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Full Length Article

Effects of Fertilizer Levels and Plant Density on Chlorophyll Contents, its Fluorescence and Grain Yield of *Setaria italica*

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Abstract

Maximizing the use of light is essential to increase grain yield of foxtail millet (*Setaria italic* L.). However, extensive research has been undertaken to optimize the cultivation of conventional foxtail millet by single-factor or orthogonal test. In this study, a quadratic general rotation combination design with five factors was employed to evaluate the effects of fertilizer levels (N, P_2O_5 and K_2O) and plant density (row and plant spacing) on leaf photosynthesis and grain yield of a hybrid, Zhangzagu 5. Leaf chlorophyll contents (Chl, SPAD value), and fluorescence induction were measured at the mid-filling stage, and grain yield was determined at harvest. The N effect was found significant for Chl, effective photochemical quantum yield of photosystem II (Y(II)), coefficients of Chl fluorescence quenching (photochemical-qP and non-photochemical-qN), and maximum photochemical quantum yield (Fv/Fm). With increasing N, Chl, Y(II) and qP first increased and then declined, opposite to qN; Fv/Fm elevated linearly. The P_2O_5 effect was significant for Y (II) and qN, while the K_2O effect reached significance for Y(II) and qP. With decreasing row spacing, Y(II) and qP displayed an increase followed by decrease, opposite to qN. Grain yield was more affected by N and row spacing compared with P_2O_5 and K_2O. N × K_2O, N × plant spacing, and K_2O × plant spacing showed significant effects on Y(II), qP and yield, while the effect of P_2O_5 × row spacing was significant for Fv/Fm. Multivariate quadratic regression analysis revealed a relationship between these factors and grain yield, which can be used for crop production forecasts. Recommended cultivation conditions were: 186 kg ha^{-1} N, 95 kg ha^{-1} P_2O_5, 60 kg ha^{-1} K_2O, 23 cm row spacing, and 13 cm plant spacing to achieve an expected yield of 6,683 kg ha^{-1}. © 2018 Friends Science Publishers

Keywords: Foxtail millet hybrid; Leaf photosynthesis; Grain yield; Response surface methodology; Fertilizer; Plant density

Introduction

Foxtail millet (*Setaria italica* L.) is a nutritious crop that has been mostly used for foods in Africa, Asia, and Central and South America (Antony *et al*., 1996; Zhang *et al*., 1997). Compared with staple crops such as wheat and maize, foxtail millet has been gradually ignored because of its relatively low yield associated with low use efficiency of light energy. Since 90% of the dry matter of millet grain yield comes from the products of photosynthesis (Yang *et al*., 2013), maximizing the photosynthetic light use efficiency is the primary method to increase grain yield of this crop (Ahmed *et al*., 2012; Covshoff and Hibberd, 2012).

Nitrogen (N), phosphorus (P) and potassium (K) fertilizers are critical determinants of photosynthesis (Zou *et al*., 2007; Shehu *et al*., 2010; Zhang *et al*., 2011). An effective way to improve crop photosynthetic function is the scientific use of nutrient control (Bradford and Tsiao, 1982). Additionally, appropriate plant density contributes to the formation of high population yield (Ariapour and Afrougheh, 2008; De Bruin and Pedersen, 2008; Jost and Cothren, 2000), because the setting of row and plant spacing affects light interception and ventilation of crop canopy (Zhu *et al*., 1998). Plant density can effectively control canopy structure, the distribution and accumulation of photosynthetic products, and leaf senescence (De Bruin and Pedersen, 2008; Gözübenli, 2010). Therefore, it is of reference value to evaluate the effects of fertilizer levels and plant density on the photosynthetic characteristics of foxtail millet.

An approach of chlorophyll fluorescence induction (CFI) has been widely used to assess the physiological state of the photosynthetic apparatus (PSA) in plants (Ptushenko *et al*., 2014). The CFI approach is available for solution of fundamental problems in the biochemistry and biophysics of photosynthesis and plant physiology, as well as in ecological and agricultural studies (Strasser and Tsimill-Michael, 2004). CFI studies on the PSA are non-invasiveness, allowing for express analysis under field conditions and the use of sensitive parameters to practically important stress factors of the environment (Buonasera *et al*., 2011; Liu *et al*.,

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2012). Non-monotonic changes in the fluorescence intensity of leaves have been detected in different plants (Ptushenko *et al*., 2014), such as wheat, rice, and arboreous plants (Ptushenko *et al*., 2014).

