

# Effects of fertilization on leaf photosynthetic characteristics and grain yield in tartary buckwheat Yunqiao1

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## Abstract

Tartary buckwheat (*Fagopyrum tataricum* Gaertn) has been praised as one of green foods for humans in the 21<sup>st</sup> century. Effects of fertilization on leaf photosynthetic characteristics and grain yield of tartary buckwheat has not been yet reported in detail. Our experiment was set as a split-plot factorial. The main plots and subplots were designed by fertilizer ratio and rate as: NPK 1:1:1 (A1), NPK 1:4:2 (A2), NPK 1:2:3 (A3), and 300 (B1), 450 (B2), and 600 (B3) kg (NPK) ha<sup>-1</sup>. Our results showed that the grain yield was significantly and positively correlated with the net photosynthetic rate ( $P_N$ ), stomatal conductance ( $g_s$ ), transpiration rate ( $E$ ), PAR, stomatal limitation value ( $L_s$ ), chlorophyll content (SPAD value), and leaf area index (LAI), while significantly and negatively correlated with intercellular CO<sub>2</sub> concentration ( $C_i$ ) and water-use efficiency (WUE). The grain yield,  $P_N$ ,  $g_s$ ,  $E$ , PAR,  $L_s$ , SPAD, and LAI increased and then decreased with enhanced fertilization, and their maximum values appeared in the A2B2 treatment. The  $C_i$  and WUE decreased and then increased with enhanced fertilization, and their minimum values appeared in the A2B2 treatment. Our results suggested that fertilization had significant effects on the leaf photosynthetic capacity and grain yield of tartary buckwheat Yunqiao 1, and the best fertilization strategy was 450 kg ha<sup>-1</sup> with NPK 1:4:2.

*Additional key words:* chlorophyll; fertilizer; leaf area index; net photosynthetic rate; productivity.

## Introduction

Tartary buckwheat (*Fagopyrum tataricum* Gaertn) is a dicotyledonous cereal belonging to the Polygonaceae, which originated in eastern Tibet or northwestern Yunnan in China and is grown only in Asia, Europe, and North America (Adachi *et al.* 1989, Li *et al.* 2010). It has been praised as one kind of green food material and medicinal plant because of the health-promoting properties of its grains. Tartary buckwheat grains contain a variety of nutrients, the main compounds being: rutin, polyphenols, proteins, polysaccharides, dietary fibre, lipids, microelements, and macroelements (Kim *et al.* 2004, Christa and Soral-Šmietana 2008). Tartary buckwheat is cited as a plant of origin for rutin, which is a kind of flavonol glycoside compound used in preventing edema, haemorrhagic diseases, and stabilizing blood pressure due to its effectiveness in controlling blood vessel (Havsteen

1983, Kim *et al.* 2004).

Nitrogen (N) is one of the most important nutrients for crop production because it affects dry matter production by influencing leaf area development and maintenance as well as photosynthetic efficiency (Dordas and Sioulas 2008). N deficiency reduces the radiation interception, radiation-use efficiency, dry matter partitioning to reproductive organs, and leaf area index (Shangguan *et al.* 2000, Dordas and Sioulas 2008). Phosphorus (P) is closely related to photosynthetic carbon metabolism in plants, and P deficiency can result in restriction of the photo-phosphorylation process, decreases in Rubisco activity, ribulose-1,5-bisphosphate (RuBP) regeneration rates, and photosynthetic rates in leaves (Liu *et al.* 2010). Potassium (K) is involved in many physiological processes, plays an important role in stomatal regulation, and K deficiency

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*Abbreviations:* A1 – NPK 1:1:1; A2 – NPK 1:4:2; A3 – NPK 1:2:3; B1 – 300 kg (NPK) ha<sup>-1</sup>; B2 – 450 kg (NPK) ha<sup>-1</sup>; B3 – 600 kg (NPK) ha<sup>-1</sup>; Chl – chlorophyll;  $C_i$  – intercellular CO<sub>2</sub> concentration;  $E$  – transpiration rate;  $g_s$  – stomatal conductance; LAI – leaf area index;  $L_s$  – stomatal limitation value;  $P_N$  – net photosynthetic rate; WUE – water-use efficiency.

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decreases the photosynthetic carbon metabolism and the consumption of fixed carbon resources (Tsonev *et al.* 2011, Aslam *et al.* 2014). Photosynthesis is the main driving force influencing dry matter partitioning and organ formation, and it is the basis of plant production (Iqbal *et al.* 2011, Zlatev and Lidon 2012). However, fertilizers, such as N, P, and K, which regulate the capacity of plants to utilize photosynthates, are the major determinants of potential productivity (Shen and Li 2011, Xu *et al.* 2013).

Most of previous studies on fertilization have focused on the yield and quality of tartary buckwheat. Li *et al.* (2006) concluded that the grain yield of tartary buckwheat increased significantly by applying both manure and chemical fertilizers, and the optimum combination for fertilizers is one part of manure with one part of chemical

fertilizer. Zhang *et al.* (2008) reported that fertilizer ratio had significant effects on the growth and yield of tartary buckwheat, and the optimum fertilizer ratio is NPK 1:2:3. Besides, Zhao *et al.* (2012) found that the yield and quality of tartary buckwheat increased and then decreased with the increase of fertilization. However, little is known about application of fertilizer on the physiological characteristics of tartary buckwheat, especially regarding leaf photosynthetic characteristics. Therefore, we tried to elucidate the effects of fertilization on leaf photosynthetic characteristics and grain yield in tartary buckwheat Yunqiao 1. The results could provide a theoretical basis for improvement of the leaf photosynthetic capacity in order to increase the grain yield and improve fertilization strategy in tartary buckwheat production.

