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Chlorophyll Fluorescence Responses to Application of New Herbicide ZJ0273 in Winter Oilseed Rape Species

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ABSTRACT

For suppressing weeds in the rapeseed fields, a newly developed post-emergence herbicide propyl 4-[2-(4, 6-dimethoxypyrimidin-2-yloxy) benzylamino] benzoate (ZJ0273) is recently becoming popular in China. To monitor the resistance patterns in crops in a non-destructive, real-time and efficacious way, two cultivars of rapeseed *Brassica napus* cv. ZS 758 and *B. rapa* cv. Xiaoyoucai were tested by a foliar spray of ZJ0273 at the rates of 100, 500 and 1000 mg L^{-1}. The study revealed that maximum quantum yield (Fv/Fm), non-photochemical quenching (NPQ), electron transport rate (ETR) and photochemical utilization [Y(II)] inhibition were significantly increased with herbicide application increasing, while photochemical quenching (qP), NPQ, Y(II) and regulated heat dissipation [Y(NPQ)] were significantly decreased with herbicide application and increase in leaf age. However, the resistant *B. napus* species started to recover after 5 days. Moreover, the concentration-inhibiting curves of Y(II) and ETR suggested that 500 mg L^{-1} was a safer dose for *B. napus*. These results showed that NPQ, Y(II) and ETR inhibiting rate could be appropriate for herbicide phytotoxicity detector. They also seem to be the key factors for establishing a prospective resistance monitoring tests in field herbicide bioassays. The 2nd fully expanded leaf could reflect directly the phytotoxicity of herbicide to the rape plant with more accurate distinction. © 2011 Friends Science Publishers

Key Words: Bioassay; *Brassica napus*; *B. rapa*; Electron transport rate; Non-photochemical quenching; Photochemical utilization

Abbreviations: ZJ0273, Propyl 4-[2-(4, 6-dimethoxypyrimidin-2-yloxy) benzylamino] benzoate; PAM, pulse amplitude modulation; CCD, charge coupled device; LED, light emitting diode; NIR, near infra-red; F, minimum fluorescence yield of light-adapted leaf; Fm, maximum fluorescence yield of dark-adapted leaf; Fm′, maximum fluorescence yield of light-adapted leaf; Fo, minimum fluorescence yield of dark-adapted leaf; Fv/Fm, maximum quantum yield; NPQ, non-photochemical quenching; qN, non-photochemical quenching; qP, photochemical quenching; Y(II), photochemical utilization; Y(NPQ), regulated heat dissipation (a loss process serving for protection); Y(NO), non-regulated heat dissipation (a loss process due to PS II inactivity); RLC, rapid light curve; PAR, photosynthetically active radiation; ETR, electron transport rate; α, light-limited quantum efficiency; Ek, minimum saturated irradiance

INTRODUCTION

Oilseed rape (*Brassica* spp.) is the key source of edible oil in most of the countries especially in China (Momoh *et al.,* 2002). It contains large amounts of lipids and also accounts for the major portion of the global edible oil supply. Weeds including both narrow and broad-leaf types reduce the yield of oilseed rape directly by 15.8-50% (Zhou *et al.,* 2004; Song *et al.,* 2005; 2006) and also cause heavy damage to oilseed rape production indirectly through harbouring insects in the field (Broatch *et al.,* 2008). Sustainable crop production depends on successful weed control, a number of weed management strategies for crucifer crops including physical control and chemical control were studied and discussed all over the world (Subrahmaniyan *et al.,* 2008). Recently, chemical control has gained more popularity in the large-scale agricultural production systems due to its effectiveness (Qasem, 2007).

Due to the troublesome weed infestation in winter oilseed rape (Barnes & Oliver, 2004), various herbicides have been registered for use in oilseed rape in China. In spite of the number of herbicides applied in rape field, the new herbicide still appeared necessary for a wider weed spectrum especially for broad-leaf weeds. Furthermore, with the increasing application of herbicide, weed tolerant and environmental pollution have become more seriously, it is imperative to explore new efficient rapeseed field herbicide with the advantages of low application rate, less mammalian toxicity, broad spectrum weeding action and favorable environmental profile. Therefore, the use of a new effective

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oilseed rape herbicide propyl 4-[2-(4, 6-dimethoxypyrimidin-2-yloxy) benzylamino] benzoate (ZJ0273) is becoming popular in the rapeseed field in China (Zhang *et al.,* 2008). This novel herbicide ZJ0273 is derived from a precursor pesticide compound (2-pyrimidinyloxy-*N*-aryl benzoate, registered in USA), possessing a similar biological activity and harmful symptoms of the weeds as those of ALS (acetolactate synthase, EC 4.1.3.18) inhibiting herbicides (Liu *et al.,* 2008; Zhang *et al.,* 2009). It not only improves crop production, but also provides effective weed control, as well as environmental safety.

