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Effects of 5-Aminolevulinic Acid on Oilseed Rape Seedling Growth under Herbicide Toxicity Stress

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Abstract Weeds are one of the major constraints in oilseed *Brassica* production. Use of effective herbicides to control weeds in the fields is one of the major objectives of agronomists. To improve weed control efficacy and minimize the application costs, complex combinations of 5-aminolevulinic acid (ALA) and a new postemergence herbicide, propyl 4-(2-(4,6-dimethoxypyrimidin-2-yloxy)benzylamino)benzoate (ZJ0273), were used to investigate their combined effects in relation to seedling growth and development of oilseed rape (*Brassica napus* cv. ZS 758). *Brassica* seeds were treated with different concentrations of ZJ0273 [100 (normal dose for rape), 200, 500, and 1000 mg/L] and ALA (0.1, 1, 10, and 50 mg/L). ALA was applied as pre- and post-treatment alone and in combination with ZJ0273. We found that ZJ0273 stress imposed negative effects on rape seedling growth. Shoot fresh weight, shoot length, and root fresh weight were inhibited significantly under ZJ0273 stress, and the rate of decline increased consistently with increased ZJ0273 concentration. Root oxidizability was also inhibited significantly under ZJ0273 stress conditions, and the higher the concentration of the herbicide ZJ0273, the lower the oxidizability. Herbicide ZJ0273 treatment produced a gradual decrease in antioxidant enzymes (peroxidase, superoxide dismutase, and ascorbate peroxidase) and an increase in peroxidation substance (malondialdehyde accumulation). The increase and decrease were consistent with the ZJ0273 dosage. Our results indicated that pre- and post-treatments with a lower dosage of ALA (1 mg/L) improved rape seedling growth and root oxidizability parameters, whereas a higher concentration of ALA (50 mg/L) depressed growth. We also found that plants treated with 1 mg/L ALA produced the highest shoot fresh weights, shoot lengths, root fresh weights, and root oxidizability when the seeds were treated with different concentrations of ZJ0273. Lower dosages of ALA improved the activities of antioxidant enzymes, whereas the highest dosage of ALA increased the accumulation of peroxidation substance. These results indicate that ALA has promotive effects in the recovery of growth and development of rape seedlings under herbicide ZJ0273 toxicity stress.

Keywords *Brassica napus* · New herbicide propyl 4-(2-(4,6-dimethoxypyrimidin-2-yloxy)benzylamino) benzoate · 5-Aminolevulinic acid · Synergism · Antioxidants

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Introduction

Oilseed rape (*Brassica napus* L.) is one of the world's major oilseed crops and the most important source of edible oil in China (Zhou 2001; Momoh and others 2002). Weeds are a significant threat to oilseed rape production resulting in 15.8–50% loss of oilseed production (Su and Song 1996; Zhou and others 2004; Song and others 2005, 2006). These weeds include both narrow- and broad-leaf types. Optimum crop production depends on successful weed control. Methods of weed control in crucifer crops include cultural

(Lanini and others 2002) and crop rotation (Zaragoza 2003), mechanical and hand-weeding (Kroonen-Backbeir and Zwart-Roodzant 1998), mowing (Zaragoza 2003), physical such as mulching (James and others 1989; Barberi 2002; Subrahmaniyan and others 2008; Subrahmaniyan and Zhou 2008), soil solarization with chicken manure (Haidar and Sidahmad 2000), and chemical control (Qasem 2007).

Recently, chemical control of weeds has become more popular, and various herbicides have been registered for use in oilseed rape in China and provide pre- or postemergence weed control (Agricultural Chemicals Verification Institute, China Ministry of Agriculture 2000). In spite of the large number of herbicides reported for use in the rape field, sequential applications of these herbicides appeared necessary for wider weed spectrum control, especially for broad-leaf weed control. Propyl 4-(2-(4,6-dimethoxypyrimidin-2-yloxy) benzylamino)benzoate (ZJ0273), derived from a precursor compound pesticide (a derivative of 2-pyrimidinyloxy-*N*-aryl benzoate) with novel structure and efficient biological activity (Lu and others 2004), is a new oilseed rape herbicide with the advantages of low dosage, low mammalian toxicity, broad weeding spectrum, and environmental compatibility (Tang and others 2005). It provides effective control of both monocotyledonous and dicotyledonous weeds in the oilseed rape field (Tang and others 2005).

Some plant growth regulators can accelerate the absorption and translocation of herbicides in weeds when they are used together with herbicides (Carson and Bandeen 1975; Hykaliuk and others 1982; Jerey and Michael 1989) and improve the effect of weed control (Li 1994; Qiu 1994; Zhu and others 2003). On the other hand, some other plant growth regulators can alleviate herbicide effects on rice (Zhou 2003). 5-Aminolevulinic acid (ALA) is a key precursor in the biosynthesis of porphyrins such as chlorophyll and heme and is found in all plants and animals. ALA has diverse uses as it has practical medical applications for cancer treatment, tumor diagnosis, and other clinical uses and is used in agriculture as a herbicide, pesticide (Duke and Rebeiz 1994), and growth-promoting factor for plants *in vivo* (Weinstein and Beale 1985; Hotta and others 1997b) and *in vitro* (Zhang and others 2006). Recently, it was found that a low concentration of ALA has a promotive effect on growth and yield of several field crops, including vegetables (Hotta and others 1997a, b; Watanabe and others 2000). ALA was also found to increase cold and salt tolerance in rice and potato (Hotta and others 1998; Zhang and others 2006).

To our knowledge, no information is available regarding the effects of ALA on seedling growth, lipid peroxidation, and activities of the antioxidant system in oilseed rape (*Brassica napus* L.), with respect to toxicity stress of the new herbicide ZJ0273. The objectives of the present investigation were to study the effect of ALA on seedling growth and the physiologic and biochemical responses in oilseed rape with or without herbicide ZJ0273 stress and to determine the possible beneficial effects of the application of ALA on the growth of oilseed rape seedlings under herbicidal stress.

