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Original article

Interactive effect of nitrogen fertilizer and plant density on photosynthetic and agronomical traits of cotton at different growth stages

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ABSTRACT

Individual effects of application of nitrogen (N) and plant densities (PD) were reported in various studies; however an interactive effect of N and PD in cotton was not studied. To explore the benefits of interactive effects of N fertilizer and PD to increase the quality of cotton. This study was carried out in randomized complete block design (RCBD) with split plot arrangement. In split plot arrangement, main plot was consisted of N application rate and in sub plots different PD were done. There were two nitrogen levels; low N level (F1) 120 kg ha^{-1} and high N level (F2) 180 kg ha^{-1} and three planting densities; 8 plants m^{-2} as low density (LD), 10 plants m^{-2} as medium density (MD) and 12 plants m^{-2} as high density (HD). In this study we observed the interactive effect of N application levels and PD on cotton photosynthetic and agronomic traits of various stages of development. Results showed that cotton growth and N contents was varied among treatments on different development stages. Plant biomass production, photosynthetic rate (Pn), intercellular CO_2 (C_i), water use efficiency (WUE) and N contents were unaffected at the seedling stage by N application rate and PD, however, the highest Pn, C_i and N contents was at squaring stage followed by blooming stage. Higher seed cotton yield and lint yield were obtained F1 with HD, and F2 with MD yielded the highest N contents and cotton yield among treatments. We found that the squaring stage was more critical, followed by the blooming stage when considering N rate and PD.

1. Introduction

For efficient cotton production, it is very important to understand the response of cotton growth, development and yield to various management practices. Management Practices include cultivars, planting density, ecological conditions, nutrients application (Dong et al., 2006; Ciampitti and Vyn, 2011; Hoffmann et al., 2009; Shah et al., 2017a). For sustainable cotton production nutrients application and plant density plays a vital role (Yang et al., 2014; Hafeez et al., 2019).

Nitrogen (N) is a major nutrient and it is essential constituent during period for sustainable production of cotton (Shah et al., 2017b). Nitrogen is very important constituent for photosynthesis and enlargement of canopy area (Zhang et al., 2002; Ali et al., 2018). Moreover N applications enhance cotton production by enhancing bolls number $plant^{-1}$ (Bondada et al. (1996); Chen et al. (2018)). Lokhande and Reddy, 2015 stated that application of N from the optimal level decreases, then cotton growth and yield to decrease at significant level. Similarly, Jaynes et al. (2001) investigated due to N deficiency stunted growth of cotton with reduce leaf area, fruiting branches and lint yield. Excess N application

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caused in late maturity and also reduces of lint yield per unit area (Saleem et al., 2010; Tung et al., 2018). The plant needs of N, particularly in the squaring stage if failure to secure N on that stage it may cause to a significantly decrease in yield (Moore, 2008). Moreover rising costs of N fertilizer and also emissions of greenhouse gases have provoked better consideration toward the proficient utilization of nitrogen inputs (Rochester et al., 2007). Rochester et al. (2009) stated extra N fertilizers have been applied in cotton fields that about 50 to 60 kg N ha^{-1} and 15–25% N inputs, these can be decreased if manage practices properly.

Second main factor is plant density (PD) which influence on cotton yield. Highest production attained at optimal PD which depends upon cultivar, ecological circumstance and cropping system (Halemani and Hallikeri, 2002). Enhance in PD increasing bolls number $plant^{-1}$ as well as 100-boll weight, on the other hand some say the opposite increase in PD decrease bolls number and 100 bolls weight of a plant (Boquet, 2005), although seed cotton yield per hectare improved because of more plants ha^{-1} (Ali et al., 2011). Moreover high PD consequences in higher dry matter partitioning to the fruits, thus resulting greater bolls number per unit area. PD, crop cultivar and standard cultural practices vary with regions, (Bozbek et al., 2006). Maximum cotton production was achieved as of PD (m^{-2}) 21.5 plants and 12.6 plants between 3.6, 9.0, 12.6 in Georgia (Bednarz et al., 2005), 10 plants in Arizona (USA) (Kawakami et al., 2012). Suggested plant density in Mali 8–25 plants (Barrabé et al., 2007), in India 5.5 plants (Blaise and Ravindran, 2003), in Yangtze River valley china 2.0 plants (Yang and Zhou, 2010) and 22.5 plants in Northwest China (Han et al., 2009).