Also, continuously increasing use of herbicides in the crop fields contributes to pollution in large areas on one hand and on the other poses severe consequences for non-target plants (Wibawa *et al.,* 2009). Herbicides can also leave un-desired residues in soil that would harm the agro-ecosystem (Riaz *et al.,* 2007). However, Wibawa *et al.* (2009) reported that if an appropriate herbicide is applied at recommended rate, it would not leave phytotoxic residues in the soil. As herbicides bring changes in chlorophyll fluorescence parameters, therefore measurement of chlorophyll fluorescence is considered as an effective method for quickly evaluating the phytotoxicity of applied herbicides (Merkel *et al.,* 2004).

A recently developed innovative bioassay, viz., imaging pulse-amplitude-modulated chlorophyll fluorometer (imaging PAM) is being used to assess the pollution intensity via growth of the plant and applied as an effect detector. To our knowledge, fluorescence imaging is largely used in aquatic environment so far. However, no information regarding the new herbicide ZJ0273 phytotoxicity on oilseed rape has been reported to date. In this investigation, we used the Imaging-PAM as an effective monitoring method in short-term to bioassay with respect to susceptibility and resistance of the two rapeseed species i.e., *B. rapa* and *B. napus*. We also studied the excitation flux affected by the herbicide ZJ0273 stress and illustrated the differential response of *B. rapa* and *B. napus*, by assessing Fv/Fm (maximum photosystem II quantum yield), NPQ (non-photochemical quenching), qP (photochemical quenching), RLCs (rapid light curves), Y(II) (effective photosystem II quantum yield), Y(NPQ) (quantum yield of regulated energy dissipation in PSII) and Y (NO) (quantum yield of non-regulated energy dissipation in PSII). These results would be useful for the in field monitoring of growth status and herbicide effect on oilseed rape crop, as well as the sorting of resistant and susceptible crop species.

MATERIALS AND METHODS

Plant materials and treatments: Two cultivars of oilseed rape (*B. napus* cv. ZS 758, *B. rapa* cv. Xiaoyoucai) with different herbicide susceptibility were grown in the field of a silt-loam soil (with 1.8 g kg^{-1} total nitrogen, 17.5 g kg^{-1} organic matter & 63 mg kg^{-1} soil available phosphorus) at the Zhejiang University farm, Hangzhou, China (30° 10´N, 120° 12´E). Experiment was laid out in randomized complete block design (RCBD) with 3 replications in each case. The new herbicide namely propyl 4-[2-(4, 6-dimethoxypyrimidin-2-yloxy) benzylamino] benzoate (ZJ0273) was supplied by the Shanghai Institute of Organic Chemistry, China. Various concentrations of ZJ0273 (0, 100, 500 & 1000 mg L^{-1}) were foliar applied at the three leaf stage (two weeks after sowing). Measurements were carried out at 1, 3, 5 and 7 days after herbicidal treatment. The whole plants were harvested at 1, 3, 5 and 7 days after spray. All measurements were undertaken on the adaxial surface of the leaves.

Chlorophyll fluorescence imaging: Components of Imaging-PAM include a high-speed charge coupled device (CCD) camera, which can image an area of approximately 10×13 cm at a spatial resolution of 50 µm; light emitting diodes (LED) array (contained 112 LEDs, blue LEDs, red and near-infrared LEDs), which provide irradiance for measuring chlorophyll fluorescence parameters; a control unit to synchronize light emission by the LED array with the shutter opening of the camera linked with a PC.

Images of Imaging-PAM chlorophyll fluorometer (Heinz Walz GmbH, Effeltrich, Germany) were used to determine the heterogeneity in photosynthetic attributes of both oilseed rape genotypes at room temperature according to the principle of Schreiber (2004). First of all, F, F_o (minimum fluorescence yield of the light-adapted & dark-adapted leaves, respectively) and Fm′, Fm (maximum fluorescence yield of the light & dark-adapted leaves) were obtained with the application of a saturation pulse. Maximum photosystem II quantum yield [(Fm-Fo)/Fm =Fv/Fm] and effective quantum yield of PSII [Y(II) =(Fm′-F)/Fm′] were calculated according to Genty *et al.* (1989).

Electron transport rate of PS II [ETR = Y(II) × PAR × 0.5 × PAR absorptivity] was calculated automatically by Imaging PAM (Walz) for all images. The co-efficient 0.5 is a factor assuming an equal distribution of absorbed photons between PSII and PSI (Björkman & Demmig, 1987). The quantum yield of regulated energy dissipation of PSII, Y (NPQ) = 1-Y(II)-1/(NPQ + 1+ qL (Fm/Fo- 1)), as a fraction non-photochemical quenching value was NPQ = (Fm-Fm′)/Fm′, the value of NPQ was divided by 4 to keep values between 0-1. The parameters qL = qP Fo′/F and qP = (Fm′-F)/(Fm′-Fo′). The quantum yield of non-regulated energy dissipation of PSII was estimated according to Kramer *et al.* (2004) as Y(NO)=1-Y(II)-Y(NPQ). According to Oxborough and Baker (1997), the Fo′ fluorescence yield was considered, when PSII was under light adapted state [Fo′=Fo(Fv/Fm+Fo/Fm′)]. These parameters were employed as indicators for fraction of open PSII reaction centre participating in electron transport and were evaluated through analytical software IMAGING-WIN (Walz, Effeltrich, Germany).

