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Photosynthesis and physiology responses of paired near-isogenic lines in waxy maize (*Zea mays* L.) to nicosulfuron

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Abstract

Nicosulfuron is a post-emergence herbicide used for weed control in fields of maize (*Zea mays* L.). We used a pair of nearly isogenic inbred lines, SN509-R (nicosulfuron-resistant) and SN509-S (nicosulfuron-sensitive), to study the effect of nicosulfuron on waxy maize seedling. After the nicosulfuron treatment, net photosynthetic rate, stomatal conductance, transpiration rate, leaf maximum photochemical efficiency of PSII, photochemical quenching of chlorophyll fluorescence, and the actual photochemical efficiency of PSII were significantly lower in SN509-S than those of SN509-R, contrary to intercellular CO_2 concentration, stomatal limitation, and nonphotochemical quenching. Compared to SN509-R, antioxidant enzyme activities in SN509-S decreased significantly in response to the nicosulfuron treatment, while SN509-S exhibited an increased malondialdehyde content, which was associated with lower antioxidant enzyme activities. These results collectively suggest that the nicosulfuron-resistance mechanism was associated with photosynthetic rate, reactive oxygen species metabolism, and protective mechanisms.

Additional key words: antioxidant enzymes; chlorophyll florescence; nicosulfuron; photosynthesis; waxy maize.

Introduction

Nicosulfuron [2-[(4,6-dimethoxypyrimidin-2-ylcarbamoyl) sulfamoyl]-*N*,*N*-dimethylnicotinamide] is an effective, broad-spectrum, inner-absorption conducting sulfonylurea herbicide that is mainly used for the control of annual weeds and broadleaf weed species in maize production (Stall and Bewick 1992, Williams and Harvey 2000). Nicosulfuron residues in soils and rivers are degraded by hydrolysis and microbial activity, so the toxicity of nicosulfuron has a minor effect on the next crop (Wang *et al.* 2011). The effectiveness of nicosulfuron makes up for the paucity of post-emergence herbicides in corn, and it has been also important for the development of the herbicide industry in China.

The differences in susceptibility of weeds and crops to nicosulfuron explain its ability to control the growth of weeds effectively. Nicosulfuron selectively kills weeds, while causing minimal harm to crops (Dobbels and Kapusta 1993, Kapusta *et al*. 1995). Nicosulfuron is a sulfonylurea herbicide that inhibits the activity of acetolactate synthase (ALS), reduces the synthesis of branched chain amino acids, and kills weeds (Ray 1984). By contrast, nicosulfuron can be degraded into inactive substances and can lose its activity when it is combined with glucose in maize (Pataky *et al*. 2008, Meyer *et al*. 2010).

Recent nicosulfuron studies have concentrated on the degradation of nicosulfuron residue in soil and its environmental impacts, but a few studies related to plant photosystem damage have been reported. Some studies have indicated that nicosulfuron can reduce chlorophyll (Chl) contents and photosynthesis, thereby inhibiting plant growth (Saladin *et al*. 2003, Belgers *et al*. 2007, Bigot *et al*. 2007, Macedo *et al*. 2008, Hussain *et al*. 2010). Herbicide application damages the photosynthetic apparatus within the PSI and PSII, thus reducing the maximum photochemical efficiency of PSII (F_v/F_m), the photochemical quenching coefficient (q_P), and the

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Abbreviations: APX – ascorbate peroxidase; CAT – catalase; Chl – chlorophyll; C_i – intercellular CO_2 concentration; DAT – days after herbicide treatment; ETR – electron transport rate; F_0 – minimal fluorescence yield of the dark-adapted state; F_m – maximal fluorescence yield of the dark-adapted state; F_0' – minimal fluorescence yield of the light-adapted state; F_m' – maximal fluorescence yield of the light-adapted state; F_v – variable fluorescence; F_v/F_m – maximal quantum yield of PSII photochemistry; g_s – stomatal conductance; L_s – stomatal limitation; MDA – malondialdehyde; NPQ – nonphotochemical quenching; NILs – nearly-isogenic lines; NBT – nitroblue tetrazolium chloride; P_N – net photosynthetic rate; POD – peroxidase; q_P – photochemical quenching coefficient; ROS – reactive oxygen species; SOD – superoxide dismutase; Φ_{PSII} – effective quantum yield of PSII photochemistry.
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quantum yield of PSII electron transport (Φ_{PSII}) (Kaňa *et al*. 2004, Tan *et al*. 2012, Hu *et al*. 2014, Debona *et al*. 2016). This is due to the excessive reactive oxygen species (ROS) which are produced by photosynthesis under the stress conditions. The Q_A and primary electron acceptor of PSII are reduced, leading to the direct attack of ROS on the photochemical reaction center of PSII (Vass *et al*. 1992). However, with the constant evolution of plants, herbicide toxicity can be minimized by self-defense mechanisms. One of these protective mechanisms is the antioxidant system, an important plant defense mechanism. It can be combined with a variety of enzymes to remove excessive ROS so that the PSI and PSII are not affected (Jiang and Yang 2009). Hwang *et al.* (2004) used a superoxide dismutase (SOD) inhibitor to treat leaves of chilling-resistant spinach, which reduced the activity of the PSI by 50%. At the same time, a large number of studies have confirmed that the antioxidant system plays a key role in the protection of the PSI by scavenging hydroxyl radicals, superoxide, and hydrogen peroxide (Jakob and Heber 1996, Sonoike 1996, Aroca *et al.* 2001, Tjus *et al.* 2001).

The effects of nicosulfuron on photosynthesis and antioxidant enzyme activities in waxy maize leaves have not been reported yet. Accordingly, we investigated the effects of nicosulfuron on the net photosynthetic rate, fluorescence characteristics, and antioxidant enzymes in a pair of nicosulfuron-resistant and nicosulfuron-sensitive nearly-isogenic lines (NILs) of maize, SN509-R and SN509-S. We selected these paired NILs in order to minimize effects caused by differences in genetic background. Our study helped us further understand the mechanism of physiological defense in different sensitive inbred lines, which provides a strong theoretical basis for the innovation, screening, and conservation of maize germplasm resources that are resistant to nicosulfuron.

