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RESEARCH ARTICLE

Responses of photosynthesis, antioxidant enzymes, and related gene expression to nicosulfuron stress in sweet maize (*Zea mays* L.)

Zhen-Xing Wu[1] · Ning-Wei Xu[2,3] · Min Yang[2] · Xiang-Ling Li[2] · Jin-Ling Han[2] · Xiao-Hu Lin[2] · Qing Yang[2] · Gui-Hua Lv[1] · Jian Wang[2]

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Abstract

Weed control in maize (*Zea mays* L.) crops is usually undertaken using the postemergence herbicide nicosulfuron. The toxicity of nicosulfuron on maize, especially sweet maize, has been widely reported. In order to examine the effect of nicosulfuron on seedling photosynthetic characteristics, chlorophyll fluorescence, reactive oxygen species production, antioxidant enzyme activities, and gene expressions on sweet maize, nicosulfuron-tolerant "HK310" and nicosulfuron-sensitive "HK320" were studied. All experiment samples were subjected to a water or 80 mg kg^{-1} of nicosulfuron treatment when sweet maize seedlings grow to the stage of four leaves. After treatment with nicosulfuron, results for HK301 were significantly higher than those for HK320 for net photosynthetic rate, transpiration rate, stomatal conductance, leaf maximum photochemical efficiency of PSII, photochemical quenching of chlorophyll fluorescence, and the electron transport rate. These results were contrary to nonphotochemical quenching and intercellular CO_2 concentration. As exposure time increased, associated effects also increased. Both $O_2^{\cdot-}$ and H_2O_2 detoxification is modulated by antioxidant enzymes. Compared to HK301, SOD, POD, and CAT activities of HK320 were significantly reduced as exposure time increase. Compared to HK320, the gene expression for the majority of *SOD* genes, except for *SOD2*, increased due to inducement by nicosulfuron, and it significantly upregulated the gene expression of *CAT* in HK301. Results from this study indicate that plants can improve photosynthesis, scavenging capabilities of ROS, and protective mechanisms to alleviate phytotoxic effect of nicosulfuron. Future research is needed to further elucidate the important role antioxidant systems and gene regulation play in herbicide detoxification in sweet maize.

Keywords Nicosulfuron · Sweet maize · Photosynthesis · Chlorophyll florescence · Reactive oxygen species · Antioxidant enzymes · Gene expression

Introduction

Nicosulfuron, a sulfonylurea herbicide that is active at low doses, has a low propensity to contaminate groundwater and it has a low toxicity to mammals (Corbett et al. 2005). This herbicide not only has a strong control over a variety of annual and perennial grasses, but also has a level of control over a number of broadleaf weeds. Therefore, the combination of nicosulfuron with broadleaf herbicides provides effective weed control over a broad spectrum (Stall and Bewick 1992; Dobbels and Kapusta 1993; Williams and Harvey 2000).

Nicosulfuron acts to reduce branched-chain amino acid synthesis, inhibiting acetolactate synthase (ALS) enzyme activity in sensitive plants (Rey-Caballero et al. 2016). Crops have different tolerances to sulfonylurea herbicides as their rates of metabolism to herbicides differ; plants sensitive to

Responsible Editor: Gangrong Shi

✉ Gui-Hua Lv
lvgh@zaas.ac.cn

✉ Jian Wang
wangjian1989@live.cn

[1] Institute of Maize and Featured Upland Crops, Zhejiang Academy of Agricultural Sciences, Dongyang 322100, China

[2] College of Agronomy and Biotechnology, Hebei Key Laboratory of Crop Stress Biology, Hebei Normal University of Science &Technology, Qinhuangdao 066000, China

[3] College of Landscape and Tourism, Hebei Agricultural University, Baoding 071000, China

these herbicides detoxify the herbicides slower than plants with a tolerance. Studies have shown that the sensitivity or tolerance of sweet corn to several herbicides metabolized by cytochrome P450 is a simple genetic trait. Previous studies have highlighted that sensitivity of maize to various P450 herbicides is mainly regulated by a single CYP locus or a group of tightly linked CYP locus on the short arm of chromosome 5S (Pataky et al. 2008, 2009). A single gene sensitive to nicosulfuron was identified from the field maize inbred line W703A and named *nsf1* (Kang 1993). A single-gene cross-sensitivity to nicosulfuron and bentazon was identified in the field corn inbred GA209 and named *ben1* (Barrett et al. 1997; Bradshaw et al. 1994; Fleming et al. 1988). The allele of *nsf1* locus on chromosome 5S of nicosulfuron-resistant field maize inbred line B73 contains a highly conserved heme-binding sequence found in most cytochrome P450 genes (Williams et al. 2006). The alleles in field maize inbred lines GA209 and W703a contained the same 392 bp insertion relative to B73 in the gene sequence. Therefore, this insertion mutation seems to lead to a non-functional P450 allele, leading to herbicide sensitivity, and *nsf1* and *ben1* genes are the same mutants of CYP gene. In the sweet corn population produced by the hybridization of Cr1 (herbicide sensitive inbred line) and Cr2 (herbicide tolerant inbred line), the chromosomal positions responsible for herbicide cross-sensitivity, including nicosulfuron, are closely related to *nsf1* (Nordby et al. 2008). In addition, 29 sweet corn inbred lines and 45 sweet corn hybrids have the same or closely linked genes with Cr1, resulting in cross sensitivity to a variety of postemergence herbicides including nicosulfuron (Pataky et al. 2009). *nsf1* plays an important role in herbicide resistance, and its functional relationship with herbicide resistance is being continuously established (Williams et al. 2006; Liu et al. 2019; Choe and Williams 2020). However, we still lack knowledge whether it may be associated to the complex nature of CYP450 genes, especially as these genes are difficult to isolate and functionally identify due to their sequence complexity and their low abundance of transcripts (Battett 2000; Nelson et al. 2004).

Nicosulfuron is not rapidly metabolized in plants with a sensitivity, and it will exist as a toxic substance. Herbicide residues in plants (as an abiotic stress) induce oxidative stress through the electron transport chain of the PSI and PSII systems (Hussain et al. 2010; Wang et al. 2018). Stress that destroys electron transport and other metabolic processes in photosynthetic organs can lead to an increase in O_2^- production. Previous studies have demonstrated that abiotic stresses (cold, drought, heavy metals, and salinity) induces stomatal closure, reduces the utilization efficiency of carbon dioxide in photosynthesis, and increases electron transfer to O_2, thereby increasing the rate of $O_2^{\cdot-}$ production in chloroplasts (Vaahtera et al. 2014; Zarepour et al. 2010). Although $O_2^{\cdot-}$ is very active, it cannot diffuse into adjacent organelles far from its production site, and it can be rapidly converted into H_2O_2 by SOD; H_2O_2 is another slightly stable reactive oxygen species (ROS) (Bienert and Chaumont 2014). Plants have evolved complex antioxidant defense mechanisms to scavenge ROS, including enzymes and non-enzymatic substances. Biotic and abiotic stresses have been recorded to induce changes in plant antioxidant enzyme activity on the physiological and biochemical levels (Hossain et al. 2015). However, data relating to isozyme encoding due to the impact of nicosulfuron on gene expression in higher plants involved in the antioxidant enzyme system are rare.

Widening our understanding of gene expression involved in antioxidant enzyme activities will advance our knowledge of plant mechanisms to molecular adaptation and detoxification against various biotic and abiotic stresses. Expression of genes associated with antioxidants and other stresses can modulate the overproduction of ROS in plant cells. For example, stress related to drought, salinity, oxidative, and cold results in upregulation in the transcript level of antioxidative enzyme genes (Li et al. 2017; He et al. 2017; Zhang et al. 2017). Reports on ROS generation, elevated transcript levels of antioxidant enzymes, and an increase in antioxidant enzyme activity have also been recorded in response to various abiotic stresses (Zhao et al. 2019; AbdElgawad et al. 2020; Han et al. 2021). However, after treatment with nicosulfuron, regulatory mechanisms associated to antioxidant system genes family in sweet maize have not been reported. Our previous study have indicated that in different resistant sweet maize, C_4 photosynthetic enzymes activity and key gene expression play a critical role in enhancing the adaptability of plants to nicosulfuron stress at a photosynthetic physiological level (Wang et al. 2021). In this study, therefore, we continued using a pair of sister lines differing in nicosulfuron tolerance to (1) understand related photosynthetic physiological mechanisms, (2) investigate ROS accumulation and the change of antioxidant enzyme activity in plants after spraying with nicosulfuron, and (3) investigate the expression level of antioxidant enzyme–related genes induced by nicosulfuron. Our results will increase our understanding of oxidative stress responses of sweet maize, and how gene expression and enzyme activity systems of sweet maize adapt to nicosulfuron stress.

Materials and methods

Plant materials

In this study, a pair of sister lines (nicosulfuron-tolerant HK301 and nicosulfuron-sensitive HK320) was used as plant material. After spraying with nicosulfuron, preliminary results indicated that HK301 recorded normal growth

patterns, exhibiting a better level of tolerance; HK320 recorded high mortality rates and its growth was found to be inhibited.

