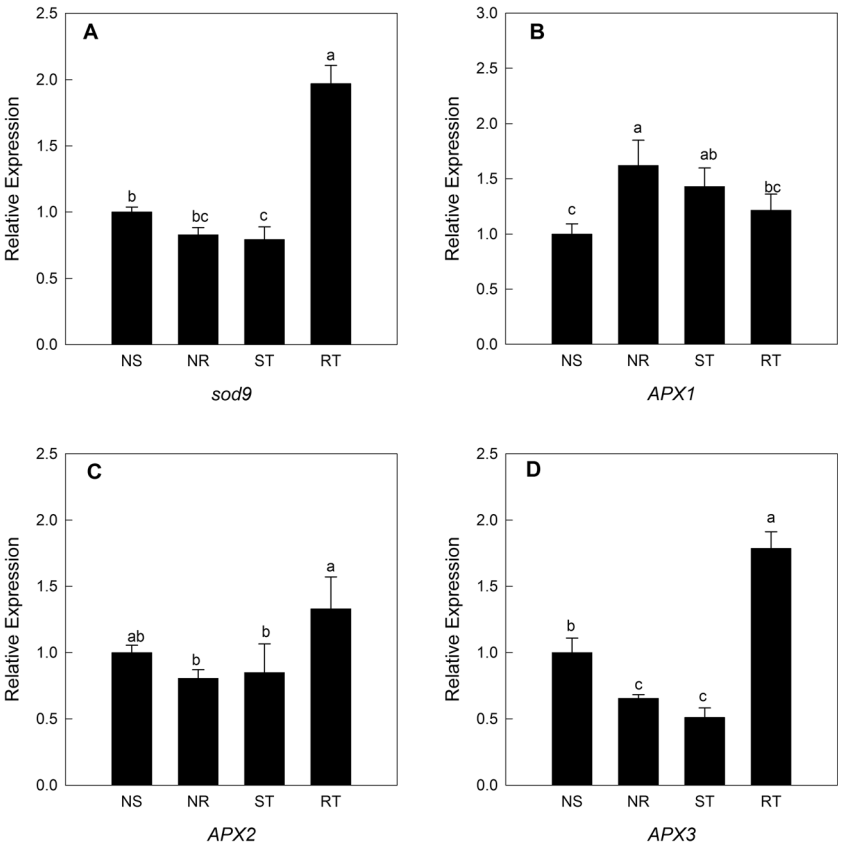

Fig. 7 **a**–**d** Transcriptional levels of different antioxidant enzymes, expressed relative to the control, in leaves of maize seedlings exposed to nicosulfuron (A. *Cu/Zn SOD*, B. *APX1*, C. *APX2*, D. *APX3*). NR, water treatment in SN509-R; RT, nicosulfuron 80 mg kg^{-1} treatment in SN509-R; NS, water treatment in SN509-S; ST, nicosulfuron 80 mg kg^{-1} treatment in SN509-S. Values are mean of three independent biological replicates. Vertical lines represent standard error. Asterisk refers to significant levels ($P < 0.05$)