Materials and Methods

Plant Materials and Treatments

Seeds of oilseed rape (*B. napus* cv. ZS 758, a popular cultivar in China) were obtained from the College of Agriculture and Biotechnology of Zhejiang University. Seeds were washed with distilled water and air-dried before use. The herbicide propyl 4-(2-(4,6-dimethoxypyrimidin-2-yloxy)benzylamino) benzoate (ZJ0273) was supplied by the Shanghai Institute of Organic Chemistry, Chinese Academy of Sciences, China, and 5-aminolevulinic acid (ALA) was purchased from Cosmo Oil Co. Ltd. (Japan). Seeds were treated with different combinations of ZJ0273 and ALA as per the following procedure:

1. ALA pretreatment: Fifty seeds were sown in Petri dishes filled with two pieces of filter paper to which 6 ml ALA solutions (0, 0.1, 1, 10, and 50 mg/L) had been added; 24 h later the excess solutions were removed and 6 ml ZJ0273 solutions (0, 100, 200, 500, and 1000 mg/L) were added to the Petri dishes. The normal dose for rape is 100 mg/L of ZJ0273, as recommended for field use.
2. ALA post-treatment: Fifty seeds were sown in Petri dishes filled with two pieces of filter paper to which 6 ml ZJ0273 solutions (0, 100, 200, 500, and 1000 mg/L) had been added; 24 h later the excess solutions were removed and 6 ml ALA solutions (0, 0.1, 1, 10, and 50 mg/L) were added to the Petri dishes.
3. After another 24 h, the excess solutions were discarded, seeds and filter paper were transferred onto a sponge floating in a high-wall white porcelain plate filled with half-strength Hoagland solution. Seeds were cultured in the incubator at a temperature of 20°C under a 16-h photoperiod (light intensity of 140 μmol m^{-2} s^{-1}) and high relative humidity (85-90%). Seven days later plants were divided into shoots and roots for the determination of fresh weights and physiologic characters. All treatments were replicated four times.

Determination of Biomass and Physiologic Characters

Fresh weights of shoots (10 plants) and roots (50 plants) and shoot lengths of rape plants were measured. However, seedlings under 1000 mg/L ZJ0273 treatment alone and in different doses of ALA with 1000 mg/L herbicide ZJ0273 were not large enough for fresh weight and shoot length measurements.

Antioxidants, superoxide dismutase (SOD) and peroxidase (POD) activities, and malondialdehyde (MDA) accumulation were determined according to Zhang (1992) with some modifications. Shoots were ground with a mortar and pestle under chilling conditions in the homogenization buffer specific for each enzyme. The homogenate was centrifuged at 10,000*g* for 20 min at 4°C, and the supernatants were used for enzyme assays (Leul and Zhou 1999).

Superoxide dismutase (SOD, EC 1.15.1.1) activity was assayed by using the photochemical NBT method. Samples (0.5 g) were homogenized in 5 ml extraction buffer consisting of 50 mM phosphate, pH 7.8. The assay mixture in 3 ml contained 50 mM phosphate buffer, pH 7.8, 26 mM L-methionine, 750 μM NBT, 1 μM EDTA, and 20 μM riboflavin. The photoreduction of NBT (formation of purple formazan) was measured at 560 nm and an inhibition curve was made against different volumes of extract. One unit of SOD is defined as the amount present in the volume of extract that causes inhibition of the photoreduction of NBT by 50% (Zhou and others 1997).

Peroxidase (POD, EC 1.11.1.7) activity was measured with guaiacol as the substrate in a total volume of 3 ml. The reaction mixture consisted of 50 mM potassium phosphate buffer (pH 6.1), 1% guaiacol, 0.4% H_2O_2, and enzyme extract. Increases in the absorbance due to oxidation of guaiacol were measured at 470 nm. Enzyme activity was calculated in terms of μmol of guaiacol oxidized min^{-1} g^{-1} fresh weight at 25 ± 2°C (Zhou and Leul 1999).

The assay for ascorbate peroxidase (APX, EC 1.11.1.11) activity was carried out in a reaction mixture of 3 ml containing 100 mM phosphate (pH 7), 0.1 mM EDTA-Na_2, 0.3 mM ascorbic acid, 0.06 mM H_2O_2 and 100 μl enzyme extract. The change in absorption at 290 nm was recorded 30 s after addition of H_2O_2 (Nakano and Asada 1981).

The level of lipid peroxidation was expressed in terms of malondialdehyde (MDA) content and was determined as 2-thiobarbituric acid (TBA) reactive metabolites, as previously described by Zhou and Leul (1998). Plant fresh tissues (0.2 g) were homogenized and extracted in 10 ml of 0.25% TBA made in 10% trichloroacetic acid (TCA). The extract was heated at 95°C for 30 min and then quickly cooled on ice. After centrifugation at 5000*g* for 10 min, the absorbance of the supernatant was measured at 532 nm. Correction of nonspecific turbidity was made by subtracting the absorbance value taken at 600 nm. The level of lipid peroxidation was expressed as μmol g^{-1} fresh weight by using an extinction coefficient of 155 mM cm^{-1}.

Root oxidizability was measured by the triphenyl tetrazolium chloride (TTC) reduction method (Zhou and Ye 1996). A 500-mg sample of roots from each treatment was placed in a 25-ml test tube and sealed with a rubber stopper. The tubes were treated with 5 ml of 0.4% TTC solution and 5 ml of 1/15 M phosphate buffer (pH 7.0). After incubation for 1 h at 37°C, the tubes were treated with 2 ml of 2 N H_2SO_4. The roots were ground with ethyl acetate (a total of 10 ml) to extract red triphenylformazane and the extract was read at 485 nm.

All data presented are mean values. Measurements were performed on four replicates. Data were analyzed using the statistical program SAS and the analysis of variance (ANOVA) was followed by Fisher's protected LSD test to identify homogeneous groups within the means. Significant differences among treatments were considered at the $P \leq 0.05$ level.

Results

Figure 1a–d shows the germination of oilseed rape (*B. napus* cv. ZS 758) in response to the new herbicide ZJ0273 and bioregulator ALA. In the control, seedling development was significantly better than all the treated samples (Figure 1a). Germination was severely inhibited under 1000 mg/L ZJ0273 stress conditions (Figure 1b). However, the addition of 1 mg/L ALA with 1000 mg/L ZJ0273 improved germination (Figure 1c), but no germination was observed with the addition of the higher concentration of ALA (50 mg/L) with 1000 mg/L herbicide ZJ0273.

Pretreatment with 1 mg/L ALA significantly increased the shoot fresh weight of oilseed rape seedlings in comparison to all other ALA treatments (Table 1). Post-treatment with 0.1 and 1.0 mg/L ALA also produced significantly higher shoot fresh weights compared with other treatments. Both pre- and post-treatments of ALA at 50 mg/L produced significantly lower shoot fresh weights than the controls. The fresh weight of shoots was significantly lower under ZJ0273 stress conditions. The rate of decline in shoot weight was consistently higher with each successive increase in ZJ0273 concentration. As expected, the highest shoot fresh weight was achieved by the controls, whereas the lowest weight was observed with higher doses of ZJ0273. Seeds pretreated with 0.1 and 1 mg/L ALA produced significantly higher biomass when the seeds were subsequently treated with different ZJ0273 dosages. Similar results were observed in the 1-mg/L ALA post-treatment.

