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ORIGINAL ARTICLE

# Leaf gas exchange, source–sink relationship, and growth response of cotton to the interactive effects of nitrogen rate and planting density

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**Abstract** Nitrogen (N) rate and plant density (PD) are important factors for sustainable cotton production. The objective of this study is to examine the effects of nitrogen rate and plant density on plant growth, source–sink relationship, and cotton yield. A split-plot arrangement was used in the field experiment with the main plots assigned to N rate (120 and 180 kg/ha), and the sub-plots assigned to plant density (8, 10, and 12 plants/m$^2$). Results showed significant N and PD interaction on plant growth, leaf gas exchange, and yield. Higher plant growth and cotton yield were noted under low nitrogen rate and high planting density than other treatment combinations. Leaf photosynthesis, stomatal conductance, intercellular CO$_2$, transpiration rate, and water use efficiency were considerably influenced by planting density and nitrogen rate. Maximum values of these traits were obtained under low nitrogen rate with high planting density or high nitrogen rate with medium planting density, while the least values were under low nitrogen rate with low planting density. Correlation analysis revealed highly significant and positive relation between leaf gas exchange and cotton yield.

**Keywords** Cotton phenology · Crop growth rate · Leaf area · Photosynthesis · Mineral nutrition · Cotton lint yield

## Introduction

Cotton is one of the most important industrial crops and the most widely fiber crop grown under a very broad range of climates, soils, and cultivation practices (Wang et al. 2011; Shah et al. 2016). Cotton fiber is widespread used in our daily life due to its excellent rendering and great production in the world, mainly in China. Worldwide, it is grown as annual crop primarily for oil seed, lint, and meal for animal feed (Constable and Bange 2015). Cotton has generally familiar history of planting with multipurpose usages. Cotton is being cultivated in 76 countries, which occupies 32 million hectares of the land (Saranga et al. 2001). In the world, China has become one of the high yielding country in cotton. In the world, during 2013, the average lint yield was 1438 kg/ha and was in China, which was higher than other countries, such as India, Pakistan, and US (USDA 2013).

Nitrogen fertilization has always been the most applied macronutrient, supplied in various amounts, applied in different ways at different timings, but what is common in all those methods and dates is the high cost of the fertilizer itself and the high consumption (Zhu and Chen 2002; Shah et al. 2016). It is essential to manage the N application in a balanced (Cisneros and Godfrey 2001; Fritschi et al. 2004; Reddy et al. 2004). Nitrogen has a pivotal role in cotton growth and yield. Nitrogen had boosting effect on the root length and surface area (Xie et al. 2009; Tang et al. 2012). It is a vital ingredient for canopy development and photosynthesis (Bondada and Oosterhuis 2001; Zhang et al. 2002). Failure to secure the plant needs of nitrogen,

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especially in the squaring stage leads to a remarkable decline in yield (Moore 2008). N has indirect effects on boll and seed formation through its effects on the canopy's size and creating a bigger source for nutrient that increases the size and weight of the bolls and seed (Saleem et al. 2010; Rashidi and Gholami 2011).

The high-density cotton planting system (HDPS) has been more popularized over the world; using ultra-narrow rows (UNR) introduced by Jost and Cothren (2000), the advantage of this system is earliness in cotton as UNR needs low boll number/plant to attain yields as in conventional planting system (Rossi et al. 2004). UNR cotton produces small bolls relative to conventional planting system but retains a higher percentage of bolls at the first sympodial position (Clawson et al. 2006). Better light interception, efficient leaf area development, and reduced weed competitiveness by early canopy closure are observed by (Wright et al. 2011). Increase in planting density results in seed cotton yield by increasing number of bolls plant$^{-1}$ and 100-boll weight; however, some reports contradicted that with an increase in plant density resulted in decreasing boll weight and number of bolls plant$^{-1}$ (Sawan et al. 1993; Bednarz et al. 2006), but per hectare seed cotton yield increased due to more number of plants ha$^{-1}$ (Dong et al. 2006a, b). Relative water contents (RWC) explain turgidity of cells; however, reduction in turgidity results in reduced leaf water contents and declined water use efficiency (González and González-Vilar 2001). Studies reported that measurement RWC is a good indicator of plant growth and plant leaf gas exchange ability (Jensen et al. 2000; Anjum et al. 2016).

Both these factors are of individual importance; however, their interactive effects are of great concern in optimizing planting density and N application. Therefore, this study was conducted (1) to assess yield and its components under the interactive effects of nitrogen application and plant density, (2) to reveal the effects of nitrogen and planting density on source and sink relationship, and (3) to examine leaf gas exchange of the fourth leaf and whole plant at different intervals of time in response to varied nitrogen application rates and planting density treatments. The present study attempts to highlight the leaf gas exchange, source–sink relationship, and growth response of cotton to the interactive effects of nitrogen rate and planting density.