## Materials and methods

**Site description:** The field experiments were carried out in 2013 and 2014 at the Xiema Experimental Station (19°51'N, 106°37'E) of Southwest University, Beibei, Chongqing, China. The station is located 10 km south of Southwest University at 350 m a. s. l., and the area is classified as having a subtropical monsoon climate. The soil was a sandy loam, and the 0–100 mm soil layer contained 12.6 g(organic matter) kg<sup>-1</sup>, 73.2 mg(available N) kg<sup>-1</sup>, 21 mg(available P) kg<sup>-1</sup>, 106 mg(available K) kg<sup>-1</sup>, 0.84 g(total N) kg<sup>-1</sup>, 0.46 g(total P) kg<sup>-1</sup>, and 17.5 g(total K) kg<sup>-1</sup>(soil). The pH value of the soil was 5.8.

**Experimental design:** The experiment was a split-plot factorial, on the basis of randomized complete block design with three replications. The main plots represented a fertilizer of three ratios: NPK 1:1:1 (A1), NPK 1:4:2 (A2), and NPK 1:2:3 (A3). The subplots were fertilizer rates with three concentrations: 300 (B1), 450 (B2), and 600 (B3) kg ha<sup>-1</sup>. N fertilizer (urea, contained 46% N) was obtained from *Sichuan Lutianhua Co., Ltd.* (Luzhou, Sichuan Province, China). P fertilizer (calcium superphosphate, contained 12% P<sub>2</sub>O<sub>5</sub>) was obtained from *Sinochem Chongqing Fuling Chemical Co., Ltd.* (Fuling, Chongqing, China). K fertilizer (potassium sulfate, contained 53% K<sub>2</sub>O) was obtained from *National Investment Xinjiang Lop Nor potassium Co., Ltd.* (Hami, Xinjiang Province, China). All fertilizers were mixed and then applied as a basal fertilizer, when the seeds were sown, and the details of treatments were presented in the table below. Yunqiao 1, a tartary buckwheat cultivar, with a high photosynthetic capacity (Wang *et al.* 2013) and widely cultivated in local production, was used in the experiment. Tartary buckwheat seeds were obtained from *College of Agronomy and Biotechnology, Southwest University* (Beibei, Chongqing, China). Sowing was performed on 28 August 2013 and 25 August 2014, respectively, and subsequently thinned at the four-leaf stage to a uniform density of 900,000 plants ha<sup>-1</sup>. The plot size was 5 m long and 2 m wide with row spacing of

approximately 33 cm, and consisted of six rows. There were two board rows around each plot, and plants in these rows were not included for any sampling.

Treatment	N [kg ha <sup>-1</sup> ]	P <sub>2</sub> O <sub>5</sub> [kg ha <sup>-1</sup> ]	K <sub>2</sub> O [kg ha <sup>-1</sup> ]
A1 × B1	100	100	100
A1 × B2	150	150	150
A1 × B3	200	200	200
A2 × B1	43	171	86
A2 × B2	64	257	129
A2 × B3	86	343	171
A3 × B1	50	100	150
A3 × B2	75	150	225
A3 × B3	100	200	300

**Indicators and measuring method:** At the anthesis stage (coexistent stage of vegetative growth and reproductive growth) of tartary buckwheat, ten plants in each plot were selected and the third functional or fully expanded leaf from the top of plants were labeled with red thread, in order to investigate the photosynthetic parameters, chlorophyll (Chl) content (SPAD value), and leaf area index. The photosynthetic parameters were measured with *LI-6400* photosynthesis system (*Li-Cor Inc.*, Lincoln, USA) during 09:00–11:00 h on sunny day once a year on 15 October 2013 and 12 October 2014. Light intensity, temperature, CO<sub>2</sub> concentration, flow rate, and relative humidity were maintained at 1,000 μmol(photon) m<sup>-2</sup> s<sup>-1</sup>, 30°C, 380 μmol mol<sup>-1</sup>, 500 mL min<sup>-1</sup>, and 70%, respectively. The net photosynthetic rate ( $P_N$ ), stomatal conductance ( $g_s$ ), transpiration rate ( $E$ ), and intercellular CO<sub>2</sub> concentration ( $C_i$ ) were automatically recorded. Water-use efficiency (WUE) was calculated as  $P_N/E$  (Ou *et al.* 2015). Stomatal limitation value ( $L_s$ ) was calculated according to the methods of Farquhar and Sharkey (1982). The PAR was measured with a quantum sensor *LI-190* (*Li-Cor Inc.*, Lincoln, USA) according to the method of Su *et al.* (2014). At the same time, ten labeled leaves in each plot

were chosen to measure the Chl content (SPAD value) with a *Minolta SPAD-502* Chl meter (*Minolta*, Japan) according to the method of Abdelhamid *et al.* (2003). The measurement was done five times for each leaf, and the mean was calculated as the SPAD value of the given leaf. Next, the total leaf area of each labeled plant was measured with disc method according to the description of Tao and Lin (2006). The leaf area index (LAI) was calculated as total leaf area of one plant ( $\text{m}^2$  per plant)  $\times$  plant density ( $\text{plant m}^{-2}$ ) (Su *et al.* 2014). Plants were hand-harvested when 70–80% of total seeds changed their color from green to black. For the grain yield, seeds were air-dried for two weeks before measurement.