Materials and methods

Experimental design: The experiment was designed at the south farm of Shenyang Agricultural University (41°49'N, 123°34'E), 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(organic matter) kg^{-1}, 2.45 g(total nitrogen) kg^{-1}, 110.75 mg(alkaline-hydrolyzed nitrogen) kg^{-1}, 11.09 mg (available phosphorous) kg^{-1}, and 105.74 mg(available potassium) kg^{-1}. A pair of NILs, SN509-R and SN509-S, were developed by the Institute of Speciality Corn of Shenyang Agricultural University. The SN509-R line grew normally after being sprayed with nicosulfuron. By contrast, nicosulfuron inhibits the growth of the SN509-S inbred line, which ultimately causes the plant to die. A split-plot experimental design was used with the nicosulfuron treatment defining the 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 in each plot, with each plot measuring 28.8 m^2 in area.

Pesticide treatments: In 2015, 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 being rinsing with a small volume of distilled water in culture dishes. After 7 d, uniformly sized buds (0.01 m) were transferred to the new culture dishes with filter paper. Following nicosulfuron concentrations: 0 mg kg^{-1} (control), 8, 40, 80, 120, 160, 240, 320, and 400 mg kg^{-1} were applied to each of the cultures to screen the resistance of SN509-R and SN509-S under a wide range of herbicide concentrations. Fifty buds of each line were evenly spread into culture dishes, with three replicates. After 2 d, buds were transferred to the field, and the survival rate was investigated at the five-leaf stage. This screening revealed that when the concentration of nicosulfuron was 80 mg kg^{-1}, SN509-R plants were able to attain normal growth *via* self-defense mechanisms, while SN509-S plants wilted or died (Table 1). In 2016, an experiment was conducted in a field. The 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 effects on photosynthetic and physiological parameters and antioxidant enzyme activities of maize seedlings. Because the seedlings of SN509-S either died or wilted after 15 d after herbicide treatment (DAT), data were determined every 2 d after 0 DAT.

Gas exchange: The *LI-6400* portable optical instrument (*LI-COR Biosciences Inc*., Lincoln, NE, USA) was used to measure several parameters at a light intensity of 1,200 ± 50 μmol(photon) m^{-2} s^{-1}, CO_2 concentration of 360 ± 20 μmol mol^{-1}, and air temperature of 25 ± 1°C, including the net photosynthetic rate (P_N), stomatal conductance (g_s), intercellular CO_2 concentrations (C_i), and transpiration rate (E).

Chlorophyll (Chl) fluorescence parameters were analyzed with a *PAM-2500* pulse modulated fluorometer (*Walz*, Germany) connected to a computer with the data acquisition software *PAMwin3*. Prior to the measurements, the leaves were adapted to darkness. The minimal fluorescence yield of the dark-adapted state (F_0) was determined using a very low modulated light [<0.1 μmol(photon) m^{-2} s^{-1}]. The maximal fluorescence yield of the light-adapted state (F_m) and maximal quantum yield of PSII photochemistry (F_v/F_m) were determined by using a 600-ms saturated light pulse. Then, the leaves were illuminated with an actinic light [800 μmol(photon) m^{-2} s^{-1}] in order to determine the steady state (F_s) and the maximal fluorescence yield of the light-adapted state (F_m').

Table 1. Effect of nicosulfuron on germination rate of waxy maize seed of SN509-R and SN509-S. * – differences under different days after herbicide treatment at $P<0.05$, according to the least significant difference (LSD) test. CK – Control.

Spaying concentration [mg k^{-1}]	Germination percentage SN509-R	Than CK [%]	SN509-S	Than CK [%]	Survival rate after germination 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*	-	

The following Chl fluorescence parameters were collected: the maximal quantum yield of PSII photochemistry (F_v/F_m), calculated according to van Kooten and Snel (1990); the effective quantum yield of PSII photochemistry (Φ_{PSII}) and electron transport rate (ETR), calculated according to Genty *et al.* (1989); the photochemical quenching coefficient (q_p), calculated as $q_P = (F_m - F_s)/(F_m - F_0)$; nonphotochemical quenching (NPQ), calculated as $NPQ = (F_m - F_m')/F_m'$.

Assay of antioxidant enzyme activities (superoxide dismutase, peroxidase, catalase, and ascorbate peroxidase): After the nicosulfuron treatment, 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 by a *UNICO™ UV-2000* spectrophotometer (*UV-2000*, *UNICO*, USA).

Enzyme extraction: Enzymes were extracted by grinding 0.5 g of leaf samples in 5 ml of phosphate buffer (pH 7.5) containing 0.05 mM EDTA. The homogenate was centrifuged at 10,000 × *g* at 4°C for 20 min and the supernatant was collected for measurement of enzyme activities.

Superoxide dismutase (SOD, EC 1.15.1.1) activity: The method described by Giannopolitis and Ries (1977) was used to measure the SOD activity. The 3 ml of 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 absorbance at 560 nm. The enzyme activity was caculated as 50% inhibition expressed in unit g^{-1}(fresh mass, FM). One unit of SOD activity was defined as the enzyme activity that inhibited the photoreduction of NBT to purple formazan by 50%.

Peroxidase (POD, EC 1.11.1.7) activity: The method described by Cakmak and Marschner (1992) was used to determine guaiacol peroxidase activity. The catalyzed reaction system consisted of 1.0 ml of phosphate buffer (100 mM, pH 6.1), 0.5 ml of guaiacol, 0.5 ml of H_2O_2, and 0.1 ml of enzyme extract. Changes in the absorbance of the reaction solution described above at 470 nm (extinction coefficient = 26 mM^{-1} cm^{-1}) caused by the formation of tetraguaicol were recorded for 3 min. The enzyme activity was calculated and expressed in μmol(guaicol reduced) min^{-1} g^{-1}(FM).

Catalase (CAT, EC 1.11.1.6) activity: Catalase activity was assayed according to the method described by Aebi (1984). A decrease in the absorbance due to the disappearance of H_2O_2 was measured at 240 nm (extinction coefficient = 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 μmol(H_2O_2 oxidised) min^{-1} g^{-1}(FM).

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 the enzyme activity. The enzyme activity of APX was expressed in μmol(product) min^{-1} g^{-1}(FM) (Nakano and Asada 1981).

Malondialdehyde (MDA) content: After the nicosulfuron treatment, the seedling leaves were sampled and placed in liquid nitrogen, and then stored at –80°C until analyses were performed. The thiobarbituric acid method was used to determine the MDA content of the 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 4,500 × *g* for 10 min and the supernatant was collected for measurement of MDA. The 2 ml of extract and 3 ml of 0.5% thiobarbituric acid (TBA) containing 5% trichloroacetic acid (TCA) were boiled for 10 min, and the solution was

cooled to room temperature. The mixture was then centrifuged at 4,500 × *g* for 10 min. The absorbance of the supernatant was recored at 450, 532, and 600 nm with a *UNICO™ UV-2000* spectrophotometer (*UV-2000*, *UNICO*, USA). MDA was expressed as nmol g^{-1}(FM), and the extinction coefficient (155 mM^{-1} cm^{-1}) was used to calculate lipid peroxidation.