The ZJ0273 dosage-inhibiting curves of Fv/Fm, NPQ, Y(II), ETR were calculated using Eqs. 1, 2, 3 and 4, respectively.

Eq. 1: % Fv/Fm Inhibition = [1- $(Fv/Fm)_{sample}$ /

$(Fv/Fm)_{control}] \times 100\%$

Eq. 2: % NPQ Inhibition = $(1- NPQ_{sample} / NPQ_{control}) \times 100\%$

Eq. 3: % Y (II) Inhibition = $(1- Y_{sample} / Y_{control}) \times 100\%$

Eq. 4: % ETR Inhibition = $(1- ETR_{sample} / ETR_{control}) \times 100\%$

Rapid light curves (RLCs) were produced by the Imaging-PAM through the application of a series of 10^{-s} light exposure with increasing irradiance (0, 10, 35, 80, 230, 335, 460, 610, 800 & 925 μmol photons $m^{-2} s^{-1}$) (White & Critchley, 1999). Measured RLCs were fitted to a double exponential decay function (Hill *et al.*, 2004):

$$P=Pm\,(1-e^{-\alpha\,PAR/Pm})\,e^{-\beta\,PAR/Pm}$$

Three parameters were derived from the fitted curves equation: α, the initial slope of the RLCs before the onset of saturation; Pm, the maximal photosynthesis at light-saturation; β, characterises the slope of the RLC beyond the onset of photoinhibition. These values were then used to calculate the irradiance at the onset of saturation (Ek = Pm/α) as described by Ralph (2005).

Statistical analysis: Variation in descriptive attributes of chlorophyll fluorescence for each species was tested using one-factor analysis of variance (ANOVA) technique. These analyses were performed using the statistical programme SAS (version 8.0).

RESULTS

ZJ0273 dosage-inhibiting curves of Fv/Fm and NPQ: After day one, herbicide treatments significantly declined Fv/Fm and NPQ in *B. rapa* except 100 mg L^{-1} herbicide (ZJ0273), compared to that of the control. In *B. napus*, both aforementioned parameters were also significantly declined. In *B. rapa*, 19% inhibition was recorded for Fv/Fm under 1000 mg L^{-1} ZJ0273, whereas it was only 5.3% for *B. napus*, showing the tolerant nature of the genotype. However, it is worth mentioning that herbicide inhibited the NPQ more seriously in *B. napus* than *B. rapa* for initial few days, NPQ inhibition was 27.1% and 22.9% under 1000 mg L^{-1} ZJ0273 in *B. napus* and *B. rapa*, respectively (Fig. 1-1 d).

After day three, both parameters continued to decline gradually in *B. rapa* and *B. napus* with the increasing dose of ZJ0273. The inhibition of Fv/Fm in *B. rapa* was still increasing significantly at higher dosages of ZJ0273 (especially at 1000 mg L^{-1} ZJ0273), however Fv/Fm inhibition was only 0.24%- 4% in *B. napus* (100-1000 mg L^{-1} ZJ0273). In addition, NPQ inhibition in both species became more severe and the inhibition was still more serious in *B. napus* than that in *B. rapa* (Fig. 1B-3d).

After day five, Fv/Fm inhibition was lessened in *B. napus* compared to the three days after treatment. However, the inhibition of Fv/Fm became more serious in *B. rapa* treated with 500 and 1000 mg L^{-1} dose of ZJ 0273 than that at three days (Fig. 1A-3 d, 5 d). At the same time, an increasing inhibition of NPQ was detected progressively in both species except 100 mg L^{-1} ZJ0273 stress for *B. napus*. Moreover, NPQ inhibition was more severe in *B. rapa* than *B. napus* at higher herbicide dosage (Fig. 1B-5 d).