## Materials and methods

### Experimental site description

An experiment was conducted during 2015 at Huazhong Agricultural University located at 114°21-E Longitude, 30°37-N Latitude, and 23 m altitude, Wuhan, China. The soil was high fertility containing 128.64 mg/kgN, 16.01 mg/kg $P_2O_5$, and 97.09 mg/kg $K_2O$.

### Experimental design and treatment

The experiment was using split-plot design and having four replications. There were two factors: nitrogen rate and planting density. Main plot assigned to different nitrogen rates, while sub-plots assigned to different planting densities. Nitrogen was applied at the rate of 120 kg/ha (low application rate) and 180 kg/ha (high application rate), and plant density included 8 plants/m$^2$ (LD), 10 plants/m$^2$ (MD), and 12 plants/m$^2$ (HD). Nitrogen was supplied using urea (46% N) and applied one time at the beginning of flowering.

### Data collection

#### *Cotton growth, phenology, nitrogen uptake, and yield*

Five successive and uniform plants from each replication were marked after emergence, and then, the number of days is taken to complete different stages counted. Plant fresh and dry weight was measured at 80 days after emergence (DAE). Four plants were pulled out slowly from soil and took fresh weight and then separated the leaves, stem, and roots and enveloped them separately. After that put those pieces in oven for 70 °C and then weighted them to measure dry weight. Regarding morphological traits, leaf area of the fourth leaf and whole plant was measured using Yaxin1241 leaf area meter and was computed at 60, 80, and 100 DAE, leaf area index (LAI), and crop growth rate (CGR) which were measured using the following formulae:

$$\text{Leaf area index} = \frac{\text{Leaf area}}{\text{Land area}}$$

$$\text{CGR} = \frac{W_2 - W_1}{T_2 - T_1} \times \frac{1}{\text{Land area}} \,(\text{g/m}^2/\text{d})$$

where $W_1$ and $W_2$ are plant dry weights taken at time $T_1$ and $T_2$, respectively. Relative water contents (RWC) were measured from 20 days after emergence (DAE), to 120 DAE with an interval of 20 days, and then, average was taken to examine final RWC in cotton under studied treatments. For RWC measurement, first, leaf fresh weight (Wf) was measured; following this saturated weight of leaves (Ws) was measured after floating leaves in water for 18 h. Leaf dry weight (Wd) was measured (at 70 °C) after drying the leaves in oven for 72 h (Anjum et al. 2016). The RWC (%) were computed as

$$\mathrm{RWC} = \frac{\mathrm{Wf} - \mathrm{Wd}}{\mathrm{Ws} - \mathrm{Wd}} \times 100\,(\%)$$

For nitrogen percentage, micro-Kjeldahl method (Nelson and Sommers 1972) was utilized and five fully expanded leaves mature leaves from main stem from each treatment were used to measure nitrogen contents. Seed cotton of each matured boll was picked on the 4th day after boll opening and was weighed after drying for boll weight and the total weight of all the bolls was the seed cotton yield of the plant. Lint yield was obtained by weighing the lint of each plant after seed cotton was ginned. Bolls (matured only) per plant were counted on the last sampling.

### *Cotton leaf gas exchange and SPAD value*

Cotton leaf gas exchange of the fourth leaf was measured at 40, 60, and 100 DAE, while leaf gas exchange at whole plant level was measured at 100 DAE. Determination of Net photosynthetic rate, stomatal conductance, intercellular $CO_2$ concentration, transpiration rate, and water use efficiency (using Licor-6400 portable photosynthesis system 9:00–10:00 a.m.). Fifteen leaves were selected for each treatment with the following adjustments: air flow per unit leaf area 378.22 mmol/$m^2$/s; PAR at leaf surface was up to 1299 µmol/$m^2$/s; water vapor pressure was 3.20 mbar; temperature of leaf ranged from 33.17 to 40.11 °C; ambient temperature was 39.69–42.68 °C; ambient $CO_2$ concentration was 358 mmol/$m^2$/s; and relative humidity (RH) was 47.67%. Determination of chlorophyll content was carried out using SPAD (Minolta SPAD 502 Chlorophyll Meter) at 100 DAE. The Minolta SPAD 502 Chlorophyll Meter is a hand held meter or device which records the leaf chlorophyll content which absorbs light at a wavelength of 430 and 750 nm when passed through the leaf (Wood et al. 1992).

### *Statistical analysis and experimental design*

The experiment was conducted with split-plot design with four replications. Data were analyzed by the analysis of variance using the Statistix 8.1 (Analytical Software, Tallahassee, FL, USA) software. The mean differences between treatments were separated using least significance difference (LSD) test at 0.05 probability level. Graphical presentation was made using Sigma Plot 10.0 (Systat Software Inc., San Jose, CA, USA).