Proline content: Samples of 0.5 g from leaves were homogenized with 5 ml of 3% sulfosalicylic in a boiling water bath for 10 min, and then the homogenate was filtered through filter paper. A reaction mixture containing 2 ml of glacialacetic acid, 3 ml of acid-ninhydrin, and 2 ml of extract was boiled in a water bath for 40 min. After cooling, the solution was mixed with 5 ml of methylbenzene and stirred well. The absorbance of the red methylbenzene supernatant was measured at 520 nm with a *UNICO™ UV-2000* spectrophotometer (*UNICO*, USA). Proline content was expressed as μg g^{-1}(FM).

Data analysis: *Microsoft Excel* and *SigmaPlot 12.5* were used for data processing and mapping, and each reported data point is 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 analysis of variance (*ANOVA*), and comparisons among mean values were made by least significant difference (LSD) test at a $P<0.05$ significance threshold.

Results

***P*N**: Nicosulfuron decreased P_N differently in the two inbred lines (Fig.1). Compared to 0 DAT, nicosulfuron significantly decreased the P_N values of SN509-R at 5, 7, and 9 DAT by up to 5.0, 3.0, and 11.0%, respectively. Nicosulfuron also significantly reduced the P_N values of SN509-S at 1, 3, 5, 7, and 9 DAT by up to 3.3, 3.9, 28.1, 99.6, and 99.8%, respectively. In general, the effect of nicosulfuron on P_N was much higher in SN509-S than that in SN509-R. After 1 DAT, the P_N value of SN509-R was 28.31–24.23 μmol m^{-2} s^{-1}, with an average of 26.62 μmol m^{-2} s^{-1}; in contrast, the P_N value of SN509-S was 0.06–26.64 μmol m^{-2} s^{-1}, with an average of only 14.62 μmol m^{-2} s^{-1}. The average P_N of SN509-R was 45.1% higher than that of SN509-S. Compared to the SN509-S line, the SN509-R line maintained a higher photosynthetic capacity after spraying with nicosulfuron and completed its normal growth and development.

***g*s, *E*, L*s*, and *C*i**: Nicosulfuron had little effect on the photosynthetic parameters of SN509-R, in contrast to those of SN509-S. The g_s of SN509-R was first reduced, and then increased by each day following treatments (Table 2), while the g_s of SN509-S continued to decline after 5 DAT. Compared to 0 DAT, nicosulfuron significantly decreased the g_s of SN509-S by up to 27.8, 94.4, and 94.4% by 5, 7, and 9 DAT, respectively. Nicosulfuron had no significant effect on the C_i, E, and L_s of SN509-R, in contrast to those of SN509-S. After 9 DAT, the C_i and L_s of SN509-S reached their maximum values of 321.74 μmol(CO_2) mol^{-1} and 0.87, respectively, which were was 171.8 and 248% higher than those at 0 DAT, respectively. The E of SN509-S reached a minimum value of 0.19 mmol(H_2O) m^{-2} s^{-1}, which was 95.4% lower than that at 0 DAT. The photosynthetic parameters were compared between the two inbred lines: compared to SN509-R, the nicosulfuron treatment significantly reduced the g_s and E of SN509-S by 40.7 and 46.9%, on average, respectively. The C_i and L_s of SN509-S were remarkably higher than those of SN509-R. Our study showed that the L_s might be the main reason for the decline of P_N in SN509-S, and it was also the main response of SN509-S to the nicosulfuron treatment.

F_v/F_m: represents the photochemical conversion efficiency and potential activity in plant leaves. Nicosulfuron had little effect on the F_v/F_m of SN509-R, while F_v/F_m of SN509-S declined (Fig. 2*A*). Compared to 0 DAT, F_v/F_m of SN509-S declined by 0.7, 8.5, 32.4, and 27.6% by 3, 5, 7, and 9 DAT, respectively; F_v/F_m of SN509-R increased by an average of 1.5% from 3 DAT to 9 DAT. Additionally, the F_v/F_m of SN509-R was significantly higher than that of SN509-S at 3, 5, 7, 9 DAT, with increases of 2.3, 3.9, 29.6, and 29.1%, respectively. The F_v/F_m indicated that the SN509-R had a greater capacity to adapt to herbicide stress.

Φ_{PSII} reflects the actual photochemical activity of the PSII (Govindjee 2002). The Φ_{PSII} values of the inbred lines had

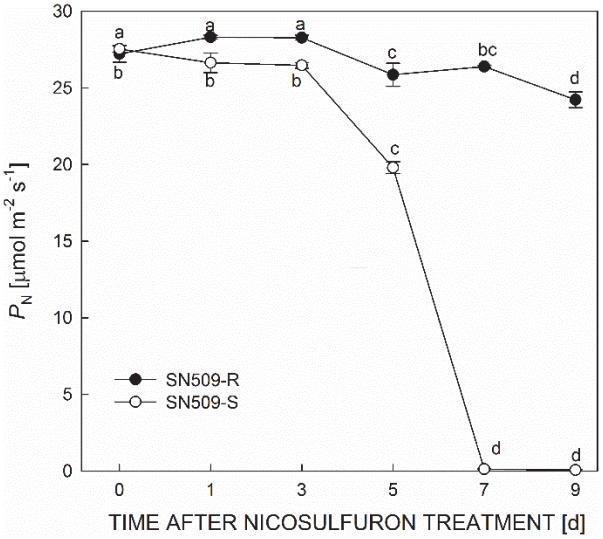

Fig. 1. Effects of nicosulfuron on the net photosynthetic rate (P_N) in leaves of maize seedlings. *Vertical bars* represent the SE (n = 5). *Small letters* indicate differences between values obtained on different days after nicosulfuron treatment ($P<0.05$) according to a least significant difference (LSD) test.

Table 2. Effect of nicosulfuron on photosynthetic indexes of waxy maize leaves in SN509-R and SN509-S. * indicate differences under different days after herbicide treatment at $P < 0.05$, according to the least significant difference (LSD) test. C_i – intercellular CO_2 concentration; DAT – days after nicosulfuron treatment; E – transpiration rate; g_s – stomatal conductance; L_s – stomatal limitation.

