



## Planting density and sowing date strongly influence growth and lint yield of cotton crops



Aziz Khan<sup>a</sup>, Ullah Najeeb<sup>b</sup>, Leishan Wang<sup>a</sup>, Daniel Kean Yuen Tan<sup>b</sup>, Guozheng Yang<sup>a,\*</sup>,  
Fazal Munsif<sup>c</sup>, Saif Ali<sup>a</sup>, Abdul Hafeez<sup>a</sup>

<sup>a</sup> MOA Key Laboratory of Crop Eco-physiology and Farming System in the Middle Reaches of the Yangtze River, Huazhong Agricultural University, Wuhan, 43007, PR China

<sup>b</sup> Faculty of Science, Plant Breeding Institute, Sydney Institute of Agriculture, School of Life and Environmental Sciences, The University of Sydney, Sydney, NSW 2006, Australia

<sup>c</sup> Department of Agronomy, Amir Muhammad Khan Campus Mardan, The University of Agriculture, Peshawar, Khyber Pakhtunkhwa, 23200, Pakistan

### ARTICLE INFO

#### Keywords:

Cotton lint yield  
Sowing date  
Planting density  
Photosynthesis  
Nitrogen partitioning  
Assimilation

### ABSTRACT

This study assesses the effects of plant population density (PPD) and sowing date (SD) on growth, physiology and lint yield of a cotton crop. Seedling transplanting is one of the most dominant cotton production systems in China. But on the other hand, the net benefit is decreasing because the system is labor intensive. Therefore, a shorter cotton growing season is urgently needed to reduce the production costs through management practices such as adjusting sowing date and PPD. The following hypothesis was tested; would cotton yield and physiology from a late sowing be compensated by plant density? Field experiments were conducted with two sowing dates (S<sub>1</sub>, May 20; S<sub>2</sub>, June 04) as the main plot and three PPDs (D<sub>1</sub>, low; 7.5 × 10<sup>4</sup>; D<sub>2</sub>, moderate; 9.0 × 10<sup>4</sup> and D<sub>3</sub>, high; 10.5 × 10<sup>4</sup> ha<sup>-1</sup>) as the sub-plot. Early-sown plants produced 23%, 32%, 55%, 77% and 14%, taller stems more nodes, leaves and fruits, respectively, than the late-sown plants. Consequently, S<sub>1</sub> produced 26% higher lint yield than S<sub>2</sub>. This increase in lint yield was mainly attributed to a relatively longer cropping season, which allowed utilization of available resources. Growth and fruit production in S<sub>1</sub> plants were further increased by an increased photosynthetic rate (Pn) and N acquisition. Across the plant densities, 13% and 6% more lint yield was achieved under D<sub>2</sub> than the D<sub>3</sub> and D<sub>1</sub>, respectively. Moderate PPD increased lint yield by 13% and 6% over high and low, respectively. Nitrogen (N) acquisition was 45%, 33%, higher for S<sub>1</sub> sown crop compared with S<sub>2</sub>, respectively. S<sub>1</sub>D<sub>2</sub> had higher average (3.5 V<sub>T</sub> kg ha<sup>-1</sup> d<sup>-1</sup>) and maximum (4.5 V<sub>M</sub> kg ha<sup>-1</sup> d<sup>-1</sup>) rates of N accumulation in reproductive organs at the fastest accumulation point among other treatments. Our data suggest that for both sowing dates moderate PPD is a promising option, which allows light interception and penetration to the lower canopy, efficient N utilization and assimilate distribution to reproductive structures.

### 1. Introduction

Cotton (*Gossypium hirsutum* L.) is grown globally as a major source of natural fiber (Constable and Bange, 2015). Due its indeterminate growth habit, the crop shows morphological adaptations to its growing environment such as modification in canopy structure in response to sowing date (SD) and plant population density (PPD) (Mao et al., 2014; Zhang et al., 2003). These morphological adaptations in terms of canopy development, light interception, source sink relationship and assimilates partitioning are the major determinant of lint yield and quality (Yang et al., 2014a,b). Hubei is one of the major cotton growing

provinces in China, contributing 12.3% of the total national lint production in less than 9.4% of the planting area (Yang and Zhou, 2010a,b). Despite introduction of high yielding varieties, cotton yield per unit area in this region is stagnant for the last decade (Yang et al., 2014a,b). Cotton planting is a laborious practice in this region due to raised bed sowing and transplantation into open field (Lu et al., 2017). This situation will worsen due to an accelerated migration of farm labor towards cities since 1990 (Zhou, 2004). Therefore crop management techniques such as late sowing and high plant population density are often practiced to overcome input costs without sacrificing yield. With the introduction of row planting (Briggs et al., 1967), the concept of

**Abbreviation:** PPD, plant population density; SD, sowing date; Pn, net photosynthetic rate; AVG, aminoethoxyvinylglycine; FB/FN, fruiting branch to fruiting node ratio; RH%, percent relative humidity; FAP, fastest accumulation point; VT, average rate; VM, maximum rate; t<sub>1</sub>, t<sub>2</sub> beginning and terminating days of the fast accumulation period; CNP, cotton plant nitrogen VON vegetative organ nitrogen; ROP, reproductive organ nitrogen; DAE, days after emergence

\* Corresponding author.

E-mail address: [ygzh9999@mail.hzau.edu.cn](mailto:ygzh9999@mail.hzau.edu.cn) (G. Yang).

<http://dx.doi.org/10.1016/j.fcr.2017.04.019>

Received 16 March 2017; Received in revised form 28 April 2017; Accepted 29 April 2017

Available online 09 May 2017

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high density planting system (HDPS) has become popular in the cotton production systems worldwide. However, dense populations ( $> 10$  plants  $m^{-2}$ ) and subsequent shading may lead to disease infestation, reduced boll size, fruit shedding, delayed maturity and decreased individual plant development (Yang et al., 2014a,b; Bednarz et al., 2006). Current recommended PDD in China was is 22.7 plants  $m^{-2}$  in the Northwest (Han et al., 2009), 5.3–7.5 plant  $m^{-2}$  in the Yellow River Valley (Dong et al., 2012) and 3.0 plants  $m^{-2}$  in the Yangtze River Valley (Yang et al., 2014a,b).