Experimental design and treatments

Field experiment

Our experiment was conducted at Changli Farm of Hebei Normal University of Science and Technology (39°25′N, 118°45′E), and Dongyang experimental station of Zhejiang Academy of Agricultural Sciences (28°63′N, 120°31′E). The Changli Farm is located in a warm temperate zone, having a continental, monsoon-affected semi-humid climate, while the Dongyang experimental station is located in a subtropical monsoon climate zone with enough light and rainfall. Experimental analysis was undertaken from 2018 to 2019 using a herbicide concentration screening test. In order to screen HK301 and HK320, a pilot study was initially undertaken in Changli and Dongyang. A split-plot experimental design was used with nicosulfuron treatment defining the main plots and inbred lines within subplots, with three replicates. Each inbred line was sown in fifteen rows measuring 5 m in length, with 60 cm between rows and 14.9 cm between plants. Two seeds were planted in each hole to ensure 500 seedlings per plot were grown. Maize seedlings at the four-leaf stage were sprayed with nicosulfuron at effective concentrations of 0 (control), 20, 40, 80, 120, 160, 200, 240, 280, and 320 mg kg^{-1} using an electric backpack sprayer with a nozzle. The survival rate of seedlings was recorded after spraying (Table 1).

In 2019–2020, we designed a field experiment in Changli, Hebei Province, China. The experiment was designed as a randomized complete block design with three replicates. Each inbred line was sown in four rows measuring 5 m in length, with 60 cm between rows and 14.9 cm between plants. Two seeds were planted in each hole. When maize seedlings reached the four-leaf stage, an electric backpack sprayer with a nozzle was used to spray nicosulfuron, with water used as a control. We sprayed seedling plots at an effective concentration of 80 mg kg^{-1}. When the effective concentration of the nicosulfuron was 80 mg kg^{-1}, HK301 plants were able to attain normal growth, while HK320 plants either wilted or completely died. Field investigation and sampling were conducted 1, 3, 5, and 7 days after nicosulfuron treatment (DAT).

Table 1 Effect of nicosulfuron on survival rate of sweet maize seed of HK301 and HK320. * indicates differences under different herbicide concentration treatment at $P < 0.05$, according to the least significant difference (LSD) test. *CK* control

Province	Spraying concentration (mg kg^{-1})	HK301			HK320		
		CK	Number of seedlings after spraying	Survival rate (%)	CK	Number of seedlings after spraying	Survival rate (%)
Hebei	0	449	449	100.00	446	446	100.00
	20	447	442	98.88	446	7	1.57*
	40	443	430	97.07	444	0	0*
	80	442	431	97.51	445	0	0*
	120	444	396	89.19*	444	–	
	160	439	338	76.99*	450	–	
	200	445	188	42.25*	447	–	
	240	449	102	22.72*	445	–	
	280	442	41	9.28*	445	–	
	320	442	23	5.20*	448	–	
Zhejiang	0	444	444	100.00	449	449	100.00
	20	448	441	98.44	447	5	1.12*
	40	447	432	96.64	442	0	0*
	80	442	420	95.02	446	0	0*
	120	446	400	89.69*	447	–	–
	160	445	321	72.13*	449	–	–
	200	448	190	42.41*	443	–	–
	240	449	108	24.05*	448	–	–
	280	448	40	8.93*	447	–	–
	320	446	20	4.48*	447	–	–

Pot experiment

Maize seeds were soaked for 24 h at 25 °C before being germinated for 24 h at 27 °C until white. In order to avoid the impact of the environment on growth, all samples were placed in an artificial climate chamber with a photoperiod of 24 h, an illumination intensity of 12,000 lx, a culture temperature of 25 °C during the day and 22 °C at night, and a relative humidity of 70%. The experiment utilized a randomized complete block design with three replicates of five pots each. Three seeds were planted in each pot (20 cm in diameter, 18 cm in depth) filled with soil. The soil was obtained from the top 0–15-cm soil layer of the Changli experimental station. All samples were watered daily during the culture period. At the four-leaf stage, the maize seedlings were sprayed with nicosulfuron; a water treatment was used as a control. As the best response time for maize to nicosulfuron herbicide is 24 h (Liu et al. 2015), 24 h after the samples had been sprayed they were frozen in liquid nitrogen and stored at − 80 °C for gene expression determination.

Gas exchange properties

Gas exchange properties for all leaf samples were analyzed for net photosynthetic rate (P_n), intercellular CO_2 concentrations (C_i), stomatal conductance (G_s), and transpiration rate (E) using a *LI-6400* portable optical instrument (*LI-COR Biosciences Inc*., Lincoln, NE, USA). Measurements were undertaken from 9:00 to 11:00 a.m. under ambient temperatures between 23 and 27 °C, and relative air humidity between 60 and 70%. No differences between the treatments were recorded during the experiment.

Chlorophyll fluorescence parameters

A *PAM-2500* pulse modulated fluorometer (*Walz*, Germany) was used to measure chlorophyll fluorescence parameters, using *PAMwin3* as the data acquisition software. By using the equations of Maxwell (2002), maximal quantum yield of PSII photochemistry (F_v/F_m), the effective quantum yield of PSII photochemistry (Φ_{PSII}), and electron transport rate (ETR) were calculated. Calculations were also undertaken to determine the photochemical quenching coefficient (qP) and nonphotochemical quenching (NPQ), respectively: $qP = (F_m - F_s)/(F_m - F_0)$; $NPQ = (F_m - F_m')/F_m'$.

O_2^- production rate, H_2O_2,and lipid peroxidation assay

The method of Jiang and Zhang (2002) was used to calculate $O_2^{\cdot -}$ production rate. The methods of Jena and Choudhuri (1981) were used to determine H_2O_2 content. The methods of Heath and Packer (1968) were used to calculate malondialdehyde (MDA) content in the samples.

Proline content

Determination of proline content by Wang's method (Wang et al. 2018).

Measurement of antioxidant enzyme activities

Enzymes were extracted by grinding of 0.5 g leaf sample in 5 ml phosphate buffer (pH 7.5) that contained 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 determine the enzyme activity. The method of Giannopolitis and Ries (1977) was used for the estimation of SOD activity. The amount of enzyme required to inhibit the photoreduction of NBT to purple formazan by 50% was defined as one unit of SOD activity. The method of Cakmak and Marschner (1992) was used to measure guaiacol peroxidase (POD, EC 1.11.1.7) activity by observing the increase in absorbance at 470 nm. Catalase (CAT, EC 1.11.1.6) activity levels were assayed by observing the decrease in absorbance at 240 nm (Aebi 1984).

RNA isolation and RT-PCR

Sample RNA was extracted by using total RNA extraction reagents (TaKaRa, Japan), and the experimental procedures were performed according to the product specifications. RT-PCR was used to detect the target gene primers. The following primers were synthesized in Beijing Invitrogen Company (Table 2). In this experiment, only primers with high amplification efficiency (more than 90%) were used. Reverse transcription of cDNA was performed using PrimeScript™ RT reagent Kit with gDNA Eraser, and experimental procedure was performed according to the product manual. According to the manufacturer's instructions (CFX Connect Optics Module, Singapore), the RT-PCR reaction was performed on the target gene and the internal reference (*actin*) of each sample in the real-time PCR detection system, and each sample was tested for 3 replicates. Data were analyzed using $2^{-\Delta\Delta ct}$ method.

Statistical analysis

Data processing and mapping was undertaken using Microsoft Excel and SigmaPlot 12.5. Analysis of variance (ANOVA) and mean values were compared using the least significant difference (LSD) test in SPSS (V. 12.0; SPSS Inc., Chicago, IL, USA). Significant differences were identified at the $P < 0.05$ threshold.

Table 2 List of primers used for the real-time RT-PCR

Name	Sequence ID	Forward primer	Reverse primer	Product (bp)
SOD	*GRMZM2G081585*	TTGAACTTCACTGGGGTAAGC	ACAAAAGACTCTGCACGCATC	246
SOD1	*GRMZM2G106928*	TTCGCCGCTCCCTATTCC	GTCCTGTCGATATGCACCCA	282
SOD2	*GRMZM2G173628*	GCCTACAACAATGGCAATCC	AGACAAGCCAAACCCAACC	151
SOD3	*GRMZM2G124455*	TTTTGGAAGAACCTCAAGCCTAT	CCCAGACATCAATCCCCAAC	267
SOD9	*GRMZM2G058522*	GGCTGTTGCTGTGCTTGGTA	CTTGCTCGCAGGATTGTAGTG	195
CAT1	*GRMZM2G088212*	CAGGCTGTCGTGAGAAGTGC	GAGATCCAAATGGTACGGTGTT	165
CAT2	*GRMZM2G090568*	CCCCAACTACCTGCTGCTAC	TGGTTATGAACCGCTCTTGC	274
Actin	*GRMZM2G126010*	GATGATGCGCCAAGAGCTG	GCCTCATCACCTACGTAGGCAT	168

Results and discussion

Gas exchange properties

Apart from results at 5 DAT, nicosulfuron treatment resulted in no significant changes in P_n and G_s in HK301 (Fig. 1). Compared to HK301-CK, P_n in HK301 decreased by 17.64% at 5 DAT. Results for HK320 recorded a significant decrease in P_n and G_s in maize seedling leaves after 3 DAT due to the addition of nicosulfuron. Compared to HK320-CK, the addition of nicosulfuron significantly reduced P_n in HK320 at 3, 5, and 7 DAT by 35.41%, 79.05%, and 82.15%, respectively; nicosulfuron treatment significantly decreased G_s in HK320 at 3, 5, and 7 DAT by 36.18%, 71.92%, and 67.21%, respectively. In addition to 3 DAT, treatment using nicosulfuron resulted in no significant effect on C_i and E in HK301, a result contrary to those in HK320 (Fig. 2). Compared to HK320-CK, C_i in HK320 significantly increased by 65.62% (5 DAT) and 121.80% (7 DAT); E in HK320 significantly declined by 22.39% (3 DAT), 48.75% (5 DAT), and 75.57% (7 DAT).