## Results

### Cotton growth, phenology, and N uptake

Cotton growth and phenology were significantly influenced by N application and planting density (Tables 1, 2). Cotton plants under low fertilizer and high planting density took more days to complete blooming (73DAE) and boll setting (50 days) and to reach maturity (123 DAE) followed by high N fertilizer and medium planting density. Least numbers of days (119 days) were taken to reach maturity under high N fertilizer and high planting density (Table 2). Interactive effects of N application rate and planting density showed that cotton plant exhibited maximum plant fresh weight (PFW) and plant dry weight (PDW) at either low nitrogen application with high planting density or high N fertilizer with medium planting density followed by high N fertilizer with medium planting, while least PFW and PDW were at low N application with low planting density (Table 3). Furthermore, relative water contents (RWC) were significantly higher but statistically similar when cotton plants were grown under low nitrogen application with medium and high planting density and high nitrogen application with medium planting density, while least RWC were under high N application with low planting density treatment (Table 3). Data relating to cumulative crop growth rate (CGR) showed that cotton plant exhibited crop growth rate of 10.29 and 10.15 g/$m^2$ days under high N application with medium planting density and low nitrogen application with high planting density, respectively, while least CGR (7.349 g/$m^2$ day) was noted under low N application with low planting density (Table 3). Nitrogen uptake was significantly influenced by planting density and N application rate (Table 1). Among planting densities, N uptake was higher at high planting density, while among N rates N uptake increased with an increase in nitrogen application rate (Data not shown). Interactive effects of N application rate and planting density showed that the highest N uptake was under high population density with low nitrogen application rate, while least was under low nitrogen application rate with low population density (Table 3).

### Cotton lint and seed cotton yield

Cotton lint and seed cotton yield and relating traits were considerably influenced by N application and planting density (Table 1). The highest number of bolls per $m^2$ (120.41) was noted under high N application and medium planting density, followed by low N application with high planting density which gave number of bolls per $m^2$ (118.56). Least number of bolls per $m^2$ (104.10) was under low N application with low planting density, while cotton bolls gained statistically similar number of bolls per $m^2$ under low N application with medium planting density (107.49) and high N application with high planting density (107.15 g) (Table 3). A non-significant interactive effect of N × PD was observed on the boll weight (g). Higher seed cotton yield (251.22 and 249.47 g/$m^2$) and lint yield

**Table 1** Summary of the analysis of variance, showing the individual and interactive effects of nitrogen application rate and planting density on cotton growth, phenology, leaf gas exchange, and yield

| SOV | Seedling stage | Squaring stage | Boll setting stage | Total crop duration | PFW | PDW | RWC | CGR | SPAD | No. of bolls/$m^2$ | BW | Seed cotton yield | Lint yield | N uptake |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| N | * | * | * | ** | Ns | * | * | * | Ns | * | Ns | * | * | * |
| PD | * | ** | * | * | * | * | ** | * | Ns | ** | * | * | ** | * |
| N × PD | ** | * | * | ** | * | * | * | * | Ns | ** | * | * | ** | ** |

In the first column; SOV is source of variation, and N is nitrogen application rate. In other columns, PD is planting density, PFW is plant fresh weight/$m^2$, PDW is plant dry weight/$m^2$, RWC are relative water contents, CGR is crop growth rate, and BW is boll weight. ** and * are denoting significance at the 0.01 and 0.05 probability level, respectively. ns is representing non-significant

**Table 2** Interactive effects of nitrogen application and planting density on cotton phenology and growing periods

| Treatments | Growth stages (m-d/DAE) | | | | | Growing period (d) | | | Total |
|---|---|---|---|---|---|---|---|---|---|
| | Sowing | Emergence | Squaring | Blooming | Maturity | Seedling | Squaring | Boll setting | |
| F1LD | 5–22 | 5–27 | 7–13/47 | 8–5/73 | 9–22/121 | 47b | 26b | 48c | 121b |
| F1MD | 5–22 | 5–27 | 7–8/42 | 8–1/69 | 9–16/115 | 42d | 27a | 46e | 115d |
| F1HD | 5–22 | 5–27 | 7–16/50 | 8–5/73 | 9–24/123 | 50a | 23c | 50a | 123a |
| F2LD | 5–22 | 5–27 | 7–13/47 | 8–5/73 | 9–21/120 | 47b | 26b | 47d | 120bc |
| F2MD | 5–22 | 5–27 | 7–12/46 | 8–4/72 | 9–22/121 | 46c | 26b | 49b | 121b |
| F2HD | 5–22 | 5–27 | 7–12/46 | 8–4/72 | 9–20/119 | 46c | 26b | 47d | 119c |

In treatment column; F1 is showing nitrogen application @ 120 kg/ha, F2 is showing nitrogen application @ 180 kg/ha, LD is showing low planting density treatment (8 plants/$m^2$), MD is showing medium plant density (12 plants/$m^2$) treatment, and HD is showing high planting density (12 plants/$m^2$) treatment. In growth stages column, date of each growth stages is provided and values after '/' show days after emergence. Data in 'Growth period' column show the total number of days taken by cotton plants to complete given growth stage. Mean values followed by the same letter are not significantly different at 5% level by LSD