Similar to PPD planting time is an important determinant of lint yield and quality in cotton farming systems. Timely planting of crops is essential for root penetration and proliferation, and vegetative growth for optimum harvesting of available soil nutrients and solar radiation (Soler et al., 2007). Early planted crops may experience some challenges of seedling establishment due to low temperatures and high insect pest incidence (Pettigrew and Adamezyk, 2008). Late planting, in contrast, usually reduces cotton yield due to delayed physiological maturity and carbohydrate deficiency (Gwathmey and Clement, 2010). Both PPD and planting time strongly influence N status in cotton leaves, which is positively associated with canopy photosynthetic capacity (Poorter and Evand, 1998). Synchronization of crop N demands with its supply is crucial for improving crop nitrogen use efficiency (NUE). N demand for a crop is strongly related to yield potential, which in turn is associated with N supply and crop management (Yousaf et al., 2016). Since nutrient uptake and PPD are strongly associated, increasing plant density may lead to an increased N uptake in reproductive tissues. High plant population favors high N uptake and N translocation from vegetative structures to reproductive organs (Jiang et al., 2013).

A leaf with a history of low light has lower photosynthetic saturation relative to an illuminated leaf, and this is particularly important when the cotton crop is grown under dense PPD (Landivar et al., 2010). The affinity of the enzymes involved in carbon fixation e.g., rubisco increases under low light conditions (Jenson, 1986), which imbalances ethylene/sugar ratio and can lead to abscission of the reproductive structures (Zhao and Oosterhuis, 2000). The rubisco has high affinity with  $O_2$  and  $CO_2$  (Jenson, 1986), and photorespiration is increased under low light conditions. This increased ethylene/sugar ratio can lead to abscission of reproductive structures (Guinn, 1974), and cotton yield reduction (Zhao and Oosterhuis, 2000). Thus, time of sowing and plant density can be an important determinant of growth cycle of a cotton crop phenology, growth and development. However, limited information is available on their combined effects on nutrient dynamics, growth, leaf photosynthetic capacity and lint yield of cotton crop. This study explores, the role of plant density and sowing date on (1) cotton growth, lint yield, leaf photosynthetic capacity and nutrient dynamics at different phenological stages; and (2) elucidates the quantitative relationship between planting density and planting date. These data will provide crop management guidelines to cotton growers.

## 2. Materials and methods

### 2.1. Experimental site and cultivar

Field studies were conducted in 2014 and 2015 on the experimental farm of Huazhong Agricultural University, Wuhan, China ( $30^{\circ} 37' N$  latitude,  $114^{\circ} 21' E$  longitude, 23 m above sea level). Soil of the experimental site was yellowish brown and clay loam comprising of 1.2% organic matter, 81.7 mg  $kg^{-1}$  alkaline N, 21.3 mg  $kg^{-1}$   $P_2O_5$ , and 78.4 mg  $kg^{-1}$   $K_2O$ . Mean air temperature was higher during seedling establishment, vegetative growth and remained relatively lower during reproductive periods of both years. On average, 2014 was relatively cooler than 2015. Relative humidity was associated with air temperatures during earlier crop stages. It was low during early growth phases and increased as canopy gets closer (Table 1). A cotton cultivar Huazamian H318 (*G. hirsutum* L.) having moderate maturity was used for the present study.

**Table 1**  
Description of climatic parameters during 2014 and 2015.

Month	2014				2015			
	Max°C	Min°C	Mean°C	RH%	Max°C	Min°C	Mean°C	RH%
May	26.8	17.1	22.0	80.6	27.0	18.4	22.7	82.4
June	30.1	21.4	26.0	65.7	31.2	22.5	26.9	68.7
July	41.0	26.5	34.0	62.2	43.3	28.1	31.2	63.1
August	40.2	23.4	31.8	69.1	41.5	24.6	33.1	70.0
September	31.5	21.1	26.3	75.5	33.4	22.7	28.1	75.6
October	28.4	15.3	21.9	70.3	29.3	17.2	23.3	71.2
Average	33.0	20.8	27.0	70.5	34.3	22.3	28.0	71.8

### 2.2. Experimental design, treatment and crop management

The experiment was conducted in a split-plot arrangement e.g. three plant densities ( $D_1$ , low;  $7.5 \times 10^4$ ;  $D_2$ , moderate;  $9.0 \times 10^4$  and  $D_3$ , high;  $10.5 \times 10^4$   $ha^{-1}$ ) randomly assigned (sub plot) with two planting dates ( $S_1$ , early May 20;  $S_2$ , late June 04) (main plot). The split-plot arrangement with four independent replicates was used to increase the precision of comparisons. The experimental sub plot was size consisted of a 12 m long and 3.04 m wide with total plot size of 36.48  $m^2$ . Row spacing of the experimental treatment consisted of narrow row spacing (25 cm) and wide row spacing (76 cm). Plant spacing was adjusted according to the corresponding plant population density. Each sub plot was consisted of four rows with narrow row and wide row space. Cotton seeds were sown on raised bed by hand in respective plots. Seedlings were thinned two weeks after emergence to the required plant density (75,000, 90,000 and 105,000  $ha^{-1}$ ). Fertilizer, at the rate ( $kg$   $ha^{-1}$ ) of 180 N, 54  $P_2O_5$ , 180  $K_2O$ , 1.5 B with urea (46% N), superphosphate (12%  $P_2O_5$ ), potassium chloride (59%  $K_2O$ ) and borate (10% B), were applied at early flowering (66 days after emergence). Cultural management practices such as irrigation, weeding, hoeing and pesticide application were implemented to reduce competition for nutrient, light, water and spacing for a better crop stand. Mepiquat chloride was applied as a growth regulator in order to speed up boll opening and reduce excessive vegetative growth.

