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The interacting effect of urea and fenoxaprop-P-ethyl on photosynthesis and chlorophyll fluorescence in *Perilla frutescens*

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Abstract

We studied the effect of herbicide and nitrogen supply on photosynthesis in *Perilla frutescens* L. Britt. Plants were exposed to combined treatment of urea and herbicide, fenoxaprop-P-ethyl (FPE), in various concentrations. FPE reduced significantly chlorophyll (Chl) content, photosynthetic rate, and stomatal conductance, but increased significantly intercellular CO_2 concentration; thus, FPE inhibited significantly the photosynthetic capacity. In addition, FPE also decreased significantly the PSII photochemical efficiency, effective quantum yield of photochemical energy conversion in PSII, PSII potential activity, and photochemical quenching of variable Chl fluorescence. It also decreased nonphotochemical quenching. It indicated that FPE impaired PSII and blocked the electron transport in light reaction. The urea treatment at moderate concentration (1–4 g L^{-1}) could antagonize the negative effect of FPE, while the high urea concentration (8 g L^{-1}) aggravated this effect. The treatment with urea (4 g L^{-1}) and then with FPE (1.33 mL L^{-1}) enhanced Chl content index, photosynthetic rate, and stomatal conductance by 12.5, 36.1, and 28.5% compared to FPE treatment alone. Thus, we suggested to treat plants first with urea (4 g L^{-1}) and then by FPE (1.33 mL L^{-1}) as the best and the safest method to balance the fertilization and weeding.

Additional key words: herbicide; Labiatae; photosynthetic parameters.

Introduction

Perilla frutescens (L.) Britt. (Labiatae) is an annual herb of Lamiaceae widely used in the Chinese traditional medicine to treat various diseases, including liver remedy, reduction of blood pressure and lipids, prevention of platelet aggregation, thrombus and cancer formation, and it shows anti-inflammatory, -anaphylactic, and -microbial effects (Makino *et al*. 2001, Ueda *et al.* 2002, Zekonis *et al.* 2008). A recent survey indicates a large gap (60%) between the demand and supply of *P. frutescens*, and the market demands an increase by 10% per year. Therefore, an improvement in *P. frutescens* yields is imminently needed.

P. frutescens is a typical short-day plant with a poor drought resistance, which prefers humidity and tolerates cloudy weather and floods. Shading nets have been widely used in multicropping cultivation of *P. frutescens*, and even off-season transplant production because of low cost, good effect on cooling, moisturizing, and rain-proof. However, annual, monocotyledon, malignant weeds grow always fast during such a cultivation. Chemical weeding seems to be highly efficient to control weeds during *P. frutescens* production. Nevertheless, none of the registered herbicides has been found until now for *P. frutescens*. One of aryloxyphenoxypropionate herbicides, the highly selective one, FPE, has been proposed to be used for *P. frutescens* production. Yet, even recommended dose affects negatively crops and the high concentration brings about severe toxic effects.

Nitrogenous nutrition is one of the significant factors that may regulate photosynthesis, growth, and production of *P. frutescen*s (Feng *et al*. 2012). The growth rate, stem diameter, and leaf area index of *P. frutescens* enlarge with the increase in N concentration. The N fertilizer could antagonize the toxic effects resulting from herbicide on

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Abbreviations: C_i – intercellular CO_2 concentration; CCI – chlorophyll content index; Chl – chlorophyll; F_v/F_0 – PSII potential activity; F_v/F_m – maximum PSII photochemical efficiency; FPE – fenoxaprop-P-ethyl; g_s – stomatal conductance; NPQ – nonphotochemical fluorescence quenching; P_N – net photosynthetic rate; q_p – photochemical quenching of fluorescence; Φ_{PSII} – effective quantum yield of photochemical energy conversion in PSII.
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crops (Liu *et al.* 1995). A combination of fertilization and chemical weeding provides the best growth conditions for crops only when a balance between fertilization and chemical weeding is achieved (Kismanyoky and Lehoczky 2007). Thus, more and more attention has been recently paid to find out how to fertilize and use herbicide reasonably. For example, the protection of winter wheat by herbicide Granstar 75 DF, applied in doses of 18 and 24 g per ha, and leaf-feeding with urea in concentrations of 18 and 24% showed a significantly positive effect on the yield (Brzozowska and Brzozowski 2002). The application of both urea with herbicide and urea with urease inhibitor increases significantly grain protein contents compared with urea treatment alone (Dawar *et al.* 2011). However, only a few studies focused on *P. frutescens*. The objective of this study was to analyze the effect of combined application of FPE and urea on *P. frutescens* photosynthesis. Our results may set a basis for the selection of the safest dose of FPE and urea to *P. frutescens.*

Materials and methods

Materials: *P. frutescens* seeds and urea were provided by Center of Crop Chemical Control, Shanxi Agricultural University. FPE (69 g L^{-1} emulsion in water) was purchased from *Aventis Co. Ltd*. (Canada).