Leaf chlorophyll *a* (Chl *a*) of photosystem II (PSII) is the major source of fluorescence in green plants (Lambrev *et al*., 2012). The photochemical activity of PSII is often characterized using the maximal efficiency ($\phi_{PSII}^{\max}$) as measured in response to a saturating light flash given after a sufficiently long term adaptation of the leaf to darkness (from dozens of minutes to hours) (Kramer *et al*., 2004). Moreover, the operating efficiency of PSII (j_{PSII}) is an important parameter measured during illumination of the leaf or after insufficiently long-term dark adaptation. j_{PSII} is usually lower than $\phi_{PSII}^{\max}$ and decreases with increasing light intensity (Wild and Ru¨hle, 1975). The j_{PSII} value gradually increases along with adaptation to illumination and reaches the steady-state level ($\phi_{PSII}^{st.st}$). Additionally, the coefficient of non-photochemical quenching of Chl fluorescence (qN) is used rather frequently to characterize the weakening of the photochemical activity of PSII that is associated with the activation of mechanisms for PSA protection against light-induced damage (Baro´csi *et al*., 2009; Ptushenko *et al*., 2014).

Several studies have examined the relationship between photosynthetic parameters with grain yield in conventional foxtail millet cultivars. There is evidence that improving the photosynthetic performance increases dry matter accumulation and yield production of this crop (Lu *et al*., 1997; Liao and Wang, 1999). According to Yang *et al*. (2013), the photosynthetic advantage of hybrid cultivar is the primary cause of higher grain yield compared with conventional cultivar in foxtail millet. However, little research has analyzed the response of photosynthetic characteristics to fertilizer levels and plant density in foxtail millet hybrids (Fan *et al*., 2011). Thus far, the physiological mechanism of photosynthesis in wheat has been elucidated using CFI parameters, but similar studies are rarely reported in foxtail millet. Moreover, a large body of work has taken into consideration single-factor effects on cultivation of foxtail millet, while multiple-factor interaction effects are largely overlooked (Liao and Wang, 1999; Lu *et al*., 1997; Fan *et al*., 2011).

In the present study, field experiments based on 5-factor-5-level quadratic general rotary combination design were conducted to assess the effects of different fertilizer levels and plant densities on photosynthetic parameters and grain yield of hybrid millet. Based on the experimental results, the optimal cultivation conditions were determined, in order to provide reference data for yield improvement in foxtail millet hybrids.

Materials and Methods

Experimental Site and Materials

Field experiments were carried out in the Agricultural Experimental Station of Shanxi Agricultural University in Taigu County, Jinzhong City, Shanxi, China. The study site has a temperate continental climate, with the annual average temperature of 9.9°C and annual average of 462.9 mm.

The foxtail millet (*Setaria italica* L.) hybrid Zhangzagu 5 was provided by the Academy of Agricultural Sciences of Shanxi Province (Taiyuan, Shanxi, China). Zhangzagu 5 was bred by the Academy of Agricultural Sciences of Zhangjiakou (Zhangjiakou, Hebei, China) and this hybrid has become the most extensively planted hybrid millet in Shanxi over the recent years. Strawberries were cultivated for rotation with foxtail millet in the experimental site. The soil was red sandy loam in texture and medium in organic matter (18.2 g kg^{-1}) with a weak alkaline pH (8.1). Soil available N, P_2O_5 and K_2O contents before the initiation of the first-year experiment were 75, 27, and 97 mg kg^{-1}, respectively.

Experimental Design

Quadratic general rotation combination design with a 5-level-5-factor was employed to optimize fertilizer levels (N, P_2O_5, and K_2O) and plant density (row and plant spacing). The five independent factors (x_1 to x_5) were studied at five different levels (coded: −2, −1, 0, +1 and +2, respectively) (Table 1), with six repetitions at the central point and two replications at the axial and factorial points, respectively (Table 2). A total of 32 treatment combinations were run in a completely randomized block design and protection rows were set around the experimental site. Each plot was 3 m×6 m in size, with three replications.

Uniform seeds were sown on May 5, 2015 using a 2BX-3 small seeder (College of Engineering, Shanxi Agricultural University). Seedlings with at least three fully expanded leaves were thinned in accordance with plant spacing. One half of N was applied as a basal fertilizer, and the other half as a top-dressing at the jointing-booting stage. K_2O and P were applied as basal fertilizers. Fertilizers included urea (N = 460 g kg^{-1}), triple superphosphate (P_2O_5 = 420 g kg^{-1}), and sulfate of potash (K_2O = 500 g kg^{-1}). Plots were irrigated and prepared by rotary tillage before sowing. Weed control was undertaken by inter-tillage twice during the experimental period.