## Results

**Grain yield:** As shown by *ANOVA* (Table 1), the fertilizer ratio, fertilizer rate, and their interaction significantly affected the grain yield. There were significant differences in the grain yield between the fertilizer ratio treatments, and the maximum value appeared in the A2 treatment (Table 2). Compared with the A1 and A3 treatments, the A2 treatment significantly increased the grain yield by 25.3% and 10.3%, respectively. The grain yield increased and then decreased with the increase of fertilizer rate, and the maximum value appeared in the B2 treatment (Table 2). Compared to the B1 and B3 treatments, the B2 treatment significantly increased the grain yield by 11.6% and 11.5%, respectively. Interactions between fertilizer ratio and fertilizer rate (Table 3) showed that the A2B2 treatment had the highest grain yield.

**$P_N$ :** The fertilizer ratio, fertilizer rate, and their interaction had significant effects on the leaf  $P_N$  (Table 1). There were significant differences in  $P_N$  between the fertilizer ratio treatments, and the maximum value appeared in the A2 treatment (Table 2). Compared with the A1 and A3 treatments, the A2 treatment significantly increased the  $P_N$  by 30.2% and 15.1%, respectively. The  $P_N$  increased and

**Statistical analysis:** The differences in data between the two years were not significant ( $P>0.05$ ). Therefore, for all indicators, mean data of the two years were presented. Analysis of variance (one-way *ANOVA*) was performed with *SPSS 19.0* software (*SPSS Institute Inc.*, Chicago, USA), and data from each sampling data were analyzed separately. Means were tested by least significant difference at the  $P<0.05$  level ( $LSD_{0.05}$ ). Linear regression was performed by *SigmaPlot 10.0* (*Aspire Software Intl.*, Ashburn, USA) to identify the relationship between the grain yield and photosynthetic characteristics of tartary buckwheat.

then decreased with the increase of fertilizer rate, and the maximum value appeared in the B2 treatment (Table 2). Compared with the B1 and B3 treatments, the B2 treatment significantly increased the  $P_N$  by 33.1% and 14.0%, respectively. Interaction between fertilizer ratio and fertilizer rate (Table 3) showed that the A2B2 treatment exhibited the highest  $P_N$ .

**$g_s$ :** The fertilizer ratio and fertilizer rate had significant effects on the  $g_s$ , but their interaction had no significant effect (Table 1). There were significant differences in  $g_s$  between the fertilizer ratio treatments, and the maximum value was reached in the A2 treatment (Table 2). Compared to the A1 and A3 treatments, the A2 treatment significantly increased the  $g_s$  by 30.8% and 17.2%, respectively. The  $g_s$  increased and then decreased with the increase of the fertilizer rate, and the maximum value was found in the B2 treatment (Table 2). Compared to the B1 and B3 treatments, the B2 treatment significantly increased the  $g_s$  by 115.8% and 41.4%, respectively. Interaction between fertilizer ratio and fertilizer rate (Table 3) showed that the A2B2 treatment had the highest  $g_s$ .

Table 1. Analysis of variance (*ANOVA*) for the effects of fertilization on the grain yield, net photosynthetic rate ( $P_N$ ), stomatal conductance ( $g_s$ ), intercellular  $\text{CO}_2$  concentration ( $C_i$ ), transpiration rate ( $E$ ), photosynthetically active radiation (PAR), water-use efficiency (WUE), stomatal limitation value ( $L_s$ ), chlorophyll content (SPAD value), and leaf area index (LAI) in tartary buckwheat ( $F$ -value). A, B, and A $\times$ B represent fertilizer ratio, fertilizer rate, and the interaction between fertilizer ratio and fertilizer rate. \* and \*\* – significant at  $P<0.05$  and  $P<0.01$ , respectively; ns – not significant.

Variation source	Degree of freedom	Grain yield	$P_N$	$g_s$	$C_i$	$E$	PAR	WUE	$L_s$	SPAD	LAI
A	2	83.3**	139.8**	44.6**	22.7**	35.3**	3.0 <sup>ns</sup>	1.7 <sup>ns</sup>	37.3**	43.2**	87.1**
B	2	24.3**	188.4**	193.5**	8.6**	171.9**	7.3**	125.8**	89.7**	212.8**	224.7**
A $\times$ B	4	10.1**	6.8**	3.1 <sup>ns</sup>	0.3 <sup>ns</sup>	9.2**	0.3 <sup>ns</sup>	6.6*	3.4 <sup>ns</sup>	2.9 <sup>ns</sup>	15.2**

Table 2. Effects of fertilization on the grain yield, net photosynthetic rate ( $P_N$ ), stomatal conductance ( $g_s$ ), intercellular  $CO_2$  concentration ( $C_i$ ), transpiration rate ( $E$ ), photosynthetically active radiation (PAR), water-use efficiency (WUE), stomatal limitation value ( $L_s$ ), chlorophyll content (SPAD value), and leaf area index (LAI) in tartary buckwheat. Data are expressed as the mean  $\pm$  standard error ( $n = 3$ ). Values followed by *different letters* a column are significantly different ( $P < 0.05$ ). A1, A2 and A3 represent NPK 1:1:1, 1:4:2 and 1:2:3 fertilizer, respectively. B1, B2 and B3 represent fertilizer rate at three levels of 300, 450 and 600 kg ha $^{-1}$ , respectively.