The phytotoxic effect of nicosulfuron on maize, especially on sweet maize, has previously been reported (Stall and Bewick 1992; Grey et al. 2000). Our results indicated some phenotypic changes were observed on the leaves of sweet maize sprayed with nicosulfuron. Herbicides affect plant cell metabolism, chloroplast disintegration, and leaf color change in plants, ultimately affecting the photosynthetic mechanism of plants (Hess 2000; Xu et al. 2019). In our study, compared to HK301, P_n, G_s, and E in HK320 were significantly reduced after 3 DAT. In contrast, C_i in HK320 significantly increased after 5 DAT, suggesting that stomatal limitation was the main cause of P_n decrease. Although nicosulfuron targets specific areas in plants, evidence suggests that P_n, photosynthetic pigments, and photosynthesis-related protein activity of plants are also significantly reduced by nicosulfuron (Hess 2000). In the sensitive sweet inbred line HK320, C_i significantly increased and G_s significantly decreased after nicosulfuron treatment. Our results suggest that the action of nicosulfuron destroyed the chloroplast structure of sweet maize seedling leaves, resulting in a decrease in their photosynthetic carbon assimilation ability, an increase in the risk of photo oxidation damage, and a reduction in light absorption, transmission, and distribution between PS II and PSI (Murata et al. 2007), thereby affecting ATP and NADPH synthesis.

Chlorophyll fluorescence measurements

In HK320, nicosulfuron treatment caused no significant changes in F_v/F_m (Fig. 3(a)). After 3 DAT, nicosulfuron treatment significantly decreased F_v/F_m in HK320 (Fig. 3(b)). Compared to HK320-CK, F_v/F_m in HK320 significantly declined by 19.47%, 23.35%, and 23.99% at 3, 5, and 7 DAT, respectively. The actual photochemical reactivity of the PSII system is reflected using Φ_{PSII} (Govindjee 2002). After nicosulfuron treatment, Φ_{PSII} in HK301 only increased at 1 DAT while in HK320 Φ_{PSII} significantly reduced at 3 and 7 DAT (Fig. 3(c, d)). After 3 DAT, ETR in HK301 recorded its minimum value before increasing. ETR results in HK320 recorded a continuous decrease (Fig. 3(e, f)); average ETR in HK301 was 37.03% higher than that of HK320. The decline of P_n in HK320 was linked to the reduction of ETR.

In addition to 1 DAT, the addition of nicosulfuron in HK301 resulted in no significant changes in qP and a significant reduction in qP in HK320; qP in HK320 was significantly lower than that in HK301 (Fig. 4). After herbicide treatment, F_v/F_m, Φ_{PSII}, ETR, and qP in HK320 leaves were reduced. In contrast, NPQ in HK320 increased. These results indicate that higher non-radiative energy dissipation reduced the openness of the PSII system.

Light emitted by chlorophyll molecules from the excited state to the non-excited state is termed chlorophyll fluorescence. Photosynthetic energy conversion in higher plants, algae, and bacteria is typically calculated using chlorophyll fluorescence as an indicator (Chen et al. 2016). Thus, information about the potential enantioselectivity of nicosulfuron to PSII in sweet maize was gathered using chlorophyll fluorescence measurements. In the control samples, the F_v/F_m

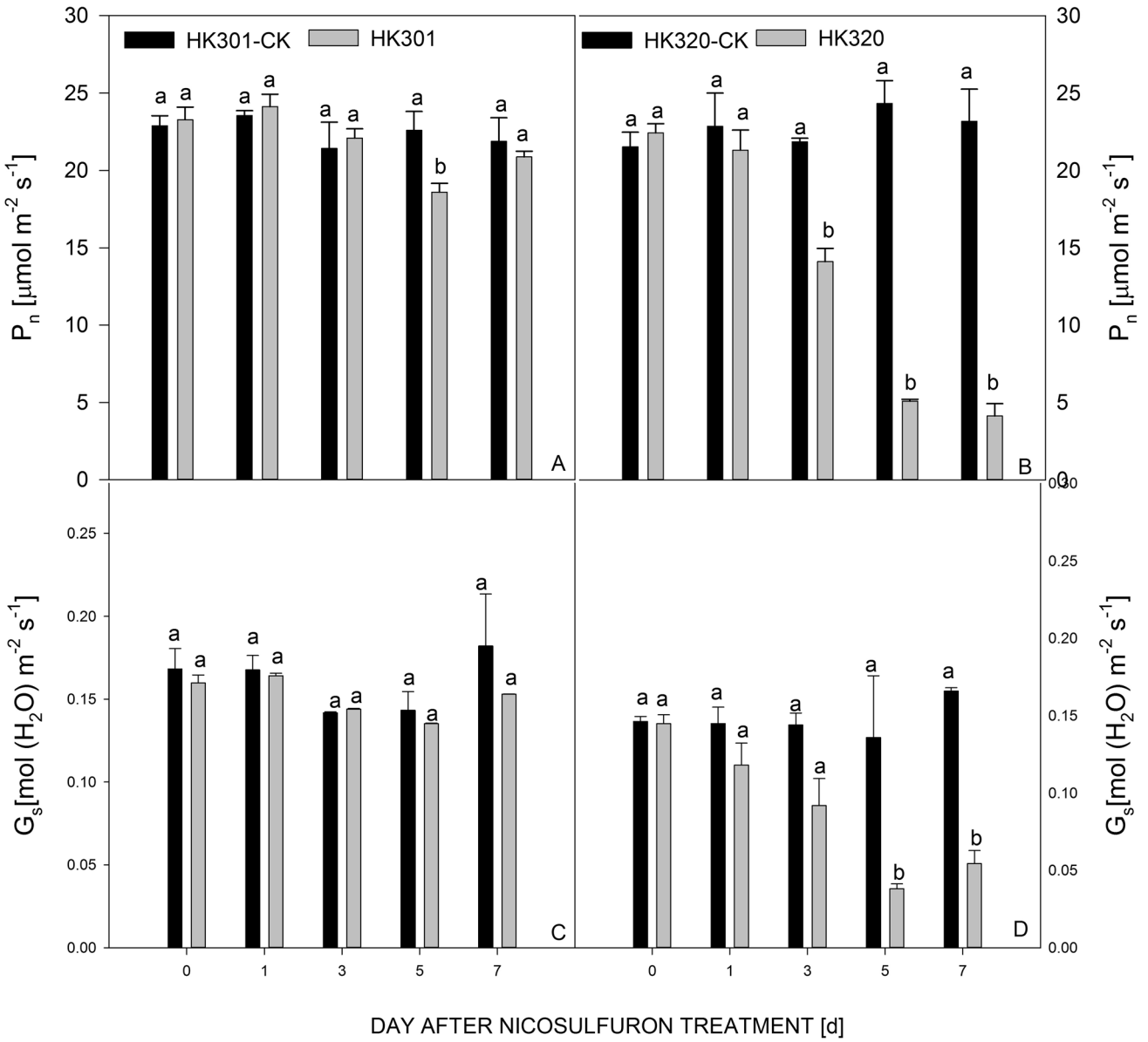


Fig. 1 Effects of nicosulfuron on the net photosynthetic rate (P_n) (**A**, **B**) and stomatal conductance (G_s) (**C**, **D**) in leaves of sweet maize seedlings. HK301-CK: water treatment in HK301; HK301: nicosulfuron 80 mg·kg^{-1} treatment in HK301; HK320-CK: water treatment in HK320; HK320: nicosulfuron 80 mg·kg^{-1} treatment in HK320; vertical bars represent the SE ($n=3$). Small letters (a, b) indicate differences between values obtained on different days after nicosulfuron treatment ($P<0.05$) according to a least significant difference (LSD) test

ratio of the two sweet inbred lines were close to 0.80, results which were in accordance with findings related to a wide range of stress-free higher plants (Johnson et al. 1993; Li et al. 2018). After nicosulfuron treatment, the F_v/F_m was significantly reduced in HK320 compared to the controls, indicating a remarkable enantioselective effect. In addition, qP, ETR, and Φ_{PSII} of HK320 were significantly reduced and NPQ significantly increased. About 50% of commercially available herbicides have been widely reported to inhibit the action of chloroplast electron transport chains (Flores et al. 2013). As their mode of action is related to disturbance of the photosynthetic electron flow, thereby affecting carbon fixation, they therefore affect plant photosynthesis and inhibit normal plant growth and development (Chen et al. 2015). In our study, the reduction of qP may lead to a decrease in openness of the PSII system, and ultimately to the decline of the utilization ratio of excitation energy for photochemistry reaction. At the same time, the increase of NPQ indicates that a reduction of PSII system activity in HK320 was related to non-energy dissipation.