DAT	g_s [mol(H_2O) m^{-2} s^{-1}]			C_i [μmol(CO_2) mol^{-1}]			E [mmol(H_2O) m^{-2} s^{-1}]			L_s		
	SN509-R	SN509-S	SN509-R than SN509-S [%]	SN509-R	SN509-S	SN509-R than SN509-S [%]	SN509-R	SN509-S	SN509-R than SN509-S [%]	SN509-R	SN509-S	SN509-R than SN509-S [%]
0	0.19 ± 0.05	0.18 ± 0.04	5.26	119.98 ± 1.61	118.37 ± 1.07	1.34	3.97 ± 0.13	4.11 ± 0.14	–3.52	0.24 ± 0.06	0.25 ± 0.03	–4.16
1	0.18 ± 0.008	0.16 ± 0.05	11.11	121.34 ± 4.77	116.06 ± 1.49	0.04	4.39 ± 0.92	4.12 ± 0.28	6.15	0.26 ± 0.07	0.33 ± 0.01	–26.92
3	0.17 ± 0.02	0.19 ± 0.04	–10.53	123.63 ± 9.12	126.52 ± 5.42	–2.33	4.43 ± 1.36	3.60 ± 0.08	18.74	0.28 ± 0.01	0.30 ± 0.01	–7.14
5	0.15 ± 0.03	0.13 ± 0.01	13.33	118.73 ± 10.83	207.51 ± 3.23	–74.77*	4.06 ± 0.61	3.04 ± 0.47	25.12	0.35 ± 0.04	0.50 ± 0.05	–42.85*
7	0.20 ± 0.02	0.01 ± 0.01	95.0*	116.34 ± 6.37	323.84 ± 1.40	–178.35*	3.70 ± 0.15	0.27 ± 0.06	92.7*	0.34 ± 0.04	0.68 ± 0.01	–100*
9	0.19 ± 0.02	0.01 ± 0.01	94.74*	124.94 ± 7.34	321.74 ± 9.04	–157.52*	4.10 ± 0.37	0.19 ± 0.08	95.36*	0.29 ± 0.02	0.87 ± 0.01	–200*

different responses to nicosulfuron over time (Fig. 2*B*). Φ_{PSII} of SN509-S was reduced, while nicosulfuron had little effect on Φ_{PSII} of SN509-R. Except for 3 DAT, the Φ_{PSII} of SN509-R was significantly higher than that of SN509-S at 1, 5, 7, and 9 DAT, with increases of 16.6, 58.5, 71.3, and 64.8%, respectively. This suggested that SN509-R resisted to injury caused by herbicide through its own regulatory mechanisms.

ETR reflects the efficiency of the apparent electron transfer under the actual light intensity, and it has a strong linear relationship with the photosynthetic rate (Zhong *et al*. 2014). In SN509-R, ETR was first reduced, and then increased with the exposure time (Fig. 2*C*), while ETR was reduced in SN509-S throughout. The average ETR of SN509-R was 39.5% higher than that of SN509-S. The reduction of ETR was a consistent explanation for the decrease of P_N in SN509-S.

q_P is commonly used to determine the openness of the PSII. Nicosulfuron caused little change in q_P of SN509-R, while q_P of SN509-S was significantly reduced (Fig. 2*D*). The q_P of SN509-R was significantly higher than that of SN509-S. The nonphotochemical quenching coefficient (NPQ) reflects the ability to dissipate excess light energy. After being sprayed with herbicide, F_v/F_m, Φ_{PSII}, ETR, and q_P decreased in leaves of SN509-S. In contrast, NPQ was enhanced (Fig. 2*E*). Nonradiative energy dissipation appeared to play a key role in the reduction of PSII activity.

Antioxidant enzyme activities: The antioxidant enzyme system plays an important role in scavenging ROS in the PSI and PSII (Jiang *et al.* 2009). Our experiment showed that nicosulfuron treatment induced significant decreases in SOD enzyme activity in two inbred lines at each sampling time. SOD activity of SN509-S was much lower than that of SN509-R. After herbicide treatment, the POD activity of SN509-R increased as the exposure time increased, while the POD activity of SN509-S was reduced as the exposure time increased. The average POD activity of SN509-R was 49.9% higher than that of SN509-S. The CAT activity in the two inbred lines was significantly reduced over the exposure time. The average CAT activity of SN509-S was 16.0% lower than that of SN509-R. After 3 DAT, the APX activity of the two inbred lines reached its maximum values, and then declined. After 9 DAT, the APX activity of SN509-R increased slightly, and was 14.5% higher than that of SN509-S (Fig. 3).

MDA content: After 5 DAT, the MDA content of SN509-R reached its maximum value, and then decreased, while the MDA content of SN509-S increased with the exposure time. Compared to SN509-R, the MDA content of SN509-S significantly increased by 28.9, 65.6, and 62.9%, respectively (Fig. 4*A*).

