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Effects of nitrogen fertilizer and planting density on the leaf photosynthetic characteristics, agronomic traits and grain yield in common buckwheat (*Fagopyrum esculentum* M.)

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ABSTRACT

Nitrogen fertilizer and planting density are two crucial factors that affect the yield of common buckwheat (*Fagopyrum esculentum* M.). Youqiao2, a common buckwheat cultivar with high photosynthetic capacity and planted widely in local production was used to investigate the effects of nitrogen fertilizer and planting density on the leaf photosynthetic characteristics, agronomic traits, and grain yield in common buckwheat by a split plot design. The main plots were assigned to three nitrogen fertilizer rates: 0, 45, and 90 kg ha^{-1}, and the subplots were assigned to three planting densities: 60, 90, and 120 plants m^{-2}. Results showed that the grain yield was significantly and positively correlated with net photosynthesis rate (P_n), stomatal conductance (G_s), transpiration rate (T_r), stomatal limitation value (L_s), chlorophyll content (SPAD value), leaf area index (LAI), plant height, stem diameter, branch number, internode number, grain number per plant, and 1000-grain weight, while significantly and negatively correlated with intercellular CO_2 concentration (C_i) and water-use efficiency (WUE). The P_n, G_s, T_r, L_s, SPAD, LAI, grain yield, stem diameter, branch number, internode number, grain number per plant, and 1000-grain weight increased and then decreased with the increase of nitrogen fertilizer and planting density, and their maximum values appeared in the nitrogen fertilization of 45 kg ha^{-1} and planting density of 90 plants m^{-2} treatment. The C_i and WUE decreased and then increased with the increase of nitrogen fertilizer and planting density, and their minimum values appeared in the nitrogen fertilization of 45 kg ha^{-1} and planting density of 90 plants m^{-2} treatment. The plant height increased with the increase of nitrogen fertilizer, while decreased with the increase of planting density. These results suggested that nitrogen fertilizer and planting density had significant effects on the leaf photosynthetic capacity, agronomic traits, and grain yield of common buckwheat, and the combination of nitrogen fertilization of 45 kg ha^{-1} and planting density of 90 plants m^{-2} is recommended for common buckwheat planting.

1. Introduction

Common buckwheat (*Fagopyrum esculentum* M.) belongs to the *Fagopyrum* of Polygonaceae, which origined from China (Yang et al., 2010). Common buckwheat is a popular traditional food in Asian countries, including China, Japan, and Korea. Recently, it has been widely grown and consumed in Europe, North America, and Canada (Tohgi et al., 2011). Common buckwheat is characterized by interesting nutritional properties, such as the presence of proteins of a high biological value, natural antioxidants, minerals, vitamins (especially those of the B group), and dietary fibre (Alamprese et al., 2007). It is a low-input plant and an important source of the antioxidant (Kreft, 1989; Kreft et al., 1999). Common buckwheat proteins may prevent gallstone formation more effectively than soy protein products, they may also

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retard mammary carcinogenesis by lowering serum estradiol, and suppress colon carcinogenesis by reducing cell proliferation (Tomotake et al., 2000; Liu et al., 2001). Besides, common buckwheat seeds are an important dietary source of Zn, Cu and Mn, and can be used as additives to improve the quality of bread or other products (Stibilj et al., 2004).

Nitrogen 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). As a key component of proteins (both structural and enzymatic) and nucleic acids, nitrogen affects organ formation, root-cap development, photosynthesis, carbon-nitrogen ratio, source-sink dynamics, and many other processes (Mi et al., 2007). Nitrogen fertilization decreases nitrogen release from the leaf after blossom, delays leaf senescence, maintains a higher photosynthetic rate, and provides carbohydrates for grain filling (Li et al., 2012). Lack of nitrogen inhibits crop growth and photosynthesis and is therefore a major cause of yield and quality decline in crops (Jin and He, 1999; Shangguan et al., 2000). In addition, nitrogen deficiency delays both vegetative and reproductive phonological development, reduces leaf emergence rate, yield, and yield components such as the number of heads per plant, the number of seeds per head, the single seed weight, and the number of seeds per plant (Jones and Tucker, 1968; Steer and Harrigan, 1986). Moreover, nitrogen deficiency reduces the radiation interception, radiation use efficiency, dry matter partitioning to reproductive organs, leaf area index, protein content of the plant, and the seed (Dordas and Sioulas, 2008). A closely positive correlation was found between the photosynthetic capacity of leaves and nitrogen fertilizer (Sage and Pearcy, 1987).

Planting density is an important crop management that affects the grain yield by regulating growth, yield components, and photosynthesis, which are the target traits closely related to the ideotype of crops (Peng et al., 2008; Ciampitti and Vyn, 2011). High planting density results in strong competition and also increases the potential for cooperation, thus creating a difference between individual and group performance that can be utilized (Ma et al., 2014). In general, the leaf is the photosynthetic source before anthesis, which receives less radiation due to the development of the plant canopy in high planting density, and results in the decrease of the leaf photosynthetic capacity and grain yield (Will et al., 2005; Dong et al., 2006; Liu et al., 2015). Thus, the optimization of planting density is an important step toward improving grain yield.

