Synergism of new herbicide pyribambenz-propyl and 5-aminolevulinic acid in relation to seedling growth and antioxidant activity in oilseed rape

ZHANG Wenfang[1], FENG Haiqiang[1], ZHANG Fan[1], JIN Zonglai[1], TANG Qinghong[2], LU Long[2], YE Qingfu[1], ZHOU Weijun[1]

[1] College of Agriculture and Biotechnology, Zhejiang University, Hangzhou 310029, China
[2] Shanghai Institute of Organic Chemistry, Chinese Academy of Sciences, Shanghai 200032, China
Email: wjzhou@zju.edu.cn

Abstract

Field experiments were conducted to investigate the combining effects of novel herbicide pyribambenz-propyl {propyl 4-[2-(4,6-dimethoxy-2-pyrimidinyloxy) benzyl amino] benzoate, PP; 100 mg/L as recommended} and 5-aminolevulinic acid (ALA; 1, 10 and 100 mg/L) in relation to seedling growth and development of oilseed rape (*Brassica napus* cv. ZS 758). The plant pre-treated by ALA retained higher dry matter when the seedlings were subsequently sprayed 100 mg/L PP, and even better result was observed from 10 mg/L ALA pre-treatment relative to the control (neither PP nor ALA treatment). In ALA post-treatment experiment, all ALA treatments showed a significant increase over the control with the highest dry matter being observed from 100 mg/L ALA treatment following PP application. The chlorophyll contents of 10 and 100 mg/L ALA pre-treatment were significantly higher than that of PP treatment control (non-ALA) and even recovered to the level of the control. In ALA post-treatment, the highest chlorophyll content was observed from 100 mg/L ALA treatment, which showed a marked increase as compared to PP treatment control and even the control. Malondialdehyde (MDA) decreased significantly after ALA (10 and 100 mg/L) and PP (100 mg/L) were both applied subsequently in ALA pre- and post-treatment experiments. The highest SOD activity was obtained from 10 mg/L ALA pre-treatment, followed by 100 and 1 mg/L ALA, which all showed the significant increase over the control. Similarly, in ALA post-treatment, all ALA treatments exhibited a significant increase over the control with the highest SOD activity being observed from 100 mg/L ALA treatment following PP application. Therefore, it is feasible to apply subsequently the herbicide and plant growth regulator with the synergistic effects on plant growth and development.

Key words: *Brassica napus*, new herbicide pyribambenz-propyl, 5-aminolevulinic acid, synergism, antioxidants

Introduction

Plant growth regulators play an important role in crop production and are being used increasingly to manipulate plant growth and yield. 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 its concentration is regulated at low concentrations (10-300, 0.3-3 mg/L) *in vivo* (Weinstein & Beale 1985; Hotta et al. 1997b) and *in vitro* (Zhang et al. 2006b) respectively. Recently it was found that low concentrations of ALA had a promotive effect on growth and yield of several crops and vegetables (Hotta et al. 1997a, b; Roy & Vivekanandan 1998; Watanabe et al. 2000).

The novel herbicide pyribambenz-propyl {propyl 4-[2-(4,6-dimethoxy-2-pyrimidinyloxy) benzyl amino] benzoate, PP, formerly ZJ273} is a new oilseed rape field herbicide with the advantages of low dosage, low mammalian toxicity, broad weeding spectrum and compatible to environment. This new herbicide, derived from a precursor compound, was supposed to be one kind of herbicides with the inhibition in biosynthesis (Erksson et al., 1996; Scarponi et al., 1997; Cobb et al., 2000; Wu et al., 2003).

Previous studies indicated that higher concentration of the herbicide could cause damage to plants, and ALA was found to be able to increase cold and salt tolerance in rice and potato (Hotta et al. 1998; Zhang et al. 2006b). No information is available on the synergism of new herbicide PP and ALA applied in the field crop. Therefore, this study was conducted to evaluate their combining effect on seedling growth and antioxidant activity in oilseed rape.

Material and Methods

Plant material and treatments: Seeds of *B. napus* cv. ZS758 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 1.3 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 ALA was purchased from Cosmo Oil Co. Ltd. (Japan). Treatments were as follows: ALA pre-treatment: Rape seedlings at 4-leaf stage were pre-treated by ALA (1, 10 and 100 mg/L) for 48 hr and then foliar sprayed by 100 mg/L PP (recommended concentration in the field (Wang et al., 2000)) for another 48 hr. ALA post-treatment: Seedlings at 4-leaf stage were foliar applied by 100 mg/L PP for 48 hr and then foliar sprayed by ALA (1, 10

and 100 mg/L) for another 48 hr. Conventional crop management was applied during the growing period (Zhou, 2001; Zhang et al., 2006a).