Field site: A field experiment was carried out in the experimental station of Shanxi Agricultural University, China, from May 2010 to October 2011. The texture of soil was carbonate cinnamon soil developed from loess-like parent material and irrigable land. The 0–20 cm soil layer (pH 7.68) contained 1.74% of organic matter, 0.64% of total nitrogen, 6.69 mg kg^{-1} of available phosphorus, and 185.2 mg kg^{-1} of available potassium. Black shading net (45% shading) was placed 2 m above the ground to ensure a good ventilation condition and to allow observation and sampling.

Experimental design: The experiment was a three-factor, split-split plot arrangement of treatments in a randomized complete block design with 50 treatments and 3 replications. The two main plot treatments were: (*1*) Spray with urea for 24 h, and then with FPE herbicides (group A), and (*2*) Spray with FPE herbicides for 24 h, and then with urea (group B). The subplot factors were different concentration gradient of urea and FPE:

Treatment	[g(urea) L^{-1}]
N0	0
N1	1
N2	2
N3	4
N4	8
	[mL(FPE) L^{-1}]
P0	0
P1	1.33
P2	2.66
P3	5.32
P4	7.98

The area of a single experimental plot was 6 m^2 (3 m × 2 m), with a density of 90,000 plants per ha. The treatments were performed with a compression sprayer at six-leaf stage of *P. frutescens* (40–60 d after sowing), applying 270 mL per plot.

Physiological parameters: Photosynthetic gas-exchange parameters and Chl fluorescence were measured on the mature leaves (the second leaf from the bottom) of three plants for three times 3 d after treatments. Measurements were carried out at 09:00–11:00 h on sunny days. Chl content index (CCI) was estimated with a Chl meter (*SPAD-250*, *Opti-Sciences Inc*., USA). Photosynthetic rate (P_N), intercellular CO_2 concentration (C_i), and stomatal conductance (g_s) were determined with a portable photosynthesis system (*CI-203* model, *CID Inc*., USA) at 25°C under artificial light of 1,000 μmol(photon) m^{-2} s^{-1}.

Chl fluorescence was measured on the same leaves as CCI and photosynthetic parameters were obtained by using a portable pulse-amplitude-modulation fluorometer (*PAM 2500*, *Walz*, Effeltrich, Germany) after the leaves were dark-adapted at room temperature (25°C) for 20 min. The following Chl fluorescence parameters were measured: minimum in the dark-adapted state (F_0), maximum in the dark-adapted state (F_m), minimum in the light-adapted state (F_0'), maximum in the light-adapted state (F_m'), and steady-state fluorescence in the light-adapted state (F_s). Chl fluorescence parameters were calculated according to the following equations (Genty *et al.* 1989): maximum PSII photochemical efficiency (F_v/F_m) as $(F_m - F_0)/F_m$, effective quantum yield of photochemical energy conversion in PSII (Φ_{PSII}) as $(F_m' - F_s)/F_m'$, PSII potential activity (F_v/F_0) as $(F_m - F_0)/F_0$, photochemical quenching of variable fluorescence (q_p) as $(F_m' - F_s)/(F_m' - F_0')$, and nonphotochemical quenching (NPQ) as $(F_m - F_m')/F_m'$.

Statistical analysis: All data were expressed as average ± standard deviation (SD) and subjected to the one-way analysis of variance (*ANOVA*) with normal distribution (Lazár and Nauš 1998) using the *Statistical Analysis System (SAS*) software package and *Microsoft Excel 2003*. The *Duncan*'s multiple range test was used to check the significance of difference between treatments. $P<0.05$ was considered statistically significant.

Results

CCI significantly decreased with the increase in FPE concentration when the herbicide was used alone (Fig. 1*A*). When treated with urea alone, CCI increased with the increasing concentration, and reached the maximum after N3 treatment. However, CCI decreased after N4 treatment compared with control. These results showed that the Chl content was reduced by excessive FPE and urea concentrations, but enhanced by the moderate urea application.