Table 2 presents the effects of ALA and ZJ0273 on rape shoot length. Pre- and post-treatments of 1 mg/L ALA produced significantly higher shoot lengths in rape seedlings than the control, whereas 50 mg/L ALA treatments produced significantly lower shoot lengths, indicating that high concentrations of ALA depressed the growth of the seedlings. Shoot elongation was inhibited significantly under ZJ0273 stress conditions, and the higher the concentration of herbicide ZJ0273, the lower the shoot length. Plants pretreated by 1 mg/L ALA produced the longest

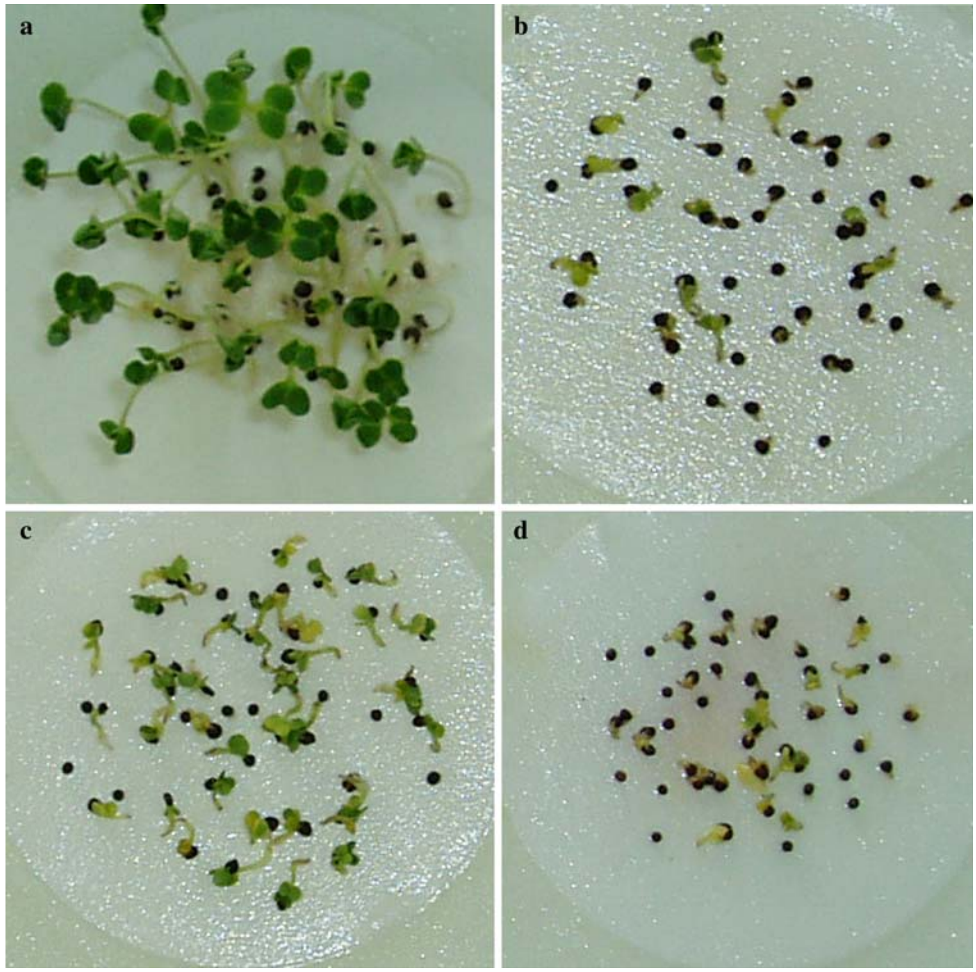

Fig. 1 Effects of treatments of propyl 4-(2-(4,6-dimethoxypyrimidin-2-yloxy)benzylamino)benzoate (ZJ0273) (1000 mg/L) and 5-aminolevulinic acid (ALA) on germination and growth of *Brassica napus* cv. ZS 758. (**a**) Control, neither ZJ0273 nor ALA. (**b**) 1000 mg/L ZJ0273. (**c**) 1000 mg/L ZJ0273 and 1 mg/L ALA. (**d**) 1000 mg/L ZJ0273 and 50 mg/L ALA

Table 1 Effects of Treatments of propyl 4-(2-(4,6-dimethoxypyrimidin-2-yloxy)benzylamino)benzoate (ZJ0273) and 5-Aminolevulinic Acid (ALA) on Shoot Fresh Weight (g FW/10 plants) of *Brassica napus* cv. ZS 758

	ALA concentration (mg/L)	ZJ0273 concentration (mg/L)			
		0	100	200	500
ALA pretreatment	0	A[y] 0.630[x] c[z]	B 0.554 b	C 0.508 c	D 0.408 b
	0.1	0.707 b	0.637 a	0.593 a	0.487 a
	1	0.811 a	0.663 a	0.620 a	0.506 a
	10	0.632 c	0.585 b	0.541 c	0.435 b
	50	0.480 d	0.465 c	0.398 d	0.350 c
ALA post-treatment	0	A 0.630 b	A 0.576 b	B 0.509 c	C 0.403 bc
	0.1	0.755 a	0.681 a	0.620 ab	0.423 b
	1	0.817 a	0.710 a	0.631 a	0.514 a
	10	0.673 b	0.610 b	0.543 bc	0.413 b
	50	0.533 c	0.458 c	0.387 d	0.349 c

[x] Each value represents the mean of four replicates of each treatment

[y] ZJ0273 treatment alone: Among four ZJ0273 (0–500 mg/L) treatments (row), means preceded by same capital letters are not significantly different by the LSD test at $P \leq 0.05$ (Completely Randomized Design, CRD)

[z] ALA treatment alone (the 3rd column from left) and the combined ALA and ZJ0273 treatments (the 4th, 5th, 6th columns from left): Among ALA pretreatment (the upper half) or ALA post-treatment (the lower half), means followed by same lower-case letters are not significantly different by the LSD test at $P \leq 0.05$ under CRD