**Table 3** Interactive effects of nitrogen application and planting density on cotton growth, yield, and total N uptake in cotton leaves

| Treatments | PFW (g/$m^2$) | PDW (g/$m^2$) | RWC (%) | CGR (g/$m^2$ day) | SPAD | No. of bolls/$m^2$ | BW (g) | Seed cotton yield (g/$m^2$) | Lint yield (g/$m^2$) | Nitrogen uptake (%) | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | 60 DAE | 100 DAE |
| F1LD | 1349.2e | 293.70e | 77.270d | 7.394d | 42.76a | 104.10e | 3.99a | 224.40d | 103.97b | 24.36d | 15.66c |
| F1MD | 1621.0bc | 325.22d | 79.664ab | 9.031c | 45.71a | 107.49d | 4.35a | 238.64b | 107.64b | 25.26d | 16.45c |
| F1HD | 1690.8ab | 405.77a | 79.581ab | 10.158a | 43.33a | 118.56b | 4.35a | 251.22a | 118.22a | 31.39a | 18.30ab |
| F2LD | 1448.0d | 334.25 cd | 78.396c | 9.349bc | 45.36a | 107.15d | 3.83a | 230.63c | 106.96b | 27.86c | 16.45c |
| F2MD | 1728.0a | 375.33b | 79.808a | 10.297a | 43.30a | 120.41a | 4.60a | 249.47a | 120.14a | 29.37b | 19.30a |
| F2HD | 1584.7c | 348.32c | 78.828bc | 9.541b | 42.51a | 115.52c | 4.12a | 217.27e | 115.93a | 29.83b | 17.47b |

In treatment column; F1 is showing nitrogen application @ 120 kg/ha, F2 is showing nitrogen application @ 180 kg/ha, LD is showing low planting density treatment (8 plants/$m^2$), MD is showing medium plant density (12 plants/$m^2$) treatment and HD is showing high planting density (12 plants/$m^2$) treatment. PFW is plant fresh weight/$m^2$, PDW is plant dry weight/$m^2$, RWC are relative water contents, CGR is crop growth rate, and BW is boll weight in grams. Nitrogen uptake in cotton leaves under different treatments is given at 60 days after emergence (DAE), and 100 DAE

Mean values followed by the same letter are not significantly different at 5% level by LSD

(118.22 and 120.14 g/$m^2$) was noted under low N application with high planting density and high N application with medium planting density, respectively. Least seed cotton yield (217.27 g/$m^2$) was observed under high N application with high planting density as compared to all other treatment (Table 3)