Leaf Chlorophyll (Chl) Measurement

Leaf Chl content (SPAD value) was measured at the mid-filling stage and the measurement was repeated three times. Ten flag leaves of uniform size were selected for each measurement, and a SPAD-502 meter was used to measure the SPAD value in the middle portion of the selected leaves. Each leaf was measured three times and the mean SPAD value was calculated per unit of leaf surface.

Table 1: Levels and codes of five experimental factors

Code	N (kg ha^{-1})	P_2O_5 (kg ha^{-1})	K_2O (kg ha^{-1})	Row spacing (cm)	Plant spacing (cm)
–2	0	0	0	10	5
–1	69	36	37.5	20	10
0	138	72	75	30	15
1	207	108	112.5	40	20
2	276	144	150	50	25
∆j	69	36	37.5	10	5

Table 2: Program and experimental results of quadratic general rotation design for chlorophyll fluorescence parameters

No	x_1	x_2	x_3	x_4	x_5	Chl (SPAD)	Y (II)	qP	qN	F_v/F_m	Yield (kg ha^{-1})
1	1	1	1	1	1	33.5	0.235	0.387	0.67	0.776	5880
2	1	1	1	-1	-1	37.4	0.260	0.434	0.682	0.776	6375
3	1	1	-1	1	-1	35.6	0.174	0.298	0.721	0.779	5955
4	1	1	-1	-1	1	34.4	0.235	0.392	0.704	0.784	5895
5	1	-1	1	1	-1	33.7	0.210	0.366	0.733	0.779	5625
6	1	-1	1	-1	1	35.9	0.251	0.431	0.712	0.773	6015
7	1	-1	-1	1	1	30.2	0.212	0.359	0.722	0.785	4995
8	1	-1	-1	-1	-1	36.5	0.200	0.334	0.69	0.778	6255
9	-1	1	1	1	-1	30.4	0.248	0.432	0.704	0.762	4935
10	-1	1	1	-1	1	31.7	0.235	0.382	0.651	0.777	5375
11	-1	1	-1	1	1	32.5	0.231	0.399	0.703	0.767	5565
12	-1	1	-1	-1	-1	36.3	0.249	0.412	0.673	0.774	5985
13	-1	-1	1	1	1	31.8	0.188	0.317	0.711	0.781	5325
14	-1	-1	1	-1	-1	30.5	0.252	0.420	0.665	0.767	5160
15	-1	-1	-1	1	-1	32.1	0.225	0.386	0.721	0.779	5320
16	-1	-1	-1	-1	1	33.9	0.229	0.385	0.671	0.765	5640
17	-2	0	0	0	0	32.5	0.245	0.423	0.694	0.762	5430
18	2	0	0	0	0	35.7	0.206	0.348	0.724	0.789	5910
19	0	-2	0	0	0	32.4	0.238	0.413	0.677	0.777	5775
20	0	2	0	0	0	36.8	0.259	0.418	0.639	0.775	6210
21	0	0	-2	0	0	38.1	0.228	0.376	0.697	0.78	6255
22	0	0	2	0	0	30.6	0.250	0.427	0.71	0.768	5475
23	0	0	0	-2	0	33.9	0.244	0.435	0.739	0.768	6030
24	0	0	0	2	0	35.3	0.217	0.390	0.733	0.783	5370
25	0	0	0	0	-2	34.2	0.264	0.441	0.707	0.762	5600
26	0	0	0	0	2	32.7	0.226	0.381	0.705	0.78	5415
27	0	0	0	0	0	39.8	0.264	0.443	0.691	0.774	6450
28	0	0	0	0	0	40.5	0.261	0.455	0.703	0.781	6240
29	0	0	0	0	0	36.1	0.271	0.454	0.692	0.777	6705
30	0	0	0	0	0	38.9	0.269	0.463	0.719	0.777	6675
31	0	0	0	0	0	41.2	0.251	0.434	0.696	0.776	6180
32	0	0	0	0	0	38.2	0.291	0.479	0.685	0.77	6765

Note: x_1–N, x_2–P_2O_5, x_3–K_2O, x_4 – row spacing, x_5– plant spacing. Chl – leaf chlorophyll content; Y(II) – effective photochemical quantum yield of photosystem II; qP – coefficient of photochemical fluorescence quenching; qN – coefficient of non-photochemical fluorescence quenching; Fv/Fm – maximum photochemical quantum yield; Yield – grain yield of foxtail millet