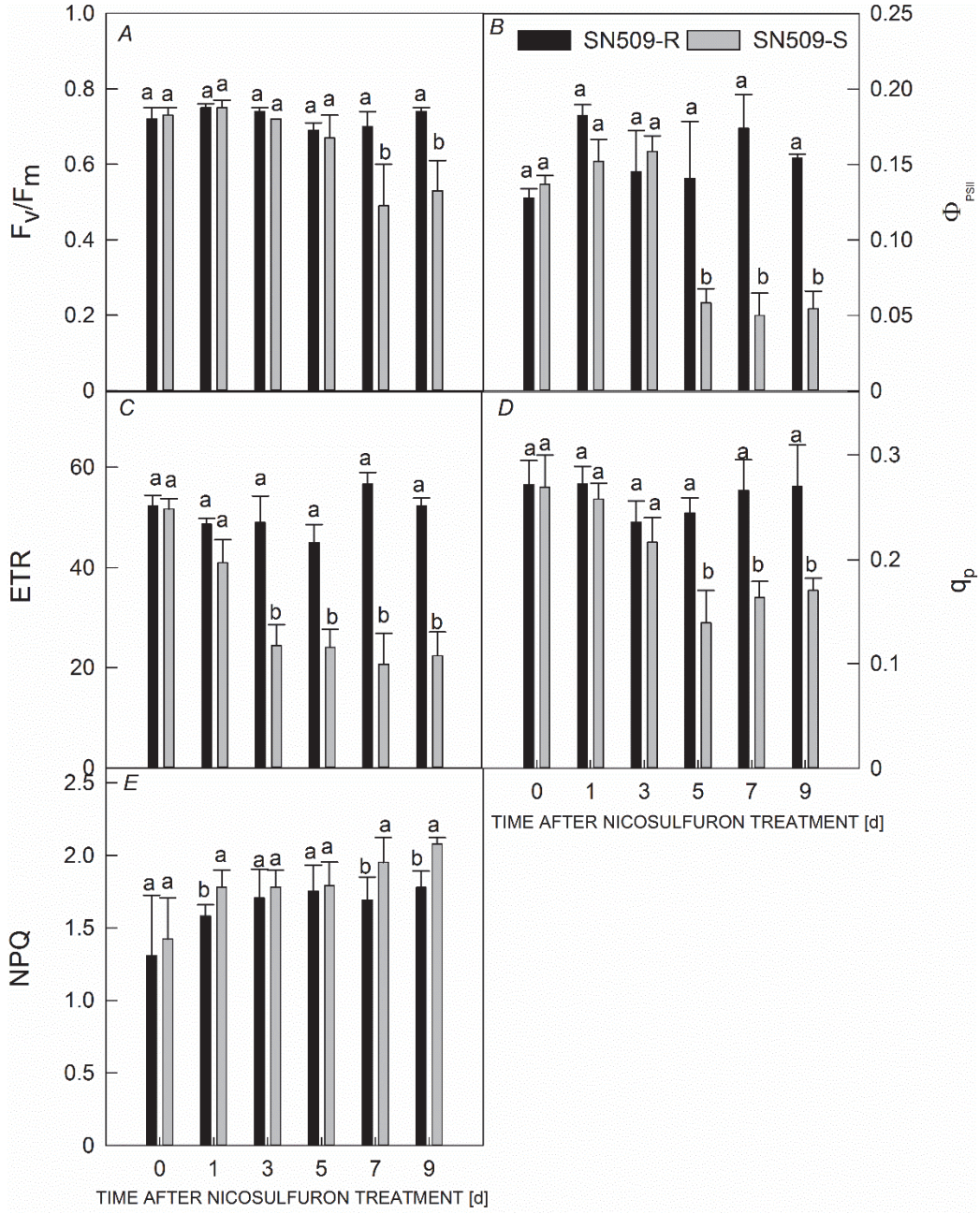

Fig. 2. Effects of nicosulfuron on leaf maximum photochemical efficiency of PSII (F_v/F_m; *A*), actual photochemical efficiency of PSII (Φ_{PSII}; *B*), electron transport rate (ETR; *C*), photochemical quenching (q_p; *D*), and nonphotochemical quenching (NPQ; *E*) in leaves of SN509-R and SN509-S. *Vertical bars* represent the SE (n = 5). *Small letters* indicate differences in values obtained on different days after nicosulfuron treatment (P<0.05) according to a least significant difference (LSD) test.

Proline content: After the herbicide treatment, the proline content of the two inbred lines increased, and the content of SN509-R increased significantly (Fig. 4*B*). Compared to SN509-S, the proline content of SN509-R increased by 18.9, 26.4, 22.3, and 34.8% at 3, 5, 7, and 9 DAT, respectively. This suggests that the increase in proline was related to the resistance of SN509-R to nicosulfuron treatment.

Discussion

Both development and popularization of herbicides were accompanied with precision agriculture and benefited agriculture. Accordingly, the total application area of herbicides has rapidly expanded since the 1970s in Europe

and America (Wu *et al*. 2004). During the past 20–30 years, the application of herbicides in America and Canada has increased 3- to 5-fold (Freemark and Boutin 1994). Since the 1990s, the herbicide industry in China has entered a period of accelerated development and the proportion of herbicides, accounting for three types of pesticides, has increased linearly. Nicosulfuron in agricultural production has been widely used as a post-emergence herbicide in maize. Many studies have demonstrated that maize cultivars differ in their sensitivity to nicosulfuron (Eberline *et al*. 1989, Wang *et al*. 2016). Therefore, the harm that nicosulfuron causes to maize crops should be further studied in order to evaluate its impact on different corn varieties, a crucial issue in maize production that urgently needs to be addressed.

Herbicide-induced damage to crops can be evaluated by measuring agronomic traits and physiological indexes. Many herbicides are photosynthesis inhibitors, that operate by interfering with the electron transport rate, which is significantly reduced in the thylakoid membranes of chloroplasts from plants treated with clomazone (Kaňa *et al*. 2004). A similar effect occurs following atrazine treatment. When atrazine binds to the D1 protein of PSII, electron transfer is blocked (Zheleva *et al*. 1994). Similarly, fluoroglycofen and acetochlor treatments reduce P_N and g_s in grape leaves (Bigot *et al*. 2007). Nicosulfuron can be absorbed by leaves and roots and can then be transported by the xylem and phloem, thus decreasing photosynthesis in leaves (Dobbels *et al.*1993, Wang *et al*. 2016). In our experiment, the NILs SN509-R and SN509-S were used to assess resistance of maize to nicosulfuron, especially, the effect of the herbicide on photosynthetic and physiological properties. Our results showed that P_N, F_v/F_m, q_P, ETR, and Φ_{PSII} decreased significantly as the exposure time increased in SN509-S, while the photosynthetic capacity of SN509-R remained high, so as to maintain the normal growth of crops. After 7 DAT, P_N and E of SN509-S significantly decreased, while C_i and L_s of SN509-S significantly increased, indicating that the decrease of P_N could be mainly attributed to stomatal limitation. We inferred that the chloroplast structure of the maize seedling leaf was destroyed due to the action of nicosulfuron, leading to a reduction in the thylakoid stacking level (Liu *et al*. 2007, Yuan *et al*. 2014). Thus, the light energy conversion efficiency of the photosynthetic apparatus was affected, and the normal growth and development of the plant were inhibited.

On the chloroplast thylakoid membrane, PSI and PSII are sensitive to changes in the surrounding environments. PSII is destroyed by changes in its external environment, resulting in substantial increases in F_0 (Demmig *et al*. 1987). Nicosulfuron has significant effects on Chl fluorescence parameters in SN509-S. F_v/F_m, q_P, ETR, and Φ_{PSII} significantly decreased, while NPQ significantly increased. Frankart *et al*. (2003) studied the effects of paraquat and norflurazon on Chl fluorescence parameters in *Lemna minor*, and found significant reductions in F_v/F_m, q_P, and Φ_{PSII}, but increased NPQ. Conversely, terbutryn, flumioxazin, and nicosulfuron decrease the NPQ in grape, *Vicia faba*, and *Radix isatidis*, respectively (Frankart *et al*. 2003, Murata *et al*. 2007, Yuan *et al.* 2014). A lower proportion of opened PSII centers results from the reduction of q_P. Furthermore, NPQ is involved in energy dissipation in the leaf. After the nicosulfuron treatment, NPQ of SN509-S significantly increased, and Φ_{PSII} decreased. These results suggest that the reduction in PSII activity was mainly caused by nonradiative energy dissipation.