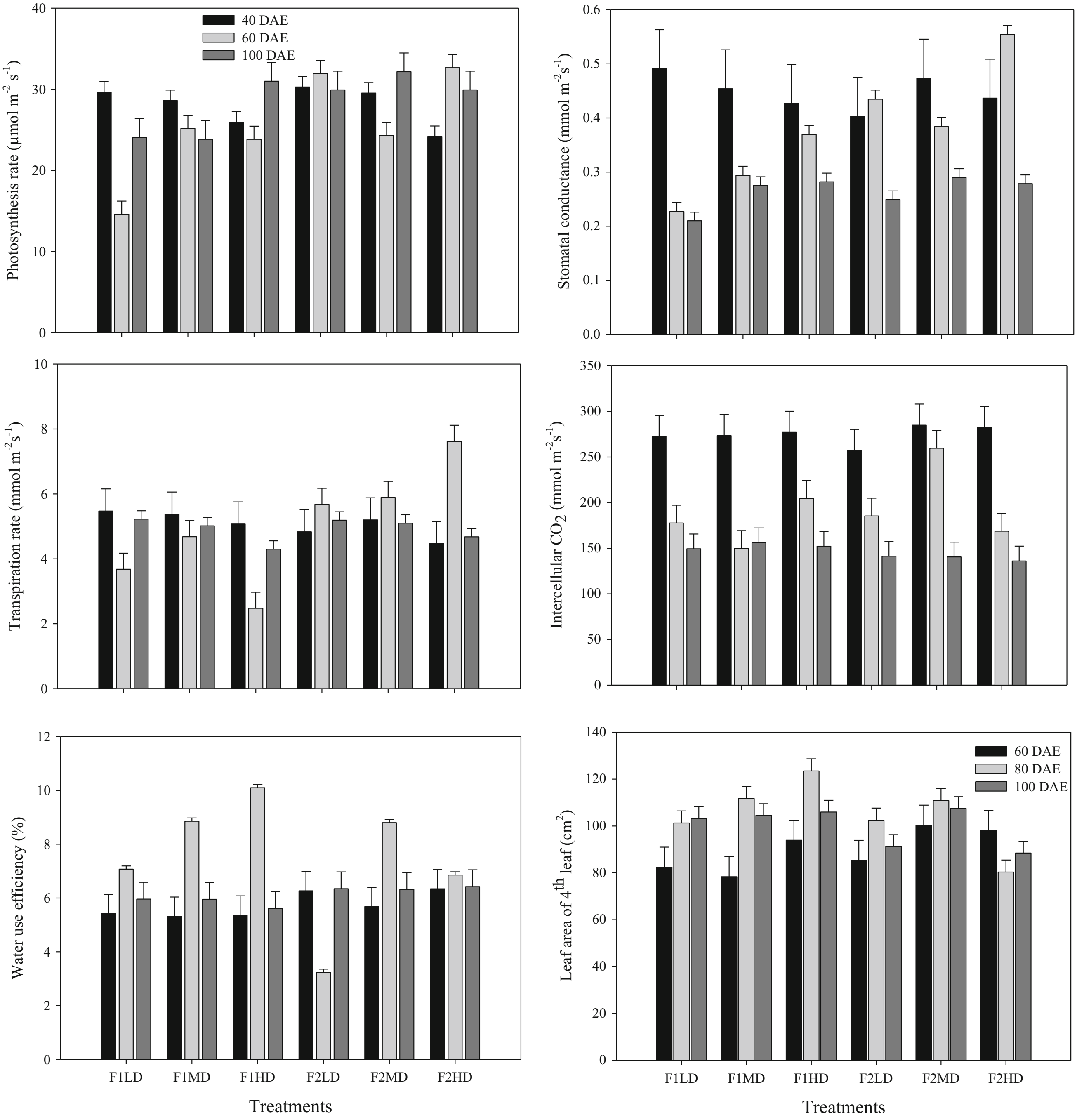
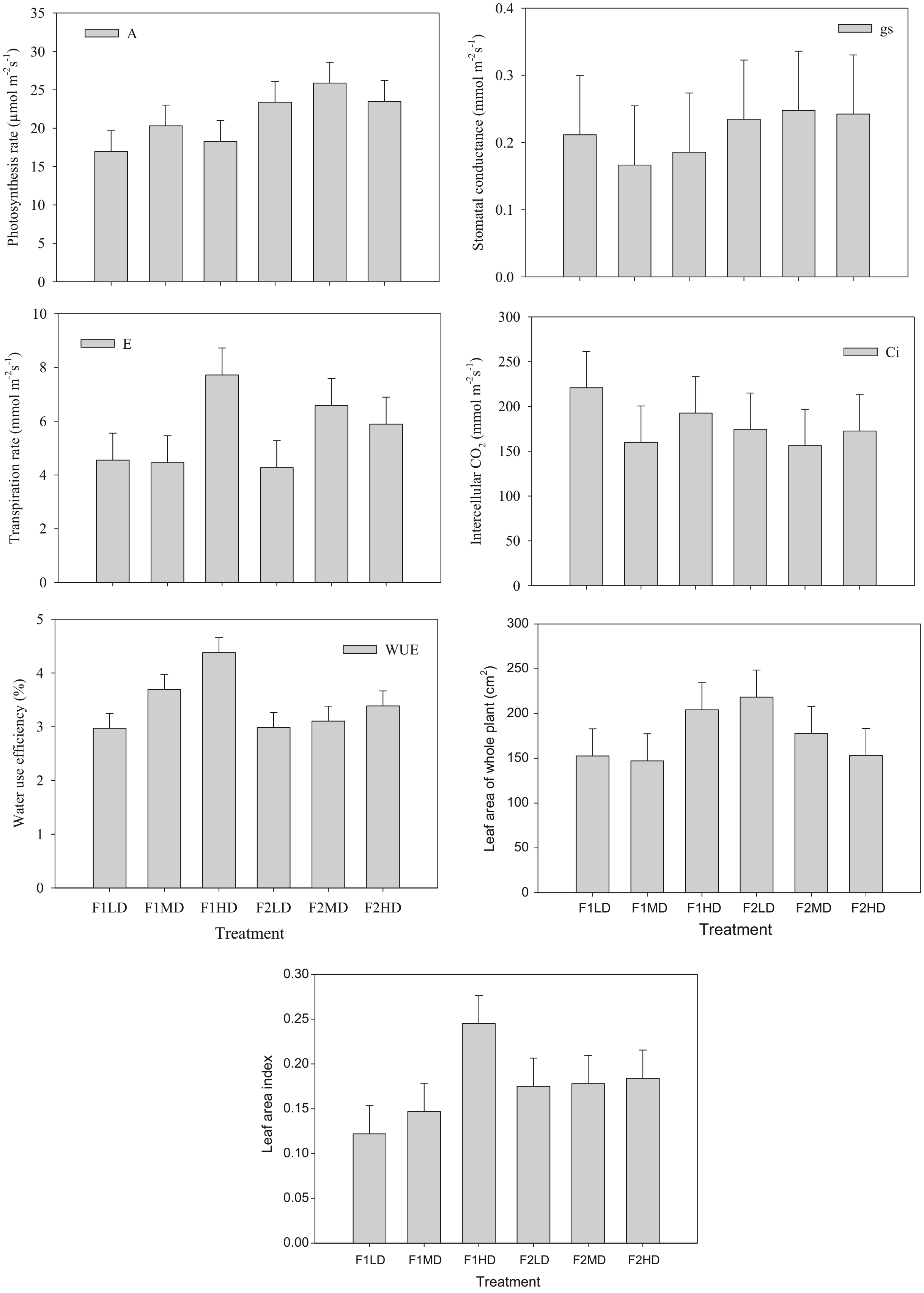