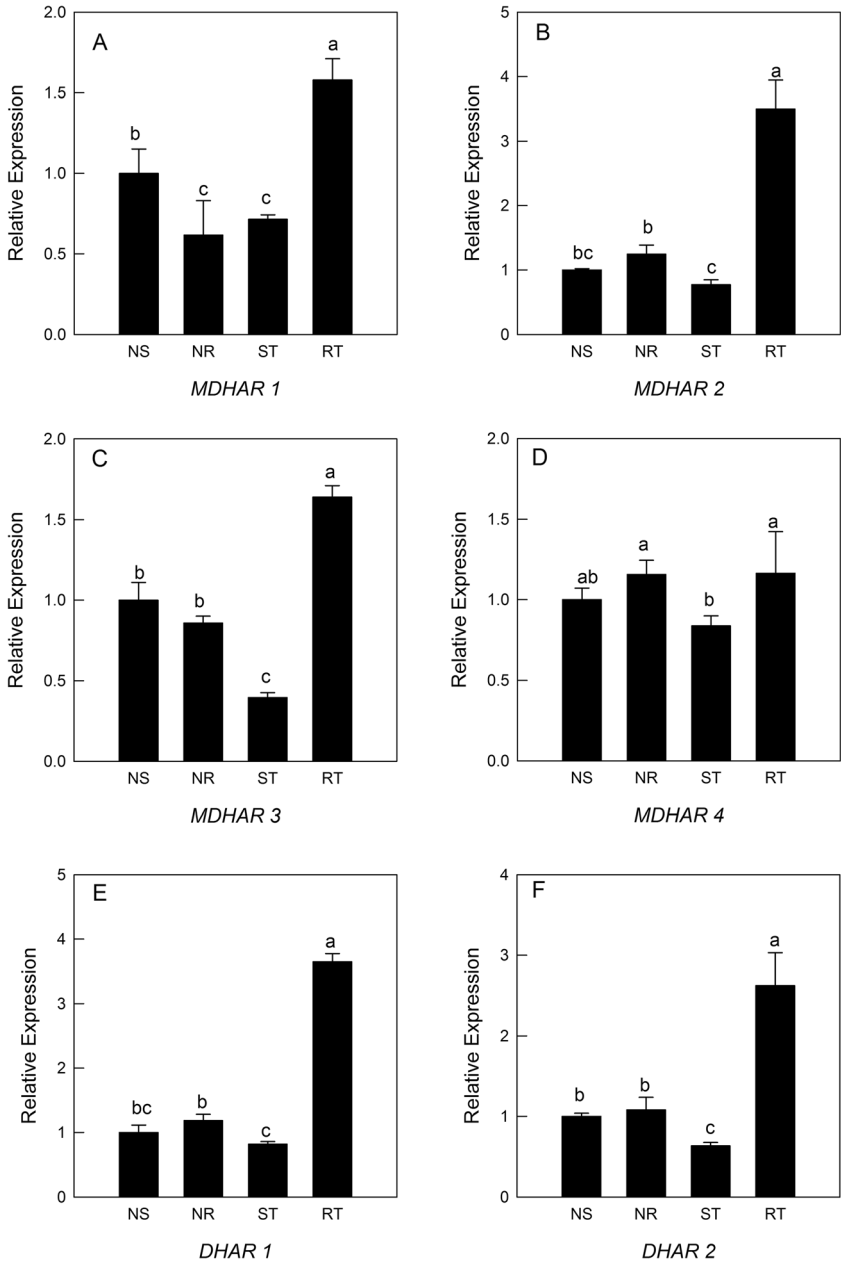

Fig. 8 **a**–**f** Transcriptional levels of different antioxidant enzymes, expressed relative to the control, in leaves of maize seedlings exposed to nicosulfuron (A. *MDHAR1*, B. *MDHAR2*, C. *MDHAR3*, D. *MDHAR4*, E. *DHAR1*, F. *DHAR2*). NR, water treatment in SN509-R; RT, nicosulfuron 80 mg kg^{-1} treatment in SN509-R; NS, water treatment in SN509-S; ST, nicosulfuron 80 mg kg^{-1} treatment in SN509-S. Values are mean of three independent biological replicates. Vertical lines represent standard error. Asterisk refers to significant levels ($P < 0.05$)

compared to the control might be associated with a low level of AA content in SN509-S, since the APX enzyme stability is closely related to the AA content (Prasad et al. 2016). Furthermore, MDHA and DHA regenerate AA via MDHAR and DHAR enzymes. The results of this study showed that the average MDHAR and DHAR activity of SN509-R was higher than that of SN509-S. Altered MDHAR and DHAR activities have been reported in the presence of patulin in maize (Ismaiel and Papenbrock 2017). The GR activity was significantly increased in SN509-R and GSH content continued to decline. However, compared to the control, the GSH content of SN509-R remained at a high level. In contrast, compared to control, the GR activity of SN509-S was not significantly different. In summary, these results suggest that nicosulfuron significantly suppresses MDHAR, DHAR, and GR activities in SN509-S, and this reduction in their activities is related to the depletion of GSH. Bashir et al. (2015) furthermore suggested that increased enzyme activity is closely related to changes in both GSH content and AA-GSH cycle activity in Indian mustard. In addition, the low level of MDHAR and DHAR activities in SN509-S may lead to a change in AA homeostasis, which is indicated by the severe decrease of the ratio of AA/DHA. Many studies have demonstrated that the regeneration of AA requires NADPH as the electron donor for