### 2.3. Observations

#### 2.3.1. Cotton plant growth characteristics

At peak boll stage (74 days after emergence), fifteen plants per plot were randomly selected to measure plant height using a specially designed ruler. Cotton fruiting branch length was measured from the point of attachment to the end of the branch. Number of fruiting branches nodes and leaves were counted from fifteen randomly selected plants in each plot. Fruiting branch length data were divided by fruiting branch number to obtain fruiting branch length to fruiting branch numbers ratio (FB/FN).

#### 2.3.2. Yield and yield contributors

Seed cotton yield (fiber and seed) was recorded three times from the manually harvested plants in each sub plot. The boll was sun dried to  $\leq 11\%$  water content (Dong et al., 2010), and ginned to obtain lint yield. Prior to second harvest one hundred fully matured open bolls were picked from each plot dried and ginned to calculate individual boll weight and lint%. Individual boll weight was calculated by total seed cotton yield of 100 bolls divided by total boll number. Lint% was assessed from the ratio of lint yield derived from 100 bolls divided by seed cotton weight of 100 bolls.

#### 2.3.3. Net photosynthetic rate (Pn)

Net photosynthetic rate (Pn) was measured at various reproductive growth stages e.g. squaring (47 days after emergence) (DAE), first bloom (66 DAE), peak bloom (74 DAE) and boll opening (121 DAE)

from the functional 4th leaf on the main stem from the apex using a gas-exchange meter (Li-6400, Li-COR Inc., NE, USA). These measurements were carried out on sunny days between 10:00 and 12:00 am in each treatment in the following conditions; light intensity of  $1800 \mu\text{mol m}^{-2} \text{s}^{-2}$ , the ambient  $\text{CO}_2$  concentration was  $366 \mu\text{mol mol}^{-1}$  and the vapor pressure was 3.5 kPa during different phenological stages at field temperatures. Three readings per leaf were replicated on three plants in each sub plot.

### 2.3.4. Nitrogen uptake

Three plants in each plot were randomly harvested at each growth stage of the crop, e.g. squaring (47 DAE), first flowering (66 DAE), peak flowering (74 DAE) boll opening (121 DAE) and plant removal (151 DAE). The plants were dissected into vegetative structures (root, stem, leaves, fruiting branch) and reproductive organs (squares, flowers, bolls). Samples were placed in an electric fan-assisted oven for quick cell killing at  $105^\circ\text{C}$  for 30 min to stop N consumption by respiration and dried at  $70^\circ\text{C}$  for at least 48 h to constant weight. The dried samples were milled with a Wiley mill and screened through a 0.5 mm sieve. Total N concentration was determined according to the micro-Kjeldahl method (Bremer and Mulvaney, 1982), and expressed on a dry weight basis. N accumulation was described by logistic function was used to describe N accumulation (Yang et al., 2011).

$$= \frac{Y}{1 + e^{-K(t-t_0)}} \quad (1)$$

In the formula  $t$  (d) shows DAE (days after emergence),  $Y$  (g) shows the biomass at  $t$ ,  $K$  (g) is the maximum biomass, and  $b$  are the constants.

$$t_1 = \frac{1}{b} \left( \frac{2 + \sqrt{3}}{a} \right), \quad t_2 = \frac{1}{b} \left( \frac{2 - \sqrt{3}}{a} \right), \quad m = \frac{1}{b} \quad (2)$$

$$V = \frac{1}{4} \quad (3)$$

$$V = \frac{2}{2^{-1}} \quad (4)$$

The period calculated 65% of the biomass uptake defined as fast accumulation period (FAP), which begins at  $t_1$  and terminates at  $t_2$ . During FAP,  $Y$  is the linearly correlated to  $t$  and the average speed of growth.

### 2.3.5. Statistical analysis

Microsoft Excel 2013 was used for data processing and figures were plotted using Sigma Plot 12.5 software. SAS 8.1 and DPS software were used to assess individual and interactive effects of sowing date and PPD on crop growth, physiology and lint yield.

## 3. Results

### 3.1. Cotton plant growth characteristics

Sowing date and plant density had a significant effect on cotton plant growth characteristics but the interaction was non-significant for all these parameters (Table 2). Early sown crops had 22.7% taller plants with 32% longer fruiting branches than the late-sown crops due to access to available resources early in the season. Similarly,  $S_1$  crops produced 54.9% and 77% more fruiting branches and nodes, respectively, compared with  $S_2$  crops. Across the population density, low PPD crops density produced 8.5% and 8.9% taller plants compared with moderate and high density crops, suggesting that competition for resources limiting crop growth and development.

Further, plants with low density had 21% and 17.2% longer fruiting branches. There was no significant difference among the plant density for number of fruiting nodes. No significant effect of sowing date was observed on ratios of fruiting branch to fruiting node. Across the population densities, plants grown with low density had 11.7% higher

fruiting branch to fruiting node ratios compared with other treatments. Crop cultivated with low density had 5.8% more number of leaves plant<sup>-1</sup> compared with other treatments. Sowing date and population density had no effect on cotton square abscission rate. The abscission rate was 8% and 6.6% higher in  $D_3$  population compared with  $D_2$  and  $D_1$ .