**Fig. 1** Leaf gas exchange and leaf area of the fourth leaf as affected by N application and planting density

## Leaf area and leaf gas exchange of the fourth leaf

Nitrogen application under different planting densities showed significant effects on leaf area and leaf gas exchange of the fourth leaf in cotton (Fig. 1). At 60DAE, leaf area of the fourth leaf (LA$^4$) was relatively higher under high N application as compared to low N application across all planting densities, while at 80 and 100 DAE, higher LA was noted under low N application as compared to high N application across planting densities (Fig. 1). On other hand, at 60DAE, less LA$^4$ was observed under high plating density across N application, but at 80 and 100 DAE, higher LA$^4$ was under high planting density as compared to low or medium planting density (Fig. 1). At 40DAE, photosynthetic rate (Pn) of the fourth leaf was reduced under higher planting density with either N application rate. Nonetheless, after 40DAE to 100DAE, higher Pn was observed under high N application rate and


A
Photosynthesis rate (μmol m-2s-1)
0
5
10
15
20
25
30
35
gs
Stomatal conductance (mmol m-2s-1)
0.0
0.1
0.2
0.3
0.4
E
Transpiration rate (mmol m-2s-1)
0
2
4
6
8
10
Ci
Intercellular CO2 (mmol m-2s-1)
0
50
100
150
200
250
300
WUE
Water use efficiency (%)
0
1
2
3
4
5
Leaf area of whole plant (cm2)
0
50
100
150
200
250
300
Leaf area index
0.00
0.05
0.10
0.15
0.20
0.25
0.30
F1LD
F1MD
F1HD
F2LD
F2MD
F2HD
Treatment

◀**Fig. 2** Leaf gas exchange, total leaf area, and leaf area index at whole plant level as affected by N application and planting density

high planting density. Least Pn was noted under low N application with low planting density at 60DAE and 100DAE (Fig. 1). Stomatal conductance (*gs*), intercellular $CO_2$ (Ci), and concentration of the fourth leaf were unaffected by treatments at 40 DAE, but at 60 DAE and 100 DAE, high *gs* and Ci increased under low N application with high planting density and/or high N application with medium planting density as compared to other treatments (Fig. 1). Likewise, transpiration rate (Ts) of the fourth leaf was unaffected at 40 DAE; however, at 60 DAE and 100DAE, Ts was decreased under high N application and high planting density, while higher WUE was only observed under low N application with high planting density at 60DAE (Fig. 1).

### Leaf area, total leaf area index, and leaf gas exchange at whole plant level

Significant interactive effects of N application and planting density were observed at leaf area index (LAI) and leaf gas exchange at whole plant level (Fig. 2). The highest final leaf area (LA) and leaf area index (LAI) were noted at under low N application with high planting density, and least was under low N application with low planting density. Regarding leaf gas exchange, interactive effects of PD and N application did not showed significant effects on stomtal conductance (gs) and intercellular $CO_2$ (Ci), while higher gs and Ci were noted under high planting density (data not shown). On other hand, higher photosynthetic rate (Pn) was noted under high N application with medium planting density as compared to all other treatments (Fig. 2). Transpiration rate (Ts) and water use efficiency (WUE) showed vice versa response to N and planting density. Lower Ts and high WUE were noted under low N application with high planting density as compared to other treatments.

### Regression analysis of leaf gas exchange and seed cotton yield and lint yield

Regression and correlation analysis showed positive and significant relation among photosynthesis rate (Pn), stomatal conductance (gs) and transpiration rate (Ts) and water use efficiency (WUE) and lint and seed cotton yield (Table 4). Nonetheless, the dependence of lint yield and seed cotton yield was varied among these leaf gas exchange parameters and plant part. Furthermore, results showed that contribution of these leaf gas exchange parameters and WUE towards lint and seed cotton yield was more by the fourth leaf as compared to whole plant level (Table 4). Correlation between Pn of the fourth leaf with lint yield and Pn of the fourth leaf with seed cotton yield was 0.9957 and 0.9944, respectively, while correlation between Pn at whole plant level with lint yield and with seed cotton yield was 0.9884 and 0.9890, respectively. Similarly, *gs*, Ts, and WUE of the fourth leaf were more as compared with whole plant level. This clearly indicated that the fourth leaf contributed in significant proportion towards cotton productivity (Table 4).