CFI Measurements

Leaf CFI curves were measured at the mid-filling stage with a portable FluorPen FP100 fluorimeter (Photon System Instruments, Czech). The effective photochemical quantum yield of PSII [Y(II)], (Y(II)), coefficients of Chl fluorescence quenching (photochemical-qP and non-photochemical-qN), and maximum photochemical quantum yield (Fv/Fm) were determined as previously described (Maxwell and Johnson, 2000). To examine the correlations between leaf Chl and CFI parameters, the measurements were performed on cuttings from dark-adapted leaves. In all the other cases, the measurements were performed on intact leaves that had not been separated from the plant in the evening (no earlier than 2 h after sunset, i.e., on dark adapted leaves). The fluorescence was excited and measured from the ventral (abaxial) side of the leaf. Each measurement was repeated three times.

Grain Yield Measurement

Foxtail millet was harvested on September 30, 2015. After being dehulled and air-dried, the yield of each plot was expressed on hectare basis.

Statistical Analysis

The experimental design, data analysis, and quadratic model building were conducted using SAS 9.0 (SAS Institute Inc., Cary, NC, USA). Quadratic regression model was used for optimizing the cultivation conditions of foxtail millet for fertilizer levels and plant density. Contour plots showing the effects of factorial interactions on grain yield were constructed using Minitab7.0 (Minitab Inc., State College, PA, USA).

Results

Effects of Fertilizer Levels and Plant Density on Leaf Chl and CFI

The results showed that the effects of N and K_2O were significant for leaf Chl (SPAD), while the effects of N, P_2O_5, and K_2O as well as row spacing were significant for Y(II) (Table 3), Both SPAD and Y(II) followed a parabola trend with increasing fertilizer levels or decreasing row/plant spacing (Fig. 1a and b).

With respect to the coefficients of Chl fluorescence quenching, qP was affected by N and K_2O (Table 3). qP followed a parabola trend with increasing N and K_2O (Fig. 1c). qN was strongly affected by N and P_2O_5, other than K_2O (Table 3). The qN first decreased and then increased with increasing N, while it followed the opposite trend with increasing P_2O_5 (Fig. 1d). Moreover, row spacing had great effects on both qP and qN, despite the opposite trends between qP and qN. The former showed an increase followed by decrease, while the latter first decreased and then increased with decreasing row spacing (Fig. 1c and d). The effect of only N reached significance for Fv/Fm, and the Fv/Fm almost linearly increased with increasing N (Fig. 1e).

The effects of N × K_2O, N × plant spacing, K_2O × plant spacing interactions reached significance for Y(II) and qP, whereas the effect of P_2O_5 × row spacing interaction was significant for Fv/Fm (Table 3).

Table 3: Test of significance for the coefficients of regression equation for leaf photosynthetic parameters and grain yield of foxtail millet

Parameters	Chl	Y(II)	qP	qN	F_v/F_m	Yield
x_1	0.0281*	0.0253*	0.0077**	0.0302*	0.0006**	0.0011**
x_2	0.1258	0.0388*	0.1167	0.0317*	0.5115	0.0385*
x_3	0.0470*	0.0182*	0.0049**	0.5289	0.0889	0.0398*
x_4	0.1751	0.0021**	0.0027**	0.0153*	0.0889	0.0016**
x_5	0.2549	0.2225	0.1124	0.5449	0.0575	0.2507
$x_1 \times x_1$	0.0087**	0.0005**	0.0001**	0.6456	0.9006	0.0006**
$x_2 \times x_2$	0.0161*	0.0420*	0.0051**	0.0027**	0.7933	0.0175**
$x_3 \times x_3$	0.0119*	0.0060**	0.0008**	0.9940	0.7742	0.0043**
$x_4 \times x_4$	0.0161*	0.0012**	0.0032**	0.0187*	0.9006	0.0007**
$x_5 \times x_5$	0.0040**	0.0193*	0.0026**	0.8306	0.2793	0.0001**
$x_1 \times x_2$	0.8045	0.4528	0.2034	0.5158	0.5458	0.3768
$x_1 \times x_3$	0.0992	0.0128*	0.0033**	0.9635	0.2385	0.0147*
$x_1 \times x_4$	0.4924	0.4231	0.1305	0.0855	0.9191	0.2437
$x_1 \times x_5$	0.2398	0.0040**	0.0013**	0.8908	0.9191	0.0482*
$x_2 \times x_3$	0.5390	0.3003	0.3818	0.1110	0.7612	0.4064
$x_2 \times x_4$	0.8818	0.9164	0.6413	0.3613	0.0047**	0.5690
$x_2 \times x_5$	0.4204	0.8136	0.9707	0.3767	0.5458	0.8660
$x_3 \times x_4$	0.5714	0.3611	0.2652	0.7491	0.8392	0.3885
$x_3 \times x_5$	0.2140	0.0335*	0.0059**	0.5956	0.1247	0.0492*
$x_4 \times x_5$	0.9014	0.7142	0.8833	0.1431	0.7612	0.3885