Determination of physiological characters: Seven days after the treatment, the third and fourth leaves from the top, except for malondialdehyde (MDA) which was taken from the first green leaf from the base of the plant were sampled for analysis of physiological characters (Leul & Zhou, 1999). Chlorophyll content was determined according to Chen (1984). MDA accumulation was determined by the thiobarbituric acid method, as previously described by Zhou & Leul (1998). Leaf superoxide dismutase (SOD) activity was analyzed by the method of Zhu et al. (1990).

Statistical analysis: In all experiments, data were analyzed using the statistical programme SAS and the analysis of variance (ANOVA) was followed by Fisher's protected LSD test to identify homogenous groups within the means. Significant differences among treatments were considered at the $P < 0.05$ level.

Results

The combining effects of new herbicide PP and ALA to promote oilseed rape seedling growth were determined in the present work. The plant pre-treated by ALA retained higher dry matter when the seedlings were subsequently sprayed 100 mg/L PP, and even better result was observed from 10 mg/L ALA pre-treatment relative to the control (neither PP nor ALA treatment) (Fig. 1 A1). Whereas in ALA post-treatment experiment, all ALA treatments showed a significant increase over the control with the highest dry matter being observed from 100 mg/L ALA treatment following PP application (Fig. 1 A2).

The ALA and PP combining responses of chlorophyll content are summarized in Fig. 1B. The chlorophyll contents of 10 and 100 mg/L ALA pre-treatment were significantly higher than that of PP treatment control (non-ALA) and even recovered to the level of the control (Fig. 1 B1). In ALA post-treatment experiment, the highest chlorophyll content was observed from 100 mg/L ALA treatment, which showed a marked increase as compared to PP treatment control and even the control (Fig. 1 B2).

Malondialdehyde (MDA), a decomposition product from the peroxidation of polyunsaturated fatty acids of biomembranes, decreased significantly after ALA (10 and 100 mg/L) and PP (100 mg/L) were both applied subsequently in ALA pre- and post-treatment experiments (Fig. 1 C1, C2).

Fig. 1D showed the combining responses of ALA and PP treatments to SOD activity. The results indicated that the highest SOD activity was obtained from 10 mg/L ALA pre-treatment, followed by 100 and 1 mg/L ALA, which all showed the significant increase over the control (Fig. 1 D1). Similarly, in ALA post-treatment experiment, all ALA treatments exhibited a significant increase over the control with the highest SOD activity being observed from 100 mg/L ALA treatment following PP application (Fig. 1 D2).

Discussion

The seedling growth was inhibited and chlorophyll content was reduced by the new herbicide PP (100 mg/L) at 7 days after the treatment in oilseed rape. This induced decline in photosynthesis and chlorophyll content by the herbicides was also observed in other plant species (David et al., 2000; Chesworth et al., 2004). ALA is a key precursor in the biosynthesis of porphyrins such as chlorophyll and heme, and is found to have a promotive effect on plant growth and yield (Roy & Vivekanandan 1998; Watanabe et al. 2000) as well as to increase cold and salt tolerance in crops (Hotta et al. 1998; Zhang et al. 2006b). The present experiment indicated that both pre- and post-ALA treatments significantly alleviated the transient stress of the plant incurred by the herbicide PP, and in fact the highest dry matter and chlorophyll content were obtained from 10 and 100 mg/L ALA treatments following PP application respectively.

Malondialdehyde (MDA) is a product of lipid peroxidation that damages enzymes and plant membranes, and is known to show greater accumulations under stress conditions (Gossett et al. 1994; Zhang et al. 2005). Our data indicated that foliar sprays of appropriate concentrations of the new herbicide PP and plant growth regulator ALA could significantly reduce MDA accumulation and increase SOD activity, which protected the rape plants from the instant damage and improved the herbicide tolerance of the plant.

In conclusion, these results offer the opportunity to foliar pre-treat 10 mg/L ALA to improve *B. napus* plant tolerance to the herbicide (100 mg/L PP) and to post-spray 100 mg/L ALA to recover rape plant from the transient stress incurred by the herbicide (100 mg/L PP). Therefore, it is feasible to apply subsequently the herbicide and plant growth regulator with the synergistic effects on plant growth and development. Further work is under the way to understand the mechanism of synergism of the novel herbicide PP and ALA by using various approaches including PP concentrations higher than recommended (as a possible solution to the potential herbicide injury).