Treatment	Grain yield [kg ha $^{-1}$ ]	$P_N$ [ $\mu\text{mol m}^{-2} \text{s}^{-1}$ ]	$g_s$ [ $\text{mol m}^{-2} \text{s}^{-1}$ ]	$C_i$ [ $\mu\text{mol mol}^{-1}$ ]	$E$ [ $\text{mmol m}^{-2} \text{s}^{-1}$ ]	PAR [ $\mu\text{mol m}^{-2} \text{s}^{-1}$ ]	WUE [%]	$L_s$	SPAD	LAI
A1	1,532 $\pm$ 14.76 <sup>c</sup>	9.6 $\pm$ 0.57 <sup>c</sup>	0.26 $\pm$ 0.02 <sup>c</sup>	337 $\pm$ 15.0 <sup>a</sup>	2.32 $\pm$ 0.33 <sup>c</sup>	976 $\pm$ 10.24 <sup>a</sup>	0.42 $\pm$ 0.03 <sup>a</sup>	0.30 $\pm$ 0.03 <sup>c</sup>	45.6 $\pm$ 5.36 <sup>c</sup>	0.75 $\pm$ 0.06 <sup>c</sup>
A2	1,919 $\pm$ 12.31 <sup>a</sup>	12.5 $\pm$ 0.83 <sup>a</sup>	0.34 $\pm$ 0.05 <sup>a</sup>	317 $\pm$ 13.3 <sup>b</sup>	3.24 $\pm$ 0.24 <sup>a</sup>	1,005 $\pm$ 15.41 <sup>a</sup>	0.40 $\pm$ 0.04 <sup>a</sup>	0.44 $\pm$ 0.04 <sup>a</sup>	55.3 $\pm$ 4.29 <sup>a</sup>	0.99 $\pm$ 0.08 <sup>a</sup>
A3	1,740 $\pm$ 15.45 <sup>b</sup>	10.8 $\pm$ 0.45 <sup>b</sup>	0.29 $\pm$ 0.03 <sup>b</sup>	325 $\pm$ 14.4 <sup>b</sup>	2.79 $\pm$ 0.18 <sup>b</sup>	991 $\pm$ 18.13 <sup>a</sup>	0.41 $\pm$ 0.06 <sup>a</sup>	0.38 $\pm$ 0.02 <sup>b</sup>	50.7 $\pm$ 6.74 <sup>b</sup>	0.89 $\pm$ 0.05 <sup>b</sup>
B1	1,666 $\pm$ 18.65 <sup>b</sup>	9.4 $\pm$ 0.32 <sup>c</sup>	0.19 $\pm$ 0.01 <sup>c</sup>	336 $\pm$ 13.8 <sup>a</sup>	2.02 $\pm$ 0.16 <sup>c</sup>	975 $\pm$ 10.35 <sup>b</sup>	0.47 $\pm$ 0.02 <sup>a</sup>	0.27 $\pm$ 0.05 <sup>c</sup>	35.4 $\pm$ 3.95 <sup>c</sup>	0.74 $\pm$ 0.03 <sup>c</sup>
B2	1,859 $\pm$ 14.29 <sup>a</sup>	12.5 $\pm$ 0.94 <sup>a</sup>	0.41 $\pm$ 0.04 <sup>a</sup>	317 $\pm$ 10.9 <sup>b</sup>	3.62 $\pm$ 0.56 <sup>a</sup>	1,010 $\pm$ 13.42 <sup>a</sup>	0.35 $\pm$ 0.03 <sup>c</sup>	0.46 $\pm$ 0.07 <sup>a</sup>	67.7 $\pm$ 4.87 <sup>a</sup>	1.02 $\pm$ 0.14 <sup>a</sup>
B3	1,667 $\pm$ 13.47 <sup>b</sup>	11.0 $\pm$ 0.79 <sup>b</sup>	0.29 $\pm$ 0.03 <sup>b</sup>	326 $\pm$ 17.1 <sup>ab</sup>	2.72 $\pm$ 0.28 <sup>b</sup>	886 $\pm$ 12.35 <sup>b</sup>	0.41 $\pm$ 0.05 <sup>b</sup>	0.38 $\pm$ 0.04 <sup>b</sup>	48.6 $\pm$ 5.86 <sup>b</sup>	0.86 $\pm$ 0.07 <sup>b</sup>

Table 3. Effects of interaction between fertilizer ratio and fertilizer rate on the grain yield, net photosynthetic rate ( $P_N$ ), stomatal conductance ( $g_s$ ), intercellular  $CO_2$  concentration ( $C_i$ ), transpiration rate ( $E$ ), photosynthetically active radiation (PAR), water-use efficiency (WUE), stomatal limitation value ( $L_s$ ), chlorophyll content (SPAD value), and leaf area index (LAI) in tartary buckwheat. Data are expressed as the mean  $\pm$  standard error ( $n = 3$ ). Values followed by *different letters* a column are significantly different ( $P < 0.05$ ). A1, A2 and A3 represent NPK 1:1:1, 1:4:2 and 1:2:3 fertilizer, respectively. B1, B2 and B3 represent fertilizer rate at three levels of 300, 450 and 600 kg ha $^{-1}$ , respectively.