The reduction of P_N and the photochemical efficiency of PSII are closely linked to a high concentration of ROS (Ramel *et al*. 2009). Many herbicides cause direct or indirect oxidative damage in plants (Qian *et al*. 2008, Jiang *et al*. 2009). The destruction of redox homeostasis causes a reduction in antioxidant enzyme activity, and these changes can be used to as an indicator of oxidative stress (Platiša *et al*. 2008). Many studies have indicated that antioxidant enzymes in various plants respond differently to different herbicides due to differences in treatment methods and treatment time and the point at which measurements are taken, and there are also concentration and time effects (Geoffroy *et al*. 2004, Yoon *et al*. 2011, Wang *et al*. 2016). SOD, POD, CAT, and APX are important antioxidant enzymes. In this study, after spraying by herbicide, SOD, CAT, and APX activities were significantly reduced in both inbred lines. However, SOD, CAT, and APX of SN509-R were higher than those of SN509-S. POD is a crucial antioxidant enzyme. A wide range of electron donors enable conversion of H_2O_2 to H_2O by POD (Xue *et al*. 2008). Furthermore, POD also can improve the defense mechanism in plant leaves and roots exposed to chemicals and other abiotic stresses (Tarchoune *et al*. 2010). After herbicide treatment, the POD activity of SN509-R increased as the exposure time increased, while the POD of SN509-S decreased as the exposure time increased. The average POD activity of SN509-R was 50% higher than that of SN509-S. Thus, the POD enzyme activity in the leaves of SN509-R showed a significantly positive correlation with the exposure time. It is possible that SN509-R could promote POD activity for protection under nicosulfuron stress. Many studies have demonstrated that membrane lipid peroxidation leads to the accumulation of MDA, and herbicide treatment causes the accumulation of MDA (Hassan and Nemat Alla 2005, Wu *et al*. 2010). In this study, the MDA content of SN509-S increased as the exposure time increased, while the content of SN509-R was significantly lower than that of SN509-S. These findings suggested that oxidative stress

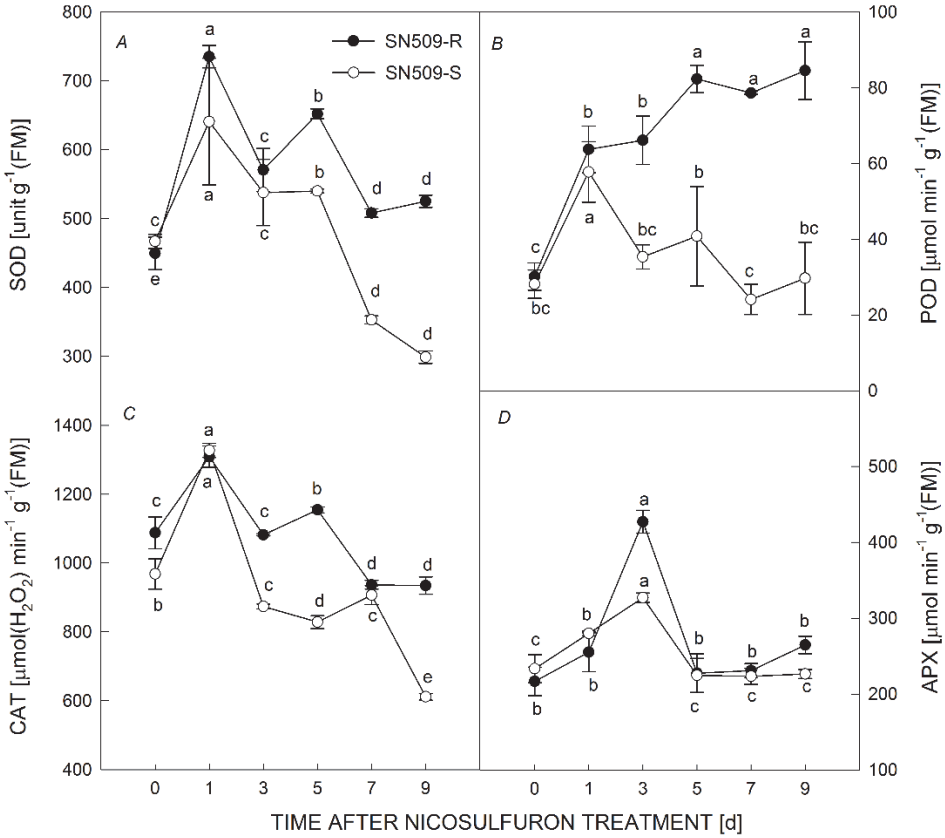

Fig. 3. Effects of nicosulfuron on superoxide dismutase (SOD; *A*), peroxidase (POD; *B*), catalase (CAT; *C*), and ascorbate peroxidase (APX; *D*) in leaves of SN509-R and SN509-S. *Vertical bars* represent the SE ($n = 5$). *Small letters* indicate differences in values obtained on different days after nicosulfuron treatment ($P<0.05$) according to a least significant difference (LSD) test.

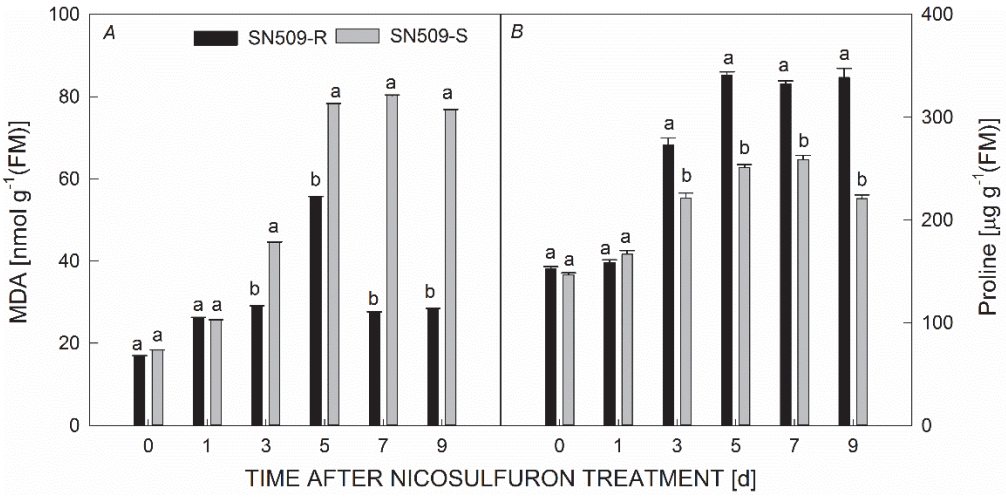

Fig. 4. Effects of nicosulfuron on the malondialdehyde content (MDA; *A*) and proline content (*B*) in leaves of SN509-R and SN509-S. *Vertical bars* represent the SE ($n = 5$). *Small letters* indicate differences in values obtained on different days after nicosulfuron treatment ($P<0.05$) according to a least significant difference (LSD) test.