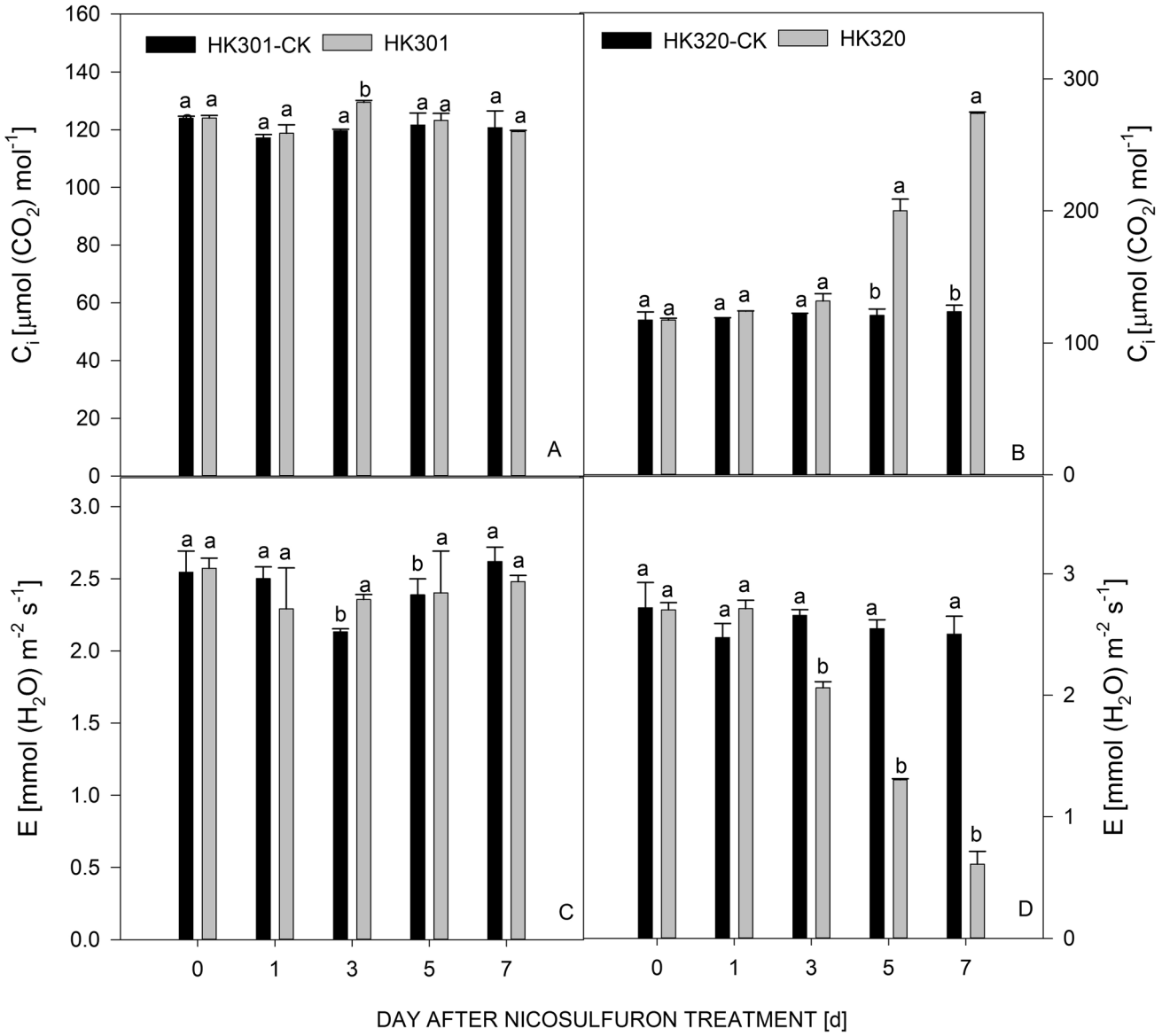
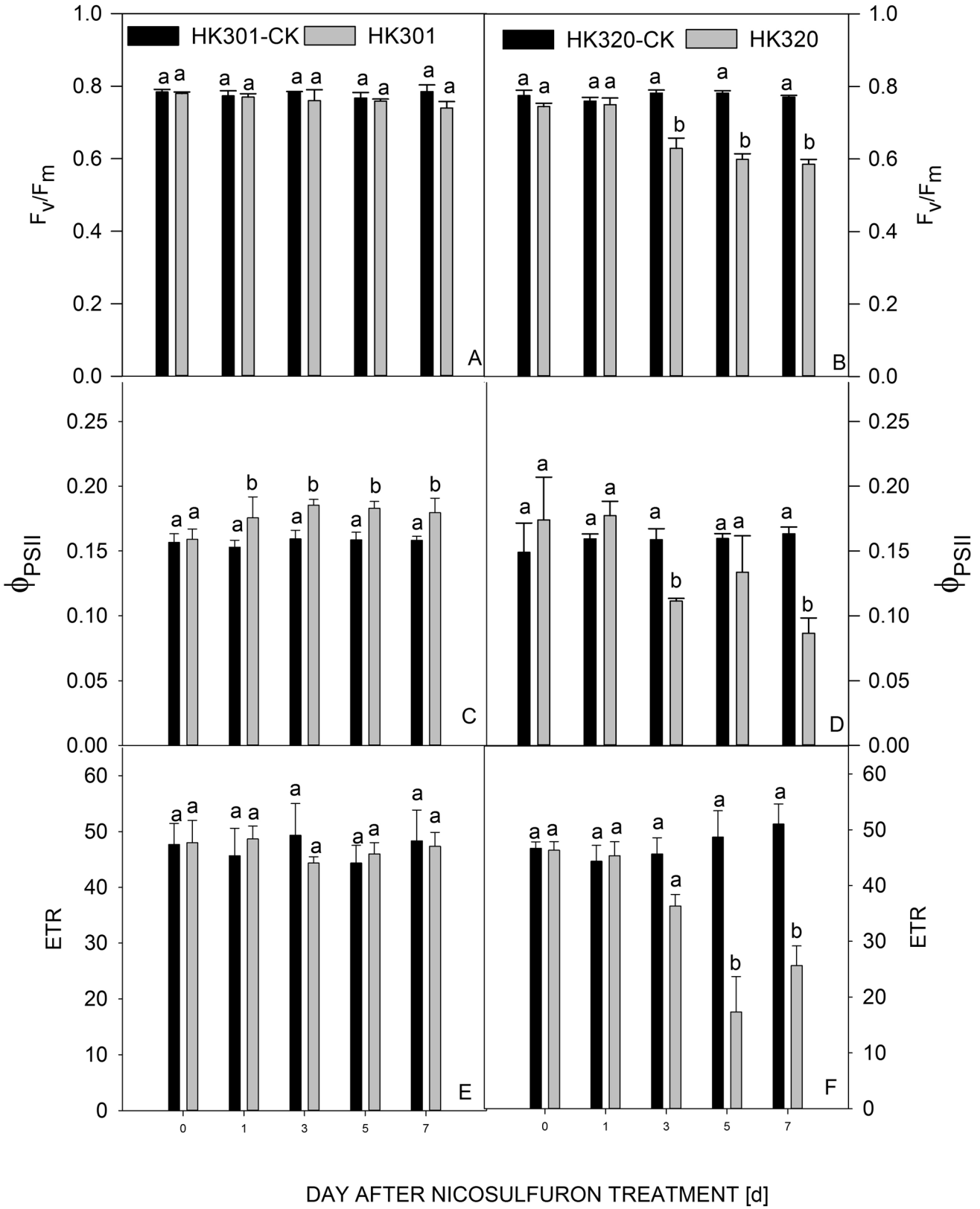


Fig. 2 Effects of nicosulfuron on the intercellular CO_2 concentrations (C_i) (**A**, **B**), and transpiration rate (E) (**C**, **D**) in leaves of sweet maize seedlings. HK301-CK: water treatment in HK301; HK301: nicosulfuron 80 mg·kg^{-1} treatment in HK301; HK320-CK: water treatment in HK320; HK320: nicosulfuron 80 mg·kg^{-1} treatment in HK320; vertical bars represent the SE ($n=3$). Small letters (a, b) indicate differences between values obtained on different days after nicosulfuron treatment ($P<0.05$) according to a least significant difference (LSD) test

Nicosulfuron-induced oxidative stress

After 3 DAT, the production rate of $O_2^{\cdot-}$ in HK301 attained its maximum value before decreasing. Compared to HK301-CK, the production rate of $O_2^{\cdot-}$ in HK301 significantly reduced by 30.06% at 5 DAT (Fig. 5(a, b)). In contrast, after 1 DAT, the production rate of $O_2^{\cdot-}$ in HK320 continued to increase. After 3 DAT, the H_2O_2 content in HK301 reached its maximum value before decreasing; H_2O_2 content in HK320 increased with exposure time (Fig. 5(c, d)). Results indicate that the addition of nicosulfuron significantly increased H_2O_2 content in HK320 by 70.32%, 70.35%, 141.91%, and 203.67% at 1, 3, 5, and 7 DAT, respectively, compared to the control.

After 1 DAT, MDA content in HK301 attained its maximum value before decreasing. MDA content in HK301 significantly increased at 1, 3, and 5 DAT by up to 16.59%, 28.47%, and 42.59%, respectively, compared with HK301-CK (Fig. 6(a, b)). After 3 DAT, MDA reached its maximum value in HK320, after which it declined. Compared to HK320-CK, MDA content in HK320 significantly increased at 1, 3, 5, and 7 DAT by up to 45.19%, 206.54% 181.67%,


HK301-CK
HK301
HK320-CK
HK320
F_v/F_m
ϕ_{PSII}
ETR
A
B
C
D
E
F
DAY AFTER NICOSULFURON TREATMENT [d]

◂**Fig. 3** Effects of nicosulfuron on the PSII photochemistry (F_v/F_m) (**A**, **B**), PSII photochemistry (Φ_{PSII}) (**C**, **D**), and electron transport rate (ETR) (**E**, **F**) in leaves of sweet maize seedlings. HK301-CK: water treatment in HK301; HK301: nicosulfuron 80 mg·kg^{-1} treatment in HK301; HK320-CK: water treatment in HK320; HK320: nicosulfuron 80 mg·kg^{-1} treatment in HK320; vertical bars represent the SE ($n=3$). Small letters (a, b) indicate differences between values obtained on different days after nicosulfuron treatment ($P<0.05$) according to a least significant difference (LSD) test

and 250.38%, respectively. MDA content in HK320 was therefore higher than that of HK301.

