Effects of new herbicide Pyribambenz-propyl on photosynthesis and chlorophyll fluorescence of different *Brassica* species

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Abstract

The impact and recovery characterized by photosynthetic and chlorophyll fluorescence parameters from exposure to the novel herbicide Pyribambenz-propyl (PP) and the control herbicide Bispyribac-sodium (BS) were assessed in three species of rapeseeds (*B. napus*, *B. juncea* and *B. rapa*) maintained in the field condition. The results indicated that the inhibiting effect of herbicide treatments on plant net photosynthetic rate (Pn) was similar with that on SPAD value and chlorophyll fluorescence parameter presented by maximal PS II efficiency. New herbicide PP treatment showed slight or no herbicide injury on plants of all three *Brassica* species with the maintaining of relatively higher Pn, SPAD value and optimal PS II efficiency. Most plants treated by various PP concentrations (100, 500 and 1000 mg/L) could recover from the herbicide stress, as their photosynthetic and chlorophyll fluorescence parameters were close to that of the control at 28 days after the treatments. Compared to PP, the control herbicide BS showed severe damage to the plants, for their Pn, SPAD value and optimal PS II efficiency after 14 days after BS treatment were not detectable due to the deterioration of the plants. 100 mg/L PP was a suitable concentration for the field application of this novel herbicide, which enhanced the photosynthesis and promoted the growth of plants. It was also suggested that among three species of *Brassica*, *B. rapa* was the most sensitive to this new herbicide.

Key words: *Brassica* species, herbicide Pyribambenz-propyl, photosynthesis, SPAD, chlorophyll fluorescence

Introduction

The novel herbicide Pyribambenz-propyl {Propyl 4-[2-(4,6-dimethoxy-2-pyrimidinyloxy) benzyl amino] benzoate, PP, formerly ZJ273}, derived from a precursor compound pesticide (a derivative of 2-pyrimidinyloxy-N-aryl benzoate) with novel structure and efficient biological activity (Lu et al., 2004), takes an effective prevention to both monocotyledonous and dicotyledonous weeds in the oilseed rape field. From previous studies, this new herbicide seems to be an ALS (acetolactate synthase) inhibitor, since its inhibition effect on sensitive plants such as wheat and sorghum, could be recovered by addition of three kind branched-chain amino acids (Cobb et al., 2000; Kee et al., 2004; Lu et al., 2004). However, PP was supposed to be a precursor herbicide, as it showed little inhibiting effect on ALS activity *in vitro* (Chen et al., 2005), and it might possess other target enzyme or affect other physiological and metabolism processes (Tang et al., 2005). These hypotheses need to be approved yet.

Photosynthetic properties of plants under herbicide stress had been shown negatively influenced by several researchers, which was often found that the low net photosynthetic rate of affected plants had been probably due to the herbicide injury on photosystem II (David et al., 2000; Chesworth et al., 2004). Currently, chlorophyll fluorescence is used in analyzing the photosynthetic apparatus and understanding the mechanism of photosynthesis and its related environmental factors including both biotic and abiotic stresses (Sayed, 2003). Chlorophyll fluorescence can also be used for screening the resistant plants. It was reported that $(F_m-F_0)/F_m$ ratio (as the optimal PSII efficiency) was not affected by plant species and growth conditions, but significantly decreased under the stress of PSII-inhibiting herbicides (Frankart et al., 2003; Masaki et al., 2004).

No information is currently available regarding the effects of PP on the characterization of photosynthetic and chlorophyll fluorescence parameters. The objectives of the present investigation were to compare the effects of PP at different concentrations on net photosynthetic rate, SPAD value (indicator of the chlorophyll content) and the maximal PS II efficiency in three rapeseed species (*B. napus*, *B. juncea* and *B. rapa*).

Materials and Methods

Plant material and herbicide treatment: Seeds of *B. napus* cv. ZS758, *B. juncea* cv. Hongyejie and *B. rapa* cv. Youbai were directly sown at Zhejiang University farm, Hangzhou (China) in early October of 2004 and 2005. The field contains 0.18% total nitrogen, 1.75% organic matter and 63 mg/kg soil available phosphorus. Each plot was 1.5 m long and 1.0 m wide, and the experiment was laid out in a randomized complete block design with 3 replications in each case. The herbicide PP was supplied by Dr. Lu (Shanghai Institute of Organic Chemistry, Chinese Academy of Sciences, China), and the control herbicide BS was purchased from the local market. Three concentrations of PP (0, 100, 500 and 1000 mg/L) were foliar applied at 5 leaf stage at the quantity of 750 kg/ha. 100 mg/L (recommended concentration in the field (Wang et al., 2000)) BS (Bispyribac-sodium, Nominee), a known ALS-inhibitor, was used as the control herbicide. Conventional crop management was applied during the growing period (Zhou, 2001).