Interaction between urea and herbicide had a similar effect with their individual treatment. Compared with control (P0-N0), CCI significantly increased after treatment with P1-N3 and P2-N3 in group A and P1-N3 in group B, suggesting that N3 treatment reversed the inhibition effect of FPE at low concentration. P1-N3 treatment increased CCI by 12.5 and 3.8% compared with treatment with FPE alone (P1-N0) and control (P0-N0), respectively.

Photosynthetic parameters: P_N (Fig. 1*B*), C_i (Fig. 2*A*), and g_s (Fig. 2*B*) were used to assess the effect of FPE and urea on photosynthetic capacity. P_N and g_s decreased gradually with the increase of FPE and significant differences were found among different concentrations. Whereas, after urea treatment, P_N and g_s followed a trend: N3 > N2 > N1 > N0 > N4, and significant differences were present among different concentrations.

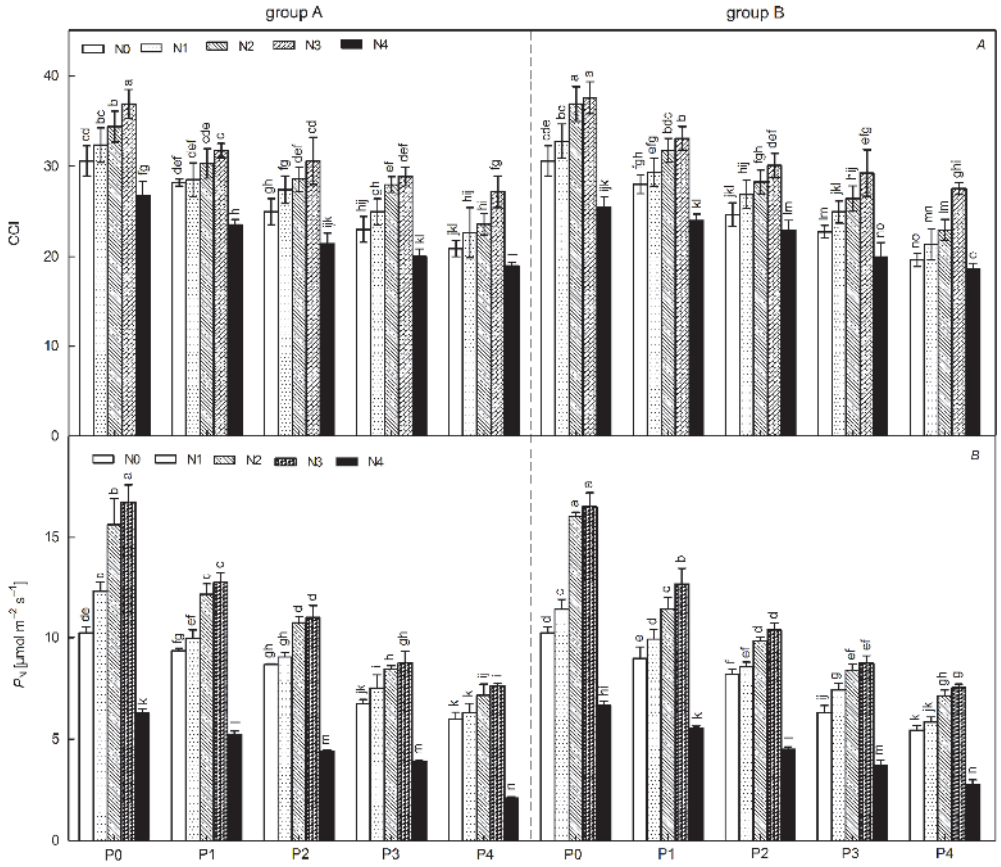

Fig. 1. Combination effects of fenoxaprop-P-ethyl (FPE) and urea on *A*: chlorophyll content index (CCI) and *B*: photosynthetic rate (P_N) of *P. frutescens* seedlings. N0 – 0 g(N) L^{-1}, N1 – 1 g(N) L^{-1}, N2 – 2 g(N) L^{-1}, N3 – 4 g(N) L^{-1}, N4 – 8 g(N) L^{-1}, P0 – 0 mL(FPE) L^{-1}, P1 – 1.33 mL(FPE) L^{-1}, P2 – 2.66 mL(FPE) L^{-1}, P3 – 5.32 mL(FPE) L^{-1}, P4 – 7.98 mL(FPE) L^{-1}. Group A: treatment with urea and 24 h-later with FPE; group B: treatment with FPE and 24 h-later with urea. Different *lowercase letters* in bar correspond to significant differences of different treatments at $p<0.05$. (*A*): *F*-value group A: F_P, 138.76**; F_N, 122.77**; $F_{P\times N}$, 0.63. *F*-value group B: F_P, 127.96**; F_N, 98.12**; $F_{P\times N}$, 1.83. (*B*): *F*-value group A: F_P, 419.66**; F_N, 520.48**; $F_{P\times N}$, 13.34**. *F*-value group B: F_P, 648.86**; F_N, 696.38**; $F_{P\times N}$, 20.87**. ** – significant at $p<0.01$. F_P – the effect of FPE; F_N – the effect of urea; $F_{P\times N}$ – the interactive effect between FPE and urea.