Table 2 Effects of Treatments of propyl 4-(2-(4,6-dimethoxypyrimidin-2-yloxy)benzylamino)benzoate (ZJ0273) and 5-Aminolevulinic Acid (ALA) on Shoot Length (cm) of *Brassica napus* cv. ZS 758

	ALA concentration (mg/L)	ZJ0273 concentration (mg/L)			
		0	100	200	500
ALA pretreatment	0	A[y] 7.09[x] c[z]	B 6.58 c	C 5.98 c	D 4.59 b
	0.1	8.50 b	7.25 b	6.45 b	4.74 ab
	1	9.27 a	8.00 a	6.99 a	4.96 a
	10	7.27 c	6.88 c	6.08 c	4.75 ab
	50	6.40 d	5.62 d	5.43 d	4.21 c
ALA post-treatment	0	A 7.09 c	A 6.81 b	B 6.12 c	C 5.02 b
	0.1	8.57 b	7.97 a	6.55 ab	5.45 a
	1	9.08 a	8.26 a	6.92 a	5.46 a
	10	7.46 c	7.01 b	6.42 bc	5.20 ab
	50	6.70 d	6.03 c	5.31 d	4.46 c

[x] Each value represents the mean of four replicates of each treatment

[y] ZJ0273 treatment alone: Among four ZJ0273 (0–500 mg/L) treatments (row), means preceded by same capital letters are not significantly different by the LSD test at $P \leq 0.05$ (Completely Randomized Design, CRD)

[z] ALA treatment alone (the 3rd column from left) and the combined ALA and ZJ0273 treatments (the 4th, 5th, 6th columns from left): Among ALA pretreatment (the upper half) or ALA post-treatment (the lower half), means followed by same lower-case letters are not significantly different by the LSD test at $P \leq 0.05$ under CRD

shoots (8.00, 6.99, and 4.96 cm) when the seeds were subsequently treated with different doses of ZJ0273 (100, 200, and 500 mg/L), respectively. Similar results were obtained with the ALA post-treatment. ALA treatment with concentrations of 0.1–1 mg/L showed a significant increase in shoot lengths over the control, with the highest shoot lengths being observed after the 1-mg/L ALA treatment following ZJ0273 application.

Pretreatment with 1 mg/L ALA significantly improved root fresh weights compared with that of the control and all other treatments (Table 3). Similarly, post-treatment with 1 mg/L ALA also produced significantly higher root fresh weights. The ZJ0273 stress imposed negative effects on root fresh weights. The rate of decline in root weights increased with the increase in ZJ0273 dosage.

Pretreatment of seeds with 0.1 and 1 mg/L ALA significantly improved the root oxidizability by 9.1 and 17.3%, respectively, whereas 50 mg/L ALA pretreatment produced significantly lower root oxidizability than the control (Table 4). Similarly, post-treatments with 0.1 and 1 mg/L ALA produced 8.2 and 15.5% increases in root oxidizability, respectively, in comparison to the control; 50 mg/L ALA produced lower oxidizability than all other treatments. All ZJ0273 treatments significantly decreased the root oxidizability of rape seedlings. The plants pre- and post-treated with 1 mg/L ALA produced the highest root oxidizability when the seeds were subsequently treated with different concentrations of ZJ0273.

MDA content showed a positive relationship with ALA treatments (both pre- and post-treatments) as the MDA accumulation increased with increasing ALA dosage and the highest accumulation was observed with the higher dosage of ALA (50 mg/L) (Table 5). Similarly, gradual increases in MDA accumulation were observed as the concentration of the herbicide was increased.

Both pre- and post-treatments with 0.1 and 1 mg/L ALA produced significantly higher SOD activity (that is, 4.2 and 10.0% for pretreatment and 4.8 and 11.8% for post-treatment over the control, respectively). A gradual decrease in SOD activity was observed in all ZJ0273 treatments. ALA pre- and post-treatments of 0.1–10 mg/L could improve the SOD activity of the plants treated together with herbicide ZJ0273, with the highest SOD activity being observed with 1 mg/L ALA (Table 6).

Table 7 shows the POD activity of the plants treated with ALA and ZJ0273 and the synergistic effect of ALA and ZJ0273. The plants pretreated with 0.1 and 1 mg/L ALA had a significantly higher POD activity, 65.1 and 87.2% over the control, respectively. Similarly, post-treatment with 0.1 and 1 mg/L ALA also significantly increased (77.4 and 104.6%, respectively) POD activity compared to the control. However, the highest dosage of ALA (50 mg/L) imposed negative effects on POD and hence produced lower POD activity than the control and all other treatments. A gradual decrease in POD activity was observed with all ZJ0273 treatments and hence the least POD was accumulated in response to the 500 mg/L ZJ0273 dose. Both pre- and post-treatments with ALA at levels of 0.1–10 mg/L could improve the POD activity of plants treated together with herbicide ZJ0273, with the highest POD activity observed from 1 mg/L ALA.

Table 3 Effects of Treatments of propyl 4-(2-(4,6-dimethoxypyrimidin-2-yloxy)benzylamino)benzoate (ZJ0273) and 5-Aminolevulinic acid (ALA) on Root Fresh Weight (g FW/50 plants) of *Brassica napus* cv. ZS 758

	ALA concentration (mg/L)	ZJ0273 concentration (mg/L)			
		0	100	200	500
ALA pretreatment	0	A[y] 0.859[x] b[z]	B 0.534 cd	C 0.404 b	D 0.293 b
	0.1	1.230 a	0.631 ab	0.423 ab	0.323 b
	1	1.256 a	0.672 a	0.436 a	0.367 a
	10	1.095 ab	0.577 bc	0.418 ab	0.300 b
	50	0.851 b	0.490 d	0.352 c	0.140 c
ALA post-treatment	0	A 0.859 bc	B 0.594 b	C 0.441 bc	D 0.302 b
	0.1	1.131 a	0.726 a	0.498 ab	0.326 ab
	1	1.168 a	0.717 a	0.525 a	0.357 a
	10	0.874 b	0.596 b	0.480 ab	0.305 b
	50	0.819 c	0.512 b	0.419 c	0.229 c

[x] Each value represents the mean of four replicates of each treatment

[y] ZJ0273 treatment alone: Among four ZJ0273 (0–500 mg/L) treatments (row), means preceded by same capital letters are not significantly different by the LSD test at $P \leq 0.05$ (Completely Randomized Design, CRD)

[z] ALA treatment alone (the 3rd column from left) and the combined ALA and ZJ0273 treatments (the 4th, 5th, 6th columns from left): Among ALA pretreatment (the upper half) or ALA post-treatment (the lower half), means followed by same lower-case letters are not significantly different by the LSD test at $P \leq 0.05$ under CRD