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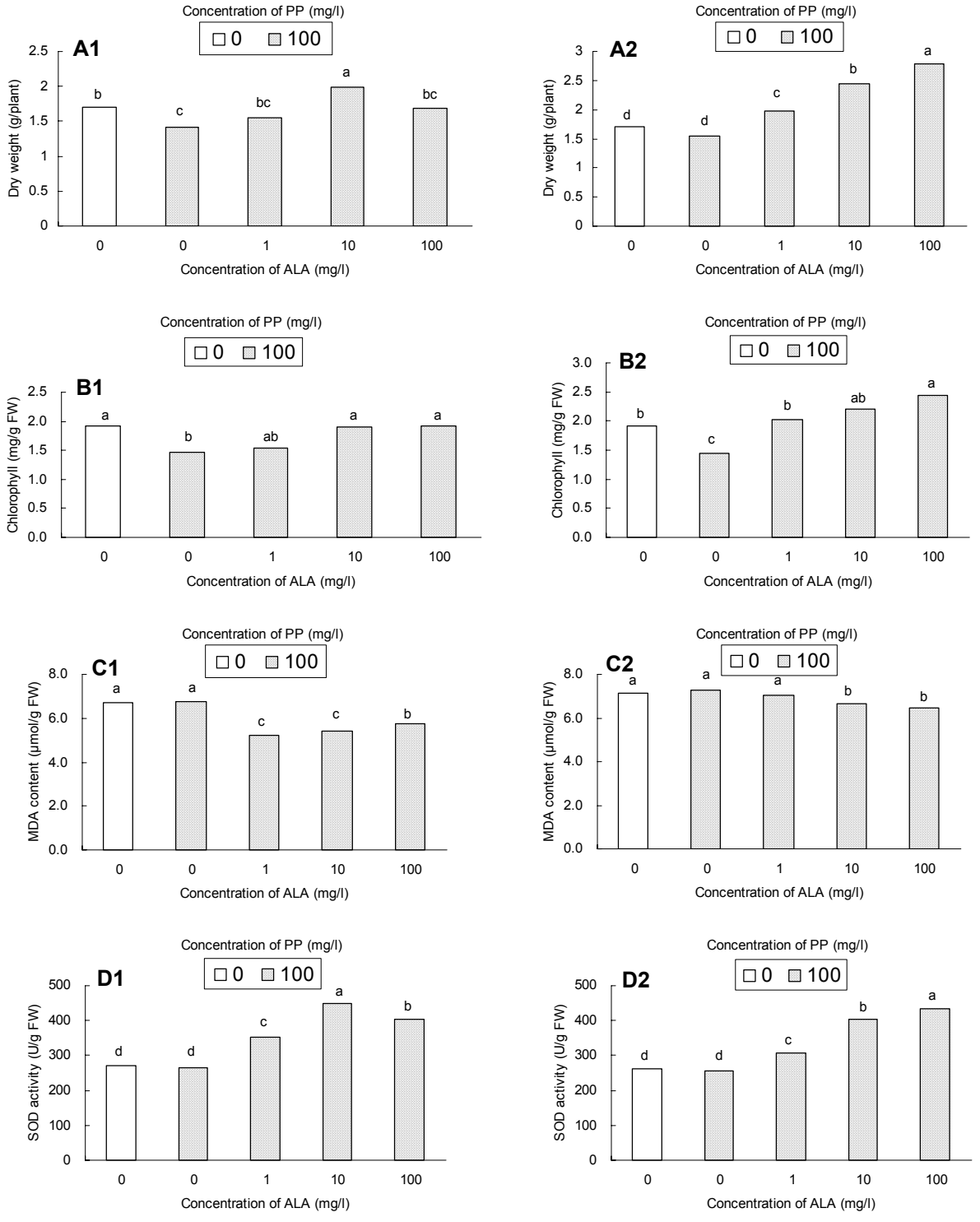

Fig. 1. Effects of combining treatments of Pyribambenz-propyl (PP) and 5-aminolevulinic acid (ALA) on biomass (A), chlorophyll content (B), MDA accumulation (C) and SOD activity (D) of *B. napus* cv. ZS 758. ALA pre-treatment (1): Rape seedlings at 4-leaf stage were pre-treated by ALA (1, 10 and 100 mg/L) for 48 hr and then foliar sprayed by 100 mg/L PP for another 48 hr. ALA post-treatment (2): Rape seedlings at 4-leaf stage were foliar applied by 100 mg/L PP for 48 hr and then foliar sprayed by ALA for another 48 hr. Data are means followed by the same letter do not differ significantly according to Fisher's protected LSD test ($P < 0.05$).

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