One week after the herbicidal treatment, Fv/Fm showed similar results as that of 5 days after spray. Both species showed recovery regarding NPQ. Plants treated with lower dosage (100 mg L^{-1} ZJ0273) had achieved the same level as that of the control. Fv/Fm inhibition continued to increase in *B. rapa* treated with the highest dosage (1000 mg

Fig. 1: Inhibition effect of time-dependent changes induced by different concentrations of ZJ0273 herbicide on Fv/Fm and NPQ in *B. rapa* and *B. napus* leaves (n = 6)

The measurements of both were carried out at the light intensity of 335 μmol photons $m^{-2} s^{-1}$; Mean values with standard error are shown (n = 6)

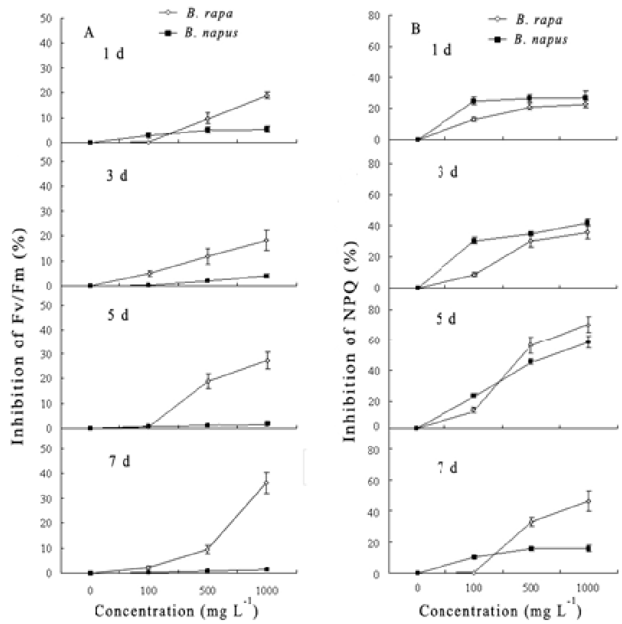


Fig. 2: Quantitative analyses of excitation energy flux at PS II induced by herbicide ZJ0273 with different concentrations (0, 100, 500 & 1000 mg L^{-1}) on *B. rapa* and *B. napus* leaves

The changes in the quantum yields Y(II) (white), Y(NO) (grey) and Y(NPQ) (black) were assessed with 6 independent individual samples after 5 days of spray

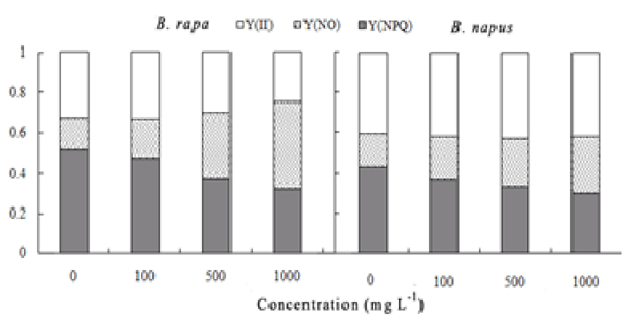


Table I: Inhibition effect of time dependent changes induced by different concentrations of ZJ0273 herbicide on ETR and Y(II) in *B. rapa* and *B. napus* leaves

Species	Character (%)	Concentration of ZJ0273 (mg L^{-1})	Days after treatment (d)			
			1	3	5	7
B. rapa	Inhibition of ETR	0	0±0 # c *	0±0 c	0±0 c	0±0 c
		100	4.69±3.69 c	12.7±0.63 b	11.6±3.09 b	10.31±1.2 b
		500	21.37±1.75 b	27.2±3.39 a	12.8±3.59 b	15.5±1.6 b
		1000	45.4±4.79 a	32.7±3.94 a	28.1±4.16 a	38.78±4.8 a
	Inhibition of Y(II)	0	0±0 d	0±0 d	0±0 d	0±0 c
		100	6.09±3.16 c	12.39±1.39 c	4.63±2.67 c	13.2±1.37 b
		500	21.72±1.93 b	24.89±2.29 b	12.72±1.54 b	14.77±2.1 b
		1000	55.17±3.30 a	33.58±2.43 a	28.6±2.67 a	30.21±1.0 a
B. napus	Inhibition of ETR	0	0±0 b	0±0 b	0±0 b	0±0 b
		100	0.98±0.67 b	2.22±0.6 b	1.62±0.69 b	1.16±0.66 ab
		500	1.06±0.88 b	2.22±0.93 b	1.86±0.76 b	1.91±0.9 ab
		1000	4.15±1.72 a	5.82±1.5 a	4.01±1.01 a	5.06±1.06 a
	Inhibition of Y(II)	0	0±0 b	0±0 c	0±0 b	0±0 c
		100	1.23±0.93 b	1.53±1.48 bc	0.57±0.44 b	3.45±3.18 bc
		500	1.01±0.96 b	2.57±0.93 b	1.3±1.17 ab	4.61±2.94 ab
		1000	4.19±1.67 a	7.44±1.52 a	2.09±1.89 a	8.01±3.18 a

* Data are mean values ± SE, n = 6. Letters within a column indicate significant differences ($P < 0.05$)
# NPQ was measured at the light intensity of 335 μmol photons m^{-2} s^{-1}