Nonetheless, China's industrialization has increased, with an increasing labor resulting in increased labor costs in agricultural production. A new planting model with low cost characterized by late sowing, high density and low fertilization but without any reduction in yield compared to conventional planting model is attractive and promising. To get a better understanding, however, why cotton can produce the similar yield with lower costs of a shorter growing season and lower fertilizer. We conducted our study to find the optimum combination of planting density and application rate of nitrogen in terms of cotton yield and to explain the mechanisms from the angles of yield formation, biomass accumulation, N contents, and photosynthetic rate as affected by nitrogen application rates and planting densities and their interactions.

2. Materials and methods

2.1. Site description

This research was conducted at Huazhong Agricultural University, Wuhan, China during growing summer season 2015. Weather data recorded by using the CMART data recorder plus (HANG ZHOU HICTECH CO., LTD) device, it had been placed at a height 30 cm high from the soil surface and readings were recorded for 24 h a day and maximum every 30 min, minimum temperature (°C) and the relative humidity (%) had been calculated for each month mentioned in (Table 1). The nutrients status of experimental soil at 20 cm depth was 128.64 mg kg^{-1} N, 16.01 mg kg^{-1} P_2O_5 and 97.09 mg kg^{-1} K_2O.

2.2. Experimental design

The experiment was carried out in RCBD with split plot arrangement. Study consists of four replications with six treatments (Table 2). Plot size was measured 30.4 m^2 (10 m × 3.4 m). Main plots were consisted of different nitrogen application rate, while various plant densities arranged in sub plots. Only one time N, P, and K fertilizer in form of Urea (46% N), calcium superphosphate (10% P_2O_5), potassium chloride (59% K_2O) respectively, and borate (10% B) was supplied at flowering stage. Cotton variety used was Huamian-3109.

2.3. Data collection

2.3.1. Cotton biomass, photosynthetic rate, intercellular CO_2, and water use efficiency

Plant fresh weight (PFW) and Plant dry weight (PDW), photosynthetic rate (Pn), intercellular CO_2 (C_i) and water use efficiency (WUE) were measured at seedling, squaring, blooming and maturity stages under 20, 50, 80 and 120 DAE (Days after emergence), respectively. For PFW, four plants from each treatment were taken out and divided the plant into different parts and wrapped separately, then put into oven for dry weight at 70 °C for 48 h.

Fifteen fully expanded 4th leaves (from top) were chosen for determination of Pn and C_i from each treatment. A portable photosynthesis system (Licor-6400) with photoactive radiation adjustments (PAR) 1500 μmol m^{-2} s^{-1} was used for its measurement, ambient temperature was 39.69 to 42.68 °C, leaf temperature was 36.67–41.91 °C and ambient CO_2 concentration was 358 mmol m^{-2} s^{-1} and data were taken in morning (9:00 am to 10:00 am).

2.3.2. Seed cotton and lint yield, harvest index (HI) and ratio of seed cotton to stalk (RSS)

At maturity, plants were harvested to measure seed cotton, lint yield and biological yield. These plants were harvested manually and divided into different parts. At 70 °C each partitioned had been dried separately dried. Measure the yields of seed cotton and stalk yield. When seed cotton retained moisture (≤11%), it was ginned by 10-saw (hand-fed laboratory gin), and after ginning lint yield (g m^{-2}) was calculated. Stalk weight plus seed cotton and plant trash were taken as biological yield (Dong et al., 2010) measured as

HI was calculated as

$$HI = \frac{\text{Lint yield}}{\text{Biological yield}} \tag{1}$$

While RSS was recorded as

$$RSS = \frac{\text{Seed cotton yield}}{\text{Stalk weight}} \tag{2}$$

2.3.3. Total nitrogen contents at different growth stages

Total nitrogen contents were measured at different growth stages and four plants per treatment were harvested on each growth stage. For determination of N contents, sample of all plant parts were grounded and pass through a 0.2 mm sieve. A procedure was followed to examine total N concentration using the micro-Kjeldahl method (Bremer and Mulvaney, 1982).