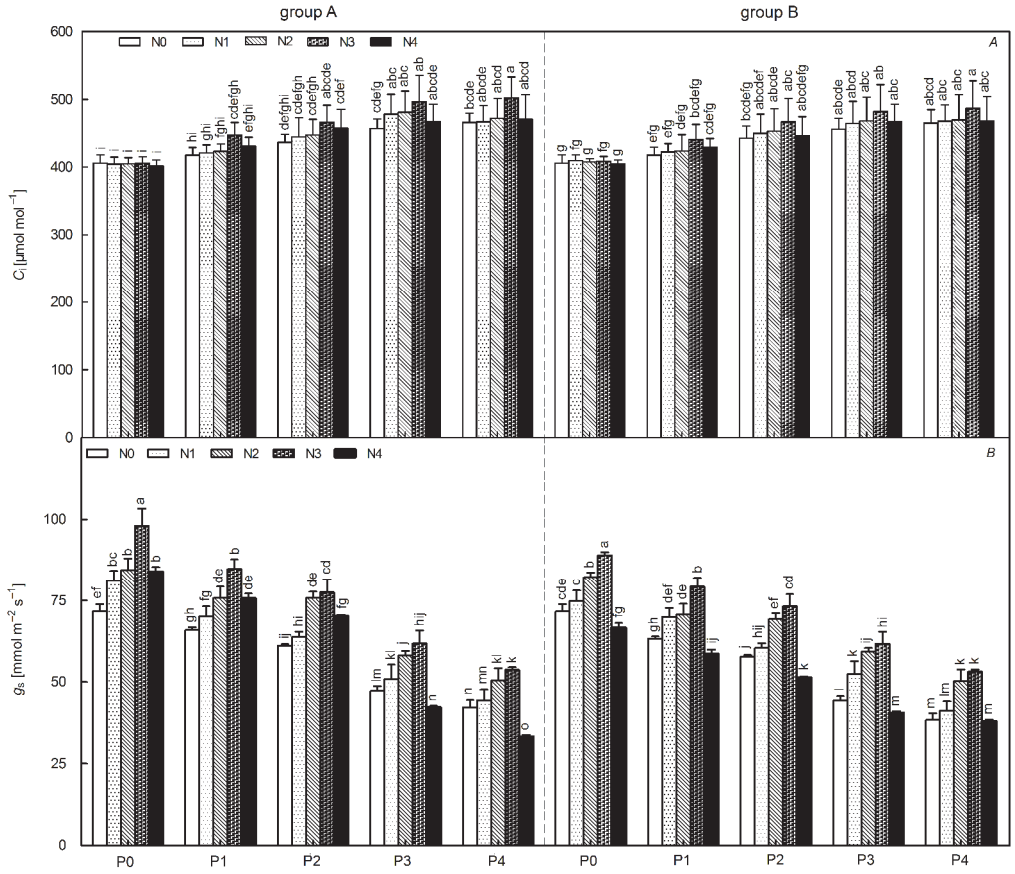

Fig. 2. Combination effects of fenoxaprop-P-ethyl (FPE) and urea on *A*: intercellular CO_2 concentration (C_i) and *B*: stomatal conductance (g_s) of *P. frutescens* seedlings. N0 – 0 g(N) L^{-1}, N1 – 1 g(N) L^{-1}, N2 – 2 g(N) L^{-1}, N3 – 4 g(N) L^{-1}, N4 – 8 g(N) L^{-1}, P0 – 0 mL(FPE) L^{-1}, P1 – 1.33 mL(FPE) L^{-1}, P2 – 2.66 mL(FPE) L^{-1}, P3 – 5.32 mL(FPE) L^{-1}, P4 – 7.98 mL(FPE) L^{-1}. Group A: treatment with urea and 24 h-later with FPE; group B: treatment with FPE and 24 h-later with urea. Different *lowercase letters* in bar correspond to significant differences of different treatments at $p<0.05$. (*A*): *F*-value group A: F_P, 28.56**; F_N, 3.07; $F_{P\times N}$, 0.34. *F*-value group B: F_P, 17.19**; F_N, 1.21; $F_{P\times N}$, 0.08. (*B*): *F*-value group A: F_P, 525.77**; F_N, 99.26**; $F_{P\times N}$, 7.18. *F*-value group B: F_P, 491.14**; F_N, 196.12**; $F_{P\times N}$, 2.13*. ** – significant at $p<0.01$; * – significant at $p<0.05$. F_P – the effect of FPE; F_N – the effect of urea; $F_{P\times N}$ – the interactive effect between FPE and urea.