After treatment with herbicide, proline content of the two sweet inbred lines significantly increased. Compared to HK301-CK, proline content in HK301 increased by 17.19%, 74.38%, 95.31%, and 91.27% at 1, 3, 5, and 7 DAT, respectively. However, average proline content of HK301 was 22.04% higher than that of HK320 (Fig. 6(c, d)), indicating that the increase in proline content was associated with sweet maize tolerance to herbicides.

Many studies have shown that the difference of maize sensitivity to nicosulfuron is related to the activity of CYP450 monooxygenase (Barrett 1995; Kreuz et al. 1996; Siminszky 2006). Tolerant plants can rapidly degrade nicosulfuron through CYP450 monooxygenase activity, resulting in the reduction of nicosulfuron in plants. In contrast, sensitive plants cannot rapidly degrade nicosulfuron. The residual nicosulfuron in plants will increase the production of ROS and may eventually damage plants (Wang et al. 2018). The effect of nicosulfuron-induced damage to the photosynthetic system and photosynthetic electron transport chain resulted in an excess of electrons to be transferred from O_2 to $O_2^{\cdot-}$. 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 (Prasad et al. 2016). In our experiment, nicosulfuron resulted in a significant increase in $O_2^{\cdot-}$ and H_2O_2 accumulation in all HK320 samples, indicating that nicosulfuron residue in plants induced the production of ROS as a toxic factor. This finding is in accordance with findings by Alla and Hassan (2007) who recorded isoproturon to significantly accelerate $O_2^{\cdot-}$ and H_2O_2 production in maize seedlings. An accumulation of excess H_2O_2 will ultimately result in membrane lipid peroxidation. Previous investigations have shown that, for phytotoxic and light-related herbicides, a key component of the development of plant phytotoxicity symptoms is the destruction of cell membrane integrity, which in turn results in severe tissue damage and eventually death (Hess 2000). Our results showed that MDA content in HK320 increased with exposure time, and MDA content in HK301 was significantly lower than that in HK320. H_2O_2 is potentially capable of inducing lipid peroxidation expressed as MDA content in plant (Wang et al. 2018). The present observations are supported by Wu et al. (2005) who found that drought stress reduced chlorophyll content and F_v/F_m which coordinately promoted the overproduction of H_2O_2 and MDA contents in cauliflower plants. Chen et al. (2018) showed that lead stress markedly altered photosynthetic efficiency of cauliflower which correlated with pronounced H_2O_2 and MDA content accumulation. It is clear from the above results that abiotic stress significantly impaired ROS homeostasis and hampered photosynthetic machinery and membrane stability. Our results suggest that nicosulfuron residue in HK320 plants induced oxidative stress on the leaves through photosynthetic electron transport pathways, destroyed plant membrane lipids, and eventually resulted in plant wilting and death.

Antioxidant enzyme activity response to oxidative stress

Significant increases in SOD enzyme activity in HK301 were induced at 1, 3, and 5 DAT due to the addition of nicosulfuron, while treatment significantly decreased SOD enzyme activity in HK320 at 3, 5, and 7 DAT (Fig. 7(a, b)). POD enzyme activity over time in the sweet inbred lines recorded different responses to the addition of nicosulfuron. Compared to the control, the addition of nicosulfuron promoted POD activity in HK301 leaves at 1, 3, 5, and 7 DAT. In HK320, POD activity initially increased before declining over time (Fig. 7(c, d)). CAT activity results for both sweet maize inbred lines attained maximum values after 1 DAT, after which they both declined. However, CAT results at 1 (58.46%), 3 (53.21%), 5 (180.49%), and 7 (191.89%) DAT in HK301 were significantly higher than the corresponding results in HK320 (Fig. 7(e, f)).

Previous studies have indicated that SOD enzymes are the first barrier to alleviate oxidative stress in plants in the intracellular antioxidant system (Foyer 2018). However, SOD enzyme activity results in HK320 after treatment were significantly lower than results in the control; SOD activity results in HK301 were significantly higher than results in HK320. This may be because the SOD activity in HK301 was increased as a result of the formation of ROS by nicosulfuron exposure. Our findings indicate that CAT activity in HK301 significantly increased after 1 DAT, and CAT activity in HK320 increased at 1 and 3 DAT before declining compared to the control. CAT enzymes eliminate H_2O_2 by breaking it down directly to form water and oxygen (Hu et al. 2012). It is likely that excess production of ROS by nicosulfuron stress can inactivate CAT activity in HK320, probably by inactivating the enzyme-bound to heme group (Chaparzadeh et al. 2004). Amor et al. (2007) reported that lower CAT activity was related to a higher H_2O_2 accumulation in the leaves of *C. maritima* under salinity stress. POD is also among the major enzymes that scavenge H_2O_2 in chloroplasts (Hu et al. 2012). However, like SOD and CAT, the POD activity was higher in HK301 versus HK320,

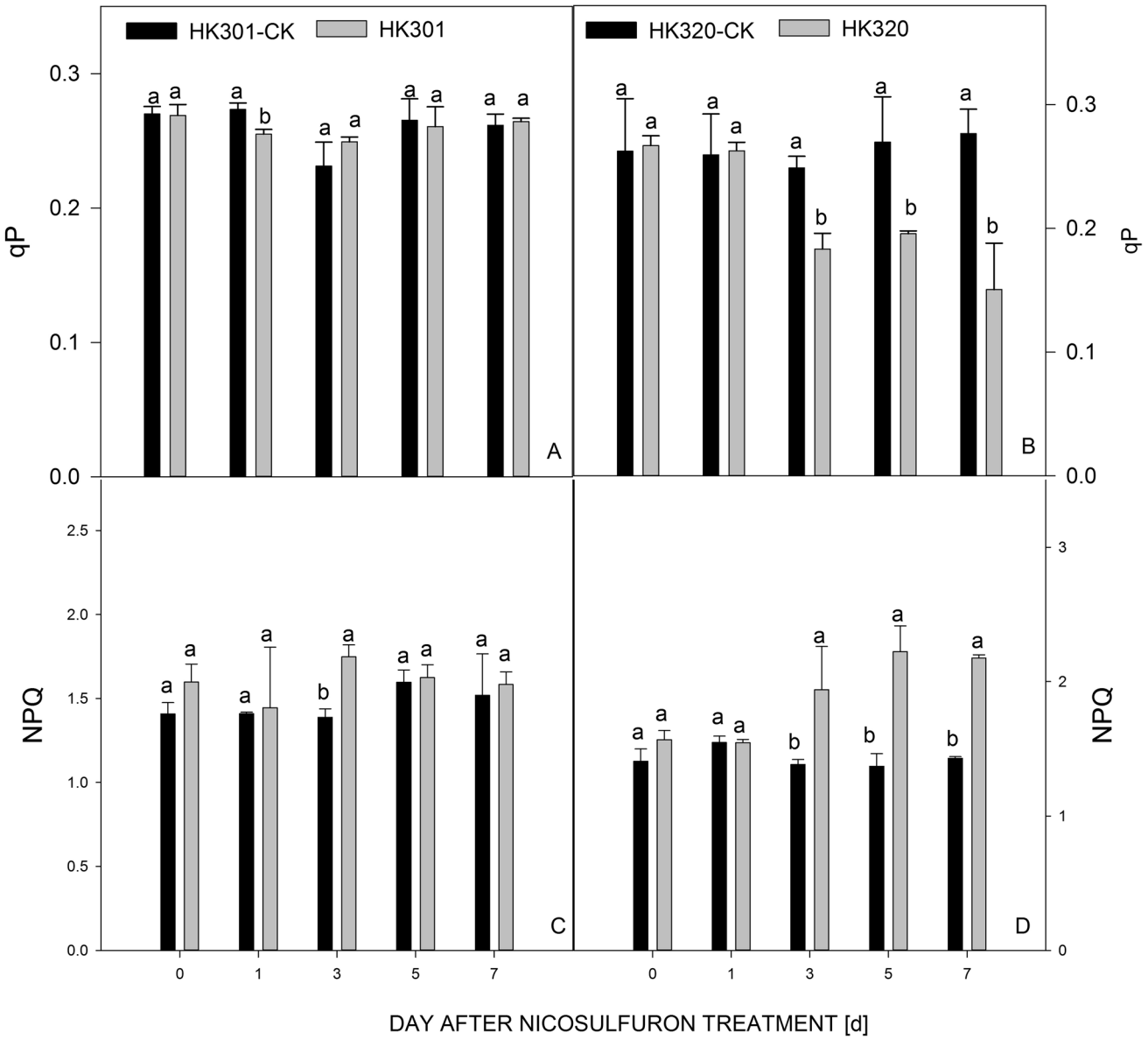


Fig. 4 Effects of nicosulfuron on the photochemical quenching coefficient (qP) (**A**, **B**), and nonphotochemical quenching (NPQ) (**C**, **D**) in leaves of sweet maize seedlings. HK301-CK: water treatment in HK301; HK301: nicosulfuron 80 mg·kg^{-1} treatment in HK301; HK320-CK: water treatment in HK320; HK320: nicosulfuron 80 mg·kg^{-1} treatment in HK320; Vertical bars represent the SE ($n=3$). Small letters (a, b) indicate differences between values obtained on different days after nicosulfuron treatment ($P<0.05$) according to a least significant difference (LSD) test

suggesting that tolerant genotype HK301 had a greater protection against the oxidative stress. Lower level of the H_2O_2 for HK301 compared with HK320 under nicosulfuron stress might be attributed to the relatively more efficient detoxifying enzymes CAT and POD. Our findings therefore suggest that increased CAT activity combined with SOD and POD activity changes in nicosulfuron-tolerant maizes play a vital protective role in the ROS-scavenging process. Oxidative stress tolerance in sweet maize due to nicosulfuron is thus partially related to the involvement of these enzymes. Our results also confirm changes in the antioxidant status and accumulation of antioxidants in response to the application of nicosulfuron.