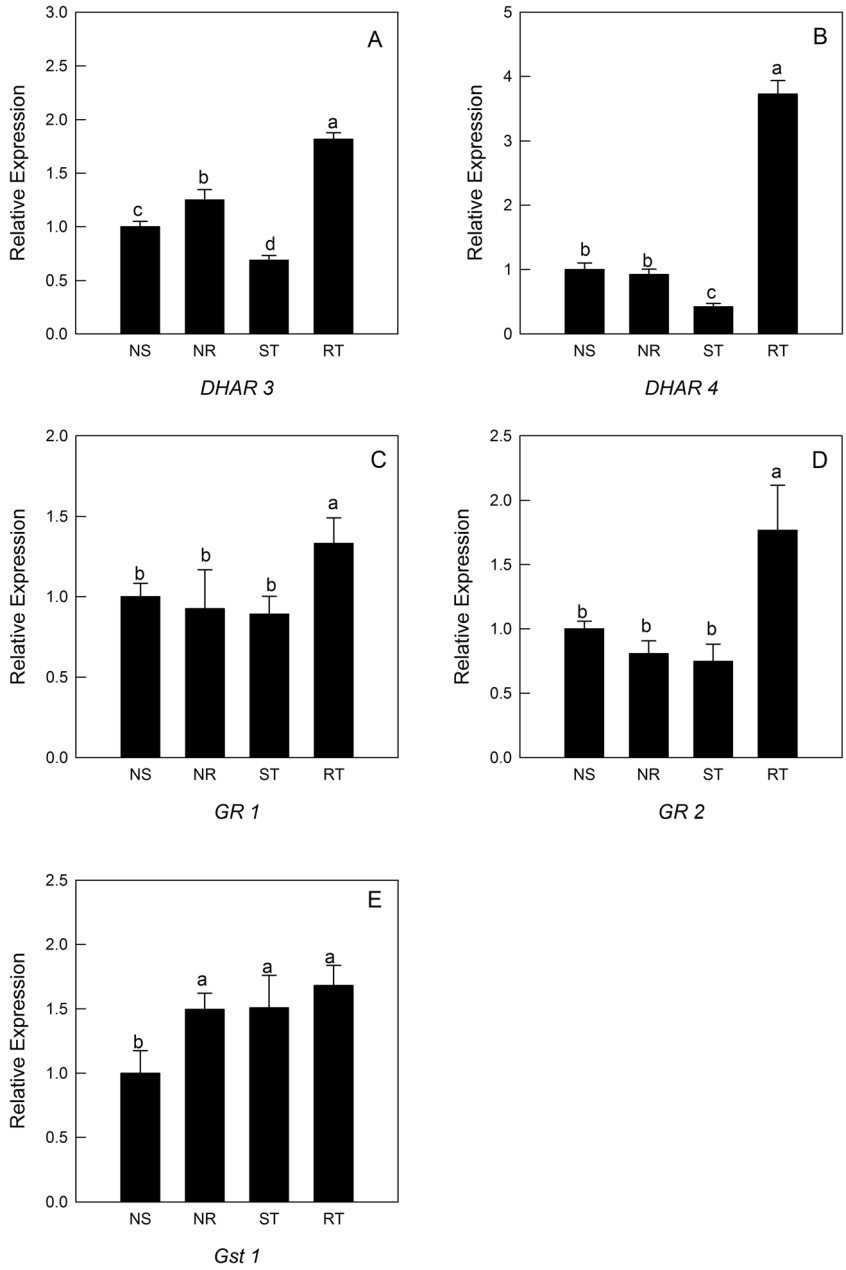

Fig. 9 **a**–**e** Transcriptional levels of different antioxidant enzymes, expressed relative to the control, in leaves of maize seedlings exposed to nicosulfuron (A. *DHAR3*, B. *DHAR4*, C. *GR1*, D. *GR2*, E. *Gst1*). NR, water treatment in SN509-R; RT, nicosulfuron 80 mg kg^{-1} treatment in SN509-R; NS, water treatment in SN509-S; ST, nicosulfuron 80 mg kg^{-1} treatment in SN509-S.Values are mean of three independent biological replicates. Vertical lines represent standard error. Asterisk refers to significant levels ($P < 0.05$)

enzyme activities (Foyer and Noctor 2011). Under nicosulfuron treatment, damage to the electron transport chain may be an important reason for the reduction of the ratio of AA/DHA. Bartoli et al. (2006) reported a direct interaction between the photosynthetic electron transport chain and AA. Therefore, disruption of photosynthetic electron transport activity by nicosulfuron may be a further reason for the decrease in AA content.