When treated with FPE and urea together, the decrease in P_N and g_s was reversed to some extent by urea at the moderate concentration (N1–N3), but promoted by urea of the high concentration (N4). Especially, P1-N3 treatment enhanced P_N and g_s by 36.1 and 28.5% compared with P1-N0 treatment and by 24.5 and 18.3% compared with control (P0-N0). Thus, P1-N3 treatment showed limited negative effects on photosynthetic capacity and it was the best treatment to balance the fertilization and weeding.

C_i increased gradually with the increase of FPE concentration (Fig. 2*A*). These results suggested that photosynthetic capacity of *P. frutescens* was reduced by FPE and reversed to some extent by the moderate increase of nitrogen concentration. However, the decrease of P_N and g_s did not result from deficiency in C_i.

Chl fluorescence: The F_v/F_0, F_v/F_m, and Φ_{PSII} were used to assess the effect of FPE and urea on PSII (Fig. 3). All three parameters were reduced after FPE treatment. Significant differences were also observed among different concentrations. With the increasing urea concentration, the Chl fluorescence parameters first increased and then decreased, with the peak value after the N3 treatment. There was a significant difference among different concentrations.

Compared with FPE sole treatment (N0), combination of urea (N1–N3) and FPE improved F_v/F_m and Φ_{PSII}, and significantly enhanced F_v/F_0. However, high urea concentration (N4) caused the opposite effect. Interestingly, the fluorescence parameters were higher after treatment of group A than that of group B. Thus, P1-N3 treatment in group A was the best selection to balance the fertilization and weeding.

The q_p and NPQ were used to assess the effect of FPE (Fig. 4*A*,*B*). The q_p decreased with the increase in the FPE concentration, while NPQ exhibited an increasing trend. Significant differences were found in different treatments.

Both q_P and NPQ were altered after urea application;