Nicosulfuron-induced alterations in antioxidant enzyme-related genes

Transcript levels of antioxidant enzymes were measured in leaves treated with nicosulfuron and control samples from maize seedlings. Compared to the control, our results

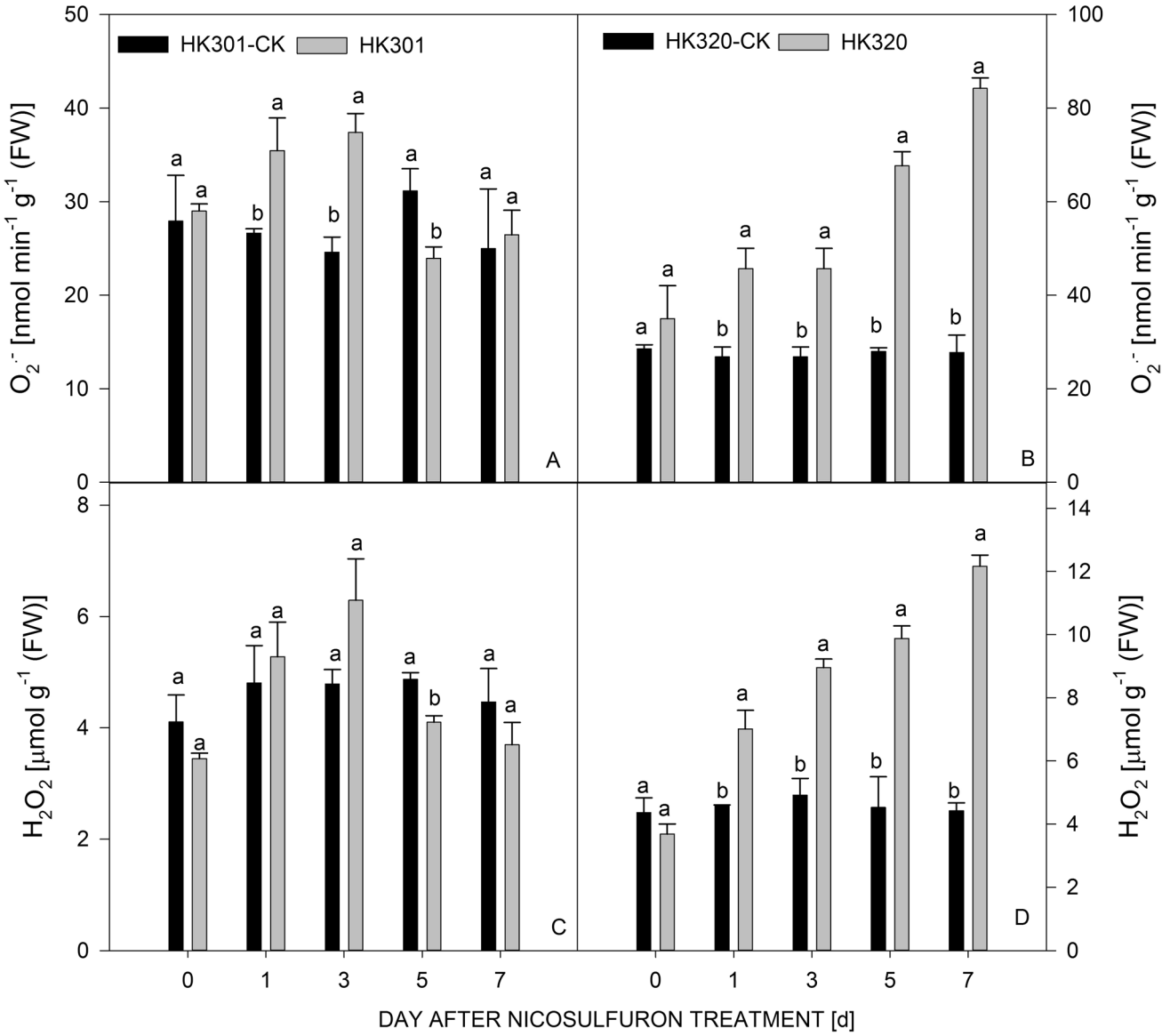


Fig. 5 Effects of nicosulfuron on the O_2^- production rate (**A**, **B**), and H_2O_2 (**C**, **D**) in leaves of sweet maize seedlings. HK301-CK: water treatment in HK301; HK301: nicosulfuron 80 mg·kg^{-1} treatment in HK301; HK320-CK: water treatment in HK320; HK320: nicosulfuron 80 mg·kg^{-1} treatment in HK320; vertical bars represent the SE ($n=3$). Small letters (a, b) indicate differences between values obtained on different days after nicosulfuron treatment ($P<0.05$) according to a least significant difference (LSD) test

demonstrated that the *Fe SOD* gene of HK301 was significantly upregulated. Compared with the control, no significant differences in the intensity of *Fe SOD2* transcript level were recorded. However, *Fe SOD* and *Fe SOD2* genes in HK320 were significantly downregulated compared to the control. After the addition of nicosulfuron, compared to the control, *Cu/Zn SOD1* and *Cu/Zn SOD9* transcripts in HK301 significantly increased, contrary to HK320. The addition of nicosulfuron significantly increased the transcript levels of *Mn SOD3* in HK301, while nicosulfuron treatment did not alter the transcription level of *Mn SOD3* in HK320 (Figs. 8 and 9(a)).

The addition of nicosulfuron had a significant effect on the transcript level of *CAT* genes (Fig. 9(b, c)). *CAT1* and *CAT2* in HK301 recorded similar responses to the addition of nicosulfuron, having strong transcript inductions. Changes in the transcript level in *CAT1* were not significant in HK320, although a slight increase was recorded in HK320. The transcript level of *CAT2* in HK320 was significantly reduced after the addition of nicosulfuron.

SOD has been previously shown to be closely related to the quenching of superoxide to H_2O_2. Three types of SODs (Cu/Zn, Fe, and Mn SODs) have been previously identified in plants, and their metal cofactors in active sites differ.

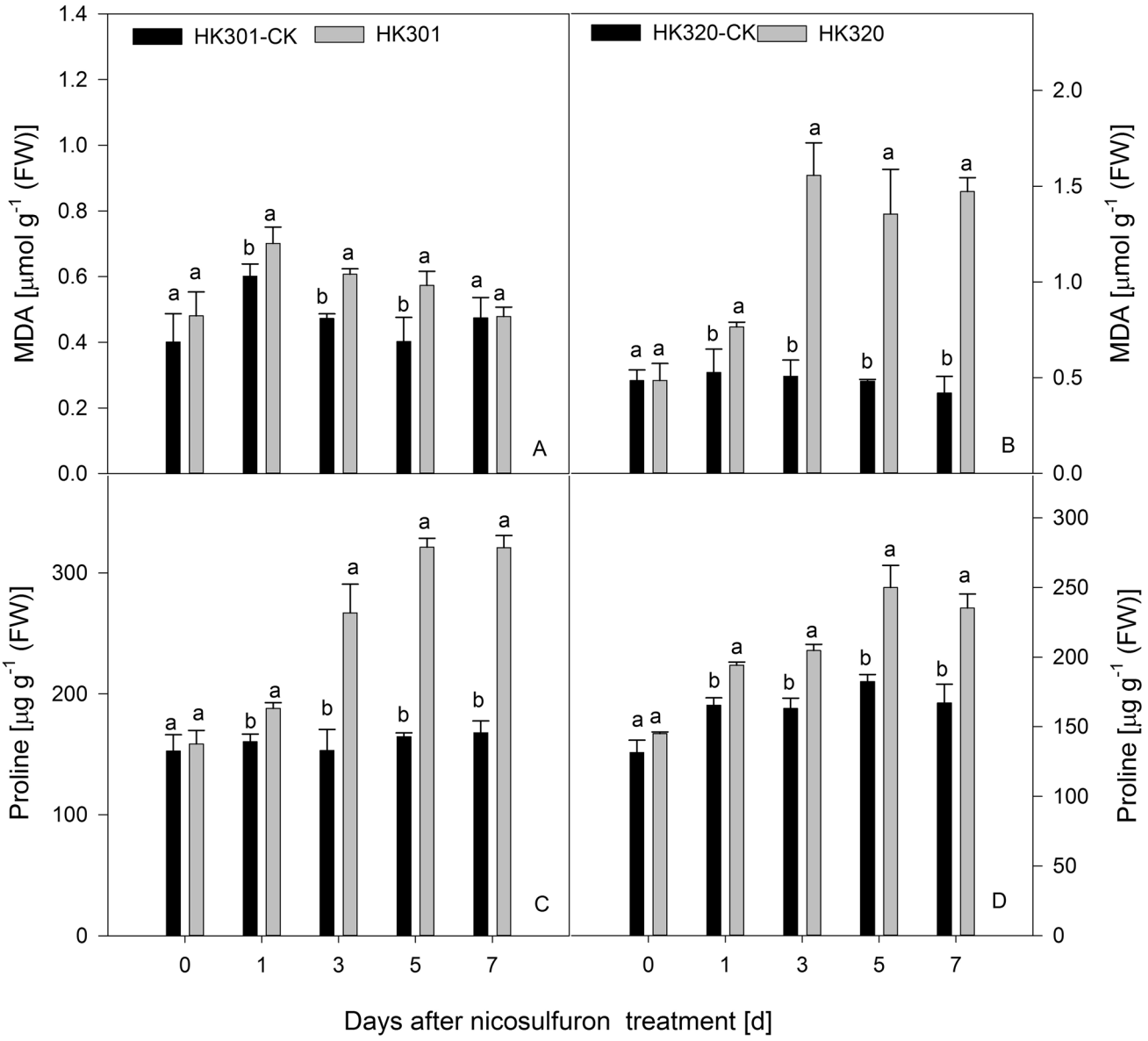


Fig. 6 Effects of nicosulfuron on the malondialdehyde (MDA) (**A**, **B**), and proline (**C**, **D**) in leaves of sweet maize seedlings. HK301-CK: water treatment in HK301; HK301: nicosulfuron 80 mg·kg^{-1} treatment in HK301; HK320-CK: water treatment in HK320; HK320: nicosulfuron 80 mg·kg^{-1} treatment in HK320; vertical bars represent the SE ($n=3$). Small letters (a, b) indicate differences between values obtained on different days after nicosulfuron treatment ($P<0.05$) according to a least significant difference (LSD) test