Determination of photosynthesis rate and SPAD value: Net photosynthetic rate (Pn) of leaves was determined by Portable Photosynthesis System (Model LI-6400, USA) and SPAD values were read by chlorophyll meter (Minolta SPAD-502, Japan). Measurements of Pn and SPAD values were carried out at 7, 14, 21 and 28 days after herbicide treatment. Three plants were determined for each analytical measurement and 3 replications of each parameter were taken. All analyses were performed on the fully expanded functional leaves (Leul & Zhou, 1998; Wu et al., 1998).

Measurement of chlorophyll fluorescence: Chlorophyll fluorescence of leaves was measured with a pulse-modulated fluorometer (Mini-PAM, Walz, Germany) at 8:00 to 11:00 a.m. After the leaves were dark-adapted for 20 min, the minimal fluorescence (F_0) with all PSII reaction centers open was determined by modulated light (< 0.1 μmol/m^2/s), and the maximal fluorescence (F_m) with all PSII reaction centers closed was measured with a 0.8 s saturating pulse at 8000 μmol/m^2/s in dark-adapted leaves (Guo et al., 2005). All measurements were replicated at least six times. The $(F_m\text{-}F_0)/F_m$ ratio was considered to be a measure of the maximal photochemical efficiency of PSII in the dark (Maxwell & Johnson, 2000). All data were analyzed by the statistical programme SAS using Fisher's protected LSD test, and significant differences among treatments were considered at the $P < 0.05$ level.

Results

The net photosynthetic rate (Pn) and SPAD values of rape leaves from different rape species (*B. juncea*, *B. napus* and *B. rapa)* were compared in the presence of different treatments of two herbicides. The results indicated that in general the similar effects of herbicide treatments on Pn and SPAD values were observed (Fig. 1). As a control herbicide, BS exhibited serious herbicide damage on plants of all three *Brassica* species, as the Pn and SPAD values of BS treatment after 14 days were not detectable due to the deterioration of the plant. Compared to BS, PP treatment showed less or no herbicide injury with the maintaining of relatively higher Pn and SPAD values, and the most plants treated by different PP concentrations (100, 500 and 1000 mg/L) could recover from the herbicide stress at 28 days after the treatments (Fig. 1).

The treatment of PP at lower concentration (100 mg/L) in *B. napus* had higher Pn than other treatments and even than the control during the 14 to 21 days (Fig. 1 A-1), and similar trends were observed of the effect on SPAD value (Fig. 1 A-2). PP treatments at higher concentration (500 mg/L) showed significantly higher Pn than other treatments at 7 days and higher SPAD value at 21 days after the treatment. No significant difference was observed of the Pn and SPAD values between the PP treatments and the control at 28 days after the treatment (Fig. 1 A-1, A-2).

Based on the data from *B. juncea* it was indicated that at the early stage (7 or 14 days after the treatment) lower Pn was found from PP treatments. But at 21 days after the treatment, Pn of 100 mg/L PP treatment was significantly higher than that of the control (Fig. 1 B-1). Lower SPAD value was also observed from PP treatments at the first 7 days. From 14 days, there was no obvious difference of SPAD value among the PP treatments and the control. No difference in Pn and SPAD values was found between 100 mg/L PP treatment and the control at 28 days (Fig. 1 B-1, B-2). Compared to *B. napus* and *B. juncea*, *B. rapa* exhibited the most susceptible to the herbicide as both Pn and SPAD values of treated plants were significantly lower than that of the control at 21 and 28 days, while other two species were almost recovered from the herbicide stress (Fig. 1 C-1, C-2).

Further study of chlorophyll fluorescence parameters indicated that much severe damage was also exhibited from BS herbicide treatment than the PP treatments as presented by maximal PS II efficiency, and the data of F_m and F_0 from BS treatment was not detectable due to the badly deterioration of the plant at 14 and 28 days (Fig. 2). At the first 7 days, the maximal PS II efficiency of PP treatments of *B. napus* was significantly lower than that of the control. 1000 mg/L PP treatment increased the maximal PS II efficiency in the followed 7 days, although there was no significant difference between the one and the other treatments (including the control). At 28 days, the optimal PS II efficiency of 100 mg/L PP treatment was recovered to the level of the control in *B. napus* (Fig. 2 A).