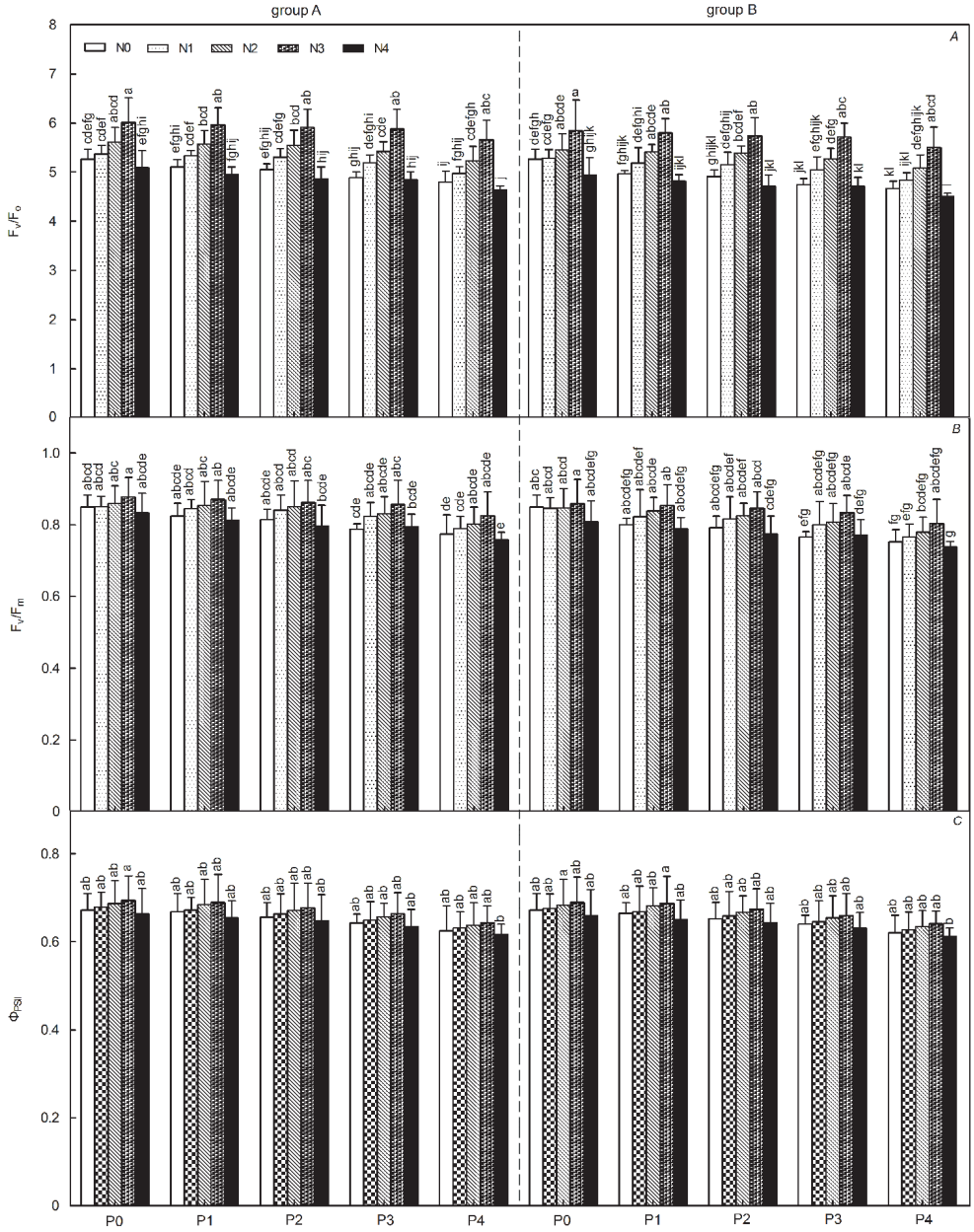

Fig. 3. Combination effects of fenoxaprop-P-ethyl (FPE) and urea on *A*: PSII potential activity (F_v/F_0), *B*: maximum PSII photochemical efficiency (F_v/F_m), and *C*: effective quantum yield of photochemical energy conversion in PSII (Φ_{PSII}) of *P. frutescens* seedlings. N0 – 0 g(N) L^{-1}, N1 – 1 g(N) L^{-1}, N2 – 2 g(N) L^{-1}, N3 – 4 g(N) L^{-1}, N4 – 8 g(N) L^{-1}, P0 – 0 mL(FPE) L^{-1}, P1 – 1.33 mL(FPE) L^{-1}, P2 – 2.66 mL(FPE) L^{-1}, P3 – 5.32 mL(FPE) L^{-1}, P4 – 7.98 mL(FPE) L^{-1}. Group A: treatment with urea and 24 h-later with FPE; group B: treatment with FPE and 24 h-later with urea. Different *lowercase letters* in bar correspond to significant differences of different treatments at $p<0.05$. (*A*): *F*-value group A: F_P, 5.29**; F_N, 34.37**; $F_{P\times N}$, 0.08. *F*-value group B: F_P, 5.74**; F_N, 31.30**; $F_{P\times N}$, 0.15. (*B*): *F*-value group A: F_P, 4.00**; F_N, 3.77**; $F_{P\times N}$, 0.07. *F*-value group B: F_P, 5.30**; F_N, 4.11**; $F_{P\times N}$, 0.11. (*C*): *F*-value group A: F_P, 2.69**; F_N, 0.99; $F_{P\times N}$, 0.003. *F*-value group B: F_P, 2.96*; F_N, 1.08; $F_{P\times N}$, 0.004. ** – significant at $p<0.01$; * – significant at $p<0.05$. F_P – the effect of FPE; F_N – the effect of urea; $F_{P\times N}$ – the interactive effect between FPE and urea.

in q_p, it followed a trend: N3 > N2 > N1 > N0 > N4; in NPQ, the trend was: N3 < N2 < N1 < N0 < N4. Significant differences were present among different concentrations, but absent in the combination of urea and FPE. The q_p was the greatest after P1-N3 treatment of group A after combined treatment with urea and FPE, providing the most energy for photosynthesis. Whereas, NPQ was the lowest after P1-N3 treatment of group A, suggesting that under such condition the lowest dissipation to heat was needed in the PSII antenna complexes. These results also indicated that P1-N3 treatment was the best selection to balance the fertilization and weeding.

Discussion

Generally, the application of most herbicides does not harm greatly crops, but it could affect their physiological activity and normal metabolism (Qian *et al.* 2012). Our results demonstrated that FPE could inhibit the photosynthetic capacity and affect chlorophyll fluorescence of *P. frutescens* seedlings. With the increase in FPE concentration, CCI, P_N, g_s, F_v/F_m, Φ_{PSII}, F_v/F_0, and q_p decreased, while NPQ exhibited an increasing trend.