Many studies have demonstrated that SOD was strongly related to the quenching of superoxide into H_2O_2 (Baek and Skinner 2003; Sun et al. 2018; Xu et al. 2017). There are three types of SOD enzymes, including *MnSOD*, *FeSOD*, and *Cu/ZnSOD*. However, *Cu/ZnSOD* is more stable than *MnSOD* and *FeSOD* (Bueno et al. 1995). In this study, the expression of genes encoding antioxidative enzyme variations was assayed after nicosulfuron treatment in 12 h. After herbicide treatment, compared to control, the expression of *sod9* in SN509-R was strongly upregulated, while the expression of *sod9* was strongly downregulated in SN509-S. A similar result has been reported for *Arabidopsis thaliana*, maize, and rice (Yu et al. 2014; Xu et al. 2017). In contrast, the expression of *Cu/ZnSOD* in Kentucky bluegrass was downregulated under drought stress (Bian and Jiang 2009). This difference may be due to the different plant species used and different stress conditions.

Under nicosulfuron stress, the expression of different *APX* genes showed different responses, indicating that separate genes may respond to specific conditions. Compared to control, *APX2* and *APX3* of SN509-R showed the strongest responses to the nicosulfuron treatment, with 1.5–2-fold transcript increases at 12 h after treatments, while the expression of *APX3* in SN509-S was strongly downregulated; however, the expression of *APX1* remained unaltered in both inbred lines. MDHAR is the second enzyme in the AA-GSH cycle, which exhibited upregulation under a series of oxidative stresses and thus plays a critical role in plant defense against ROS (Begara-Morales et al. 2015; Johnston et al. 2015). Nicosulfuron also significantly increased the transcript levels of *MDHAR1*, *MDHAR2*, and *MDHAR3* in SN509-R following 12 h of nicosulfuron treatment. In contrast, the nicosulfuron treatment led to significantly reduced transcript levels of *MDHAR1*, *MDHAR2*, and *MDHAR3* in SN509-S. However, the transcript levels of *MDHAR4* slightly decreased in both inbred lines. This suggested that *MDHAR1*, *MDHAR2*, and *MDHAR3* may play more central roles to protect plants against nicosulfuron-induced oxidative stress compared to *MDHAR4*.

It has been demonstrated that DHAR overexpression also increases plant resistance to various oxidative stresses (Shin et al. 2013; Zhang et al. 2015). In this study, nicosulfuron treatment significantly increased the transcript levels of *DHAR1*, *DHAR2*, *DHAR3*, and *DHAR4* in SN509-R, in contrast to SN509-S. These findings suggested that DHAR responded stronger to nicosulfuron stress caused after 12 h of nicosulfuron treatment. It was also shown that GR plays an important role in regulating a variety of oxidative stresses, and the overexpression of *GR* enhanced plant tolerance to heavy metal, salt, and drought stress (Seo et al. 2006; Torres-Franklin et al. 2008; Wu et al. 2015). We also observed that nicosulfuron increased the transcription of *GR* genes in SN509-R significantly, while the expression of *GR* genes in SN509-S remained unaltered, indicating the vital function of *GR* in the nicosulfuron stress tolerance of maize.

For the evaluation of detoxification capacity in a pair of nearly isogenic lines (NILs) of waxy maize, both activity and the transcript level of GST of maize treated with nicosulfuron were studied. Our results indicate that the average GST activity of SN509-R was significantly higher than that of SN509-S. Furthermore, the transcriptions of *Gst1* were increased in both inbred lines. The abiotic stress caused an increase of GST activity and transcript, which is in agreement with previous studies. The overexpression of the *Gst1* gene in transgenic potatoes can significantly increase the activity of GST and GPX enzymes and enhanced the resistance of transgenic plants to oxidative stress (Gill and Tuteja 2010).