### 3.2. Cotton yield and yield components

Cotton yield and yield components were significantly influenced by sowing date and PPD during both years (Table 3). Early planted ( $S_1$ ) crop produced 45% and 26% more bolls and lint yield, respectively, compared with late planted crop ( $S_2$ ). Further, earlier crop had 26% more lint yield compared with  $S_2$  crop. Across PPD,  $D_2$  crop produced 27% and 13% more bolls than that of  $D_1$  and  $D_3$  crops, respectively. No significant effect of PPD was observed on individual boll weight. High boll production in  $D_2$  contributed to 13% and 6% more lint yield compared with  $D_1$  and  $D_3$  crops, respectively. A significant interaction was observed between SDs and PPD for lint yield during both years. The  $S_1$  crop with moderate PPD ( $D_2$ ) produced maximum lint yield followed by  $S_1$  with  $D_2$  and  $D_3$ , respectively in 2014. Although, lint yield was lower for  $S_1D_3$  relative to  $S_1D_2$  and  $S_1D_1$  treatment in 2015.

### 3.3. Net photosynthetic rate ( $P_n$ )

Net photosynthetic rate ( $P_n$ ) of cotton leaves was significantly affected by sowing date and plant density during all growth stages, except at peak bloom phase, where it remained unaffected by the changes in sowing dates (Table 4). In general,  $P_n$  increased as the crop transitioned from one stage to another but it decreased during boll opening stage during both years. The  $S_1$  plants exhibited 10% and 16% higher  $P_n$  than  $S_2$  plants at squaring and first bloom stage, respectively. Across PPD, the  $D_2$  plants had highest  $P_n$ , followed by  $D_1$  and  $D_3$  plants during all the growth phases. No significant interaction was observed between sowing date and PPD for leaf  $P_n$  at any crop growth phase.

### 3.4. Cotton plant nitrogen (CPN) accumulation

Cotton plant nitrogen (CPN) uptake increased as the plants grew following a normal sigmoidal growth curve (Fig. 1, A). There was no significant effect of sowing dates on N status of plants at squaring and first bloom stages; but it was significantly influenced during all other reproductive phases. The  $S_1$  plants accumulated 45%, 33.2% and 28.8% higher CPN accumulation at peak bloom, boll opening and plant removal compared with  $S_2$  plants. Across PPD,  $D_3$  plants were most efficient in CPN accumulation than  $D_1$  and  $D_2$  plants during all the growth stages. The interaction between sowing date and plant density for CPN accumulation was significant during all growth stages. The  $D_3$  plants accumulated higher CPN both under early and late plantings.

Vegetative organ N (VON) acquisition increased as the plant transitioned from one growth phase to another phase. It was also significantly influenced by changes in SD during all growth phases except squaring. Further, VON uptake increased as the plant transitioned from one stage to another. For example,  $S_1$  plants accumulated 24%, 36.4%, 36% and 33%, respectively, more VON accumulation than  $S_2$  plants at first bloom, peak bloom, boll opening and plant removal (plants was uprooted prior to last harvest), respectively (Fig. 1, B). Across the PPD,  $D_3$  had the highest VON followed by  $D_2$  and  $D_1$ . Both factors had an interactive effect on vegetative tissues N accumulation, and  $S_1 D_3$  combination was superior for N acquisition followed by  $S_2 D_3 > S_1 D_2 > S_2 D_2 > S_1 D_1$ .

Neither SD nor PPD had a significant effect on reproductive organ nitrogen (RON) acquisition at squaring and first bloom stages, although, these factors significantly influenced RON at later growth phases e.g. peak bloom, boll opening and withdrawal stages (Fig. 1, C). The  $S_1$  plants accumulated 27%, 33%, and 22.6% more RON than  $S_1$

**Table 2**  
Changes in cotton growth in response to different plant density and sowing date during the year 2014.

Treatment	Plant height (cm)	Fruiting branch length (cm)	Fruiting branch no/m <sup>-2</sup>	Fruiting nodes no. m <sup>-2</sup>	FB/FN ratio	Leaves no. plant <sup>-1</sup>	Fruit abscission%
Sowing date							
S <sub>1</sub> (early sowing)	96.2a	15.6a	98.1a	207.1a	2.1a	21.6a	63.8a
S <sub>2</sub> (late sowing)	74.7b	10.6b	44.2b	47.6b	1.1b	18.5b	64.6a
Plant density							
D <sub>1</sub> (low)	90.7a	15.1a	62.6c	120.6a	1.7a	20.7a	62.4b
D <sub>2</sub> (moderate)	83.0b	11.9b	70.4b	123.5a	1.5b	19.9ab	61.3bc
D <sub>3</sub> (high)	82.6b	12.5b	80.4a	138.0a	1.5b	19.5b	66.8a
Source of variance							
Sowing date	< 0.0001	0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.3987
Density	0.0415	0.0146	< 0.0001	0.2756	0.0407	0.0487	0.0035
S*D	0.9055	0.9973	0.1996	0.3804	0.2175	0.7788	0.5758

Means within a column followed by same letters did not significantly different at ( $P < 0.05$ ) according to Duncan multiple range test. FB/FN = fruiting branch length to fruiting branch node ratio.

**Table 3**  
Cotton lint yield and its contributors in response to sowing date and plant density during the year 2014 and 2015.