Table 4 Effects of Treatments of propyl 4-(2-(4,6-dimethoxypyrimidin-2-yloxy)benzylamino)benzoate (ZJ0273) and 5-Aminolevulinic Acid (ALA) on Root Oxidizability (TTC mg/g FW/h) of *Brassica napus* cv. ZS 758

	ALA concentration (mg/L)	ZJ0273 concentration (mg/L)			
		0	100	200	500
ALA pretreatment	0	A[y] 0.329[x] c[z]	AB 0.309 c	B 0.291 c	C 0.178 b
	0.1	0.359 b	0.328 b	0.321 b	0.200 ab
	1	0.386 a	0.356 a	0.334 a	0.220 a
	10	0.346 bc	0.318 bc	0.301 c	0.191 b
	50	0.268 d	0.215 d	0.175 d	0.143 c
ALA post-treatment	0	A 0.329 c	AB 0.314 c	B 0.299 c	C 0.186 c
	0.1	0.356 b	0.332 b	0.320 b	0.211 ab
	1	0.380 a	0.360 a	0.343 a	0.229 a
	10	0.336 bc	0.319 c	0.314 b	0.192 bc
	50	0.303 d	0.270 d	0.168 d	0.148 d

[x] Each value represents the mean of four replicates of each treatment

[y] ZJ0273 treatment alone: Among four ZJ0273 (0–500 mg/L) treatments (row), means preceded by same capital letters are not significantly different by the LSD test at $P \leq 0.05$ (Completely Randomized Design, CRD)

[z] ALA treatment alone (the 3rd column from left) and the combined ALA and ZJ0273 treatments (the 4th, 5th, 6th columns from left): Among ALA pretreatment (the upper half) or ALA post-treatment (the lower half), means followed by same lower-case letters are not significantly different by the LSD test at $P \leq 0.05$ under CRD

The 0.1–10-mg/L ALA treatment significantly increased the APX activity of the oilseed rape seedlings (Table 8). The 1-mg/L ALA treatment significantly increased APX activity of rape seedlings over all other dosages, whereas the 50-mg/L ALA treatment imposed significantly negative effects on APX activity and hence produced the lowest amount of APX. A gradual decrease in APX activity was observed in response to ZJ0273 dosages. The plants pretreated with 0.1–10 mg/L ALA produced higher APX activities when seeds were subsequently treated with ZJ0273, and an even better result was observed with 1-mg/L ALA pretreatment relative to the control. Similar results were obtained in the ALA post-treatment.

Table 5 Effects of Treatments of propyl 4-(2-(4,6-dimethoxypyrimidin-2-yloxy)benzylamino)benzoate (ZJ0273) and 5-Aminolevulinic Acid (ALA) on MDA Content (μmol/g FW) of *Brassica napus* cv. ZS 758

	ALA concentration (mg/L)	ZJ0273 concentration (mg/L)			
		0	100	200	500
ALA pretreatment	0	D[y] 5.43[x] b[z]	C 6.45 b	B 7.30 b	A 8.14 c
	0.1	4.97 bc	5.68 c	6.11 c	6.62 d
	1	4.34 c	5.28 c	6.04 c	6.37 d
	10	5.68 b	6.36 b	7.61 b	8.71 b
	50	7.55 a	8.64 a	9.62 a	10.18 a
ALA post-treatment	0	D 5.43 b	C 6.35 b	B 6.95 bc	A 8.09 bc
	0.1	4.78 c	5.65 bc	6.29 c	7.74 cd
	1	4.58 c	5.47 c	6.20 c	7.45 d
	10	5.71 b	6.47 b	7.31 b	8.26 b
	50	8.40 a	8.48 a	9.38 a	10.21 a

[x] Each value represents the mean of four replicates of each treatment

[y] ZJ0273 treatment alone: Among four ZJ0273 (0–500 mg/L) treatments (row), means preceded by same capital letters are not significantly different by the LSD test at $P \leq 0.05$ (Completely Randomized Design, CRD)

[z] ALA treatment alone (the 3rd column from left) and the combined ALA and ZJ0273 treatments (the 4th, 5th, 6th columns from left): Among ALA pretreatment (the upper half) or ALA post-treatment (the lower half), means followed by same lower-case letters are not significantly different by the LSD test at $P \leq 0.05$ under CRD

Table 6 Effects of Treatments of propyl 4-(2-(4,6-dimethoxypyrimidin-2-yloxy)benzylamino)benzoate (ZJ0273) and 5-Aminolevulinic Acid (ALA) on SOD Activity (U/g FW) of *Brassica napus* cv. ZS 758

	ALA concentration (mg/L)	ZJ0273 concentration (mg/L)			
		0	100	200	500
ALA pretreatment	0	A[y] 355.9[x] c[z]	B 342.2 c	C 310.0 bc	D 267.3 b
	0.1	370.9 b	352.4 b	321.1 b	279.2 b
	1	391.5 a	375.7 a	341.3 a	308.1 a
	10	361.7 c	349.2 bc	314.9 b	272.0 b
	50	333.7 d	315.5 d	299.9 c	251.6 c
ALA post-treatment	0	A 355.9 c	B 344.3 c	C 305.8 c	D 271.6 c
	0.1	372.9 b	358.1 b	333.2 b	282.5 b
	1	397.9 a	374.7 a	359.2 a	313.3 a
	10	359.8 bc	352.1 bc	310.1 c	281.8 b
	50	330.0 d	305.0 d	279.0 d	245.0 d

[x] Each value represents the mean of four replicates of each treatment

[y] ZJ0273 treatment alone: Among four ZJ0273 (0-500 mg/L) treatments (row), means preceded by same capital letters are not significantly different by the LSD test at $P \leq 0.05$ (Completely Randomized Design, CRD)

[z] ALA treatment alone (the 3rd column from left) and the combined ALA and ZJ0273 treatments (the 4th, 5th, 6th columns from left): Among ALA pretreatment (the upper half) or ALA post-treatment (the lower half), means followed by same lower-case letters are not significantly different by the LSD test at $P \leq 0.05$ under CRD

Discussion

Weeds play an important role in yield reduction of many crops, including oilseed *Brassica*. Propyl 4-(2-(4,6-dimethoxypyrimidin-2-yloxy)benzylamino)benzoate (ZJ0273) is a new oilseed rape herbicide offering effective prevention from both monocotyledonous and dicotyledonous weeds. Its use at concentrations of 100 mg/L or more is effective under severe weed conditions (Tang and others 2005). Our preliminary research showed that 1000 mg/L ZJ0273 imposed negative effects on oilseed rape germination; therefore, only 100–500 mg/L ZJ0273 was used in the present studies.