To digest the plant samples, 0.2 g of each components of the powder was weighted and put in a 250 ml digestive tube with 5–6 ml H_2SO_4 and 2 ml H_2O_2 were added and heated for 60–90 min at 300 °C. Every 15 min 3–4 drops of H_2O_2 were added to make sure the digesting solution was clear which means the digesting process was over perfectly. The liquid was poured into a 50 ml volume pitcher after the digestion had been completed, and the volume was completed to 50 ml using distilled water. One ml of the diluted liquid was taken and placed into a 10 ml tube and finished with distilled water to 10 ml with distilled water, this solution was used for the tests.

The total nitrogen content was calculated from:

$$N\% = \frac{(V - V0) \times N(\text{of acid}) \times 1.4007}{W} \tag{3}$$

Table 1
Average daily maximum, minimum, mean temperature and relative humidity (RH) during the growing season 2015, Wuhan (China).

Months	Max °C	Min °C	Mean °C	RH%
May	27.1	18.6	22.9	82.5
June	31.3	22.6	27.0	67.6
July	42.3	27.1	34.7	63.1
August	41.2	24.5	32.9	70.0
September	33.3	22.5	27.9	75.5
October	29.5	17.3	23.4	71.0
November	22.5	19.2	20.9	67.5
Average	32.5	21.7	27.1	71.0

Table 2
Description about treatments.

Treatments abbreviations	Treatment description
F1	N application at 120 kg ha^{-1} as low N application
F2	N application at 180 kg ha^{-1} as high N application
LD	8 plants m^{-2} as low plant density
MD	10 plants m^{-2} as medium plant density
HD	12 plants m^{-2} as high plant density

In which V is volume (ml) of standard acid required for sample, V0 is volume (ml) of standard acid required for blank, N is normality of acid standard, 1.4007 = milliequivalent weight of N $\times$ 100 and W is sample weight in grams.

2.4. Statistical analysis

All statistical analysis was performed by using SPSS 18.0 (SPSS inc., USA). Mean differences among treatments were calculated using LSD at ($p < 0.05$). Figures were organized using Sigma Plot 10.0 software.

3. Results

3.1. Biomass production, photosynthetic rate, intercellular CO_2, and water use efficiency at different growth stages

Different PD and N application rates significantly influenced cotton biomass production (Fig. 1). Overall, cotton plants under F1HD and F2MD treatments exhibited relatively higher PFW and PDW as compared with other treatment while least PFW and PDW was at F1LD. Nonetheless, PFW and PDW were considerably affected by PD and N application rates at seedling, squaring, blooming and maturity stages while maximum PFW and PDW were observed at maturity stage (Fig. 1).

The effects of PD and N application rates were significantly impacted by Pn, Ci and WUE (Fig. 2). The highest Pn was found under F2MD at squaring stage and the lowest was found under F1LD at squaring stage while the highest Ci was observed under F2MD at seedling stage and the lowest was observed under F1LD at maturity level. Though higher WUE was under F1HD at blooming stage relative to other growth stages, less was found under F1LD at blooming stage (Fig. 2).

3.2. Cotton yield, ratio of seed cotton to stalk and harvest index

Seed cotton and lint yields were significantly influenced by N application rate and PD (Fig. 3). Higher cotton yield was observed under F1HD and F2MD, respectively. Least seed cotton yield was observed under F1LD compared with all other treatment (Fig. 3). While N application and PD had a significant influence on the ratio of seed cotton to stalk and harvest index. Under F1MD the highest seed cotton to stalk ratio was noted followed by F1LD and F2LD. Under F1MD the highest harvest index was noted followed by F1LD and F2LD. The F1HD and F2HD respectively noted the lowest ratio of cotton seed to stalk and harvest index (Fig. 3).

3.3. Total nitrogen contents in cotton

Interactive effect of N application rates and PD considerably influenced N contents in cotton (Table 3). At seedling stage, N contents remained unaffected in response to PD and N application rate. Nonetheless at squaring stage, the highest N contents were observed under F2HD. At blooming stage and maturity stage, F1HD gave the highest N contents followed by F2MD as compared with other treatments. Minimum N contents were noted in F1LD treatment at all growth stages except seedling stage (Table 3).