Treatment	Bolls m <sup>-2</sup>	Boll weight (g)	Lint%	Lint yield kg ha <sup>-1</sup>
Year 2014 Sowing date (SD)				
S <sub>1</sub> (early sowing)	81.9a	4.4a	50.1a	1795 a
S <sub>2</sub> (late sowing)	56.3b	4.1b	45.3b	1049 b
Planting density (PD)				
D <sub>1</sub> (low)	60.9b	4.3a	48.9a	1287 b
D <sub>2</sub> (moderate)	77.5a	4.3a	47.6ab	1606 a
D <sub>3</sub> (high)	68.6ab	4.3a	46.5b	1373 b
SD × PPD (interaction)				
S <sub>1</sub> D <sub>1</sub>	71.4b	4.4a	51.6a	1619 b
S <sub>1</sub> D <sub>2</sub>	97.8a	4.4a	49.7a	2134 a
S <sub>1</sub> D <sub>3</sub>	76.5b	4.4a	48.9ab	1631 b
S <sub>2</sub> D <sub>1</sub>	50.4d	4.1b	46.1bc	954 d
S <sub>2</sub> D <sub>2</sub>	50.7d	4.1b	45.5c	1077 cd
S <sub>2</sub> D <sub>3</sub>	60.7cd	4.2b	44.2c	1115 c
Source of variance				
SD	< 0.0001	0.0018	0.0002	< 0.0001
PPD	0.0008	ns	ns	0.0056
SD × PPD	0.0171	ns	ns	0.0279
Year 2015				
S <sub>1</sub>	66.6a	4.0a	48.6a	1254 a
S <sub>2</sub>	60.6b	4.0a	46.3a	1124 b
D <sub>1</sub>	64.0b	4.1a	47.8a	1233a
D <sub>2</sub>	68.6a	4.0a	46.8a	1283a
D <sub>3</sub>	58.3c	3.9a	46.3a	1051b
S <sub>1</sub> D <sub>1</sub>	69.3ab	4.1a	48.3a	1360a
S <sub>1</sub> D <sub>2</sub>	70.2a	3.9ab	47.0a	1299a
S <sub>1</sub> D <sub>3</sub>	60.2c	3.8b	47.6a	1103b
S <sub>2</sub> D <sub>1</sub>	58.8 cd	4.0ab	47.3a	1106b
S <sub>2</sub> D <sub>2</sub>	66.9b	4.0ab	46.7a	1267a
S <sub>2</sub> D <sub>3</sub>	56.4d	3.9ab	45.0a	998 b
Source of variance				
SD	0.0002	ns	ns	0.0011
PPD	< 0.0001	ns	ns	0.0002
SD × PPD	0.0214	ns	ns	0.0240

Means within a column followed by same letters did not significantly different at ( $P < 0.05$ ) according to Duncan multiple range test.

crops at peak bloom, boll opening and withdrawal (plant removal) stages, respectively. Among different PDD, D<sub>2</sub> plants had higher RON at all growth stages followed by D<sub>1</sub> and D<sub>3</sub> plants.

### 3.5. Simulation of nitrogen accumulation

Simulation of N accumulation with cotton growth stages was calculated using Formula (1). The function of logistic was followed by N accumulation as a normal sigmoidal growth pattern since all P

**Table 4**  
Net photosynthetic rate of cotton leaves as influenced by various sowing date and planting density at different growth stages during 2014 and 2015.

Treatment	Squaring (47 DAE)	First bloom (66 DAE)	Peak bloom (74 DAE)	Boll opening (121 DAE)
Sowing date				
S <sub>1</sub> (early sowing)	27.6a	29.8a	32.1a	24.1a
S <sub>2</sub> (late sowing)	25.2b	25.7b	30.6a	22.5b
Density				
D <sub>1</sub> (low)	27.3a	27.6b	31.0b	23.2b
D <sub>2</sub> (moderate)	26.6ab	29.6a	34.0a	24.7a
D <sub>3</sub> (high)	25.4b	25.2b	28.1c	21.2b
Source of variance				
Sowing date	< 0.0001	< 0.0001	0.0776	0.0019
Density	0.0159	0.0092	0.0472	0.0018
S*D	0.0624	0.6842	0.8832	0.7132
2015				
Sowing date				
S <sub>1</sub> (early sowing)	29.6a	32.3a	33.1a	26.1a
S <sub>2</sub> (late sowing)	26.2b	28.5b	30.2b	23.6b
Density				
D <sub>1</sub> (low)	26.3a	28.2b	31.0b	24.2b
D <sub>2</sub> (moderate)	26.7ab	30.6a	35.0a	25.7a
D <sub>3</sub> (high)	24.6b	26.2b	28.1c	21.2b
Source of variance				
Sowing date	< 0.0001	< 0.0001	0.0276	0.0019
Density	0.0148	0.0072	0.0047	0.0014
S*D	0.0724	0.6942	0.8732	0.8232

Means within a column followed by same letters did not significantly different at ( $P < 0.05$ ) according to Duncan multiple range test. DAE = days after emergence; P<sub>n</sub> = rate of photosynthesis ( $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ).

values were  $< 0.005$  (Table 5).

Data obtained by Formula (2)–(4), based on Table 5 exhibited the beginning and termination day of cotton plant nitrogen (CPN) uptake for earlier (S<sub>1</sub>) and later (S<sub>2</sub>) sowing are presented in (Table 6). S<sub>1</sub> plants had higher rates of CPN acquisition in average and maximum than S<sub>2</sub> plants during the whole growing period. Further, S<sub>1</sub> plants with higher density (S<sub>1</sub>D<sub>3</sub>) showed fast CPN accumulation at 64 days after emergence (DAE) and terminated at 83 DAE 7-d, 6-d later compared with earlier and moderate (S<sub>1</sub>D<sub>2</sub>) and earlier and lower (S<sub>1</sub>D<sub>1</sub>) density, respectively. Further, both average ( $6.7 V_M \text{ kg ha}^{-1} \text{ d}^{-1}$ ), and maximum ( $7.7 V_M \text{ kg ha}^{-1} \text{ d}^{-1}$ ) CPN accumulation rate in S<sub>1</sub>D<sub>3</sub> crops were higher than S<sub>1</sub>D<sub>1</sub> and S<sub>1</sub>D<sub>2</sub>. A similar trend was observed in S<sub>2</sub> sowing, S<sub>2</sub> D<sub>3</sub> had maximum rate ( $4.8 V_M \text{ kg ha}^{-1} \text{ d}^{-1}$ ), CPN accumulation during FAP compared with S<sub>2</sub>D<sub>2</sub> and S<sub>2</sub>D<sub>1</sub>, respectively (Table 6).