Fig. 3: Rapid light curves derived from *B. rapa* and *B. napus* leaves treated with different concentrations (0, 100, 500 & 1000 mg L^{-1}) of herbicide ZJ0273 after 5 days of spray
Mean values with standard error are shown (n = 6)

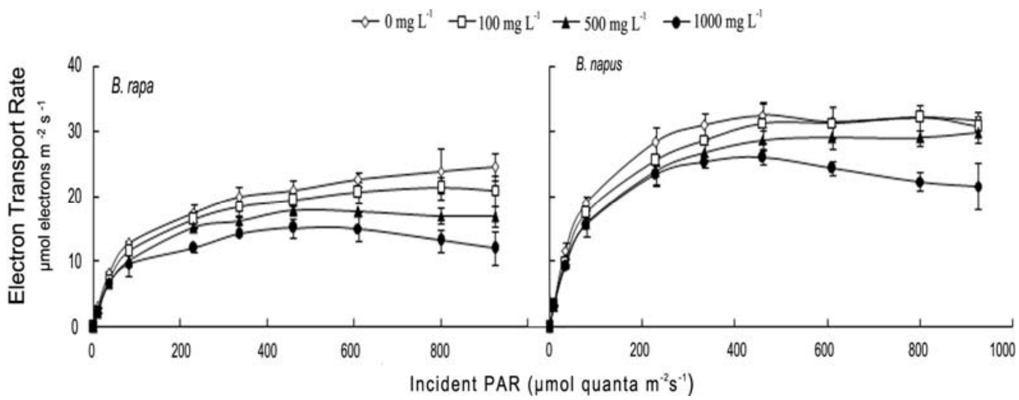
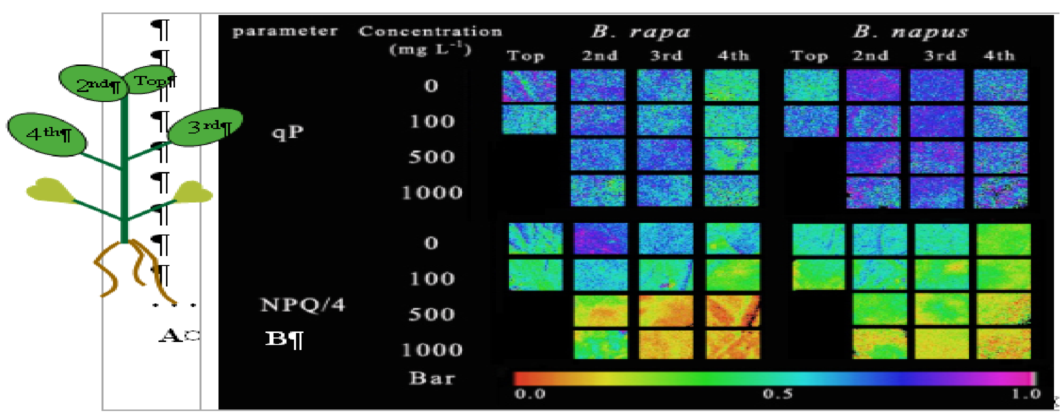


Fig. 4: Chlorophyll fluorescence images of two rape species (*B. rapa* & *B. napus*) under various concentrations of herbicide ZJ0273 (0, 100, 500 & 1000 mg L^{-1}) stress after 5 days of spray showing the photochemical quenching (qP) and the non-photochemical quenching co-efficient (NPQ/4)
All plants were exposed to an irradiance of 335 μmol quanta m^{-2} s^{-1}. Relative values of each parameter ranging from 0 to 1 are displayed using an identical false color scale (Bar is at the bottom of image) (B). Due to the high concentrations of herbicide (500 & 1000 mg L^{-1}), the growth of plant was inhibited that only three leaves emerged were presented here. The leaf age was described as Top, 2nd, 3rd and 4th (A)



L^{-1} ZJ0273), compared to that at five days (Fig. 1A-5 d, 7 d). NPQ inhibition in *B. rapa* treated with higher dose ZJ0273 (500, 1000 mg L^{-1}) was still more serious than that in *B. napus* (Fig. 1B-7 d).

The above results showed that the dosage-inhibiting curves of Fv/Fm and NPQ can be used as the biomarkers for rapid herbicide phytotoxicity assessment, to monitor the crops' herbicide phytoxicity without any damage to the plant.

ZJ0273 dosage-inhibiting effect on Y(II) and ETR: The changes of herbicide dosage-inhibiting effect on Y(II) and (ETR) in *B. napus* showed a similar trend i.e., the inhibition of effective photosystem II quantum yield [Y(II)] and relative electron transport (ETR) in response to the new herbicide ZJ0273 stress, maintained a lower level (Table I). However, a significant inhibitive effect was observed at 1000 mg L^{-1} ZJ0273 and it was only 8.01% as the highest one [Table I, 7 d, Y(II) inhibition of *B. napus*]. From these results we can deduce that the herbicide ZJ0273 imposed a slight negative impact on photosystem II of resistant species like *B. napus* and the concentration of 500 mg L^{-1} ZJ0273 seems to be a safe dose for the photosystem II of such species.