We found that ZJ0273 toxicity imposed negative effects on rape seedling growth. Fresh weights of shoots and root and shoot lengths were inhibited significantly under ZJ0273 treatments. The rate of decline was consistently higher with increasing ZJ0273 concentration. ZJ0273-induced stress significantly depressed the root oxidizability of the oilseed rape

Table 7 Effects of Treatments of propyl 4-(2-(4,6-dimethoxypyrimidin-2-yloxy)benzylamino)benzoate (ZJ0273) and 5-Aminolevulinic Acid (ALA) on POD Activity (OD_{470}/g FW/min) of *Brassica napus* cv. ZS 758

	ALA concentration (mg/L)	ZJ0273 concentration (mg/L)			
		0	100	200	500
ALA pretreatment	0	A[y] 15.72[x] c[z]	A 13.80 b	B 11.50 cd	B 10.36 cd
	0.1	25.96 b	21.90 a	16.28 b	12.95 b
	1	29.43 a	24.80 a	21.43 a	18.00 a
	10	17.28 c	16.19 b	12.99 c	11.80 bc
	50	11.82 d	10.16 c	9.48 d	8.74 d
ALA post-treatment	0	A 15.72 bc	B 14.72 b	C 12.18 c	D 10.67 bc
	0.1	27.89 a	23.22 a	15.72 b	14.68 a
	1	32.17 a	25.81 a	22.60 a	16.07 a
	10	17.95 b	16.04 b	13.13 c	11.60 b
	50	11.50 c	9.50 c	9.33 d	8.48 c

[x] Each value represents the mean of four replicates of each treatment

[y] ZJ0273 treatment alone: Among four ZJ0273 (0–500 mg/L) treatments (row), means preceded by same capital letters are not significantly different by the LSD test at $P \leq 0.05$ (Completely Randomized Design, CRD)

[z] ALA treatment alone (the 3rd column from left) and the combined ALA and ZJ0273 treatments (the 4th, 5th, 6th columns from left): Among ALA pretreatment (the upper half) or ALA post-treatment (the lower half), means followed by same lower-case letters are not significantly different by the LSD test at $P \leq 0.05$ under CRD

Table 8 Effects of Treatments of propyl 4-(2-(4,6-dimethoxypyrimidin-2-yloxy)benzylamino)benzoate (ZJ0273) and 5-Aminolevulinic Acid (ALA) on APX Activity (μmol/g FW) of *Brassica napus* cv. ZS 758

	ALA concentration (mg/L)	ZJ0273 concentration (mg/L)			
		0	100	200	500
ALA pretreatment	0	A[y] 47.22[x] c[z]	B 42.25 c	C 37.70 b	D 34.60 b
	0.1	52.39 b	46.50 b	38.53 b	35.47 b
	1	56.81 a	50.39 a	46.12 a	40.37 a
	10	50.88 b	45.25 b	38.00 b	35.56 b
	50	40.25 d	33.85 d	29.14 c	22.98 c
ALA post-treatment	0	A 47.22 c	B 42.71 c	C 36.74 bc	C 34.58 b
	0.1	51.15 b	47.36 b	37.87 b	36.11 b
	1	56.29 a	50.07 a	45.85 a	43.04 a
	10	50.92 b	44.72 c	41.58 ab	35.26 b
	50	40.74 d	35.50 d	30.93 c	22.78 c

[x] Each value represents the mean of four replicates of each treatment

[y] ZJ0273 treatment alone: Among four ZJ0273 (0–500 mg/L) treatments (row), means preceded by same capital letters are not significantly different by the LSD test at $P \leq 0.05$ (Completely Randomized Design, CRD)

[z] ALA treatment alone (the 3rd column from left) and the combined ALA and ZJ0273 treatments (the 4th, 5th, 6th columns from left): Among ALA pretreatment (the upper half) or ALA post-treatment (the lower half), means followed by same lower-case letters are not significantly different by the LSD test at $P \leq 0.05$ under CRD

seedlings. Decreased root oxidizability and root growth may have depressed water and nutrient absorption. Reactive oxygen species (ROS) formation under several abiotic stress conditions such as herbicides has been reported as related to oxidative stress (Bowler and others 1992; Romero-Puertas and others 2004). We found that herbicide ZJ0273 treatment had gradual negative effects on antioxidant enzymes (POD, SOD, APX); however, ZJ0273 stress showed consistent positive effects on the peroxidation substance (MDA accumulation).

ALA is the first key precursor of heme biosynthesis in all organisms (Castelfranco and Beale 1983; Von Wettstein and others 1995). Its herbicidal activity has been reported to increase accumulation of several chlorophyll intermediates such as protochlorophyllide, protoporphyrin IX, and Mg-protoporphyrin IX, when plants are treated with exogenous ALA at relatively high concentrations (5–40 mmol/L). In addition, it is assumed that the accumulated chlorophyll intermediates act as a photosensitizer for the formation of singlet oxygen ($^1O_2$), triggering photodynamic damage of ALA-treated plants (Rebeiz and others 1984; Chakraborty and Tripathy 1992). However, low ALA concentrations (0.06–0.60 mmol/L) appear to promote rather than damage plant growth by increasing nitrate reductase activity (Mishra and Srivastava 1983), increasing fixation of CO_2 in light, and suppressing the release of CO_2 in darkness (Hotta and others 1997a). Our results indicated that a lower dosage (1 mg/L) of ALA improved rape seedling growth (shoot fresh weight, shoot length, and root fresh weight), whereas a higher concentration of ALA decreased the growth. Our findings are similar to Hotta and others (1997b, 1998) who demonstrated that the yields of plants, including kidney bean, barley, potato, and garlic, are improved by 10–60% by ALA treatment at low concentrations. ALA has also been reported to improve salt tolerance in cotton (Watanabe and others 2000), watermelon (Liu and others 2006), and potato (Zhang and others 2006) and cold resistance in rice (Hotta and others 1998) and melon (Wang and others 2004). We also found that the plants pre- and post-treated with 1 mg/L ALA produced the highest shoot fresh weights, shoot lengths, root fresh weights, and root oxidizability when the seedlings were treated with different concentrations of ZJ0273. The improvement in seedling growth could possibly be due to the dominating effects of ALA over ZJ0273.