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References

Aebi H (1984) Catalase in vitro. Methods Enzymol 105:121–126

Alla MMN, Hassan NM (2007) Changes of antioxidants and GSH-associated enzymes in isoproturon-treated maize. Acta Physiol Plant 29:247–258. https://doi.org/10.1007/s11738-007-0031-8

Anjum NA, Gill SS, Gill R, Hasanuzzaman M, Duarte AC, Pereira E, Ahmad I, Tuteja R, Tuteja N (2014) Metal/metalloid stress tolerance in plants: role of ascorbate, its redox couple, and associated enzymes. Protoplasma 251:1265–1683. https://doi.org/10.1007/s00709-014-0636-x

Baek KH, Skinner DZ (2003) Alternation of antioxidant enzyme gene expression during cold acclimation of near-isogenic wheat line. Plant Sci 165:1221–1227. https://doi.org/10.1016/S0168-9452(03)00329-7

Bartoli CG, Yu J, Gomez F, Fernandez L, McIntosh L, Foyer CH (2006) Inter-relationships between light and respiration in the control of ascorbic acid synthesis and accumulation in *Arabidopsis thaliana* leaves. J Exp Bot 57:1621–1631. https://doi.org/10.1093/jxb/erl005

Bashir H, Ibrahim MM, Bagheri R, Ahmad J, Arif IA, Baig MA, Qureshi MI (2015) Influence of sulfur and cadmium on antioxidants, phytochelatins and growth in Indian mustard. AoB Plants 7:plv001. https://doi.org/10.1093/aobpla/plv001

Basu S, Giri RK, Benazir I, Kumar S, Rajwanshi R, Dwivedi SK, Kumar G (2017) Comprehensive physiological analyses and reactive oxygen species profiling in drought tolerant rice genotypes under salinity stress. Physiol Mol Biol Plants 23(4):837–850. https://doi.org/10.1007/s12298-017-0477-0

Beckie HJ, Tardif FJ (2012) Herbicide cross resistance in weeds. Crop Prot 35:15–28

Begara-Morales JC, Sánchez-Calvo B, Chaki M, Mata-Pérez C, Valderrama R, Padilla MN, López-Jaramillo J, Luque F, Corpas FJ, Barroso JB (2015) Differential molecular response of monodehydroascorbate reductase and glutathione reductase by nitration and S-nitrosylation. J Exp Bot 66(19):5983–5996. https://doi.org/10.1093/jxb/erv306

Bian SM, Jiang YW (2009) Reactive oxygen species, antioxidant enzyme activities and gene expression patterns in leaves and roots of Kentucky bluegrass in response to drought stress and recovery. Sci Hortic 120:264–270. https://doi.org/10.1016/j.scienta.2008.10.014

Bueno P, Varela J, Gimenez-Gallego G, Del Rio LA (1995) Peroxisomal copper, zinc superoxide dismutase. Characterization of the isoenzyme from watermelon cotyledons. Plant Physiol 108:1151–1160. https://doi.org/10.1104/pp.108.3.1151

Cakmak I, Strbac D, Marschner H (1993) Activities of hydrogen peroxide-scavenging enzymes in germinating wheat seeds. J Exp Bot 44:127–132. https://doi.org/10.1093/jxb/44.1.127

Diaz-Vivancos P, Faize L, Nicolás E, Clemente-Moreno MJ, Bru-Martinez R, Burgos L, Hernández JA (2016) Transformation of plum plants with a cytosolic ascorbate peroxidase transgene leads to enhanced water stress tolerance. Ann Bot 117(7):1121–1131. https://doi.org/10.1093/aob/mcw045

Dixon DP, Skipsey M, Edwards R (2010) Roles for glutathione transferases in plant secondary metabolism. Phytochemistry 71(4):338–350. https://doi.org/10.1016/j.phytochem.2009.12.012

Dobbels AF, Kapusta G (1993) Postemergence weeds control in corn (*Zea mays*) with nicosulfuron combinations. Weed Technol 7:844–850

Foyer CH, Noctor G (2011) Ascorbate and glutathione: the heart of the redox hub. Plant Physiol 155:2–18. https://doi.org/10.1104/pp.110.167569

Foyer CH, Descouvrières P, Kunert KJ (1994) Protection against oxygen radicals: an important defence mechanism studied in transgenic plants. Plant Cell Environ 17:507–523. https://doi.org/10.1111/j.1365-3040.1994.tb00146.x

Giannopolitis CN, Ries SK (1977) Superoxide dismutases: I. occurrence in higher plants. Plant Physiol 59:309–314. https://doi.org/10.1104/pp.59.2.309

Gill SS, Tuteja N (2010) Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. Plant Physiol Biochem 48:909–930. https://doi.org/10.1016/j.plaphy.2010.08.016

Gossett DR, Millhollon EP, Cran LM (1994) Antioxidant response to NaCl stress in salt-tolerant and salt-sensitive cultivars of cotton. Crop Sci 34:706–714. https://doi.org/10.2135/cropsci1994.0011183X003400030020x