Treatments	Grain yield [kg ha $^{-1}$ ]	$P_N$ [ $\mu\text{mol m}^{-2} \text{s}^{-1}$ ]	$g_s$ [ $\text{mol m}^{-2} \text{s}^{-1}$ ]	$C_i$ [ $\mu\text{mol mol}^{-1}$ ]	$E$ [ $\text{mmol m}^{-2} \text{s}^{-1}$ ]	PAR [ $\mu\text{mol m}^{-2} \text{s}^{-1}$ ]	WUE [%]	$L_s$	SPAD	LAI
A1 $\times$ B1	1,306 $\pm$ 11.56 <sup>e</sup>	8.4 $\pm$ 0.15 <sup>e</sup>	0.16 $\pm$ 0.01 <sup>f</sup>	346 $\pm$ 17.3 <sup>a</sup>	1.82 $\pm$ 0.15 <sup>g</sup>	964 $\pm$ 13.05 <sup>c</sup>	0.46 $\pm$ 0.05 <sup>a</sup>	0.17 $\pm$ 0.02 <sup>f</sup>	31.1 $\pm$ 1.29 <sup>f</sup>	0.66 $\pm$ 0.06 <sup>f</sup>
A1 $\times$ B2	1,738 $\pm$ 11.32 <sup>c</sup>	10.6 $\pm$ 0.33 <sup>c</sup>	0.35 $\pm$ 0.02 <sup>c</sup>	324 $\pm$ 12.4 <sup>cd</sup>	2.79 $\pm$ 0.58 <sup>d</sup>	989 $\pm$ 16.42 <sup>bc</sup>	0.38 $\pm$ 0.01 <sup>c</sup>	0.41 $\pm$ 0.04 <sup>bc</sup>	59.2 $\pm$ 6.37 <sup>c</sup>	0.81 $\pm$ 0.06 <sup>d</sup>
A1 $\times$ B3	1,553 $\pm$ 12.12 <sup>d</sup>	9.8 $\pm$ 0.03 <sup>d</sup>	0.27 $\pm$ 0.05 <sup>d</sup>	340 $\pm$ 10.5 <sup>ab</sup>	2.36 $\pm$ 0.47 <sup>ef</sup>	974 $\pm$ 11.81 <sup>bc</sup>	0.41 $\pm$ 0.02 <sup>b</sup>	0.32 $\pm$ 0.05 <sup>de</sup>	46.6 $\pm$ 3.38 <sup>d</sup>	0.76 $\pm$ 0.07 <sup>e</sup>
A2 $\times$ B1	1,906 $\pm$ 8.53 <sup>ab</sup>	10.5 $\pm$ 0.28 <sup>e</sup>	0.23 $\pm$ 0.02 <sup>e</sup>	326 $\pm$ 13.2 <sup>bcd</sup>	2.18 $\pm$ 0.12 <sup>f</sup>	986 $\pm$ 14.64 <sup>bc</sup>	0.48 $\pm$ 0.04 <sup>a</sup>	0.35 $\pm$ 0.03 <sup>de</sup>	39.8 $\pm$ 4.29 <sup>e</sup>	0.85 $\pm$ 0.05 <sup>d</sup>
A2 $\times$ B2	2,017 $\pm$ 18.53 <sup>a</sup>	14.7 $\pm$ 0.15 <sup>a</sup>	0.49 $\pm$ 0.05 <sup>a</sup>	310 $\pm$ 11.5 <sup>d</sup>	4.32 $\pm$ 0.59 <sup>a</sup>	1,033 $\pm$ 13.47 <sup>a</sup>	0.33 $\pm$ 0.03 <sup>d</sup>	0.52 $\pm$ 0.07 <sup>a</sup>	75.9 $\pm$ 8.36 <sup>a</sup>	1.15 $\pm$ 0.14 <sup>a</sup>
A2 $\times$ B3	1,835 $\pm$ 19.78 <sup>bc</sup>	12.3 $\pm$ 0.22 <sup>b</sup>	0.32 $\pm$ 0.01 <sup>c</sup>	316 $\pm$ 13.3 <sup>d</sup>	3.23 $\pm$ 0.12 <sup>c</sup>	996 $\pm$ 15.38 <sup>abc</sup>	0.38 $\pm$ 0.02 <sup>c</sup>	0.44 $\pm$ 0.02 <sup>b</sup>	50.3 $\pm$ 1.73 <sup>d</sup>	0.96 $\pm$ 0.08 <sup>e</sup>
A3 $\times$ B1	1,785 $\pm$ 13.43 <sup>c</sup>	9.4 $\pm$ 0.10 <sup>d</sup>	0.19 $\pm$ 0.01 <sup>ef</sup>	335 $\pm$ 14.8 <sup>bce</sup>	2.06 $\pm$ 0.33 <sup>fg</sup>	976 $\pm$ 13.45 <sup>bc</sup>	0.46 $\pm$ 0.03 <sup>a</sup>	0.30 $\pm$ 0.08 <sup>e</sup>	35.2 $\pm$ 6.38 <sup>ef</sup>	0.71 $\pm$ 0.04 <sup>ef</sup>
A3 $\times$ B2	1,821 $\pm$ 16.34 <sup>bc</sup>	12.2 $\pm$ 0.34 <sup>b</sup>	0.39 $\pm$ 0.03 <sup>b</sup>	316 $\pm$ 12.5 <sup>d</sup>	3.74 $\pm$ 0.26 <sup>b</sup>	1,009 $\pm$ 15.94 <sup>ab</sup>	0.34 $\pm$ 0.01 <sup>d</sup>	0.46 $\pm$ 0.01 <sup>b</sup>	68.0 $\pm$ 2.15 <sup>b</sup>	1.09 $\pm$ 0.05 <sup>b</sup>
A3 $\times$ B3	1,614 $\pm$ 8.63 <sup>d</sup>	10.9 $\pm$ 0.85 <sup>c</sup>	0.28 $\pm$ 0.02 <sup>d</sup>	322 $\pm$ 14.6 <sup>cd</sup>	2.56 $\pm$ 0.55 <sup>de</sup>	988 $\pm$ 10.59 <sup>bc</sup>	0.42 $\pm$ 0.06 <sup>b</sup>	0.37 $\pm$ 0.04 <sup>cd</sup>	48.8 $\pm$ 3.64 <sup>d</sup>	0.86 $\pm$ 0.07 <sup>d</sup>