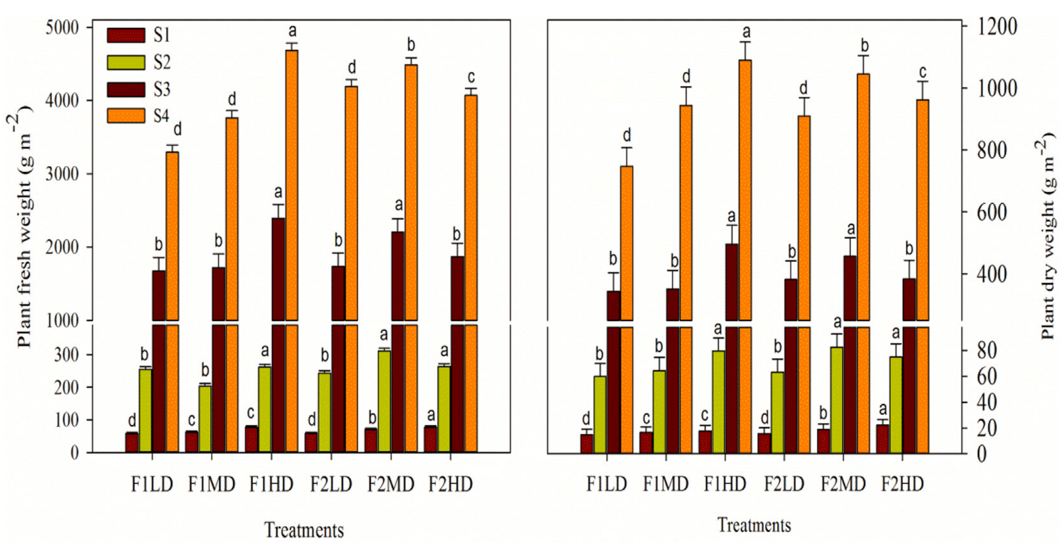

Fig. 1. Interactive effects of planting density and nitrogen application rate on plant fresh and dry weight. In treatments, F1 is showing low nitrogen application rate 120 kg ha^{-1}, F2 is showing high nitrogen application rate 180 kg ha^{-1}, LD is showing low planting density (8 plants m^{-2}), MD is showing medium planting density (10 plants m^{-2}) and HD is showing high planting density (12 plants m^{-2}). In graph; S1 shows seedling stage, S2 is showing squaring stage, S3 is showing blooming stage and S4 is showing maturity stage. Mean values followed by the same letters are not significantly different using least significance difference test (LSD) at 0.05 probability level.