Contrary to *B. napus*, a severe negative effect of herbicide ZJ0273 on Y(II) and ETR was detected in *B. rapa*. Data recorded one day after the treatment, depicted a sharp increase in inhibitive affect on Y(II) and ETR and a visible climbing from 6.09% to 55.17% of Y(II) inhibition (Table I, 1 d, *B. rapa*) was observed with the increasing herbicide dose from 100 mg L^{-1} to 1000 mg L^{-1} ZJ0273, respectively. The inhibitive affect persisted for a while and then treated plants started to recover and the inhibition was decreased. At day three, the inhibition under the influence of 500 mg L^{-1} ZJ0273 was at its highest, nevertheless, inhibition under 1000 mg L^{-1} ZJ0273 started to decline, while the one of, 24.89%, 27.2% inhibiting ratio were obtained for inhibitions of Y(II) and ETR, respectively.

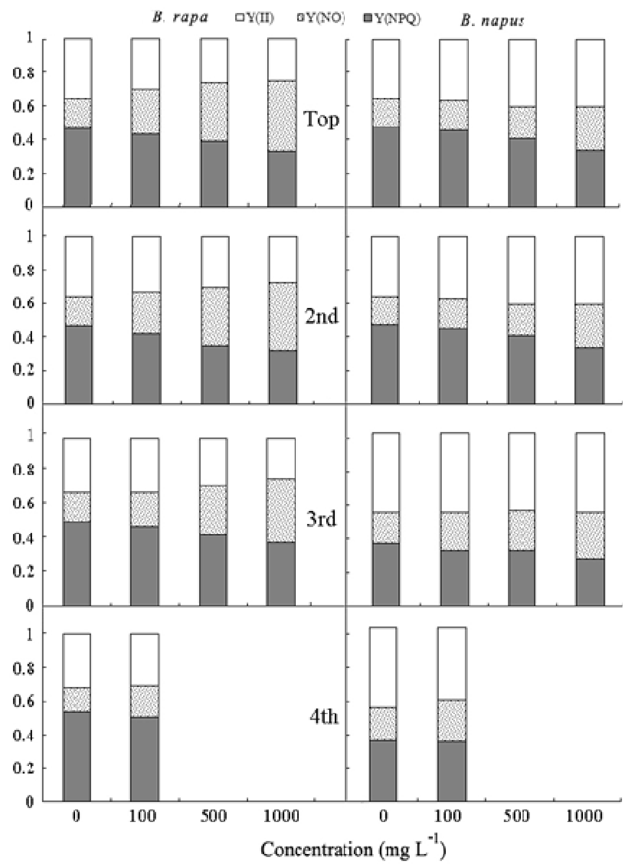
After 3 days, the highest value (12.7%) was found at the concentration of 100 mg L^{-1} ZJ0273 on ETR inhibition comparing with the one at 1, 5 and 7 days. It was surprising to note that the same rise was detected at the concentration of 100 mg L^{-1} ZJ0273 on Y(II) inhibition at 7 d, and reached 13.2% (Table I, B. rapa). Since then no detectable difference was observed between 100 and 500 mg L^{-1} herbicide. It shows that the changes of herbicide dosage-inhibition effect on Y(II) and ETR are more convenient and expeditious to monitor the crops' herbicide phytoxicity and to distinguish the crops on the basis of susceptibility/tolerance without any damage to the plant.

Excitation energy flux and light curves in PS II: Excitation energy fluxes are described by the quantum yields Y(II), Y(NPQ) and Y(NO), namely photochemical utilization, regulated heat dissipation (a loss process serving for protection) and non-regulated heat dissipation (a loss process due to PS II inactivity), respectively. They were allowed to assess the excitation energy flux at PS II, which add up to unity (Kramer *et al.,* 2004). Excitation energy fluxes in these three different pathways could easily be assessed by imaging-PAM chlorophyll fuorometer. Three fluxes recorded after five days of herbicide ZJ0273 application with various concentrations confirmed the differential herbicide susceptibility response of both the oilseed rape species (Fig. 2). Apparently *B. rapa* showed more susceptible than *B. napus*. Decreases of Y(II) and Y(NPQ) were equalled by an increase of Y(NO) in *B. rapa,* however the reduction of Y(II) was not detectable in *B. napus*, whereas Y(NPQ) decreased somewhat with a slight increase of Y(NO) in *B. napus*, which reflected inhibition of photosynthesis (Fig. 2).