**$C_i$ :** The fertilizer ratio and fertilizer rate significantly affected the  $C_i$ , but their interaction had no significant effect (Table 1). There were differences in  $C_i$  between the fertilizer ratio treatments, and the minimum value appeared in the A2 treatment (Table 2). Compared to the A1 and A3 treatments, the A2 treatment decreased the  $C_i$  by 5.8% and 2.2%, respectively. The  $C_i$  declined and then was elevated with the increase of fertilizer rate, and the minimum value appeared in the B2 treatment (Table 2). Compared to the B1 and B3 treatments, the B2 treatment decreased the  $C_i$  by 5.6% and 2.8%, respectively. Interaction between fertilizer ratio and fertilizer rate (Table 3) showed that the A2B2 treatment exhibited the lowest  $C_i$ .

**$E$ :** The fertilizer ratio, fertilizer rate, and their interaction had significant effects on the  $E$  (Table 1). Significant differences in  $E$  were found between the fertilizer ratio treatments with the maximum value in the A2 treatment (Table 2). Compared to the A1 and A3 treatments, the A2 treatment increased significantly the  $E$  by 39.7% and 16.1%, respectively. The  $E$  increased and then decreased with the increase of fertilizer rate, and the maximum value appeared in the B2 treatment (Table 2). Compared to the B1 and B3 treatments, the B2 treatment significantly increased the  $E$  by 79.2% and 33.1%, respectively. Interaction between fertilizer ratio and fertilizer rate (Table 3) showed that the the highest  $E$  was found after the A2B2 treatment.

**PAR:** As shown by *ANOVA* (Table 1), fertilizer rate had significant effect on the PAR at the third functional or fully expanded leaf from the top of plants, but fertilizer ratio and their interaction had no significant effects. No differences in PAR were observed between the fertilizer ratio treatments, and the maximum value was found after the A2 treatment (Table 2). Compared to the A1 and A3 treatments, the A2 treatment increased the PAR by 2.97% and 1.4%, respectively. The PAR increased and then decreased with the increase of fertilizer rate, and the maximum value appeared in the B2 treatment (Table 2). Compared with the B1 and B3 treatments, PAR after the B2 treatment significantly increased by 3.6% and 2.4%, respectively. Interaction between fertilizer ratio and fertilizer rate (Table 3) showed that the A2B2 treatment had the highest PAR.

**WUE:** The fertilizer ratio had no significant effect on the WUE, while the fertilizer rate and their interaction significantly affected the WUE (Table 1). There were no significant differences in WUE between fertilizer ratio treatments. The WUE decreased and then increased with

the increase of the fertilizer rate, and the minimum value appeared in the B2 treatment (Table 2). Compared to the B1 and B3 treatments, the B2 treatment significantly decreased the WUE by 25.5% and 14.6%, respectively. Interaction between fertilizer ratio and fertilizer rate (Table 3) showed that the A2B2 treatment had the lowest WUE.

**$L_s$ :** The fertilizer ratio and fertilizer rate had significant effects on the  $L_s$  of leaf, but their interaction had no significant effect (Table 1). There were significant differences in  $L_s$  between fertilizer ratio treatments, and the maximum value was reached in the A2 treatment (Table 2). Compared to the A1 and A3 treatments, the A2 treatment significantly increased the  $L_s$  by 46.7% and 15.8%, respectively. The  $L_s$  increased and then decreased with the increase of fertilizer rate, and the maximum value appeared in the B2 treatment (Table 2). Compared to the B1 and B3 treatments, the B2 treatment significantly increased the  $L_s$  by 70.37% and 21.05%, respectively. Interaction between fertilizer ratio and fertilizer rate (Table 3) showed that the A2B2 treatment had the highest  $L_s$ .