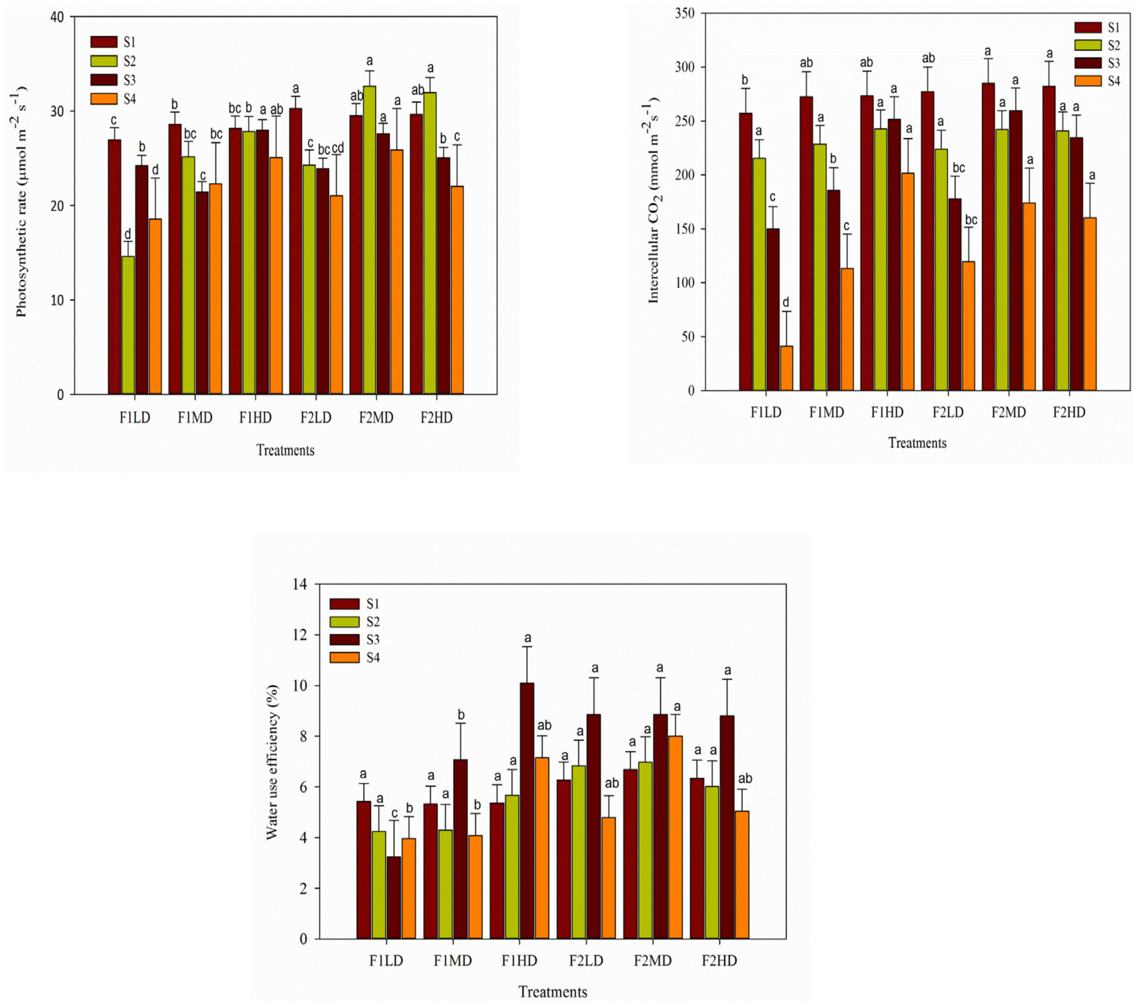

Fig. 2. Interactive effects of planting density and nitrogen application rate on photosynthetic rate, intercellular CO_2 and water use efficiency. In treatments, F1 is showing low nitrogen application rate 120 kg ha^{-1}, F2 is showing high nitrogen application rate 180 kg ha^{-1}, LD is showing low planting density (8 plants m^{-2}), MD is showing medium planting density (10 plants m^{-2}) and HD is showing high planting density (12 plants m^{-2}). In graph; S1 shows seedling stage, S2 is showing squaring stage, S3 is showing blooming stage and S4 is showing maturity stage. The same letters are not significantly different using least significance difference test (LSD) at 0.05 probability level and bar values above mean values are the LSD values.

4. Discussion

In the present study, we observed the effect of different N application rate and PD on cotton biomass production, the ratio of seed cotton to stalk, harvest index, cotton yield and total N contents at various growth stages. The overall findings of this study showed differential growth and yield response of cotton, which was linked photosynthetic activity and total N contents at different growth stages. We found that cotton growth also increases in terms of PFW and PDW, with an increase in PD. But the interaction of N and PD gave dissimilar results. In current study results showed cotton plant growth better under F1HD and F2MD combinations as contrast to other treatments (Fig. 1).

Better cotton growth on these combinations, because of better distribution of N in cotton plant, as contrast to other combinations. Better cotton growth was associated with the higher leaf area, crop growth rate and leaf gas exchange. This also showed that PD and N application rate could compensate each other in improving cotton growth, like as HD with F1 may compensate growth response, in same way as MD with F2 gave better results as contrast with other treatments (Fig. 1). For the period of the reproductive growth biomass partitioning is a vital determinant of yield and influenced by PD and N (Hussain et al., 2000; Wang et al., 2011).

Assimilates partitioning into excess vegetative structures at the expense of fruit can considerably decrease lint yield of cotton (Heitholt, 1994). Dry matter partitioning into vegetative structures relative to reproductive forms tends to increase under HD (Kerby et al., 1990; Heitholt, 1994) and similar outcome was also examined in current study that PFW and PDW were significantly high in F1HD. In a study, cotton cultivar Xiangzamian 8 performance was observed in various PD and result found with enhance in PD, lint yield and N uptake were enhanced (Xue et al., 2013).