As a species with strong tolerance to herbicide-stress, *B. juncea* showed better adaptability to high concentration PP treatments (500 and 1000 mg/L PP), since their optimal PS II efficiencies exhibited no significant difference to the control at 7 and 28 days. There was also no obvious difference of the maximal PS II efficiency between the 100 mg/L PP treatment and the control at 14 and 28 days (Fig. 2 B). It was found that the maximal PS II efficiency of *B. rapa* had been greatly declining with the increasing of PP concentrations and growth intervals (Fig. 2 C). At the 28 days, the optimal PS II efficiencies of PP treatments were still lower than that of the control. These results also suggested that among three species of *Brassica*, *B. rapa* was the most sensitive to this novel herbicide.

Discussion

The herbicides which affected photosynthesis via different approaches by accepting electron from PSI, inhibiting carotenoids and protein biosynthesis or blocking PSII electron transport, often induced the decline of Pn and chlorophyll content (Hendry & Price, 1993; David et al., 2000; Chesworth et al., 2004). The present experiment indicated that most plants treated by various PP concentrations (100, 500 and 1000 mg/L) could recover from the herbicide stress, as their photosynthetic and chlorophyll fluorescence parameters were close to that of the control at 28 days after the treatments. Generally, as a steady parameter, $(F_m\text{-}F_0)/F_m$ (the optimal PSII efficiency) that was not affected by species and growth conditions, would be significantly impaired only when plants were under the stress (Sayed, 2003). Present experiment indicated that the optimal efficiency of PSII was not significantly affected by herbicide PP in most treatments, suggesting that the PP treatment did not induce the stress to rapeseed plants. As this new herbicide PP treatment showed slight or no herbicide injury on plants of all three *Brassica* species with the maintaining of relatively higher Pn, SPAD value (indicator of chlorophyll content) and stable $(F_m\text{-}F_0)/F_m$, it indicated that PP was not a PSII-inhibiting herbicides. Compared to PP, BS that known to be an ALS inhibitor caused the severe injury in photosystem for their Pn, SPAD value and optimal PS II efficiency were not detectable as early as 14 days after the treatment due to the deterioration of plants under serious herbicide-stress. These results also suggested that the novel herbicide PP was quite safe to the oilseed rape plants.