on the leaves of SN509-S by nicosulfuron increased as the exposure time increased. A vital defense mechanisms in the leaves of SN509-R could protect plants from nicosulfuron damage. Based on our analysis, herbicide-resistance was associated with a high photosynthetic rate, ROS metabolism, and protective mechanisms. These are crucial physiological mechanisms underlying differences in nicosulfuron resistance between SN509-R and SN509-S.

Conclusion: After spraying plants with nicosulfuron, P_N, E, and g_s of SN509-S were lower than those of SN509-R. Compared to SN509-R, F_v/F_m, q_P, ETR, and Φ_{PSII} significantly decreased in SN509-S, but NPQ significantly increased. Our study showed that activities of SOD, POD, CAT, and APX of SN509-S were significantly lower than those of SN509-R. The higher amounts of ROS resulted in a reduction in P_N, which might be associated with lower antioxidant enzyme activities. These results clarify the crucial physiological mechanisms explaining plant death in nicosulfuron-sensitive maize.

References

Aebi H.: Catalase *in vitro*. – Methods Enzymol. **105**: 121-126, 1984.

Aroca R., Irigoyen J.J., Sánchez-Díaz M.: Photosynthetic characteristics and protective mechanisms against oxidative stress during chilling and subsequent recovery in two maize varieties differing in chilling sensitivity. – Plant Sci. **161**: 719-726, 2001.

Belgers J.D.M., van Lieverloo R.J., van der Pas L.J.T. *et al*.: Effects of the herbicide 2,4-D on the growth of nine aquatic macrophytes. – Aquat. Bot. **86**: 260-268, 2007.

Bigot A., Fontaine F., Clément C., Vaillant-Gaveau N.: Effect of the herbicide flumioxazin on photosynthetic performance of grapevine (*Vitis vinifera* L.). – Chemosphere **67**: 1243-1251, 2007.

Cakmak I., Marschner H.: Magnesium deficiency and high light intensity enhance activities of superoxide dismutase, ascorbate peroxidase, and glutathione reductase in bean leaves. – Plant Physiol. **98**: 1222-1227, 1992.

Debona D., Nascimento K.J.T., Gomes J.G.O. *et al*.: Physiological changes promoted by a strobilurin fungicide in the rice-*Bipolaris oryzae* interaction. – Pestic. Biochem. Phys. **130**: 8-16, 2016.

Demmig B., Winter K., Krüger A., Czygan F.C.: Photoinhibition and zeaxanthin formation in intact leaves: A possible role of the xanthophyll cycle in the dissipation of excess light energy. – J. Plant Physiol. **84**: 218-224, 1987.

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

Eberline C.V., Rosow K.M., Geadelmann J.L., Openshaw S.J.: Differential tolerance of corn genotypes to DPX-M6316. – Weed Sci. **37**: 651-657, 1989.

Frankart C., Eullaffroy P., Vernet G.: Comparative effects of four herbicides on non-photochemical fluorescence quenching in *Lemna minor*. – Environ. Exp. Bot. **49**: 159-168, 2003.

Freemark K., Boutin C.: Nontarget-plant risk assessment for pesticide registration. – Environ. Manage. **18**: 841-854, 1994.

Genty B., Briantais J.M., Baker N.R.: The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. – Biochim. Biophys. Acta **990**: 87-92, 1989.

Geoffroy L., Frankart C., Eullaffroy P.: Comparison of different physiological parameter responses in *Lemna minor* and *Scenedesmus obliquus* exposed to herbicide flumioxazin. – Environ. Pollut. **131**: 233-241, 2004.

Giannopolitis C.N., Ries S.K.: Superoxide dismutases: I. occurrence in higher plants. – Plant Physiol. **59**: 309-314, 1977.

Govindjee: A role for a light-harvesting antenna complex of photosystem II in photoprotection. – Plant Cell **14**: 1663-1668, 2002.

Hassan N.M., Nemat Alla M.M.: Oxidative stress in herbicide-treated broad bean and maize plants. – Acta Physiol. Plant. **27**: 429-438, 2005.

Heath R.L., Packer L.: Photoperoxidation in isolated chloroplasts. I. kinetics and stoichiometry of fatty acid peroxidation. – Arch. Biochem. Biophys. **125**: 189-198, 1968.

Hu H.J., Shi Z.S., Lv X.L. *et al*.: [Effect of herbicide nicosulfuron on photosynthesis traits and chlorophyll fluorescence parameters of wax maize.] – J. Maize Sci. **22**: 77-80, 2014. [In Chinese]

Hussain M.I., González L., Reigosa M.: Phytotoxic effects of allelochemicals and herbicides on photosynthesis, growth and carbon isotope discrimination in *Lactuca sativa*. – Allelopathy J. **26**: 157-174, 2010.

Hwang H.J., Kim J.H., Eu Y.J. *et al*.: Photoinhibition of photosystem I is accelerated by dimethyldithiocarbamate, an inhibitor of superoxide dismutase, during light-chilling of spinach leaves. – J. Photoch. Photobio. B. **73**: 79-85, 2004.

Jakob B., Heber U.: Photoproduction and detoxification of hydroxyl radicals in chloroplasts and leaves and relation to photoinactivation of photosystems I and II. – Plant Cell Physiol. **37**: 629-635, 1996.

Jiang L., Yang H.: Prometryne-induced oxidative stress and impact on antioxidant enzymes in wheat. – Ecotox. Environ. Safe. **72**: 1687-1693, 2009.

Jiang Z., Ma B.B., Erinle K.O. *et al*.: Enzymatic antioxidant defense in resistant plant: *Pennisetum americanum* (L.) K. Schum during long-term atrazine exposure. – Pestic. Biochem. Phys. **133**: 59-66, 2016.

