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# Cotton reproductive and fiber quality responses to nitrogen nutrition

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#### Abstract

Nutrient (N) stress affects cotton growth, primary physiological processes and fiber properties. This study utilized two sunlit growth chambers to compare cotton (cv. TM-1) responses to two levels of N nutrition imposed at the onset of flowering stage of development, 100 and 0% of optimum N, in plants grown under otherwise optimal temperature and soil moisture conditions. Flowers and bolls were tagged daily to estimate boll maturation period (BMP). Leaf N concentration was determined every four days from flowering to maturity. Plant height and main stem nodes were determined every four days from emergence to 25 days after treatment (DAT) and photosynthetic measurements were recorded weekly from 0 to 56 DAT. Plant and boll-component dry weights were recorded at end of the experiment. Fiber quality was determined in samples of lint that were grouped based on average leaf N concentration during the BMP. Total plant biomass was reduced 23% by N deficient treatment and these plants produced 14 bolls per plant as compared with 21 bolls in N sufficient plants. Stress-induced decrease in leaf N was associated with linear decreases in leaf photosynthesis (r<sup>2</sup>=0.92) and stomatal conductance  $(r^2=0.86)$ . Fiber length and strength increased linearly with increase in lean N concentration, while fiber micronaire and uniformity declined linearly with increase in leaf N concentration. Among the measured fiber properties, fiber micronaire was the most sensitive to changes in leaf N followed by strength, length and uniformity. Knowledge of the functional relationship between leaf N concentration and a fiber property can be used to develop a fiber quality submodel for cotton under optimal temperature and water conditions.

Keywords: Cotton; Nitrogen deficiency; Fiber length; Fiber strength; Micronaire.

192 S.B. Lokhande & K. Raja Reddy / International Journal of Plant Production (2015) 9(2): 191-210

#### Introduction

In the last six decades, cotton yields have been consistently increased by improving genetics and various management practices including N fertility (Stewart et al., 2005). More attention has been given to N, as it is an essential primary plant nutrient and a key factor in biomass production and is needed in relatively larger amounts than other nutrients. While N is the single most growth-limiting factor in production agriculture (Shah, 2008), applying N in excess of crop needs can have global effects of greenhouse gas emissions (Snyder et al., 2007). In cotton, excessive or deficient N has detrimental effects on several plant processes (Gerik et al., 1998), so it is important to apply N fertilizers responsibly and provide the required optimum amounts of N to enable high yield potential. Nitrogen deficiency results in stunted cotton growth and development as cotton appears to require a consistent and optimal supply of N during the growing season (Jaynes et al., 2001). Nitrogen availability during flowering decides the physiological stature of plant and reproductive development. Cell division, cell expansion and leaf production are decreased by N limitation (Chapin, 1980), restricting plant growth and developmental processes. It has been argued that during reproductive growth in cotton, growing bolls have priority for plant assimilates and, as a result, the vegetative growth is suppressed.

Major portion of the leaf N is in chloroplasts, so in C<sub>3</sub> plants like cotton, lowering N content reduces leaf chlorophyll content (Zhao et al., 2003) which subsequently affects the functionality of photosynthesis apparatus. It has been reported that cotton leaves accumulate about 44 g kg<sup>-1</sup> of N (Reddy et al., 2004) under well fertilized conditions. The strong relationship between leaf N and photosynthesis in cotton is widely recognized and reported, as N deficiency decreases leaf area and chlorophyll content which lowers net photosynthesis rate (Radin and Boyer, 1982). Net photosynthesis and stomatal conductance were positively correlated with leaf N and in cotton, the assimilation rate increased by  $0.6 \ \mu mol \ m^{-2} \ s^{-1}$  per unit increase in N (Reddy et al., 1996) as rubisco activities declined. Prior studies indicated strong correlation between leaf N and photosynthesis in cotton (Shiraiwa and Sinclair, 1993), with N deficiency adversely affecting lint yield through reductions in stem elongation, leaf expansion (Lu et al., 2001), photosynthetic and metabolic activities (Ciompi et al., 1996) and biomass production (Fritschi et al., 2003). Also, studies on the development of fruiting structures have reported that N deficient cotton has modified

flowering patterns and reduced boll number and weights, as compared with N-sufficient cotton (Gerik et al., 1998).

Fiber is the primary and economically important product of the cotton crop and is one of the natural prime sources for the textile industry. Maintaining fiber quantity and quality is a one of the great challenges to the farm managers. Cotton fiber is the elongated and thickened single cell of seed epidermis which achieves its maximum length in the early period of anthesis; by 15-20 days after anthesis, followed by cellulose deposition on secondary wall giving rise to strength and maturity (Davidonis et al., 2004). During the fiber development process, the stage at which the cotton plant is under N stress is crucial for fiber quality (Bradow and Davidonis, 2000). It has been reported that N deficiency decreased fiber length and strength (Read et al., 2006) and increased the micronaire value (Reddy et al., 2004). A positive relationship between fiber strength and N fertility was reported by Fritschi et al. (2003). Additionally, the timing and intensity of N stress is equally important in impacting fiber quality. Although several studies have focused on N nutrition effects on cotton reproductive performance and yield (Pettigrew and Meredith, 1997; Bondada and Oosterhuis, 2001), few studies have extended to incorporate effects on fiber quality (Reddy et al., 2004; Read et al., 2006).