Note: x_1 – N, x_2 – P_2O_5, x_3 – K_2O, x_4 – row spacing, x_5 – plant spacing. Chl – leaf chlorophyll content; Y(II) – effective photochemical quantum yield of photosystem II; qP – coefficient of photochemical fluorescence quenching; qN – coefficient of non-photochemical fluorescence quenching; Fv/Fm – maximum photochemical quantum yield; Yield – grain yield of foxtail millet. Values are *P* values by a *t*-test. **P* <0.05: mean significant difference at the 5% level; ***P* <0.01: mean significant difference at the 1% level

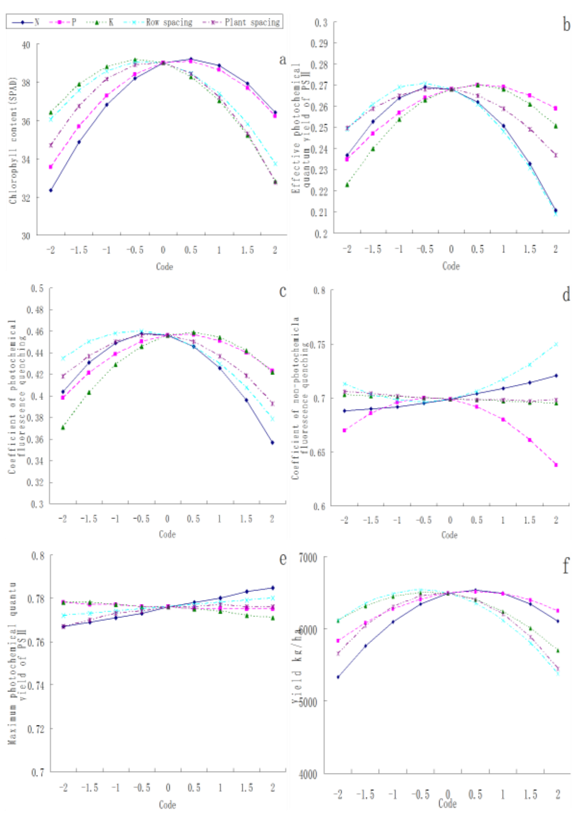

Fig. 1: Effects of single factors on leaf chlorophyll content, chlorophyll fluorescence parameters, and grain yield of Zhangzagu 5. a: leaf chlorophyll content (SPAD); b: effective photochemical quantum yield of PSII; c:coefficient of photochemical fluorescence quenching; d: coefficient of non-photochemical fluorescence quenching; e: maximum photochemical quantum yield of PSII; f: grain yield

Effects of Fertilizer Levels and Plant Density on Grain Yield

Except for plant spacing, the effects of fertilizer levels and row spacing on grain yield were significant. The significance of the effect on grain yield was highest for N (P =0.0011), followed by row spacing (P = 0.0016); P_2O_5 came the third (P = 0.0385); K_2O was the last (P = 0.0398; Table 3). In the design range, the effects of the five factors on grain yield showed a parabolic trend (Fig. 1f). With increasing N or decreasing row spacing, grain yield first rapidly increased; when N exceeded 0.5 or row spacing exceeded -0.5, the yield showed a slow decline. With increasing P_2O_5 and K_2O, the yield first increased slightly and then gradually decreased.

The effects of N × K_2O, N× plant spacing, K_2O × plant spacing interactions reached significance for millet yield (Table 3). The contour plots and response surface plots (Fig. 2) were mapped to show the interactive effects of N × K_2O, N × plant spacing, K_2O × plant spacing on millet yield, similar to those observed on qP and Fv/Fm (Table 3).

When P_2O_5, row spacing, and plant spacing were fixed at the zero level, the yield first decreased and then increased with the increase of N and K_2O (Fig. 2a). The effect of N was greater at higher K_2O levels than at lower K_2O levels. At higher N levels, enriching K_2O caused little change in the yield; at lower N levels, the yield first decreased slowly and then rapidly increased with the increase of K_2O.