Malondialdehyde (MDA) is a final decomposition product of lipid peroxidation and has been used as an index for lipid peroxidation. We found that MDA had a positive relationship to ALA treatments (both pre- and post-treatments) as MDA accumulation increased with increasing ALA concentration and the highest accumulation was observed in response to the highest dosage of ALA (50 mg/L).

Peroxidase (POD, EC 1.11.1.7) utilizes H_2O_2 in the oxidation of various inorganic and organic substrates (Asada 1994). Liu and others (2006) found that treatment with 15–30 mg/L ALA could promote seed germination and seedling growth under 125 mmol/L NaCl stress, and the promotion of exogenous ALA treatment on germination under salt stress might be associated with the increased activities of antioxidant enzymes, especially POD. Plants pre- and post-treated with 0.1 and 1 mg/L ALA had significantly increased POD activities. ALA treatments could have improved the POD activity of the plants treated together with herbicide ZJ0273. Our findings are supported by the earlier reports of Nishihara and others (2003).

The activity of superoxide dismutase (SOD, EC 1.15.1.1), which catalyzes the first step in scavenging reactive oxygen species (ROS) by the dismutation of O^{2-} to H_2O_2 and O_2 was examined. We found that both pre- and post-treatments with ALA at 0.1 and 1 mg/L together with the herbicide ZJ0273 produced a significantly higher SOD activity over the control. APX activity was also improved with lower dosages of ALA in oilseed rape seedlings. Nishihara and others (2003) suggested that ALA induced the activity of antioxidant enzymes such as superoxide dismutase and APX; this was associated with the increased salt tolerance of spinach seedlings. ALA-treated peas showed improved cytochrome and peroxidase activity. Furthermore, the activities of APX, which are composed of heme, were temporarily increased (Van Huystee 1976, 1977).

Other mechanisms may be involved in influencing the effects of ALA and ZJ0273 in *Brassica napus*. The effects of exogenous ALA in promoting seedling growth can be completely eliminated by levulinic acid, suggesting that the effect of ALA is dependent on its conversion into porphyrin (Wang and others 2005). However, the mechanisms of these effects have not been thoroughly elucidated. Thus, our results clearly indicate that 1-mg/L pre- and post-ALA treatment might be used to promote the growth of oilseed rape (*Brassica napus*) seedlings under 100–500-mg/L herbicide ZJ0273 stress.

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References

Agricultural Chemicals Verification Institute, China Ministry of Agriculture (2000) Encyclopedia of agricultural chemicals registration. China Agricultural University Press, Beijing, pp 2–58

Asada K (1994) Production and action of active oxygen species in photosynthetic tissues. In: Foyer CH, Mullineaux PM (eds) Causes of photooxidative stress and amelioration of defense systems in plants. CRC Press, London, pp 77–104

Barberi P (2002) Weed management in organic agriculture: are we addressing the right issue? Weed Res 42:177–193

Bowler C, Van Montagu M, Inzé D (1992) Superoxide dismutase and stress tolerance. Annu Rev Plant Physiol Plant Mol Biol 43:83–116

Carson AG, Bandeen JD (1975) Influence of ethephon on absorption and translocation of herbicide in Canada Thistle. Can J Plant Sci 55:795–800

Castelfranco PA, Beale SI (1983). Chlorophyll biosynthesis: Recent advances and areas of current interest. Annu Rev Plant Physiol 34:241–278

Chakraborty N, Tripathy BC (1992) Involvement of singlet oxygen in 5-aminolevulinic acid induced photodynamic damage of cucumber chloroplast. Plant Physiol 98:7–11

Duke SO, Rebeiz CA (1994) Porphyrin biosynthesis as a tool in pest management: An overview. In: Duke SO, Rebeiz CA (eds) Porphyric Pesticides: Chemistry, Toxicology and Pharmaceutical Applications, ACS Symp Ser 559. American Chemical Society, Washington DC, pp 1–17

Haidar MA, Sidahmad MM (2000) Soil solarization and chicken manure for the control of *Orobanche crenata* and other weeds in Lebanon. Crop Prot 19:169–173

Hotta Y, Tanaka T, Takaoka H, Takeuchi Y, Konnai M (1997a) New physiological effects of 5-aminolevulinic acid in plants: The increase of photosynthesis, chlorophyll content, and plant growth. Biosci Biotech Biochem 61:2025–2028

Hotta Y, Tanaka T, Takaoka H, Takeuchi Y, Konnai M (1997b) Promotive effects of 5-aminolevulinic acid on the yield of several crops. Plant Growth Regul 22:109–114

Hotta Y, Tanaka T, Bingshan L, Takeuchi Y, Konnai M (1998) Improvement of cold resistance in rice seedlings by 5-aminolevulinic acid. J Pesticide Sci 23:29–33

Hykaliuk PB, Peeper TF, Basler E (1982) Stimulation of basipetal herbicide translocation with GAF 141. Weed Sci 30:6–10

James PG, Daniel AB, William MS, Dusky AJ (1989) Pre-emergence weed control in row middles of polyethylene mulched cauliflower (*Brassica oleracae*). Weed Technol 3:340–344

Jerey LF, Michael B (1989) Effect of glyphosate combinations with 2,4-D and dicamba on field bindweed (*Convolvulus arvensis*). Weed Sci 37:12–18

Kroonen-Backbeir BM, Zwart-Roodzant MH (1998) Integrated cultivation of cabbage is competitive. PAV Bull Akkerbouw 5:23–26

Lanini WT, Bell CE, Smith RF, LeStrange M, Cudney DW (2002) Weeds. University of California, Pest Management Guidelines: Cole Crops. Division of Agriculture and Natural Resources Publication No. 3442. Available at http://www.ipm.ucdavis.edu

Leul M, Zhou WJ (1999) Alleviation of waterlogging damage in winter rape by uniconazole application: Effects on enzyme activity, lipid peroxidation and membrane integrity. J Plant Growth Regul 18:9–14

Li BX (1994) The synergism effect of gibberellin on herbicide in wheat fields. Chinese J Weed Sci 8:31–35

Liu H, Kang L, Wang LJ (2006) Promotion of 5-aminolevulinic acid on seed germination of watermelon (*Citrullus lanatus*) under salt stress. Acta Hortic Sin 6:854–859

Lu L, Chen J, Wu J (2004) 2-Pyrimidinyloxy-n-aryl-benzylamine derivatives, their processes and uses. US Patent 6,800,590 B2