Cotton showed varied growth response to different N and PD treatments at different growth stages. PFW and PDW were considerably influenced by PD and N application rates at squaring, blooming and maturity stage, however PFW and PDW were unaffected by these treatments at seedling stage. Results pertaining of C_i also confirmed that there was no significant influence of N and PD on C_i (Fig. 2). Nonetheless, maximum Pn was observed at squaring stage as compared to seedling and maturity stage and this could be due to following reasons; at seedling stage all cotton leaves were healthy and had the highest Ci as compared to other growth stage while at maturity stage, Pn was found minimum

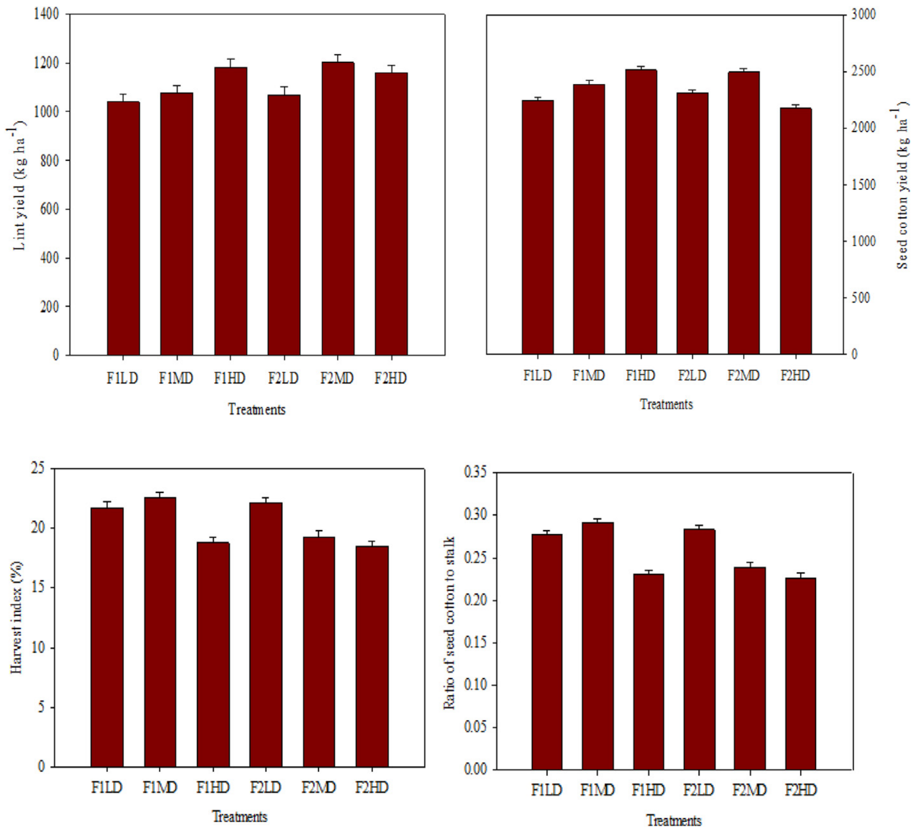

Fig. 3. Interactive effects of planting density and nitrogen application rate on lint yield, seed cotton yield, harvest index and ratio of seed cotton to stalk. In treatments, F1 is showing low nitrogen application rate 120 kg ha^{-1}, F2 is showing high nitrogen application rate 180 kg ha^{-1}, LD is showing low planting density (8 plants m^{-2}), MD is showing medium planting density (10 plants m^{-2}) and HD is showing high planting density (12 plants m^{-2}). The differences among the treatments were separated using least significance difference test (LSD) at 0.05 probability level.

Table 3
Interactive effects of planting density and nitrogen application rate on nitrogen contents mg g^{-1} in cotton.

Treatments	Seedling	Squaring	Blooming	Maturity
F1LD	35.87a	24.46d	25.86d	24.06c
F1MD	26.68a	26.00c	29.20bc	24.97c
F1HD	30.69a	29.19b	36.65a	28.42a
F2LD	32.98a	25.85c	29.51b	25.00bc
F2MD	31.64a	25.79c	35.10a	27.23ab
F2HD	32.81a	32.41a	25.96d	25.42bc

Treatments; F1 is showing low nitrogen application rate 120 kg ha^{-1}, F2 is showing high nitrogen application rate 180 kg ha^{-1}, LD is showing low planting density (8 plants m^{-2}), MD is showing medium plant density (10 plants m^{-2}) and HD is showing high planting density (12 plants m^{-2}). Seedling stage was at 20 DAE, Squaring stage was at 50 DAE, Blooming stage was at 80 DAE and Maturity stage was at 120 DAE. Mean values followed by the same letters are not significantly different using least significance difference test (LSD) at 0.05 probability level.

due to leaf senescence or yellowing of leaves. Our results further suggested that squaring is most crucial stage in cotton because of the highest Pn efficiency at squaring stage followed by blooming stage. Moreover it was observed that with a change in PD, cotton can optimize photosynthetic use efficiency and capacity by adjusting leaf mass per area, which in turn affects leaf N allocation to the photosynthetic apparatus. However medium planting density is the optimum PD due to high light utilization efficiency, superior spatial distribution of leaf N allocation to the photosynthetic apparatus and photosynthetic use efficiency of photosynthetic N in leaves within the canopy (Yao et al., 2015).