When P_2O_5, K_2O, and row spacing were fixed at the zero level, increasing N caused a dramatic yield increase at narrow plant spacing, but not at wide plant spacing. With continued increase in N application, there was a downward trend in grain yield (Fig. 2b). When plant spacing was decreased at lower N levels, the yield first increased slowly and then decreased rapidly. The opposite trend was observed in the yield with decreasing plant spacing at higher N levels.

When N, P_2O_5, and row spacing were fixed at the zero level, grain yield began to increase quickly and then dropped as plant spacing was reduced at an appropriate (or lower) level of K_2O. When plant spacing was reduced at higher K_2O levels, grain yield was rapidly reduced after a slow increase (Fig. 2c).

Response of Grain Yield to Fertilizer Levels and Plant Density

To obtain a valid model, the actual responses were fitted with existing linear, two factor interactions, cubic and quadratic model. The quadratic model was selected and validated by a few numbers of statistical evidences in the analysis of variance. The evidences included Fisher variation ratio (F value), probability value (P value), lack of fit, adjusted R-squared (R_{Adj}^2). The second-order polynomial equation for grain yield is given below:

$$y = 6486.93 + 193.75x_1 + 104.17x_2 - 103.33x_3 - 184.17x_4 - 53.75x_5 - 192.56x_1^2 - 111.93x_2^2 - 143.81x_3^2 - 185.06x_4^2 - 233.18x_5^2 + 50x_1x_2 + 156.88x_1x_3 - 66.88x_1x_4 - 120.63x_1x_5 - 46.88x_2x_3 + 31.88x_2x_4 - 9.38x_2x_5 + 48.75x_3x_4 + 120x_3x_5 + 48.75x_4x_5 \quad (1)$$

Where, y is the predicted response of grain yield; x_1, x_2,x_3, x_4, and x_5 are coded values of N level, P_2O_5 level, K_2O level, row spacing, and plant spacing, respectively.

The statistical significance of Eq. (1) was evaluated by an F-test. The analysis of variance for response surface showed that the model was statistically valid (P < 0.05), and lack of fit item test was not significant (P > 0.05). The adjusted R^2 was 0.6545.

The optimal values of the selected factors in their respective coded values were: x_1 = 0.6905, x_2 = 0.6263, x_3 = 0.4016, x_4 = -0.6847, and x_5 = -0.4814. Accordingly, the actual N level, P_2O_5 level, K_2O level, row spacing, and plant spacing were 186 kg ha^{-1}, 95 kg ha^{-1}, 60 kg ha^{-1}, 23 cm, and 13 cm, respectively. The maximum predicted yield of foxtail millet was 6,683 kg ha^{-1}. Multivariate quadratic regression indicated that the relationship between the five factors and grain yield of Zhangzagu 5 was significant, which can be used for production forecasts.

The Verification of Cultivation Conditions on Foxtail Millet

In order to verify the optimal cultivation conditions, Zhangzagu 5 was planted with eight plots of 6 m × 6 m in 2016. The yield was 6 786 kg ha^{-1}. At the zero level, the yield of foxtail millet Zhangzagu 5 was 6 423 kg ha^{-1}, which reduced by 5.7% than the optimal cultivation conditions.

Discussion

Effect of Fertilizer Levels on Leaf Photosynthesis and Grain Yield of Foxtail Millet

Fertilizers can help to maintain relatively high photosynthetic rate in plant leaves (Zou *et al*., 2007). The present study showed that the N effect was significant for CFI parameters and yield. Increasing N level led to an increase followed by decrease in Y(II), qP and yield, this result indicates that an appropriate increase of N fertilization at can enhance the light capture capacity of leaves and thereby increase PSII activity, photosynthetic efficiency, and the open proportion of the PSII reaction center. Meanwhile, it will reduce the heat dissipation of non-radiative energy and thus contribute to the effective utilization of the captured light energy for leaf photosynthesis. In this way, N fertilization promotes PSII quantity efficiency and photosynthetic rate, further promoting the translocation and accumulation of photosynthetic products to the grain (Shangguan *et al*., 2010).