Exposure to environmental stress, such as drought, salinity, heavy metals, and heat, has resulted in significant changes in SOD activity (Rasoulnia et al. 2011; Kayihan et al. 2012; Rady and Osman 2012; Tian et al. 2012). Although total SOD activity was highest in HK301 after exposure to nicosulfuron, the transcript level of these three isoforms showed quite different responses. Compared to the control, *Fe SOD*, *Cu/Zn SOD1*, *Cu/Zn SOD9*, and *Mn SOD3* had the strongest responses to nicosulfuron in HK301, with a 1.5–3.6-fold transcript increase 24 h after treatment. In contrast, the expression of *Fe SOD*, *Fe SOD2*, *Cu/Zn SOD1*, and *Cu/Zn SOD9* in HK320 was significantly downregulated. However, *Fe SOD2* transcript levels recorded a slight reduction in HK301, and the expression of *Mn SOD* remained unaltered in HK320. Plants have multiple genes encoding SOD. The isoenzymes of SOD are located in chloroplasts, mitochondria, peroxisomes, and cytoplasm. Mn SOD is most commonly found in mitochondria and peroxisomes, while Fe SOD is found in chloroplasts. When positioned in the chloroplast, Cu/Zn SODs provide less protection than Fe SODs (Bueno et al. 1995). Thus, our results suggested that chloroplast compartment might be more critical in scavenging

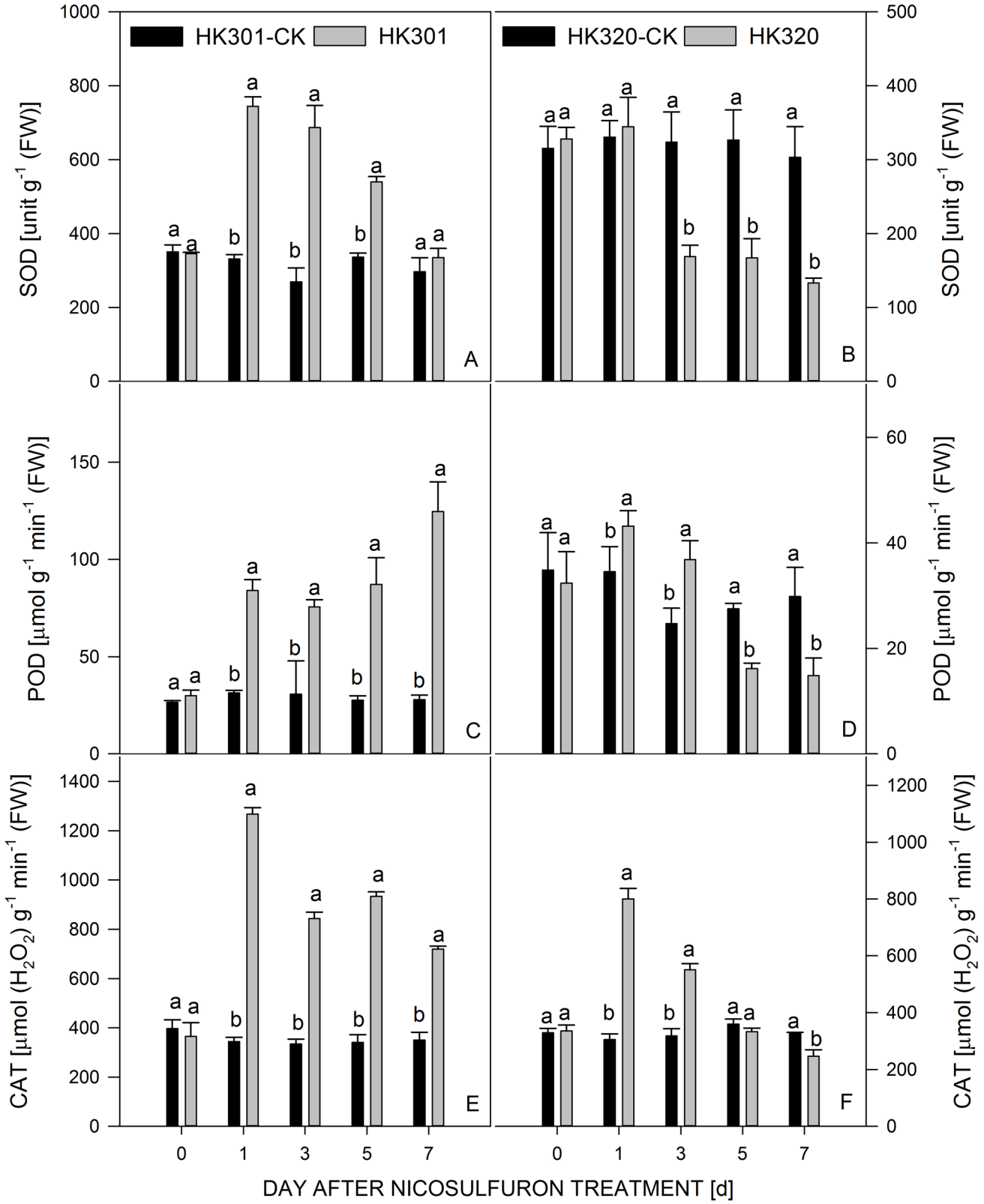


Fig. 7 Effects of nicosulfuron on the SOD (**A**, **B**), POD (**C**, **D**) and CAT (**E**, **F**) in leaves of sweet maize seedlings. HK301-CK: water treatment in HK301; HK301: nicosulfuron 80 mg·kg^{-1} treatment in HK301; HK320-CK: water treatment in HK320; HK320: nicosulfuron 80 mg·kg^{-1} treatment in HK320; vertical bars represent the SE ($n=3$). Small letters (a, b) indicate differences between values obtained on different days after nicosulfuron treatment ($P<0.05$) according to a least significant difference (LSD) test