The RLCs data exhibited typical saturation kinetics and also revealed inter-specific differences in the photosynthetic electron transport capacity between *B. rapa* and *B. napus* during exposure to increasing light levels. It reflects the significance of a certain "light pressure" for

Fig. 5: Leaf age-dependent changes in excitation energy flux at PS II induced by herbicide ZJ0273 with different concentrations (0, 100, 500 & 1000 mg L^{-1}) on *B. rapa* and *B. napus* leaves

The variation in the quantum yields Y(II) (white), Y(NO) (grey) and Y(NPQ) (black) on differently aged leaves, which was described as top, 2nd, 3rd and 4th (Fig. 4A), were assessed after 5 days of spray. Due to the high concentrations of herbicide (500 & 1000 mg L^{-1}), the growth of plant was inhibited that only three leaves emerged were presented here



assessing the difference in photosynthetic electron transport capacity between samples of control and herbicide treatment (Fig. 3). With PAR values < 80 μmol quanta m^{-2} s^{-1}, where the light is beginning to limit the electron flow, no significant changes of ETR values were found in all samples. However, the ETR values for 1000 mg L^{-1} ZJ0273 treatment were reduced evidently under the irradiance of 230 μmol quanta m^{-2} s^{-1} and then consistent reduction continued in both species. Then at the following PAR 335 μmol quanta m^{-2} s^{-1}, ETR values with 500 mg L^{-1} ZJ0273 presented a significant decline in both the species, but in *B. napus* at 610 μmol quanta m^{-2} s^{-1}, it reverted back to the control level.

Herbicide ZJ0273 at the rate of 500 mg L^{-1} exhibited a significant photo-inhibition in *B. rapa*, whereas it seems to be safer for *B. napus*, when compared with that of control on α value. At 1000 mg L^{-1} ZJ0273, ETR showed negative response at the higher photosynthetic photon flux density (>80 μmol quanta m^{-2} s^{-1}) in both species.

Leaf age-dependent changes in chlorophyll fluorescence parameters: In order to investigate heterogeneity in photosynthetic activity of *B. rapa* and *B. napus* leaves differing in age under various concentration of herbicide ZJ0273 and to find the optimum age of leaf for chlorophyll fluorescence detecting, the chlorophyll fluorescence images were produced at an irradiance of 335 μmol quanta m^{-2} s^{-1} after five days of treatment.

Brassica rapa showed differential response for low (100 mg L^{-1}) and high (500, 1000 mg L^{-1}) concentrations of ZJ0273 for qP and NPQ of different leaf age samples. The herbicide phytotoxicity was most severe in the 3rd leaf. In contrast, different leaf age samples of *B. napus* did not reveal any visible variation for the qP under various concentrations of ZJ0273. NPQ of these two rape species showed strong gradients along with increasing leaf age and herbicide concentrations. In *B. rapa* changes observed were more drastic at higher concentrations (500, 1000 mg L^{-1}) as compared to *B. napus*. However, NPQ/4 displayed pronounced heterogeneity contrast to other two parameters and was much low in all the leaf samples (Fig. 4). These results suggested that the 2nd fully expanded leaf (Fig. 4A) could reflect herbicide phytotoxicity of the rape species with more accuracy, because of the heterogeneity and senescence of the top and 3rd, 4th leaves.

Leaf age-dependent changes of excitation energy flux assessment at PS II as three fundamentally different pathways, viz. the quantum yields Y(II), Y(NPQ) and Y(NO) are shown in Fig. 5. Response of the top, 2nd and 3rd leaves for all the three parameters was quite similar and distinct, whereas it was mild in the 4th leaf of the both species. The co-efficients of Y(II) and Y(NPQ) were decreased and paralleled by an increase of Y(NO) with the increasing concentration of ZJ0273, especially, the larger changes were found at high concentrations (500, 1000 mg L^{-1} ZJ0273) in *B. rapa*. Nevertheless, the photochemical utilization Y(II) was comparatively steady and showed somewhat larger changes at high concentrations (500, 1000 mg L^{-1} ZJ0273), but did not show too much visible evidence. Further, a slight decrease in Y(NPQ) was accompanied by an increase of Y(NO) in *B. napus* (Fig. 5).

DISCUSSION

Being a crucial competitor, cruciferous weeds are hard to eradicate and are a serious threat to winter oilseed rape (*Brassica* spp.) production resulting in a massive yield loss in the field (Diepenbrock, 2000). Therefore, weed management strategies have become more and more exigent in integrated cropping systems. Under such circumstances, a viable post-emergence herbicide was needed for controlling weeds in conventional winter oilseed rape field (Merkel *et al.,* 2004). As a new post-emergence and effective herbicide, ZJ0273 allows making the best optimum weed management strategy decisions according to the actuality of rape stand. On the other hand, due to the increased application of herbicides to control weeds during recent past, weed tolerant and environmental contamination has become more and more seriously (Shaw *et al.,* 2008). To monitor and quantify the increasing contamination of both the soil and water caused by herbicides, a new fluorescence-based bioassay-Imaging-PAM is being used as an effective herbicide-detector device. Moreover, the chlorophyll fluorescence bioassay as a biomarker was carried out in greenhouse and laboratory experiments to monitor the phytotoxicity of herbicide and to develop resistance monitoring tests, so that resistant biotypes can be detected quickly and farmers may adapt their weed management (Fai *et al.,* 2007; Aper *et al.,* 2008). In this experiment, the chlorophyll fluorescence responses to application of new herbicide ZJ0273 in two winter oilseed rape species (*B. rapa* & *B. napus*) with differential susceptibilities were studied. Purposefully, the chlorophyll fluorescence imaging assays was performed to establish a resistance monitoring tests as a herbicide phytotoxicity detector for crops in a non-destructive, real-time and efficacious way.