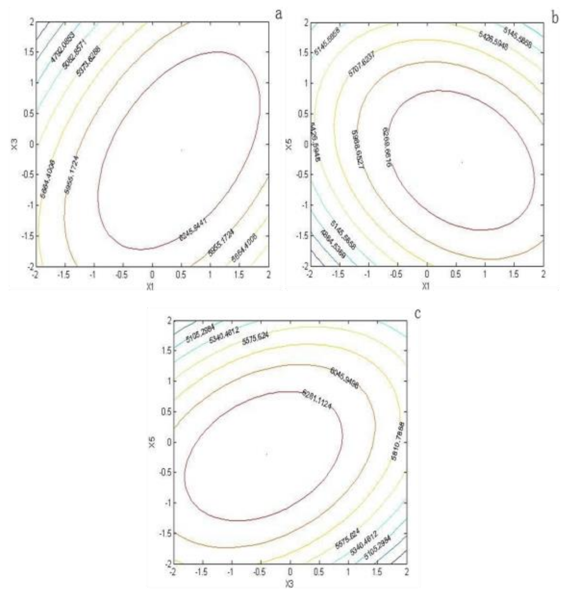

Fig. 2: Significant effects of factor interactions on grain yield of Zhangzagu 5. a: N × K_2O; b: N × plant spacing; c: K_2O × plant spacing

Compared with Y(II) and qP, qN showed the opposite trend with increasing N level, which confirmed that over-use of N fertilizer could increase the dissipation of light energy and thereby reduce the photosynthetic efficiency. This mechanism negatively affected grain yield, since 90% of dry matter of millet grain is derived from photosynthetic products (Yang *et al*., 2013). Similarly, Zhang *et al*. (2013) indicated that reasonable use of N fertilizer improves the potential activity and photochemical efficiency of PSII but reduces qN, thereby improving the photosynthetic performance and facilitating yield production of crops. Additionally, P fertilizer is known to benefit nutrient synthesis and transportation, accelerate vegetative growth and dry matter accumulation, and improve light use efficiency in plants (Xiao *et al*., 2009). In this study, the effect of P_2O_5 was significant for Y(II)，qN and yield, and the three parameters showed an increase followed by decrease with increasing P_2O_5 level. Thus, P_2O_5 level should comply with the expected yield level of foxtail millet, as overuse of P fertilizer will not increase the yield but cause waste and pollution (Xiao *et al*., 2009).

The effect of K_2O was minor for leaf photosynthesis and grain yield of conventional foxtail millet cultivar (Fan *et al*., 2003). Reversely, we found that the K_2O effect was significant for Y(II), qP and grain yield of the hybrid Zhangzagu 5. Moreover, the effect of N × K_2O interaction was significant for Y(II), qP and grain yield of Zhangzagu 5, indicating that K_2O fertilizer could indirectly promote photosynthesis and yield production of this hybrid. When K_2O was adequate, increasing N application resulted in a rapid increase in qP, indicating that K_2O promoted the N effect. However, at lower K_2O levels, continued increase of N application led to a sudden decrease in qP, indicating the necessity to control N application under low K_2O conditions. At lower N levels, the initial increase of K_2O resulted in no obvious increase in Y(II). Continued increase of K_2O application resulted in lower Y(II), qP, and Fv/Fm, indicating that excessive application of K_2O negatively affected leaf photosynthesis and thus should be avoided under low N conditions.

Moreover, at lower N level, the trend in qP was not obvious with increasing K_2O application. This result also shows that the effect of N and K_2O on photosynthesis is mainly ascribed to N, while K_2O enhances the N effect. It is recommended to combine high N and K_2O application. The effects of P_2O_5 × N and P_2O_5 × K_2Ointeractions did not reach significance for photosynthetic characteristics and yield. Therefore, reasonable fertilization for yield improvement is mainly to increase N. The ideal is to supplement K_2O by increasing N, in order to enhance the N effect by K_2O. Together with P_2O_5 application will be more conducive to enhancing photosynthesis and achieve higher grain yield of foxtail millet. This result is in agreement with the study by Kunzova and Hejcman (2009) on wheat yield.

Effect of Plant Density on Leaf Photosynthesis and Grain Yield of Foxtail Millet

Plant density can affect the nutritional state of plants, light interception and distribution of crop canopy. It can regulate the individual activity of plants, leaf photosynthetic rate in different positions, and photosynthetic carbon assimilation capacity of crop population, ultimately influencing dry matter production of the crop (Lihua *et al*., 2008; Li *et al*., 2010). When row and plant spacing was reduced, Y(II), qP and yield first increased, indicating that the use of reasonable row spacing is conducive to improving the photosynthetic performance of crop population and thereby increasing grain yield (Hang and Wang, 1984).