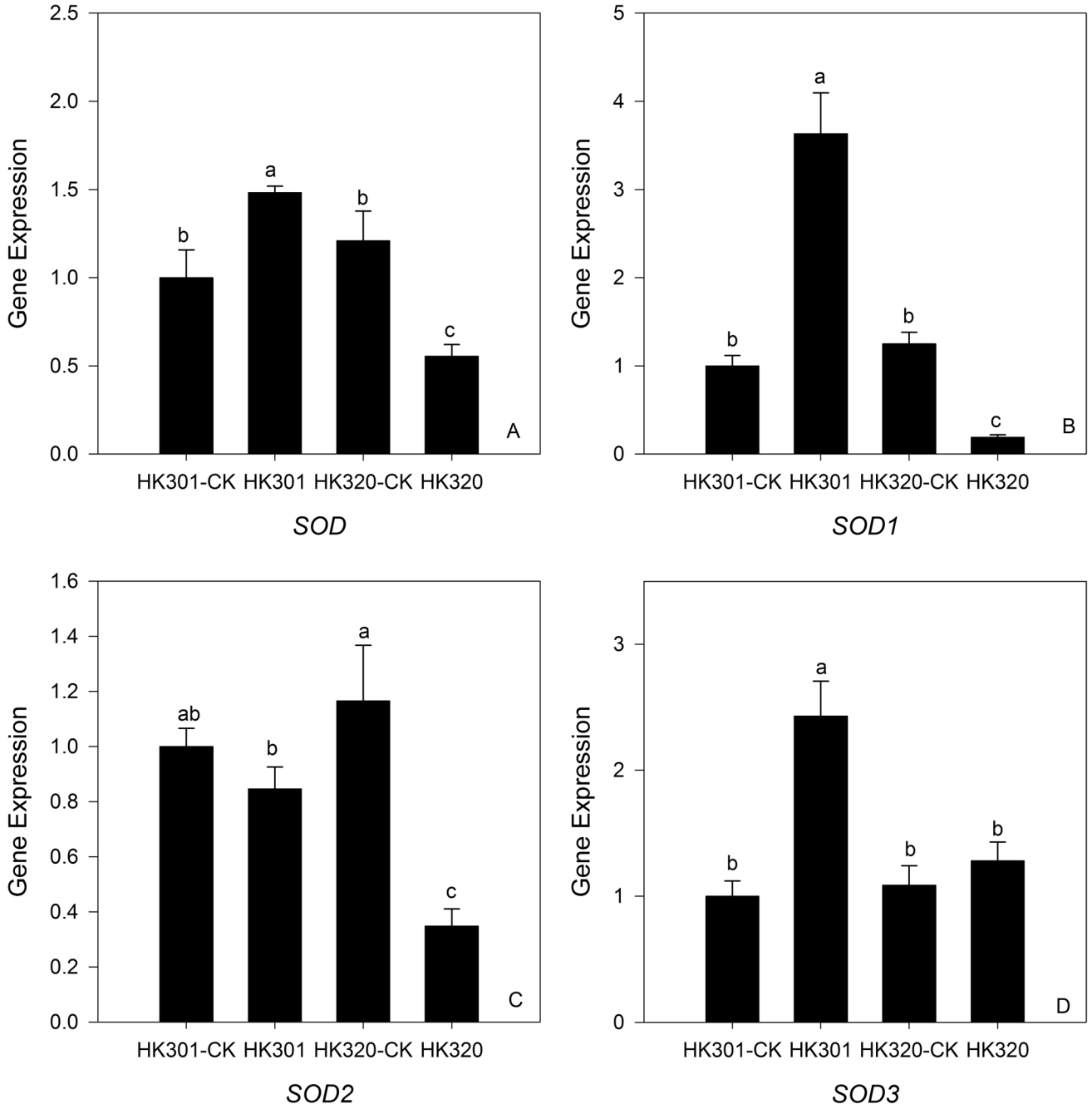


Fig. 8 Transcriptional levels of different antioxidant enzymes, expressed relative to the control, in leaves of sweet maize seedlings exposed to nicosulfuron ((**A**) *SOD*, (**B**) *SOD1*, (**C**) *SOD2*, (**D**) *SOD3*). HK301-CK: water treatment in HK301; HK301: nicosulfuron 80 mg·kg^{-1} treatment in HK301; HK320-CK: water treatment in HK320; HK320: nicosulfuron 80 mg·kg^{-1} treatment in HK320. Small letters (a, b) indicate differences between values obtained on different days after nicosulfuron treatment ($P < 0.05$) according to a least significant difference (LSD) test

$O_2^{\cdot-}$ in the stressed leaves of nicosulfuron-tolerant HK301. These results are very consistent with the study of perennial ryegrass (Hu et al. 2012); that is, under abiotic stress, the relative contribution of Fe SOD and Cu/Zn SOD to total SOD activity is higher than other SOD isoenzymes. Wang and Li (2008) studied the effects of water stress on the activities of total leaf SOD, Fe SOD, and Cu/Zn SOD in *Trifolium repens* L. and reported that the activity of SOD increased significantly under water stress. Eyidogan and Oz (2005) noted three SOD active bands (MnSOD, FeSOD, and Cu/ZnSOD) in *C. arietinum* under salt stress. In addition, the activities of Cu/ZnSOD and MnSOD isozymes increased significantly

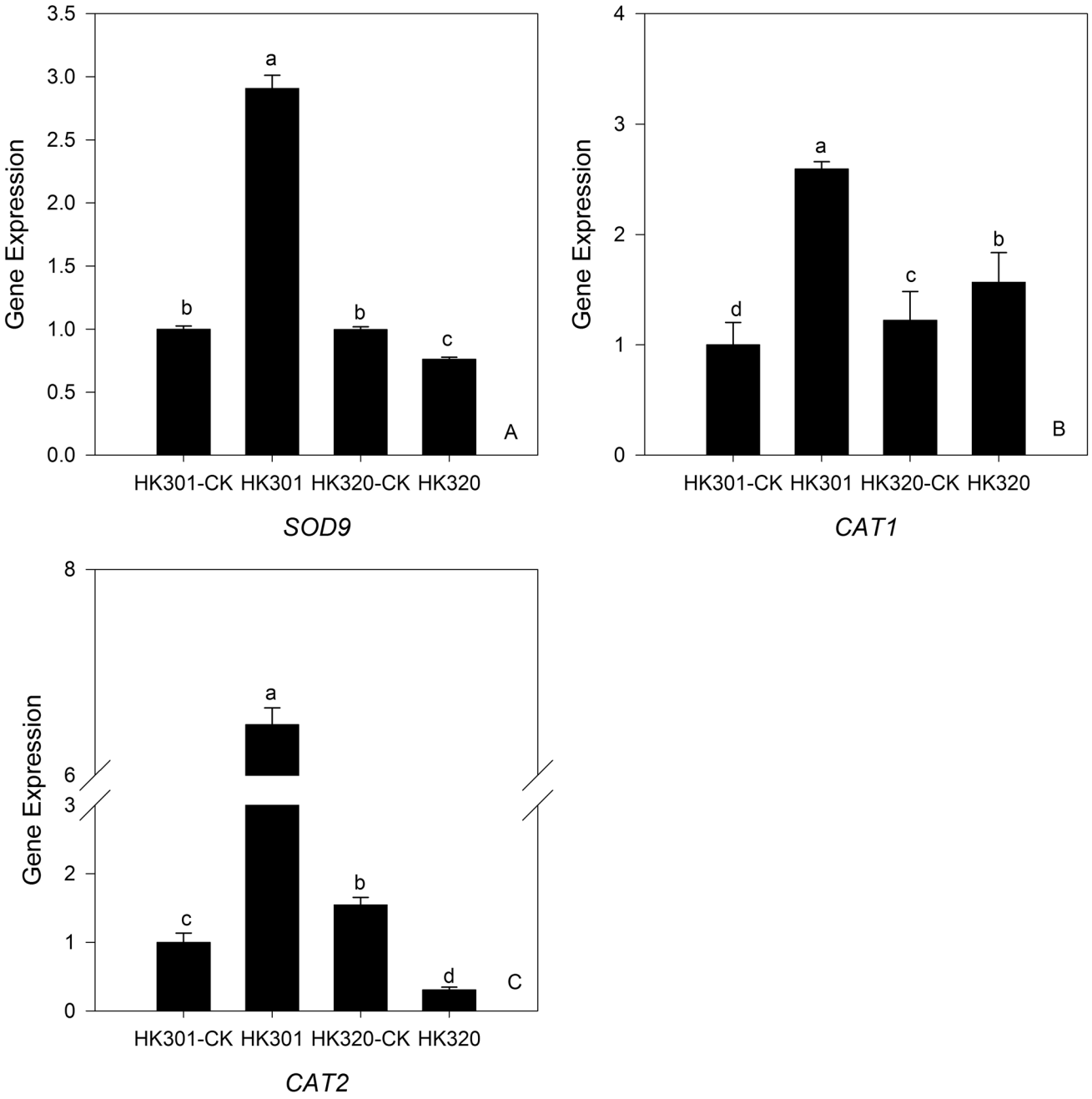


Fig.9 Transcriptional levels of different antioxidant enzymes, expressed relative to the control, in leaves of sweet maize seedlings exposed to nicosulfuron ((**A**) *SOD9*, (**B**) *CAT1*, (**C**) *CAT2*). HK301-CK: water treatment in HK301; HK301: nicosulfuron 80 mg·kg^{-1} treatment in HK301; HK320-CK: water treatment in HK320; HK320: nicosulfuron 80 mg·kg^{-1} treatment in HK320. Small letters (a, b) indicate differences between values obtained on different days after nicosulfuron treatment ($P < 0.05$) according to a least significant difference (LSD) test

under salt stress. Our research proves that *Cu/Zn SOD* and *Fe SOD* strongly responds to nicosulfuron-induced oxidative stress compared with *Mn SOD*.

Catalase, present in peroxisome, is capable of independently decomposing H_2O_2 under abiotic stress conditions (Halliwell 1981). Upregulated expression of the *CAT* gene was found in maize seedlings under drought stress (Hong et al. 2017). In our study, the addition of nicosulfuron significantly increased the transcript levels of *CAT1* and *CAT2* in HK301, compared to the control, and the expression of *CAT1* slightly increase in HK320; the transcript levels of *CAT2* was markedly reduced in HK320. This finding suggests that *CAT2* may have a more important

protective role than *CAT1* in plants against nicosulfuron-induced oxidative stress.

Conclusion

After nicosulfuron treatment, compared with HK301, P_n, E, F_v/F_m, q_P, ETR, and Φ_{PSII} of HK320 were significantly reduced, while C_i, G_s, and NPQ were significantly increased. The destruction of the PSII system in HK 320 led to higher accumulation of $O_2^{-\cdot}$ and H_2O_2, which promotes membrane lipid peroxidation. Our study showed that SOD, POD, and CAT activities of HK301 were significantly higher than those of HK320. Compared to HK320, the gene expression for the majority of *SOD* genes, except for *SOD2*, increased due to inducement by nicosulfuron, and it significantly upregulated the gene expression of *CAT* in HK301. Results from this study indicated that plants can improve photosynthesis, scavenging capabilities of ROS, and protective mechanisms to alleviate phytotoxic effect of nicosulfuron.

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Author contribution Zhen-Xing Wu: conceptualization, data curation, writing—original draft. Ning-Wei Xu: investigation, validation. Min Yang: investigation. Xiang-Ling Li: software, validation. Jin-Ling Han: visualization. Xiao-Hu Lin: formal analysis. Qing Yang: formal analysis. Gui-Hua Lv: conceptualization, supervision. Jian Wang: conceptualization, supervision, writing—review and editing.

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Data availability All data generated or analyzed during this study are included in this published article.

Declarations

Ethical approval Not applicable.

Consent to participate Not applicable.

Consent to publish Not applicable.

Conflict of interest The authors declare no competing interests.

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