Most previous studies with nitrogen fertilizer and planting density have focused on the yield and yield components of common buckwheat (Li et al., 2006; Wan, 2008; Zhang et al., 2008; Wang et al., 2013). However, little is known about application of nitrogen fertilizer and planting density on the physiological characteristics of common buckwheat, especially regarding leaf photosynthetic characteristics. The objective of this study was to investigate the effects of nitrogen fertilizer and planting density on the leaf photosynthetic characteristics, agronomic traits, and grain yield in common buckwheat. A great understanding of that information would help characterize the photosynthetic and morphological mechanism for changed grain yield under nitrogen fertilizer in modern common buckwheat production systems.

2. Materials and methods

2.1. Experimental materials

Youqiao2, a common buckwheat cultivar with high photosynthetic capacity and high lodging-resistance (Wang et al., 2013a) that is planted widely in local production, was grown in field trials at the Xiema experimental station of Southwest University (19°51′N and 106°37′E, Beibei District, Chongqing Municipality, China) during 2014 and 2015. The station is located 10 km south of Southwest University at an elevation of 350 m above sea level, and the area has a subtropical monsoon climate. The meteorological data during the growing seasons of common buckwheat in 2014 and 2015 were presented in Fig. S1 in the online version at DOI: 10.1016/j.fcr.2018.02.001. The soil type was sandy loam with a pH of 5.6, and the 0–100 mm soil layer contained 14.2 g kg^{-1} organic matter, 84.3 mg kg^{-1} available N, 19 mg kg^{-1} available P, 114 mg kg^{-1} available K, 0.96 g kg^{-1} total N, 0.54 g kg^{-1} total P, and 16.9 g kg^{-1} total K. Common buckwheat seeds were obtained from College of Agronomy and Biotechnology, Southwest University (Beibei District, Chongqing Municipality, China). Nitrogen fertilizer (urea contained 46% N) was obtained from Sichuan Lutianhua Co., Ltd. (Naxi District, Luzhou City, Sichuan Province, China).

2.2. Experimental design

The experiments were conducted using a split plot design with three replicates, giving a total of 27 subplots. The main plots were assigned to three nitrogen fertilizer rates: 0 kg ha^{-1} (A1), 45 kg ha^{-1} (A2), and 90 kg ha^{-1} (A3), applied as urea with a basal fertilizer (Wang et al., 2015). Subplots were assigned to three planting densities: 60 plants m^{-2} (B1), 90 plants m^{-2} (B2), and 120 plants m^{-2} (B3) (Wang et al., 2015). The size of each subplot was 10 m^2 (5 m long, 2 m wide, 6 rows, 33 cm row spacing). Border rows were not included for any sampling. A 0.5 m wide buffer plot with plastic film buried to a depth 150 cm was arranged between neighboring nitrogen fertilizer rate plots to prevent nitrogen transport and root extension between plots. Phosphorus, as calcium superphosphate, and potassium, as potassium sulfate, were applied for each plot as basal fertilizer at rates of 45 kg ha^{-1} P_2O_5 and 67.5 kg ha^{-1} K_2O, respectively. Three nitrogen fertilizer rates: 0 kg ha^{-1} (A1), 45 kg ha^{-1} (A2), and 90 kg ha^{-1} (A3) were mixed to 45 kg ha^{-1} P_2O_5 and 67.5 kg ha^{-1} K_2O, respectively, and then evenly applied as basal fertilizer on the formed land when the seeds were sown. Sowing were performed on 28 August 2014 and 25 August 2015, respectively, and subsequently thinned at the four-leaf stage. A prophylactic programme of fungicides, insecticides, and herbicides was applied in both seasons to control disease, pests, or weed infestation. No significant incidence of disease, pests, or weeds and no effects on common buckwheat growth were observed in both seasons. Harvests were performed on 22 November 2014 and 15 November 2015.

2.3. Measurement of the leaf photosynthetic characteristics

At the beginning of anthesis stage (26 September 2014 and 24 September 2015), five plants in each plot were selected randomly and the third functional or fully expanded leaf from the top of plants were labeled with red thread, to investigate the gas exchange, chlorophyll content (SPAD value), and leaf area index. The gas exchange was measured with LI-6400 photosynthesis system (Li-Cor Inc., Lincoln, USA) during 9:00–11:00 a.m. on sunny day once a year on 6 October 2014 and 4 October 2015. Light intensity, temperature, CO_2 concentration, flow rate, and relative humidity were maintained at 1000 μmol m^{-2} s^{-1}, 30 °C, 380 μmol mol^{-1}, 500 mL min^{-1}, and 70% respectively. The net photosynthesis rate (P_n), stomatal conductance (G_s), transpiration rate (T_r), ambient CO_2 concentration (C_a), and intercellular CO_2 concentration (C_i) were automatically recorded. Water-use efficiency (WUE) was calculated as P_n/T_r (Ou et al., 2015). Stomatal limitation value (L_s) was calculated as $1 - C_i/C_a$ (Huang et al., 2013). At the same time, five labeled leaves in each plot were chosen to measure the chlorophyll content (SPAD value) with a Minolta SPAD-502 chlorophyll meter (Minolta, Japan) according to the method of Abdelhamid et al. (2003). The measurement was done for 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 (m^2 $plant^{-1}$) × plant density (plants m^{-2}) (Su et al., 2014).