**SPAD:** The fertilizer ratio and fertilizer rate influenced significantly SPAD, while their interaction had no significant effect (Table 1). Similarly as in previous characteristics the maximum value was found in the A2 treatment (Table 2). Compared to the A1 and A3 treatments, the A2 treatment significantly increased the SPAD by 21.2% and 9.2%, respectively. The SPAD increased and then decreased with the increase of fertilizer rate, and the maximum value appeared in the B2 treatment (Table 2). Compared with the B1 and B3 treatments, the B2 treatment significantly increased the SPAD by 91.4% and 39.4%, respectively. Interaction between fertilizer ratio and fertilizer rate (Table 3) proved that the A2B2 treatment showed the highest SPAD.

**LAI:** The fertilizer ratio, fertilizer rate, and their interaction significantly affected the LAI (Table 1). There were significant differences in LAI among fertilizer ratio treatments, and the maximum value appeared in the A2 treatment (Table 2). The A2 treatment significantly increased the LAI by 32.0% and 11.2% in comparison to A1 and A3, respectively. The LAI increased and then decreased with the increase of fertilizer rate, and the maximum value appeared in the B2 treatment (Table 2). Compared with the B1 and B3 treatments, the B2 treatment significantly increased the LAI by 37.8% and 18.6%, respectively. Interaction between fertilizer ratio and fertilizer rate (Table 3) showed that the A2B2 treatment had the highest LAI.

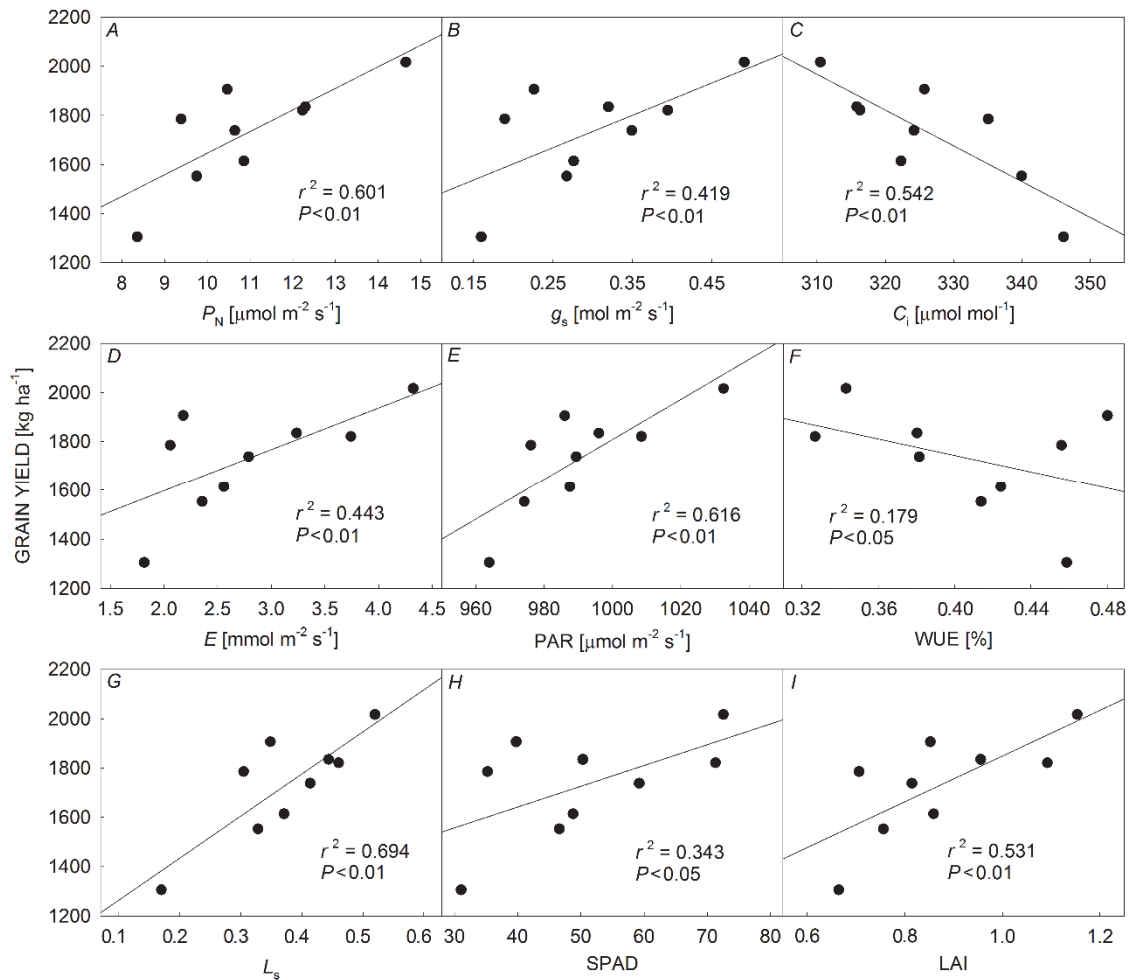


Fig. 1. Relationships of grain yield to the (A) net photosynthetic rate ( $P_N$ ), (B) stomatal conductance ( $g_s$ ), (C) intercellular  $\text{CO}_2$  concentration ( $C_i$ ), (D) transpiration rate ( $E$ ), (E) photosynthetically active radiation (PAR), (F) water-use efficiency (WUE), (G) stomatal limitation value ( $L_s$ ), (H) chlorophyll content (SPAD value), and (I) leaf area index (LAI) in tartary buckwheat.  $n = 9$ .