When row and plant spacing were reduced to a certain level, plant density conflicted with Y (II) and qP. This situation led to lower photosynthetic efficiency and eventually caused yield loss. As plant density increases, reduced light intensity within the populations weakens the photosynthetic production and thereby decreases dry matter accumulation in plants. This mechanism would cause stem diameter thinning, dry weight reduction in stem and leaf sheaths, and elongation of basal internodes (Tian *et al*., 2010). The weakened supportive power of stems becomes an internal cause for late lodging, and lodging has been implicated as a major factor negatively affecting leaf photosynthesis and grain yield of crops at higher plant density (Kelbert *et al*., 2004).

Different from the above parameters, qN first increased then decreased as row and plant spacing was reduced. This shows that initial reduction in row and plant spacing, little energy is dissipated as heat absorbed by antenna pigments of PSII. Instead, the energy is more utilized for the photosynthetic electron transport (Lu *et al*., 2001). With continued decrease in row and plant spacing, energy is increasingly dissipated as heat. This trend is in agreement with the changes in photosynthetic efficiency which first increased and then decreased with decreasing plant density. Fv/Fm of PSII was barely changed upon the reduction in plant and row spacing. Further analysis explained that Fv/Fm of PSII was less affected by the cultivation conditions in foxtail millet.

The effect of row spacing reached significance for Y(II), qP and grain yield of Zhangzagu 5. However, plant spacing had no significant effect on CFI parameters or grain yield, although its interactions with N or K_2O to some extent affected Y(II), qP and grain yield of Zhangzagu 5. Likewise, Li *et al*. (2010) suggested that the effect of row spacing, other than plant spacing, is significant for photosynthetic characteristics in wheat (Li *et al*., 2010). From the physiological point of view, we consider that the effects of row spacing on photosynthetic characteristics and millet yield are highly correlated with light use efficiency and CO_2 diffusion capacity, whereas the effects of plant spacing has a high degree of correlation with water-fertilizer competition between plants. In short, close planting at rational spacing is a way to improve light use efficiency in foxtail millet crops. The mechanism lies in that close planting increases light interception and thereby increases dry matter accumulation and yield formation of the crops.

Effect of Fertilizer Levels × Plant Density on Leaf Photosynthesis and Grain Yield of Foxtail Millet

In the present study, the interaction of plant spacing ×N (and K_2O) significantly affected Y(II), qP and grain yield of Zhangzagu 5. When low (or appropriate) levels of N and K_2O were applied, Y(II), qP and grain yield followed aparabolic trend with decreasing plant spacing, indicating that plant density can be adjusted to replace fertilizers within a certain range of fertility (Rathke *et al*., 2005). It may be useful to increase plant density and meanwhile reduce fertilizer application within a reasonable range, allowing the density to supplement the fertilizer effect.

However, as plant spacing was reduced at higher N and K_2O levels, grain yield began to increase more slowly, Y(II), qP and yield were reduced to a certain extent. Essentially, the leaves shade each other and the population has a closed canopy at high plant density. These conditions would result in lower photosynthetic efficiency and thus cause yield loss. Moreover, N and K_2O contributed more to grain yield at narrower plant spacing than at wider plant spacing, indicating that a combined use of high plant density and high fertilizer levels is conducive to improving the photosynthetic efficiency and grain yield of Zhangzagu 5.

Nonetheless, continued addition of N and K_2O fertilizers led to a remarkable reduction in the yield. At high fertilizer levels, excessive vegetative growth and serious lodging may be responsible for yield loss (Xiao *et al*., 2009; Tian *et al*., 2010; Kelbert *et al*., 2004). Only rational application of N fertilizer at appropriate plant density can effectively take advantage of their close interaction and improve the photosynthetic efficiency (Cao *et al*., 2011).

Conclusion

The proper application of fertilizer combined with reasonable plant density is required for improving leaf photosynthetic efficiency and increasing the yield in hybrid millet. The principle is to obtain the maximum number of leaves per unit area and meanwhile allow the leaves to take full use of water, fertilizer, light, and heat resources. This strategy will help to maintain a balance between the light capture and light use efficiency, thereby achieving higher photosynthetic efficiency and grain yield. Optimal cultivation conditions were obtained for Zhangzagu 5 under the experimental conditions: 186 kg ha^{-1} for N, 95 kg ha^{-1} for P_2O_5, 60 kg ha^{-1} for K_2O, 23 cm for row spacing, and 13 cm for plant spacing, for an expected yield of 6,683 kg ha^{-1}. Likewise, the regression model can be used to predict the desired fertilizer application rate and plant density according to the expected yield.

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