Regarding yield response, higher yield was noted with the combination of F1HD or F2MD (Fig. 3). Dong et al. (2010) have reported similar results; in the lesser fertility field, PD and N fertilizer were the major elements to increase the biological yield of cotton crop. This could suggest that cotton yield can be increased even with low fertilizer rate if planted with high PD. Regarding yield response, high seed cotton yield and lint yield was noted with the combination of F1HD and/or F2MD (Fig. 3). Dong et al. (2012) further supported our results and reported that considerably high lint yield (1604 kg ha^{-1}) was achieved only with a high dose of N fertilizer under low PD, but comparable yields (1693 and 1643 kg ha^{-1}) were achieved with F1MD and F1HD respectively.

In contrast, F1HD produced least HI and RSS (Fig. 3) as compared with other treatments. The least HI with this treatment was because of high biological yield as compared with other treatments. Interestingly, both seed cotton yield and biological yield increased under F1HD as compared to other treatments but seed cotton yield was not enough to increase HI. Similar results we have found (Dong et al., 2012). Furthermore, total plant biomass per unit ground area was increased by PD and N application rates (Fig. 1). Our result further agreed with previous researches that excessive N application and increased PD extensively reduced the reproductive allocation (Boquet and Breitenbeck, 2000; Boquet, 2005; Ali et al., 2009). Therefore, our results suggested that N application at low rate with high PD (120 kg ha^{-1} N and 12 plants m^{-2}) or high N application with medium PD (180 kg ha^{-1} N and 10 plants m^{-2}) are most suitable combinations for sustainable cotton production.

Overall, N contents decreased from seedling stage to maturity or even at harvesting and this could be due to decrease in availability of N in soil with time or with demand of N by cotton plant.

Among growth stages, non-significant effect of N application rates and PD were noted for N contents at seedling stage as compared with other growth stages (Table 3). At seedling stage, cotton plants were healthy enough to extract N from soil and/or at the beginning maximum N could be available in soil. Nonetheless, at squaring stage the maximum N contents were observed under F2HD while at blooming higher N contents were found under F2LD or F2MD.

This variation could be because at squaring stage, cotton plants under high N application and high PD were able to take up more N from soil and could also partly be due to higher N demand by more number of plants per unit area. At maturity, the highest N contents were noted under F1HD or under F2MD. This suggested that cotton plants can perform well under F1HD is maintained (120 kg ha^{-1} N and 12 plants m^{-2}). Moreover, it was found that high agronomic efficiency of N is associated with decrease in N application rate, which indicated that there was balanced increase in vegetative and reproductive part with increase in N level resulting in higher seed cotton (Fritschi et al., 2003). On the other hand, excessive N application may shift the balance between vegetative and reproductive growth towards excessive development, thus delaying crop maturity and reducing cotton yield (Thind et al., 2008). Our result showed that F1HD or F2MD are the best combinations of N and PD, while squaring and blooming stages are crucial for sustainable cotton development.

5. Conclusion

This study compared the response of nitrogen fertilizer and plant density on photosynthetic and agronomical traits of cotton at different growth stages. Cotton attained maximum fresh and dry biomass production with high photosynthetic rate and intercellular CO_2 under high plant density with low nitrogen level and medium plant density with high nitrogen level while least was under low nitrogen application with low plant density. Ratio of seed cotton to stalk was linearly and positively correlated with harvest index, it can be an alternative indicator of dry matter allocation. Biological yield enhanced by PD and N application rates. Among growth stages, squaring is the most crucial stage followed by blooming stage. For this purpose, the application of nitrogen 120 kg N ha^{-1} with high plant density and application of nitrogen180 kg N ha^{-1} with medium plant density should be recommended in order to boost the yield of cotton and efficiency of resource utilization.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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