There is a direct relationship between Chl content and light transformation in photosynthesis. . Previous study has proved that herbicide Sigma Broad could significantly reduce Chl content in leaves of Radix isatidis (*Isatis indigotica* Fort.) (Yuan *et al.* 2013). In agreement with above, our results also showed that CCI decreased significantly with the increasing FPE concentration.

P_N is an important parameter that can be used to reflect the photosynthetic capacity of plants. When treated with FPE, P_N significantly decreased in *P. frutescens* seedlings, suggesting that the photosynthesis was inhibited. The decreasing trend of P_N coincided with that of CCI, indicating that the decrease in photosynthetic pigment was one of the main factors that resulted in the P_N reduction (Yordanova *et al*. 2001).

F_v/F_m provides a measure of PSII photochemical efficiency and reflects the potential photochemical capacity of PSII. High values of F_v/F_m, F_v/F_0, and Φ_{PSII} imply high light transformation rate, providing more energy for CO_2 assimilation in dark reaction of photosynthesis. The F_v/F_m, F_v/F_0, and Φ_{PSII} were significantly reduced with the increasing FPE concentration indicating that PSII was impaired in *P. frutescens* leaves due to FPE stress, and the actual quantum yield of photosynthetic reaction centers was lowered (Yordanova *et al*. 2001).