Changes in sowing date also influenced progress in vegetative organ nitrogen (VON) accumulation. The fast VON accumulation period for S<sub>1</sub> D<sub>1</sub>, S<sub>1</sub> D<sub>2</sub> and S<sub>1</sub> D<sub>3</sub> started at 59 d and ended at 85 d. The S<sub>1</sub>D<sub>3</sub> had the

**Fig. 1.** (A–C) Cotton plant total (CPN), reproductive organ (RON), and vegetative organ nitrogen (VON) accumulation as influenced by various sowing date and plant density during the 2014 growing season. Plants were carefully uprooted at various growth stages e.g. squaring (47 days after emergence) (DAE), first bloom (66 DAE), peak bloom (74 DAE) and boll opening (121 DAE) and dissected into root, stem, stem leaves, fruiting branch, branch leaves and boll to determine N content. The treatments S1D1, S1D2, S1D3 indicate early sowing with lower, moderate and high density, respectively, while S2D1, S2D2, S2D3 shows late sowing with lower, moderate and high density, respectively. Error bars represent SD (n = 3).

highest average ( $3.8 V_T \text{ kg ha}^{-1} \text{ d}^{-1}$ ) and maximum ( $5.0 V_M \text{ kg ha}^{-1} \text{ d}^{-1}$ ) rate than that of other combinations S<sub>1</sub> D<sub>1</sub> and S<sub>1</sub> D<sub>2</sub>, respectively. S<sub>2</sub> D<sub>1</sub>, S<sub>2</sub> D<sub>2</sub> and S<sub>2</sub> D<sub>3</sub> showed starting and ending days of the 31-d FAP for VON, averaged across the treatments were 61 and 92 DAE, respectively (Table 6). Moreover, S<sub>2</sub> D<sub>3</sub> was superior to other treatments in average and maximum rate in FAP ( $2.6 V_M \text{ kg ha}^{-1} \text{ d}^{-1}$ ) and ( $2.9 V_M \text{ kg ha}^{-1} \text{ d}^{-1}$ ) terminated at 73 DAE.

Averaged across the treatments, the FAP for reproductive organ nitrogen (RON) uptake began at 22-d in S<sub>1</sub> crop and 26-d in S<sub>2</sub>, respectively (Table 6). Among the treatments S<sub>1</sub>D<sub>2</sub> RON accumulation initiated the FAP at 80 DAE and ended at 102 DAE with relatively higher average ( $3.5 V_T \text{ kg ha}^{-1} \text{ d}^{-1}$ ) and maximum ( $4.5 V_M \text{ kg ha}^{-1} \text{ d}^{-1}$ ) rates to S<sub>1</sub>D<sub>1</sub> and S<sub>1</sub>D<sub>3</sub>, respectively. RON accumulation in S<sub>2</sub> started day of FAP at 94.7 DAE and ended at 120.3 DAE, with higher average and maximum speed of FAP. However, S<sub>2</sub>D<sub>2</sub> had the highest average ( $2.1 V_T \text{ kg ha}^{-1} \text{ d}^{-1}$ ), and maximum

( $3.1 V_T \text{ kg ha}^{-1} \text{ d}^{-1}$ ) rates of RON uptake compared with other treatments. Significant differences existed between sowing dates for RON accumulation. S<sub>1</sub> crops had higher RON acquisition rate in both average and maximum compared with S<sub>2</sub> crops.

#### 4. Discussion

In this 2-year field study, we investigated the effects of plant density and sowing date on growth and physiology of a cotton crop. Increased PPD is practiced to maintain the number of bolls per unit area but it reduces individual plant yields. This pattern is essential when the crop growing season is short. Late planted crop with high density has the potential to increase lint yield under intensive field management (Dong et al., 2006). Late planted short season cotton with moderate plant density produced higher yields than other combinations (Dong et al., 2010). In the present study, cotton lint yield was significantly greater in D<sub>2</sub> compared with D<sub>1</sub> and D<sub>3</sub>. No significant increase in lint yield beyond D<sub>2</sub>, suggests that increasing that PPD may increase number of bolls per unit area without contributing to the total lint yield due to poor boll filling (Mao et al., 2015; Rossi et al., 2004). In contrast, to lower seeding rates, farmers tend to reduce input costs, which delays crop maturity and reduces overall lint yield (Yang et al., 2014a,b; Zhao et al., 2012; Siebert et al., 2006). Recent research has suggested that, cotton yield can be increased by increasing the PPD (Bartimote et al., 2017; Liu et al., 2015 Venugopalan et al., 2014; Ali et al., 2011; Brodrick et al., 2013).

Increased lint yield in D<sub>2</sub> crop was primarily attributed to higher root growth and activity in the soil, which in turn promoted N uptake and translocation to the developing bolls. Further, this promoted fruiting retention capacity. In contrast, poor light penetration to the lower canopy in D<sub>3</sub> crop accelerated photorespiration, leading to an increase ethylene/sugar ratio and higher abscission rate of reproductive structures (Echer and Rosolem, 2015). Elevated ethylene concentration in cotton tissues has been linked with poor yield performance (Najeeb et al., 2015) and can be regulated by blocking ethylene biosynthesis through aminoethoxyvinylglycine application (Najeeb et al., 2016). In higher density systems cotton plants produce smaller bolls due to poor boll filling compared with conventionally planted cotton (Wright et al., 2011). This suggests that, increasing lint yield is possible with high to moderate PPD but further increase in PPD may lower lint yield.