## Discussion

In the present study, significant interactive effects of N application and planting density were observed on cotton growth, phenology, and yield (Tables 1, 2, 3). We found that with an increase in planting density, cotton growth also increased in the terms of higher fresh weight, dry weight, and crop growth rate (CGR); however, interaction of N with planting density gave different results. For instance, our results showed that cotton plant exhibited better or higher growth when subjected to low N fertilizer (120 kg/ha) with high planting density (12 plants/$m^2$) and/or high N fertilizer (180 kg/ha) with medium planting density (10 plants/$m^2$) as compared to other planting densities at their respective N levels (Table 3). Better cotton growth at these N application and planting density treatments could be due to the better allocation of N in cotton plant, thus increased cotton growth as compared to other treatments. Data regarding nitrogen uptake also showed that higher N uptake was under high low nitrogen application with high planting density. This suggested that cotton plants can perform under low nitrogen and, however, require high planting density. Fritschi et al. (2003) found high agronomic use efficiency of nitrogen with decrease in N application rate and this could be further explained by balanced increment in vegetative and reproductive growth in response to high N application rates. Contrarily, higher N application from optimum level may disturb that balance between vegetative and reproductive growth thus delays crop maturity (Thind et al. 2008). Similarly Yao et al. (2015) showed that with change in planting density, cotton plants start to improve photosynthetic capacity by increasing photosynthetic N use efficiency and by adjusting leaf area per area. Nonetheless, medium planting density could be that optimum plant density might be due to high light interception and utilization and better spatial dissemination of leaf N to the carbon assimilation apparatus in leaves. Furthermore, higher yield under medium planting

**Table 4** Correlation analysis between Pn, gs, E, and WUE and cotton yield

| | *A* | *gs* | *E* | WUE |
|---|---|---|---|---|
| Correlation analysis between the fourth leaf and cotton yield | | | | |
| Seed cotton yield | $r = 0.9944$* | $r = 0.9936$** | $r = 0.9941$* | $r = 0.9972$*** |
| | $R^2 = 0.9784$ | $R^2 = 0.9835$ | $R^2 = 0.9535$ | $R^2 = 0.9792$ |
| Lint yield | $r = 0.9957$*** | $r = 0.9966$* | $r = 0.9957$** | $r = 0.9938$* |
| | $R^2 = 0.9913$ | $R^2 = 0.9931$ | $R^2 = 0.9916$ | $R^2 = 0.9878$ |
| Correlation analysis between whole plant and cotton yield | | | | |
| Seed cotton yield | $r = 0.9892$** | $r = 0.9917$** | $r = 0.9767$** | $r = 0.9895$* |
| | $R^2 = 0.9769$ | $R^2 = 0.9734$ | $R^2 = 0.9504$ | $R^2 = 0.9773$ |
| Lint yield | $r = 0.9884$* | $r = 0.9866$** | $r = 0.9749$* | $r = 0.9886$* |
| | $R^2 = 0.9788$ | $R^2 = 0.9872$ | $R^2 = 0.9882$ | $R^2 = 0.9945$ |

*A* Photosynthesis rate, *gs* stomatal conductance, *E* transpiration rate, *WUE* water use efficiency

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

density could be due to high light interception and high leaf area development (Kaggwa-Asiimwe et al. 2013).

In the present study, maximum cumulative CGR was noted under low N fertilizer (120 kg/ha) with high planting density (12 plants/m$^2$) and/or high N fertilizer (180 kg/ha) with medium planting density (10 plants/m$^2$) as compared to other planting densities at their respective N levels and was due to maximum cotton plants biomass production, as compared to other treatments. Furthermore, such high CGR under low N application with high planting density and/or high N application with medium planting density may also resulted from more efficient light harvesting as compared to other treatments. We observed that at low N application rate (120 kg N/ha), cotton plant may subject to N deficiency stress, but due to high planting density (12 plants/m$^2$), overall biomass production per unit area could be sufficient enough to give more CGR as compared to low N application with high planting density and/or high N application with medium planting density. That is why, CGR of cotton plants under low N application with high planting density was statically similar to high N application with medium planting density. Moreover, our results were also in accordance with some previous findings that plant biomass production significantly increased with increase in planting density (Boquet 2005; O'Berry et al. 2008; Dai et al. 2015).