The q_p parameter is conceived as an 'indicator' of the

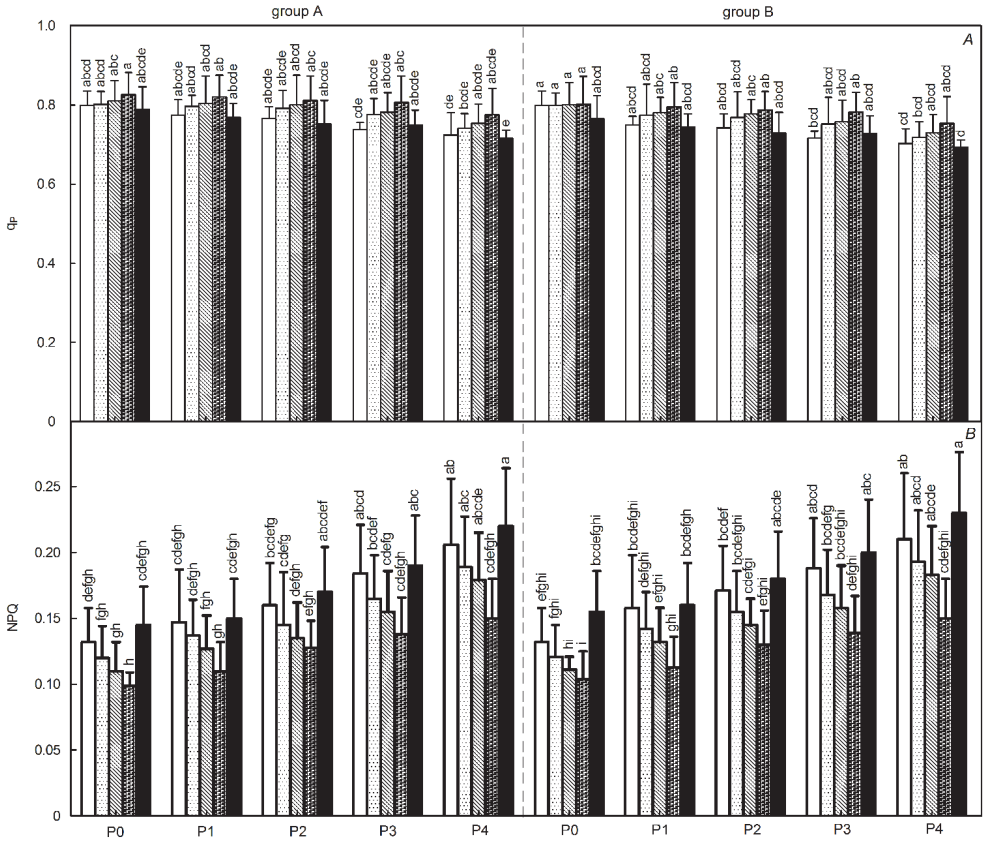

Fig. 4. Combination effects of fenoxaprop-P-ethyl (FPE) and urea on *A*: photochemical quenching of variable chlorophyll fluorescence (q_p) and *B*: nonphotochemical chlorophyll fluorescence quenching (NPQ) of *P. frutescens* seedlings. N0 – 0 g(N) L^{-1}, N1 – 1 g(N) L^{-1}, N2 – 2 g(N) L^{-1}, N3 – 4 g(N) L^{-1}, N4 – 8 g(N) L^{-1}, P0 – 0 mL(FPE) L^{-1}, P1 – 1.33 mL(FPE) L^{-1}, P2 – 2.66 mL(FPE) L^{-1}, P3 – 5.32 mL(FPE) L^{-1}, P4 – 7.98 mL(FPE) L^{-1}. Group A: treatment with urea and 24 h-later with FPE; group B: treatment with FPE and 24 h-later with urea. Different *lowercase letters* in bar correspond to significant differences of different treatments at $p<0.05$. (*A*): *F*-value group A: F_P, 3.674*; F_N, 2.934*; $F_{P\times N}$, 0.061. *F*-value group B: F_P, 4.664**; F_N, 2.776**; $F_{P\times N}$, 0.105. (*B*): *F*-value group A: F_P, 10.757**; F_N, 5.73**; $F_{P\times N}$, 0.086. *F*-value group B: F_P, 9.033**; F_N, 6.653**; $F_{P\times N}$, 0.097. ** – significant at $p<0.01$; * – significant at $p<0.05$. F_P – the effect of FPE; F_N – the effect of urea; $F_{P\times N}$ – the interactive effect between FPE and urea.

degree of openness of the PS II reaction centers, while NPQ is used to reflect the dissipation of excess excitation energy as heat protecting the photosynthetic apparatus against photodamage (Horton *et al.* 1994; Muller *et al.* 2001). Our results showed that FPE treatment resulted in a reduction in q_p and an increase in NPQ, suggesting that the openness of the PSII reaction centers decreased which further led to reduction of electrons involved in CO_2 assimilation and blockage in photosynthetic electron transport. *P. frutescens* enhanced heat dissipation to protect photosynthetic apparatus from excessive excitation energy.

There is pronounced diffusive limitation for CO_2 to reach the site of carboxylation, thus decrease in photosynthesis may result from stomatal and nonstomatal limitations. The C_i is often used to calculate the stomatal limitation. It is mainly responsible for the reduction of photosynthesis when both g_s and C_i decrease at the same time; while nonstomatal limitation is the main factor under the condition that P_N decreases while C_i increases (Zeiger and Field 1982). Thus, our results suggested that the decrease in P_N resulted from the nonstomatal limitation. This decrease might arise from the impairment of PSII and reduction of photosynthetic activity in mesophyll cells. However, the underlying mechanism requires further study.

Effect of N supply on photosynthesis is related to Chl content and electron transport in light reaction (Seemann *et al.* 1987). N nutrition control is an important way to regulate plant growth and photosynthesis and enhance tolerance to abiotic stress. Previous studies have shown that N supply affects nitrogen concentration per leaf area, and there is a positive correlation between nitrogen concentration per leaf area and Chl or the photosynthetic parameters, such as electron transport rate and maximum carboxylation (Lawlor 2002, Ripullone *et al.* 2003). The CO_2 assimilation rate increases with leaf N in wheat flag leaf, but this relationship is only true when leaf N did not exceed 125 mmol(N) m^{-2} (Evans 1983).

In accordance with these studies, our results showed that urea treatment at the moderate concentration (1 to 4 g L^{-1}) increased CCI, P_N, g_s, F_v/F_m, Φ_{PSII}, F_v/F_0, and q_p in *P. frutescens* and reduced NPQ. The combination of urea at 4 g L^{-1} and FPE at 1.33 mL L^{-1} resulted in higher CCI, P_N, g_s, and F_v/F_0 compared with FPE alone, whereas urea at 8 g L^{-1} and FPE led to the opposite results.

Although the significant difference was found only in C_i of *P. frutescens* treated with FPE alone at different concentrations, the C_i relatively decreased with the increase in N concentration, which was in accordance with previous study (Zhou *et al*. 2006).

In conclusion, electron transport in light reaction was partly inhibited and PSII was impaired in *P. frutescens* leaves after FPE treatment. Pretreatment with 4 g L^{-1} urea could enhance significantly Chl content and actual photochemical efficiency of PSII after FPE treatment, therefore it could increase the utilization of light in leaves and protect leaves from photodamage. The treatment with 4 g L^{-1} urea and 24 h later with 0.67 mL L^{-1} FPE was the best and the safest treatment to balance the fertilization and weeding and it should be recommended for practical use.

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