Sowing date is also an important determinant for cotton production. In this study, early planted crop produced significantly higher lint than late sown crop, which can be explained by the fact that S<sub>1</sub> crop took advantage of soil moisture and nutrients for longer growing season and produced more bolls. In contrast, S<sub>2</sub> crop experienced a shorter reproductive period due to increased air temperatures and reduced canopy photosynthesis due to less radiation interception. The reduction in lint yield in S<sub>2</sub> crop in 2014 was the result of many unopened bolls at harvesting. Although defoliation and tipping was practiced to enhance boll opening of late bolls in S<sub>2</sub> crop 2015, lint yield remained significantly lower than S<sub>1</sub> crop. The reproductive development in S<sub>2</sub> crop was also affected by cooler temperature and low light, which reduced photosynthetic activity carbohydrates transition to fruit structures (Gormus and Yucel, 2002; Liu et al., 2015; Zhang et al., 2014).

In this study, S<sub>1</sub> crops achieved higher leaf photosynthesis than S<sub>2</sub> crop while moderate plant density resulted in higher photosynthetic rate compared with high and low density. Lower leaf photosynthesis and assimilate production could lead to low lint yield in cotton (Constable and Oosterhuis, 2010). Recent research indicated that dense PPD can intercept higher radiation interception and increase net canopy photosynthesis rate compared with lower density (Xie et al., 2016). However, increased light capturing early in the season in dense PPD crop may not contribute to the final yield due to self-shading during the reproductive period, as observed in D<sub>3</sub> crop in this study.

The Reduction in canopy photosynthesis in the S<sub>2</sub> crops was attributed to higher leaf senescence compared with the S<sub>1</sub> crop

**Table 5**  
Regression equation of cotton plant nitrogen (N) accumulation at various sowing date and plant density.

Items	Treatment	Regression equation	P
Cotton plant	S <sub>1</sub> D <sub>1</sub>	Y = 215.4207/(1 + 10.5518e <sup>-0.143885t</sup> )	0.0036
	S <sub>1</sub> D <sub>2</sub>	Y = 260.1360/(1 + 6.3051e <sup>-0.086443t</sup> )	0.0099
	S <sub>1</sub> D <sub>3</sub>	Y = 291.8523/(1 + 7.1073e <sup>-0.102345t</sup> )	0.0037
	S <sub>2</sub> D <sub>1</sub>	Y = 176.0924/(1 + 7.1127e <sup>-0.091171t</sup> )	0.0039
	S <sub>2</sub> D <sub>2</sub>	Y = 210.2699/(1 + 4.2410e <sup>-0.054664t</sup> )	0.0004
	S <sub>2</sub> D <sub>3</sub>	Y = 235.7756/(1 + 6.1581e <sup>-0.081733t</sup> )	0.0098
Vegetative organ	S <sub>1</sub> D <sub>1</sub>	Y = 146.9431/(1 + 8.2934e <sup>-0.111388t</sup> )	0.0031
	S <sub>1</sub> D <sub>2</sub>	Y = 171.0058/(1 + 6.6382e <sup>-0.095197t</sup> )	0.0019
	S <sub>1</sub> D <sub>3</sub>	Y = 204.5176/(1 + 6.7542e <sup>-0.093538t</sup> )	0.0056
	S <sub>2</sub> D <sub>1</sub>	Y = 114.9593/(1 + 6.7824e <sup>-0.084665t</sup> )	0.0058
	S <sub>2</sub> D <sub>2</sub>	Y = 127.0314/(1 + 5.8082e <sup>-0.079451t</sup> )	0.0005
	S <sub>2</sub> D <sub>3</sub>	Y = 150.0314/(1 + 5.8082e <sup>-0.079451t</sup> )	0.0061
Reproductive organ	S <sub>1</sub> D <sub>1</sub>	Y = 67.5510/(1 + 31.3825e <sup>-0.344981t</sup> )	0.0361
	S <sub>1</sub> D <sub>2</sub>	Y = 85.8716/(1 + 14.3444e <sup>-0.152082t</sup> )	0.0006
	S <sub>1</sub> D <sub>3</sub>	Y = 80.3843/(1 + 8.6495e <sup>-0.079209t</sup> )	0.0007
	S <sub>2</sub> D <sub>1</sub>	Y = 61.6606/(1 + 26.1700e <sup>-0.268482t</sup> )	0.0004
	S <sub>2</sub> D <sub>2</sub>	Y = 81.8474/(1 + 10.0281e <sup>-0.089851t</sup> )	0.0002
	S <sub>2</sub> D <sub>3</sub>	Y = 77.8474/(1 + 10.0281e <sup>-0.089851t</sup> )	0.0008

**Table 6**  
Eigen values of cotton plant nitrogen (N) accumulation at various sowing date and plant density during 2014.

Treatment	Fast accumulation period				Fastest accumulation point	
	t <sub>1</sub> DAE	t <sub>2</sub> DAE	T d	V <sub>T</sub> kg ha <sup>-1</sup> d <sup>-1</sup>	V <sub>M</sub> kg ha <sup>-1</sup> d <sup>-1</sup>	at DAE
Cotton plant						
S <sub>1</sub> D <sub>1</sub>	57.7	88.2	30.5	4.7	5.3	72.9
S <sub>1</sub> D <sub>2</sub>	56.6	82.3	25.7	6.5	7.2	69.4
S <sub>1</sub> D <sub>3</sub>	64.2	82.5	18.3	6.7	7.7	73.3
Average	59.5	84.3	24.8	6.0	6.7	71.9
S <sub>2</sub> D <sub>1</sub>	61.5	90.7	29.2	2.8	2.9	77.6
S <sub>2</sub> D <sub>2</sub>	63.6	92.5	28.9	3.9	4.0	78.0
S <sub>2</sub> D <sub>3</sub>	59.2	91.5	32.2	4.2	4.8	75.3
Average	61.4	91.5	30.1	3.6	3.9	77.0
Vegetative organs						
S <sub>1</sub> D <sub>1</sub>	62.6	86.3	23.6	2.4	3.3	74.5
S <sub>1</sub> D <sub>2</sub>	55.9	83.6	27.7	2.8	4.1	69.7
S <sub>1</sub> D <sub>3</sub>	58.1	86.3	28.2	3.8	5.0	72.2
Average	58.9	85.4	26.5	3.0	4.1	72.1
S <sub>2</sub> D <sub>1</sub>	64.6	95.7	31.1	1.5	1.7	80.1
S <sub>2</sub> D <sub>2</sub>	56.5	89.7	33.2	2.2	2.7	75.1
S <sub>2</sub> D <sub>3</sub>	61.5	90.7	29.2	2.6	2.9	73.1
Average	60.9	92.0	31.1	2.1	2.4	76.1
Reproductive organs						
S <sub>1</sub> D	81.6	107.8	26.3	1.4	1.6	95.2
S <sub>1</sub> D <sub>2</sub>	83.2	104.3	21.6	3.5	4.5	91.0
S <sub>1</sub> D <sub>3</sub>	79.7	102.0	22.3	2.4	3.1	97.3
Average	81.5	104.7	23.4	2.4	3.0	94.5
S <sub>2</sub> D <sub>1</sub>	97.0	126.3	29.3	1.2	1.4	102.6
S <sub>2</sub> D <sub>2</sub>	89.1	110.3	21.2	2.1	3.3	106.5
S <sub>2</sub> D <sub>3</sub>	98.0	124.3	27.3	1.7	1.9	104.6
Average	94.7	120.3	25.9	1.7	2.2	104.2