Habig WH, Pabst MJ, Jakoby WB (1974) Glutathione-S-transferases, the first enzymatic step in mercapturic acid formation. J Biol Chem 249:7130–7139

Hassan NM, Alla MMN (2005) Oxidative stress in herbicide-treated broad bean and maize plants. Acta Physiol Plant 27:429–438

He YY, Häder DP (2010) UV-B-induced formation of reactive oxygen species and oxidative damage of the cyanobacterium Anabaena sp.: protective effects of ascorbic acid and N-acetyl-l-cysteine. J Photochem Photobiol B Biol 66:115–124. https://doi.org/10.1016/S1011-1344(02)00231-2

He F, Sheng M, Tang M (2017) Effects of *Rhizophagus irregularis* on photosynthesis and antioxidative enzymatic system in *Robinia pseudoacacia* L. under drought stress. Front Plant Sci 8. https://doi.org/10.3389/fpls.2017.00183

Heath RL, Packer L (1968) Photoperoxidation in isolated chloroplasts. I. kinetics and stoichiometry of fatty acid peroxidation. Arch Biochem Biophys 125:189–198. https://doi.org/10.1016/0003-9861(68)90654-1

Hossain MA, Nakano Y, Asada K (1984) Monodehydroascorbate reductase in spinach chloroplasts and its participation in regeneration of ascorbate for scavenging hydrogen peroxide. Plant Cell Physiol 25:385–395. https://doi.org/10.1093/oxfordjournals.pcp.a076726

Hu HJ, Shi ZS, Lv XL, Guo JL, Lv YY, Li ZW (2014) Effect of herbicide nicosulfuron on photosynthesis traits and chlorophyll fluorescence parameters of wax maize. J Maize Sci 22:77–80 (in Chinese)

Hussain MI, GonzáLez L, Reigosa M (2010) Phytotoxic effects of allelochemicals and herbicides on photosynthesis, growth and carbon isotope discrimination in *Lactuca sativa*. Allelopath J 26:157–174

Ismaiel AA, Papenbrock J (2017) Effect of patulin from *Penicillium vulpinum* on the activity of glutathione-S-transferase and selected antioxidative enzymes in maize. Int J Environ Res Public Health 14(7):825. https://doi.org/10.3390/ijerph14070825

Jena S, Choudhuri MA (1981) Glycolate metabolism of three submerged aquatic angiosperms during aging. Aquat Bot 12:345–354

Jiang M, Zhang J (2002) Water stress-induced abscisic acid accumulation triggers the increased generation of reactive oxygen species and up-regulates the activities of antioxidant enzymes in maize leaves. J Exp Bot 53:2401–2410. https://doi.org/10.1093/jxb/erf090

Johnston EJ, Rylott EL, Beynon E, Lorenz A, Chechik V, Bruce NC (2015) Monodehydroascorbate reductase mediates TNT toxicity in plants. Science 349:1072–1075. https://doi.org/10.1126/science.aab3472

Kaur G, Kaur S, Singh HP, Batish DR, Kohli RK, Rishi V (2015) Biochemical adaptations in Zea mays roots to short-term Pb^{2+} exposure: ROS generation and metabolism. Bull Environ Contam Toxicol 95:246–253. https://doi.org/10.1007/s00128-015-1564-y

Li Z, Su D, Lei B, Wang F, Geng W, Pan G, Cheng F (2015) Transcriptional profile of genes involved in ascorbate-glutathione cycle in senescing leaves for an early senescence leaf (*esl*) rice mutant. J Plant Physiol 176:1–15. https://doi.org/10.1016/j.jplph.2014.09.020

Li Y, Chen Q, Nan H, Li X, Lu S, Zhao X, Liu B, Guo C, Kong F, Cao D (2017) Overexpression of *GmFDL19* enhances tolerance to drought and salt stresses in soybean. PLoS One 12(6):e0179554. https://doi.org/10.1371/journal.pone.0179554

Li X, Ke M, Zhang M, Peijnenburg WJGM, Fan X, Xu J, Zhang Z, Lu T, Fu Z, Qian H (2018) The interactive effects of diclofop-methyl and silver nanoparticles on Arabidopsis thaliana: growth, photosynthesis and antioxidant system. Environ Pollut 232:212–219. https://doi.org/10.1016/j.envpol.2017.09.034