Mishra SN, Srivastava HS (1983) Stimulation of nitrate reductase activity by delta aminolevulinic acid in excised maize leaves. Experientia 39:1118–1120

Momoh EJJ, Zhou WJ, Kristiansson B (2002) Variation in the development of secondary dormancy in oilseed rape genotypes under conditions of stress. Weed Res 42:446–455

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

Nishihara E, Kondo K, Parvez MM, Takahashi K, Watanabe K, Tanaka K (2003) Role of 5-amino-levulinic acid (ALA) on active oxygen-scavenging system in NaCl-treated spinach (*Spinacia oleracea*). J Plant Physiol 160:1085–1091

Qasem JR (2007) Weed control in cauliflower (*Brassica oleracea* var. *Botrytis* L.) with herbicides. Crop Prot 26:1013–1020

Qiu MH (1994) The effect of glyphosate and triacontanol in weed control. Chinese J Weed Sci 8:26–28

Rebeiz CA, Montazer-Zouhoor A, Jopen HJ, Wu SM (1984) Photodynamic herbicides: Concepts and phenomenology. Enzyme Microb Technol 6:390–401

Romero-Puertas MC, McCarthy I, Gómez M, Sandalio LM, Corpas FJ, del Río LA, Palma JM (2004) Reactive oxygen species-mediated enzymatic systems involved in the oxidative action of 2,4-dichlorophenoxyacetic acid. Plant Cell Environ 27:1135–1148

Song WJ, Zhou WJ, Jin ZL, Cao DD, Joel DM, Takeuchi Y, Yoneyama K (2005) Germination response of *Orobanche* seeds subjected to conditioning temperature, water potential and growth regulator treatments. Weed Res 45:467–476

Song WJ, Zhou WJ, Jin ZL, Zhang D, Yoneyama K, Takeuchi Y, Joel DM (2006) Growth regulators restore germination of *Orobanche* seeds that are conditioned under water stress and suboptimal temperature. Aust J Agric Res 57:1195–1201

Su SQ, Song SZ (1996) Encyclopedia of chemical control of weeds on farmland. China Agriculture Press, Beijing, p 320

Subrahmaniyan K, Zhou WJ (2008) Soil temperature associated with degradable, non-degradable plastic and organic mulches and their effect on biomass production, enzyme activities and seed yield of winter rapeseed (*Brassica napus* L.). J Sustainable Agric (In press)

Subrahmaniyan K, Kalaiselvan P, Balasubramanian TN, Zhou WJ (2008) Soil properties and yield of groundnut associated with herbicides, plant geometry and plastic mulch. Commun Soil Sci Plant Analysis (In press)

Tang QH, Chen J, Lu L (2005) An innovative research for novel rape herbicide ZJ0273. Chinese J Pest 44:496–502

Van Huystee RB (1976) A study of peroxidase synthesis by means of double labeling and affinity chromatography. Can J Bot 54:876–880

Van Huystee RB (1977) Porphyrin and peroxidase synthesis in cultured peanut cells. Can J Bot 55:1340–1344

von Wettstein D, Gough S, Kananagara CG (1995) Chlorophyll biosynthesis. Plant Cell 7:1039–1105

Wang LJ, Jiang WB, Huang BJ (2004) Promotion of 5-aminolevulinic acid on photosynthesis of melon (*Cucumis melo*) seedling under low light and chilling stress conditions. Physiol Plant 121:258-264

Wang LJ, Jiang WB, Liu H, Kang L, Hou XL (2005) Promotion by 5-aminolevulinic acid of germination of Pakchoi (*Brassicca campestris* ssp. *chinensis* var. communis Tsen et Lee) seeds under salt stress. J Integr Plant Biol 47:1084–1091

Watanabe K, Tanaka T, Hotta Y, Huramochi H, Takeuchi Y (2000) Improving salt tolerance of cotton seedlings with 5-aminolevulinic acid. Plant Growth Regul 32:99–103

Weinstein JD, Beale SI (1985) Enzymatic conversion of glutamate to δ-aminolevulinic acid in soluble extracts of the unicellular green alga, *Chlorella vulgaris*. Arch Biochem Biophys 239:454–464

Zaragoza C (2003) Weed management in vegetables. In: Labrada R (ed), Weed Management for Developing Countries (Addendum 1), FAO Document Repository. FAO Plant Production and Protection paper 120 Add. 1. Agriculture Department, Food and Agriculture Organization of the United Nations, Rome

Zhang XZ (1992) The measurement and mechanism of lipid peroxidation and SOD, POD and CAT activities in biological system. In: Zhang XZ (ed), Research Methodology of Crop Physiology. Agriculture Press, Beijing, pp 208–211

Zhang ZJ, Li HZ, Zhou WJ, Takeuchi Y, Yoneyama K (2006) Effect of 5-aminolevulinic acid on development and salt tolerance of potato (*Solanum tuberosum* L.) microtubers *in vitro*. Plant Growth Regul 49:27–34

Zhou WJ (2001) Oilseed rape. In: Zhang GP, Zhou WJ (eds) Crop production. Zhejiang University Press, Hangzhou, pp 153–178

Zhu JW, Zhu GN, Wu HM, Liu QK (2003) Effect of six plant growth regulators on phytotoxicity of glyphosate. J Shanghai Jiaotong Univ (Agric Sci) 21:160–163

Zhou WJ, Leul M (1998) Uniconazole-induced alleviation of freezing injury in relation to changes in hormonal balance, enzyme activities and lipid peroxidation in winter rape. Plant Growth Regul 26:41–47

Zhou WJ, Leul M (1999) Uniconazole-induced tolerance of rape plants to heat stress in relation to changes in hormonal levels, enzyme activities and lipid peroxidation. Plant Growth Regul 27:99–104

Zhou W, Ye Q (1996) Physiological and yield effects of uniconazole on winter rape (*Brassica napus* L.). J Plant Growth Regul 15:69–73

Zhou WJ, Yoneyama K, Takeuchi Y, Iso S, Rungmekarat S, Chae SH, Sato D, Joel DM (2004) *In vitro* infection of host roots by

differentiated calli of the parasitic plant *Orobanche*. J Exp Bot 55:899–907
Zhou W, Zhao D, Lin X (1997) Effects of waterlogging on nitrogen accumulation and alleviation of waterlogging damage by application of nitrogen fertilizer and mixtalol in winter rape (*Brassica napus* L.). J Plant Growth Regul 16:47–53
Zhou XM (2003) Effect of four plant growth regulators on rice injured by ethametsulfuron. Mod Agrochem 2:41