Kaňa R., Špundová M., Ilík P. *et al*.: Effect of herbicide clomazone on photosynthetic processes in primary barley (*Hordeum vulgare* L.) leaves. – Pestic. Biochem. Phys. **78**: 161-170, 2004.

Kapusta G., Krausz R.F., Khan M., Matthews J.L.: Effect of nicosulfuron rate, adjuvant and weed size on annual weed control in corn (*Zea mays*). – Weed Technol. **8**: 696-702, 1995.

Liu Z., Shi L., Bai L., Zhao K.: [Effects of salt stress on the contents of chlorophyll and organic solutes in *Aeluropus littoralis* var. sinensis Debeaux.] – J. Plant Physiol. Mol. Biol. **33**: 165-172, 2007. [In Chinese]

Macedo R.S., Lombardi A.T., Omachi C.Y., Rörig L.R.: Effects of herbicide bentazon on growth and maximum quantum yield of photosystem II in the marine diatom skeletonema costatum. – Toxicol. in Vitro **22**: 716-722, 2008.

Meyer M.D., Pataky J.K., Williams M.M.: Genetic factors influencing adverse effects of mesotrione and nicosulfuron on sweet corn yield. – Agron. J. **102**: 1138-1145, 2010.

Murata N., Takahashi S., Nishiyama Y., Allakhverdiev S.I.: Photoinhibition of photosystem II under environmental stress. – BBA-Biochemistry **1767**: 414-421, 2007.

Nakano Y., Asada K.: Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. – Plant Cell Physiol. **22**: 867-880, 1981.

Pataky J.K., Meyer J.D., Bollman J.D. *et al.*: 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, 2008.

Platiša J., Veljović-Jovanović S., Kukavica B. *et al*.: Induction of peroxidases and superoxide dismutases in transformed embryogenic calli of alfalfa (*Medicago sativa* L.). – J. Plant Physiol. **165**: 895-900, 2008.

Qian H., Chen W., Sheng G.D. *et al*.: Effects of glufosinate on antioxidant enzymes, subcellular structure, and gene expression in the unicellular green alga *Chlorella vulgaris.* – Aquat. Toxicol. **88**: 301-307, 2008.

Ramel F., Sulmon C., Bogard M. *et al*.: Differential patterns of reactive oxygen species and antioxidative mechanisms during atrazine injury and sucrose-induced tolerance in *Arabidopsis thaliana* plantlets. – BMC Plant Biol. **9**: 28, 2009 .

Ray T.B.: Site of action of chlorsulfuron. – Plant Physiol. **75**: 827-831, 1984.

Saladin G., Magné C., Clément C.: Impact of flumioxazin herbicide on growth and carbohydrate physiology in *Vitis vinifera* L. – Plant Cell Rep. **21**: 821-827, 2003.

Sonoike K.: Degradation of *PsaB* gene product, the reaction center subunit of photosystem I, is caused during photo inhibition of photosystem I: possible involvement of active oxygen species. – Plant Sci. **115**: 157-164, 1996.

Stall W.M., Bewick T.A.: Sweet corn cultivars respond differentially to the herbicide nicosulfuron. – HortScience **27**: 131-133, 1992.

Tan W., Li Q.L., Zhai H.: Photosynthesis and growth responses of grapevine to acetochlor and fluoroglycofen. – Pestic. Biochem. Phys. **103**: 210-218, 2012.

Tarchoune I., Sgherri C., Izzo R. *et al*.: Antioxidative responses of *Ocimum basilicum* to sodium chloride or sodium sulphate salinization. – Plant Physiol. Bioch. **48** : 772-777, 2010.

Tjus S.E., Scheller H.V., Andersson B., Møller B.L.: Active oxygen produced during selective excitation of photosystem I is damaging not only to photosystem I, but also to photosystem II. – Plant Physiol. **125**: 2007-2015, 2001.

van Kooten O., Snel J.F.H.: The use of chlorophyll fluorescence nomenclature in plant stress physiology. – Photosynth. Res. **25**: 147-150, 1990.

Vass I., Styring S., Hundal T. *et al*.: Reversible and irreversible intermediates during photoinhibition of photosystem II: stable reduced Q_A species promotes chlorophyll triplet formation. – P. Natl. Acad. Sci. USA **89**: 1408-1412, 1992.

Wang J., Zhong X.M., Lv X.L. *et al*.: [Advances in research on tolerance of differential corn cultivars to the nicosulfuron.] – Chinese J. Pestic. Sci. **18**: 282-290, 2016. [In Chinese]

Wang X.F., Guan C.H., Fan Z.W., Zhen H.J.: Application and development of sulfonylurea herbicides. – Agrochemicals **50**: 9-15, 2011.

Williams B.J., Harvey R.G.: Effect of nicosulfuron timing on wild-proso millet (*Panicum miliaceum*) control in sweet corn (*Zea mays*). – Weed Technol. **14**: 377-382, 2000.

Wu G.L., Cui J., Tao L., Yang H.: Fluroxypyr triggers oxidative damage by producing superoxide and hydrogen peroxide in rice (*Oryza sativa*). – Ecotoxicology **19**: 124-132, 2010.

Wu X.X., Wu J.C., Jin G.Y. *et al*.: [Impact of herbicides on physiology and ecology of hydrophytes.] – Acta Ecol. Sin. **24**: 2037-2042, 2004. [In Chinese]

Xue Y.J., Tao L., Yang Z.M.: Aluminum-induced cell wall peroxidase activity and lignin synthesis are differentially regulated by jasmonate and nitric oxide. – J. Agr. Food Chem. **56**: 9676-9684, 2008.

Yoon J.Y., Shin J.S., Shin D.Y. *et al*.: Tolerance to paraquat-mediated oxidative and environmental stresses in squash (*Cucurbita* spp.) leaves of various ages. – Pestic. Biochem. Physiol. **99**: 65-76, 2011.

Yuan X.Y, Zhang L.G., Ning N. *et al*.: Photosynthetic physiological response of radix isatidis (*Isatis indigotica* Fort.) seedlings to nicosulfuron. – PLoS ONE **9**: e105310, 2014.

Zheleva D., Tsonev T., Sergiev I., Karanov E.: Protective effect of exogenous polyamines against atrazine in pea plants. – J. Plant Growth Regul. **13**: 203-211,1994.

Zhong X.M., Shi Z.S., Li F.H., Huang H.J.: Photosynthesis and chlorophyll fluorescence of infertile and fertile stalks of paired near-isogenic lines in maize (*Zea mays* L.) under shade conditions. – Photosynthetica **52**: 597-603, 2014.