**Relationship between grain yield and photosynthetic characteristics:** Remarkable positive correlations were found between the grain yield and  $P_N$  (Fig. 1A),  $g_s$  (Fig. 1B),  $E$  (Fig. 1D), PAR (Fig. 1E),  $L_s$  (Fig. 1G), SPAD

(Fig. 1H), and LAI (Fig. 1I). Correlation analysis also demonstrated that the grain yield was significantly and negatively correlated with  $C_i$  (Fig. 1C) and WUE (Fig. 1F).

## Discussion

The photosynthetic capacity of leaf and LAI are key factors to determine tartary buckwheat yield.  $P_N$  directly indicates the photosynthetic capacity of single leaf (Jiang *et al.* 2004). SPAD is the greenness of a leaf, and represent the content of photosynthetic pigments (Ommen *et al.* 1999, Rajcan *et al.* 1999). LAI is the canopy photosynthetic area (Jiang *et al.* 2004).  $L_s$  is an important indicator to evaluate the limiting effects of stomatal factors on the  $P_N$  (Farquhar and Sharkey 1982).  $E$  is often used to reflect the intensity of transpiration in plants,  $g_s$  to denote the extent of opening of stomata, and  $C_i$  to indicate the assimilation ability of mesophyll cells for  $\text{CO}_2$  in plants,

which are important indices to describe the photosynthesis of plants and have close relationships with  $P_N$  (Liu *et al.* 2010). PAR and WUE are important eco-physiological factors affecting  $P_N$  in the field-grown condition (Li *et al.* 2002). As a result, these nine parameters showed significant relationship to tartary buckwheat yield (Fig. 1). Therefore, our results showed that these nine parameters could be used to evaluate the production potential of tartary buckwheat.

The photosynthetic capacity of leaf is closely related to N, P, and K fertilizers (Wang *et al.* 2008). Various works have reported that N, P, and K fertilizers in excess or

deficient could reduce the photosynthetic capacity (Ashraf *et al.* 2001, Wu *et al.* 2002, Cechin and Fumis 2004). In the present study, the  $P_N$ ,  $g_s$ ,  $E$ , PAR,  $L_s$ , SPAD, and LAI increased and then decreased with the increase of fertilizer rate, while the  $C_i$  and WUE decreased and then increased with the increase of fertilizer rate. Furthermore, our results indicated that there were significant differences in the grain yield and photosynthetic capacity of leaf between fertilizer ratio treatments, and their maximum values appeared in the A2 (NPK 1:4:2) treatment. In this treatment, tartary buckwheat showed a high capacity to take P from soil, which is in agreement with Zhu *et al.* (2002). Previous researchers have reported that the decrease of  $C_i$  and increase of  $L_s$  implies that reduction of stomatal conductance was responsible for the decrease of  $P_N$ , while the increase of  $C_i$  and decrease of  $L_s$  indicates that the decline of photosynthetic activity of mesophyll cells could account for the decrease of  $P_N$  (Farquhar and Sharkey 1982, Guan *et al.* 1995, Xu 1997, Liu *et al.* 2010). In our study, fertilization had significant effects on the  $P_N$ ,  $C_i$ , and  $L_s$  of tartary buckwheat, and the low concentration of fertilizers (A1B1 treatment) resulted in declines of  $P_N$  and  $L_s$ , and the increase of  $C_i$ . These results imply that the reduction of  $P_N$  at low nutrient level was due to nonstomatal factors, such as the decrease of photosynthetic activity of mesophyll cells.

The availability of sufficient fertilizer is essential to increase the production of tartary buckwheat. In the

present study, the leaf photosynthetic capacity and grain yield of tartary buckwheat increased and then decreased with the increase of fertilizer ratio and rate, and their maximum values appeared in the A2B2 (450 kg ha<sup>-1</sup> with NPK 1:4:2) treatment. Two reasons could be responsible for the decrease of leaf photosynthetic capacity and grain yield in this study. The first was the weak irradiance to the leaf by shading due to leaf development promoted by abundant fertilizer (Hight *et al.* 1968, Marchiori *et al.* 2014). The second was that tartary buckwheat grew excessively tall when fertilizer was abundant and became susceptible to lodging (Okuno *et al.* 2014, Wang *et al.* 2015). Therefore, appropriate fertilization strategy should be chosen in the production of tartary buckwheat. Based on the present results, we suggested that fertilization had significant effects on the leaf photosynthetic capacity and grain yield of tartary buckwheat, and the best fertilization strategy was 450 kg ha<sup>-1</sup> with NPK 1:4:2. The present findings were considered to be valuable for improvement of productivity of tartary buckwheat.

**Conclusion:** Fertilization had significant effects on the leaf photosynthetic capacity and grain yield of tartary buckwheat Yunqiao 1. The leaf photosynthetic capacity and grain yield of Yunqiao 1 increased and then decreased with the increase of fertilization, and the best fertilization strategy was 450 kg ha<sup>-1</sup> with NPK 1:4:2.

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