We found maximum seed cotton yield and lint yield under low N application with high planting density and/or high N application with medium planting density and could be due to high boll weight (Table 3). Significant effects of treatments were found on number of bolls (Table 3). Our results also showed that at to low N application with high planting density and/or high N application with medium planting density, cotton plant took less day for squaring stage while more days to complete blooming stage and boll setting period, this can explain why seed cotton yield and lint yield was higher at these treatments (low N application rate with high planting density and/or high N application with medium planting density) as compared to other treatments. Studies showed that high planting density reduced boll weight but increased number of bolls per unit area, and lint yield (Wang et al. 2004; Bednarz et al. 2006; Dong et al. 2010). This could be associated with better boll distribution and high development of inner bolls as compared with outer bolls (Gwathmey and Clement 2010). The yield advantage under to low N application with high planting density and/or high N application with medium planting density is partly also because of high photosynthetic rate (Table 4). Correlation analysis showed that leaf gas exchange traits significantly contributed towards seed cotton and lint yield (Table 4). Biomass partitioning especially during the reproductive growth phase is a critical factor and is greatly influenced by planting density (Wang et al. 2011) and N application (Hussain et al. 2000). Thus, the distribution of photo-assimilates into vegetative structures at the expense of reproductive parts can significantly reduce cotton lint yield (Heitholt 1994). Similar results were also observed in our study that PFW and PDW were significantly higher under high planting density but only with low N application. In a study, performance of a transgenic cotton cultivar; Xiangzmian 8 was examined under different planting densities and found that with increase in PD of cotton, lint yield and N uptake per 100 g lint were increased (Xu et al. 2013).

Leaf gas exchange of the fourth leaf (LA$^4$) and at whole plant level (LA$^T$) showed significant response under different N application rates and planting density treatments. Our results further showed that at whole plant level higher photosynthetic rate (Pn). WUE was noted under low N application and high planting density as compared to other treatment, while high transpiration rate was noted under high N level with either planting density level, which suggested that cotton plant was able to increase their photosynthetic capacity and WUE under low N but with high planting density. Leaf gas exchange of the fourth leaf showed at 40DAE, Pn was least, but *gs,* Ci, Ts, and WUE was unaffected; however, after 40–100 DAE, Pn, *gs*, Ci, and WUE were significantly higher under low N application with high planting density as compared to other treatments. Low Pn and unaffected other leaf gas exchange traits of the fourth leaf at 40 DAE could be, because leaves were not fully developed under high planting density as compared to low-and-medium planting density (aata not shown); however, after 40 DAE, the fourth leaf development (e.g., $LA^4$) significantly increased under low N application with high planting density level. Dong et al. (2006a, b) found significant variation in photosynthetic rate and transfer of photo-assimilates in different cotton genotypes under varied planting density. Leaf area of the fourth leaf ($LA^4$),and total leaf area ($LA^T$) of whole plant and leaf area index (LAI) were also significantly influenced by PD and N application. Our results showed that at 60 DAE, $LA_4$ was relatively higher under high N application with high planting density, while at 80 and 100 DAE, higher LA was noted under low with high planting density as compared to other treatment (Fig. 1). $LA^T$ and LAI also showed similar response and were higher under low N application with high planting density, which suggested that cotton plants exhibited better growth under high planting density with low N application rate. Initially, higher $LA^4$ at 60 DAE under high N application could be due to the reason that at early growth stage, cotton plants may require high amount of N to develop more $LA^{4,}$ but at latter stage (at 80 and 100 DAE), low N is sufficient for cotton the fourth leaf to develop significant area. We found that there was significant increase in LAI with increase in planting density and higher LAI resulted in high partitioning of photo-assimilates into reproductive parts of cotton (Heitholt 1994). In this study, the trends in LAI variation were similar to those of above ground biomass, increasing with planting density. Kerby et al. (1990) and Kaggwa-Asiimwe et al. (2013) also reported an increase in leaf area index with increased population density. Moreover, such increase in LAI and leaf area of the fourth leaf and whole plant could be due to high light interception and light use efficiency. Similar results have also been reported in other crops such as in pigeon pea (Worku and Demisie 2012) and in wheat (Wajid et al. 2004).

## Conclusion

This study showed interactive effects of nitrogen application and planting density on cotton morphology and physiology. Results of this study showed that rate of nitrogen application and planting density significantly influenced cotton growth and development by altering leaf gas exchange and source to sink relationship. Results revealed that low nitrogen application rate (120 kg/ha) with higher planting density (12 plants/m$^2$) and high nitrogen application rate (180 kg/ha) with medium planting density (10 plants/m$^2$) showed promising results in producing higher lint and seed cotton yield as compared to other treatments. Furthermore, there was a very strong and positive correlation of different leaf gas exchange traits of the fourth leaf and whole plant with lint and seed cotton yield. Therefore, we recommend that these combinations of nitrogen application rate and planting density get higher cotton yield.

**Author contribution statement** ANS and GY initiated and designed the research; ANS performed the experiment and collect the data; ANS and MT analyzed the data and wrote the manuscript; and JI edited the manuscript.

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