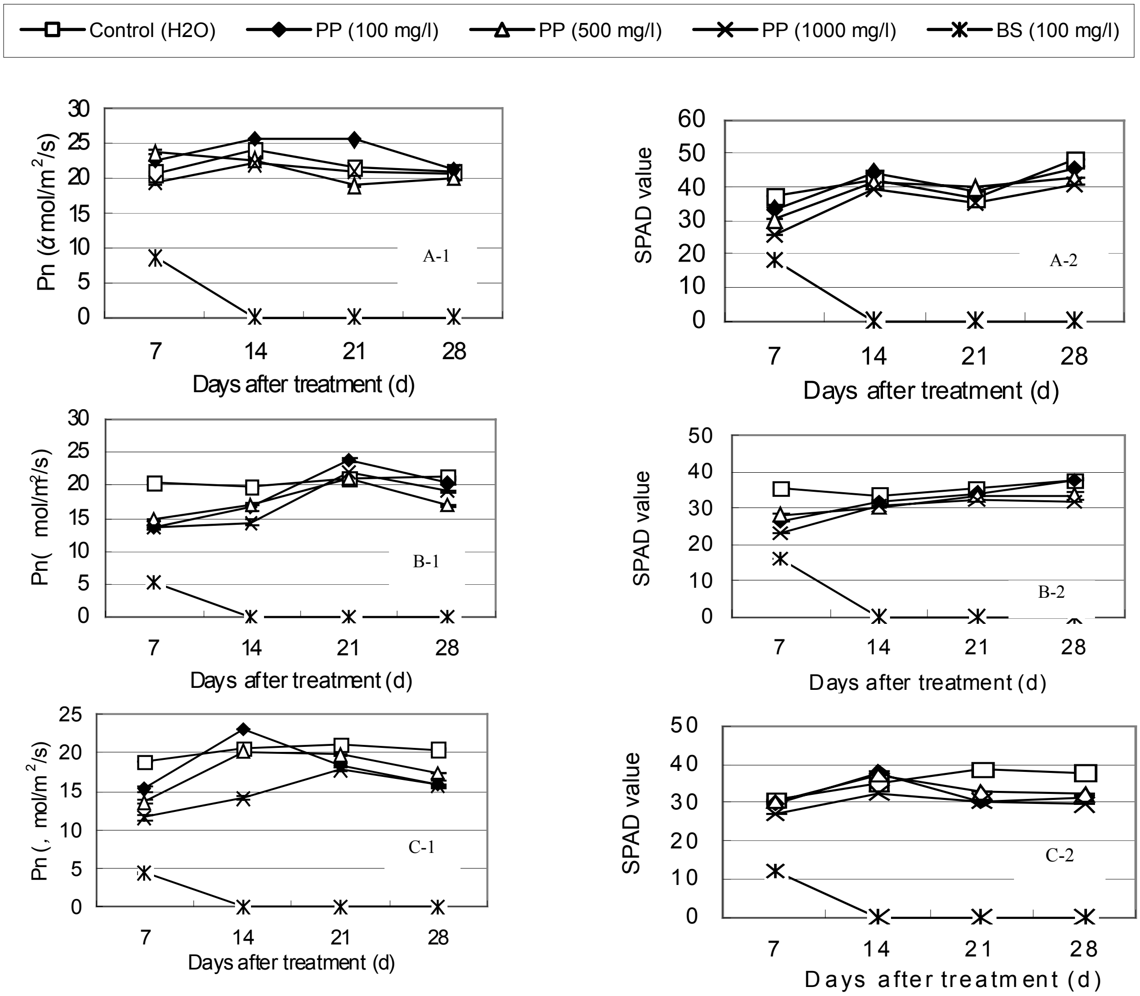

Fig. 1. Effects of herbicide treatments on net photosynthetic rate (Pn) (-1) and SPAD value (-2) of rape leaves measured at 7, 14, 21 and 28 days after the treatment in *B. napus* cv. ZS 758 (A), *B. juncea* cv. Hongyejie (B) and *B. rapa* cv. Youbai (C). Bars indicate SE.

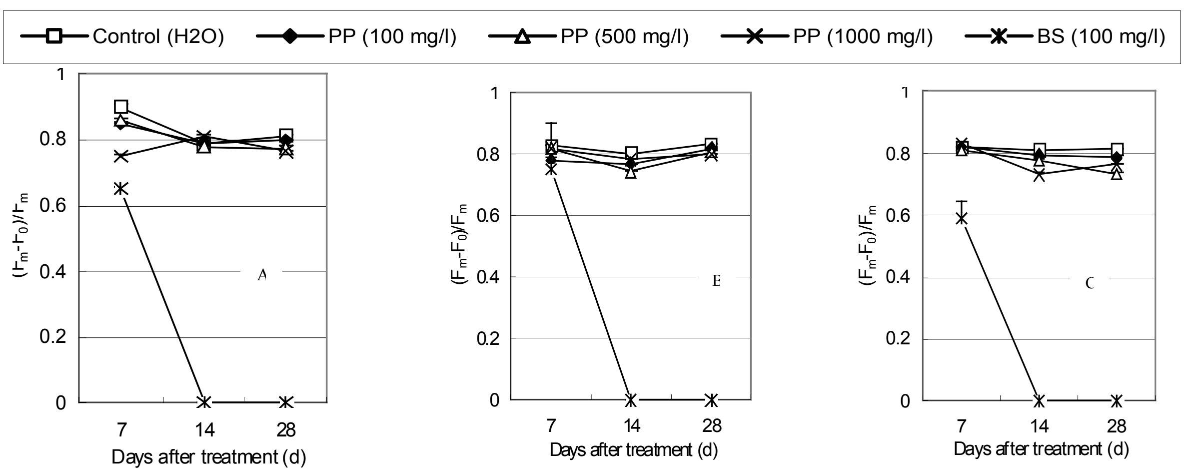

Fig. 2. Effects of herbicide treatments on maximal PS II efficiency, calculated by the formula of $(F_m\text{-}F_0)/F_m$. Data of F_m and F_0 were determined from rape leaves at 3 measurement intervals (7, 14 and 28 days after treatment) in *B. napus* cv. ZS 758 (A), *B. juncea* cv. Hongyejie (B) and *B. rapa* cv. Youbai (C). Bars indicate SE.

Furthermore, 100 mg/L PP was a suitable concentration for the field application of this new herbicide, which enhanced the photosynthesis and promoted the growth of plants. Comparing the three species of *Brassica*, *B. rapa* was relatively more sensitive to PP than other two species. More research would be necessary to further understand the mechanism of herbicide PP on the translocation and metabolism in various crops and weeds, as well as the investigation in the crop safety and environmental soundness from an ecological and molecular perspective.

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