Dosage-inhibiting curves have been studied prevalently following the widespread development of phytotoxicity assays and reported in a variety of ways (Escher *et al.,* 2006). Photosynthetic electron transport rate of resistant biotypes was less affected than that of susceptible biotypes under metamitron stress (Aper *et al.,* 2008). Moreover, Riethmuller-Haage *et al.* (2006) reported that measurement of the quantum efficiency for electron transport appeared to be an early detection method to assess the phytotoxicity of metsulfuron (ALS-inhibiting herbicide). Keeping in view the literature, the dosage-inhibiting curves of Fv/Fm and NPQ (Fig. 1) indicated that PSII was impaired by herbicide as described by Bigot *et al.* (2007) and also demonstrated that the inhibition of Fv/Fm, NPQ were more severely in *B. rapa* than that in *B. napus*. However, we observed a gradual decline in both two parameters for both rape cultivars after three days, especially for NPQ, which could be taken as an index of herbicide phytotoxicity detector (Frankart *et al.,* 2003). They reported that among the fluorescence parameters, NPQ used as a biomarker was the most suitable indicator for herbicide phytotoxicity in laboratory and in field herbicide bioassays.

Declining trend in the change of herbicide dosage-inhibiting effect on Y(II) and ETR (Table I) showed a very different response that was attributed to the diverse susceptibility of the two species. Accordingly, Y(II) or ETR inhibiting rate are the key indices to establish a prospective resistance monitoring tests. Based on it, susceptible biotypes can be detected quickly, which could be beneficial for the farmers to develop the weed management strategy for their *Brassica* fields.

In order to expound and perfect the resistance monitoring tests and herbicide phytotoxicity detector ulteriorly, the quantum yields Y(II), Y(NPQ) and Y(NO) described as photochemical utilization, regulated heat dissipation and non-regulated heat dissipation, as well as RLCs (rapid light curves) were assessed at PS II (Fig. 2 & 3). This experiment was conducted at 5 days after herbicide application because *B. napus* started visible recovery compared to the *B. rapa* at this stage. A similar change as described previously (Fig. 1) were detected with a gradual decrease in *B. rapa* (Fig. 2 & 3), while the effect of ZJ0273 in *B. napus* was negligible, except for the treatment with 1000 mg L^{-1} herbicide. The similar changes were also reported by Bonfig *et al.* (2006), who found that decrease Y(II) and Y(NPQ) was paralleled by an increase of Y(NO) in *Arabidopsis* leaves infiltrated by *P. syringae* and with increasing the amount of the virulent or avirulent strain of *P. syringae*, it became more serious.

Photosynthesis is variable in different spatial location of a leaf (Enríquez *et al.,* 2002) and this heterogeneity was affected by light, temperature, humidity surrounding the leaf and also influenced by the absorption, metabolizing ability and the transport of the herbicide (Catský & Sesták, 2005). Our results are in conformity with these findings as shown by the Fig. 4. The fluorescence imaging bioassay was firstly conducted to measure the spatial distribution of photosynthesis by Omasa *et al.* (1987) and to assess the spatial impact of herbicides (Ralph *et al.,* 2004). In our research, for the sake of choosing a suitable leaf for resistance monitoring and herbicide phytotoxicity evaluation, leaf age-depended changes of chlorophyll fluorescence imaging and Y(II), Y(NPQ) and Y(NO) were produced and analysed (Fig. 4 & 5). These changes revealed that 2nd fully expanded leaf (Fig. 4A) could reflect accurately the phytotoxicity of herbicide to the rape plant; as due to heterogeneity and senescence of the top, 3rd and 4th leaves, they were not considered for this purpose.

CONCLUSION

Results with herbicide ZJ0273 showed that 100 mg L^{-1} ZJ0273 concentration seemed to be safe for PSII of both the cultivars and even 500 mg L^{-1} ZJ0273 was safe to some extent for *B. napus*. Based on these results, we can deduce that Fv/Fm, NPQ, Y(II) and ETR inhibiting curves could be used as key indices for establishing the resistance monitoring tests as well as herbicide phytotoxicity detector.

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