DAE = days after emergence (d), t<sub>1</sub> and t<sub>2</sub> are the beginning and terminating days of the fast accumulation period. T indicates the duration of FAP, T = t<sub>1</sub>–t<sub>2</sub> and V<sub>T</sub> and V<sub>M</sub> are the average and maximum biomass accumulation rates during the FAP respectively.

(Table 1). Carbon assimilation in the S<sub>2</sub> crop was further impaired due to reduced day length and radiant energy (Bauer et al., 2000). This indicates that leaves on the main stem plays a pivotal role in cotton growth and development through light interception and radiation use efficiency. Lower Pn was expected in plants with higher PPD due to self-shading, which inhibited light penetration to the lower leaves, which also occurred in the present study (Zhang et al., 2012; Echer and

Rosolem, 2015; Pettigrew and Adamezyk, 2008). The absorption of CO<sub>2</sub> in leaves of D<sub>1</sub> and D<sub>2</sub> was greater than D<sub>3</sub> PPD. The CO<sub>2</sub> absorption of cotton leaves was 2.2 times greater when cotton was grown under higher radiation (361 μmol CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup>) relative to low radiation (63 μmol CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup>) as reported by (Xie et al., 2016; Smith and Longstreth, 1994). We suggest that later sowing and higher PPD can potentially suffer photosynthesis reduction due to shorter day length, increased competition for resources more leaf senescence and poor light penetration to lower canopy leaves which in turn lower lint yield. This also suggests that leaf photosynthetic rate can be adjusted by modifying plant architecture through plant density and sowing date.

N is the basic nutrient for enhancing crop growth and development and over use of N is detrimental to the environment (Khan et al., 2017). For continuing growth, plants need an adequate nutrient supply, and nutrient absorption may vary in quantity and rate during different growth periods (Gao and Lynch, 2016). Crops with higher PPD may benefit from accelerated N uptake during initial to peak flowering (Meng et al., 2013). Cotton growth and yield is positively associated with nutrient uptake by roots (Bange et al., 2004). The N uptake mechanisms are complex and involve a series of bio-chemical reactions. Both the root architecture and the effect of nitrate and ammonium synchronized by N form and concentration, diurnal fluctuations, and temperature fluctuations affect N acquisition by roots transport system (Garnett et al., 2009). Further, management practices such as PPD and SD also influence N availability, accumulation and utilization (Yadvinder-Singh et al., 2005; Witt et al., 2000). In this study, cotton plants with higher PPD accumulated more N in vegetative tissues during peak bloom and maturity stages, probably due to inter-plant competition per unit area for resources leading to faster nutrients accumulation. Less nutrients were translocated towards reproductive tissues compared with medium PPD plants, resulting in poor boll filling. Despite an increased rate of nutrient accumulation per unit area, plants with higher PPD might have accumulated fewer nutrients on a per plant basis (Xue et al., 2013). Similar data have been reported by Liu et al. (2011), who indicated that higher PPD crops accumulated more N accumulation in vegetative organs at early flowering, and peak bloom but, plants at medium PPD accumulated more N in reproductive structures at the same stage. Increased N uptake by S<sub>1</sub> crop might be associated with higher soil available water early in the season thus allowing greater exploration of root for nutrients uptake (Caviglia et al., 2014). Environmental factors such as soil temperature and vapor pressure deficit may also promoted N uptake in S<sub>1</sub> crop (Liu et al.,

2011). Our data are in good agreement with Ehdai and Waines (2001), who also reported that early planted crop increase macro nutrients accumulation, distribution and utilization. Our data showed that higher reproductive organ N accumulation resulted in the efficient translocation of photosynthates during boll filling which led to higher yield. Higher density has the potential to increase total plant N acquisition but on the other hand decrease N partitioning to reproductive organs.

## 5. Conclusions

The present study proved that cotton lint yield significantly increased with moderate planting density under two sowing dates. Moderate density significantly altered plant architecture and consequently increased net photosynthesis and N accumulation and lint yield, for both sowing dates. This increment in lint yield was associated with higher N uptake, which promoted canopy photosynthetic capacity and assimilate translocation towards developing fruits. It is concluded that 9.0 plants m<sup>-2</sup> could be optimum plant density under both sowing dates in Hubei province China. Ethylene management in cotton canopy could promote higher lint yield by controlling fruit shedding in dense cotton population, however, further studies are needed to confirm this hypothesis.

## Acknowledgements

We are thankful to funding by The National Natural Science Foundation of China (311716600 and 31360248).

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