Meyer MD, Payaky JK, Williams MM (2010) Genetic factors influencing adverse effects of mesotrione and nicosulfuron on sweet corn yield. Agron J 102:1138–1145. https://doi.org/10.2134/agronj2010.0093

Nakano Y, Asada K (1981) Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. Plant Cell Physiol 22:867–880. https://doi.org/10.1093/oxfordjournals.pcp.a076232

O'Sullivan J, Bouw WJ (1998) Sensitivity of processing sweet corn (*Zea mays*) cultivars to nicosulfuron/rimsulfuron. Can J Plant Sci 78:151–154. https://doi.org/10.4141/P97-054

O'Sullivan J, Brammall RA, Bouw WJ (1995) Response of sweet corn (*Zea mays*) cultivars to nicosulfuron plus rimsulfuron. Weed Technol 9:58–62 http://www.jstor.org/stable/3987822

O'Sullivan J, Zandstra J, Sikkema P (2002) Sweet corn (*Zea mays*) cultivar sensitivity to mesotrione. Weed Technol 16:421–425 http://www.jstor.org/stable/3989569

Pataky JK, Meyer JD, Bollman JD, Boerboom CM, Williams MMI (2008) Genetic basis for varied levels of injury to sweet corn hybrids from three cytochrome P450-metabolized herbicides. J Am Soc Hortic Sci 133:438–447

Prasad SM, Kumar S, Parihar P, Singh R (2016) Interactive effects of herbicide and enhanced UV-B on growth, oxidative damage and the ascorbate-glutathione cycle in two *Azolla* species. Ecotoxicol Environ Saf 133:341–349. https://doi.org/10.1016/j.ecoenv.2016.07.036

Queval G, Noctor G (2007) A plate reader method for the measurement of NAD, NADP, glutathione, and ascorbate in tissue extracts: application to redox profiling during arabidopsis rosette development. Anal Biochem 363:58–69. https://doi.org/10.1016/j.ab.2007.01.005

Rey-Caballero J, Menéndez J, Giné-Bordonaba J, Salas M, Alcántara R, Torra J (2016) Unravelling the resistance mechanisms to 2,4-D (2,4-dichlorophenoxyacetic acid) in corn poppy (*Papaver rhoeas*). Pestic Biochem Physiol 133:67–72. https://doi.org/10.1016/j.pestbp.2016.03.002

Seo JS, Lee KW, Rhee JS, Hwang DS, Lee YM, Park HG, Ahn IY, Lee JS (2006) Environmental stressors (salinity, heavy metals, H_2O_2) modulate expression of glutathione reductase (GR) gene from the intertidal copepod *Tigriopus japonicus*. Aquat Toxicol 80(3):281–289. https://doi.org/10.1016/j.aquatox.2006.09.005

Sheeba SVP, Srivastava PK, Prasad SM (2011) Differential physiological and biochemical responses of two cyanobacteria *Nostoc muscorum* and *Phormidium foveolarum* against oxyfluorfen and UV-B radiation. Ecotoxicol Environ Saf 74:1981–1993. https://doi.org/10.1016/j.ecoenv.2011.07.006

Shin SY, Kim MH, Kim YH, Park HM, Yoon HS (2013) Co-expression of monodehydroascorbate reductase and dehydroascorbate reductase from Brassica rapa effectively confers tolerance to freezing-induced oxidative stress. Mol Cell 36:304–315. https://doi.org/10.1007/s10059-013-0071-4

Stall WM, Bewick TA (1992) Sweet corn cultivars respond differentially to the herbicide nicosulfuron. HortSci 27:131–133

Sun L, Wu R, Su W, Gao Z, Lu C (2017) Physiological basis for isoxadifen-ethyl induction of nicosulfuron detoxification in maize hybrids. PLoS One 12(3):e0173502. https://doi.org/10.1371/journal

Sun X, Xu Y, Zhang Q, Li X, Yan Z (2018) Combined effect of water inundation and heavy metals on the photosynthesis and physiology of Spartina alterniflora. Ecotoxicol Environ Saf 153:248–258. https://doi.org/10.1016/j.ecoenv.2018.02.010

Sytykiewicz H (2016) Expression patterns of genes involved in ascorbate-glutathione cycle in aphid-infested maize (*Zea mays* L.) seedlings. Int J Mol Sci 17:268. https://doi.org/10.3390/ijms17030268

Tan W, Li QL, Zhai H (2012) Photosynthesis and growth responses of grapevine to acetochlor and fluoroglycofen. Pestic Biochem Physiol 103:210–218. https://doi.org/10.1016/j.pestbp.2012.05.010

Torres-Franklin ML, Contour-Ansel D, Zuily-Fodil Y, Pham-Thi AT (2008) Molecular cloning of glutathione reductase cDNAs and analysis of GR gene expression in cowpea and common bean leaves during recovery from moderate drought stress. J Plant Physiol 165:514–521. https://doi.org/10.1016/j.jplph.2007.03.011

Wang XF, Guan CH, Fan ZW, Zhen HJ (2011) Application and development of sulfonylurea herbicides. Agrochemicals 50:9–15. https://doi.org/10.16820/j.cnki.1006-0413.2011.01.003

Wang J, Zhong XM, Lv XL, Li FH, Shi ZS (2016) Advances in research on tolerance of differential corn cultivars to the nicosulfuron. Chin J Pestic Sci 18:282–290. https://doi.org/10.16801/j.issn.1008-7303.2016.0038

Wang J, Zhong XM, Lv XL, Shi ZS, Li FH (Accepted) Photosynthesis and physiology responses of paired near-isogenic lines in waxy maize (*Zea mays* L.) to nicosulfuron, Photosynthetica

Williams BJ, Harvey RG (2000) Effect of nicosulfuron timing on wild-proso millet (*Panicum miliaceum*) control in sweet corn (*Zea mays*). Weed Technol 14:377–382. https://doi.org/10.1614/0890-037X(2000)014[0377:EONTOW]2.0.CO;2

Wu TM, Lin WR, Kao CH, Hong CY (2015) Gene knockout of glutathione reductase 3 results in increased sensitivity to salt stress in rice. Plant Mol Biol 87(6):555–564. https://doi.org/10.1007/s11103-015-0290-5

Xu JH, Tran T, Marcia CSP, Braun DM, Goggin FL (2017) Superoxide-responsive gene expression in *Arabidopsis thaliana* and *Zea mays*. Plant Physiol Biochem 117:51–60. https://doi.org/10.1016/j.plaphy.2017.05.018

Yang XF, Lei K, Kong CH, Xu XH (2017) Effect of allelochemical tricin and its related benzothiazine derivative on photosynthetic performance of herbicide-resistant barnyardgrass. Pestic Biochem Physiol 143:224–230. https://doi.org/10.1016/j.pestbp.2017.08.010

Yu Q, Powles SB (2014) Resistance to AHAS inhibitor herbicides: current understanding. Pest Manag Sci 70:1340–1350. https://doi.org/10.1002/ps.3710

Yu YJ, Shi ZS, Hu HJ, Li Y, Wang J (2015) Response to nicosulfuron of hydroponic seedlings of near-isogenic lines of waxy corns with different resistances. Agri Res in the Arid Areas 33:87–90. https://doi.org/10.16302/j.cnki.1000-7601.2015.02.014

Yu XZ, Zhang XH, Yue DM (2014) Alternation of antioxidative enzyme gene expression in rice seedlings exposed to methylene blue. Environ Sci Pollut Res 21:14014–14022. https://doi.org/10.1007/s11356-014-3306-9

Yuan XY, Zhang LG, Ning N, Wen YY, Dong S, Yin MQ, Guo MJ, Wang BQ, Feng L, Guo PY (2014) Photosynthetic physiological response of Radix Isatidis (*Isatis indigotica* Fort.) seedlings to nicosulfuron. PLoS One 9:e105310. https://doi.org/10.1371/journal.pone.0105310

Zhang JX, Kirkham MB (1996) Antioxidant responses to drought in sunflower and sorghum seedlings. New Phytol 132:361–373. https://doi.org/10.1111/j.1469-8137.1996.tb01856.x

Zhang YJ, Wang W, Yang HL, Li Y, Kang XY, Wang XR, Yang ZL (2015) Molecular properties and functional divergence of the dehydroascorbate reductase gene family in lower and higher plants. PLoS One 10(12):e0145038. https://doi.org/10.1371/journal.pone.0145038

Zhang L, Sun L, Zhang L, Qiu H, Liu C, Wang A, Deng F, Zhu J (2017) A Cu/Zn superoxide dismutase gene from *Saussurea involucrata* Kar. et Kir., *SiCSD*, enhances drought, cold and oxidative stress in transgenic tobacco. Can J Plant Sci 97(5):816–826. https://doi.org/10.1139/CJPS-2016-0180