Accurate prediction of growth, developmental and yield of cotton plants under a wide range of environmental conditions is important for crop management decisions (Reddy et al., 2004). Several controlled environmental studies have been carried out to quantify cotton growth and developmental aspects (Reddy et al., 1993; Reddy et al., 1997a; Reddy et al., 1997b) and some of the resulting mathematical functions were incorporated into the cotton simulation model, GOSSYM. This model was tested for field and climate change impact analysis (Thorp et al., 2014; Liang et al., 2012a; Liang et al., 2012b). However, the existing cotton models including GOSSYM model does not have a fiber quality submodel usable to effectively predict fiber properties in the production environment.

Despite several attempts to quantify N deficiency effects on fiber properties, conflicting results have been reported due to interactive effects of weather parameters, soil and genotypic variability in which the experiments were conducted (Reddy et al., 2004; Pettigrew et al., 1996; Jenkins et al., 1990). Therefore, studies are needed to completely isolate the effects of N deficiency on fiber properties. The objectives were to evaluate the effects of N stress on cotton reproductive performance and fiber properties under optimum temperature and water conditions and to develop functional algorithms between leaf nitrogen and fiber parameters that are important to the ginning industry.

## **Materials and Methods**

### *Experimental facility*

The experiment was conducted in two sunlit, controlled environment chambers known as Soil-Plant-Atmosphere-Research (SPAR) units located at the R.R. Foil Plant Science Research Center, Mississippi State University, Mississippi, USA. Each SPAR chamber consists of a steel soil bin (1 m deep by 2 m long by 0.5 m wide) to accommodate the root system, a Plexiglas chamber (2.5 m tall by 2 m long by 1.5 m wide) to accommodate plant canopy and a heating and cooling system connected to air ducts that pass conditioned air to cause leaf flutter through the plant canopy. Variable density shade cloths, designed to simulate canopy spectral properties and placed around the edges of the plant canopy, were adjusted regularly to match canopy height and to eliminate the need for border plants. During this experiment, the incoming daily solar radiation (285-2800 nm) outside of the SPAR units measured with a pyranometer (Model 4-8; The Eppley Laboratory Inc., Newport, RI, USA), ranged from 1.4 to 27.2 MJ m<sup>-2</sup> d<sup>-1</sup> with average of 15.6 MJ  $m^{-2}$  d<sup>-1</sup>. The SPAR units supported by an environmental monitoring and control systems are networked to provide automatic acquisition and storage of the data, monitored every 10 s throughout the day and night. Many details on the operation and control of SPAR chambers were described by Reddy et al. (2001).

## Nitrogen stress control and plant culture

Two N treatments of 100% and 0% N of a modified Hoagland's nutrient solution ((Hewitt, 1952) were imposed from flowering to maturity. Prior to N stress treatments, all chambers were well-watered with full strength Hoagland's nutrient solution. Irrigation was provided three times a day to maintain optimum water supply throughout the experiment. For the two different N stress treatments, the nutrient solutions were stored in separate tanks and pumped through plastic tubing to the designated SPAR unit by drip irrigation system. Day/night temperatures of 30/22 °C and carbon dioxide

concentration of 400  $\mu$ mol mol<sup>-1</sup> were maintained throughout the experiment. The temperature control in ambient air was achieved to the desired set points using chilled ethylene glycol supplied to the cooling system via several parallel solenoid valves that were opened and closed depending on the cooling requirements and an electrical resistance heater which provided short pulses of heat and a fan circulated the air through the chamber (Reddy et al., 2001). Carbon dioxide concentration in each SPAR chamber was monitored and adjusted every 10 s throughout the day and maintained at 400  $\mu$ mol mol<sup>-1</sup> during the daylight hours using a dedicated LI-6250 CO<sub>2</sub> analyzer (Li-COR, Inc., Lincoln, NE, USA). The seasonal data for daily mean temperature and daytime CO<sub>2</sub> concentration are presented in Table 1.

Table 1. The set treatments, percent of N imposed prior to flowering and measured chamber  $CO_2$  concentration from a typical day and mean temperature during the experimental period for each treatment.

Treatments	Measured variables†			
%N	$CO_2 \ (\mu mol \ mol^{-1})$	Mean Temperature (°C)		
100	409 <sup>a†</sup>	25.5 <sup>a</sup>		
0	408 <sup>a</sup>	25.6 <sup>a</sup>		

<sup>†</sup> Each value represents the mean  $\pm$  SE for one typical day for CO<sub>2</sub> and 4 August to 15 October 2009 for temperature. <sup>†</sup> Values in each row followed by same letter are not significantly different (P<0.05) according to Fisher's LSD.

Four rows with five plants per row were maintained in each chamber until harvest. All plants were harvested when they had 80% or more of the harvestable bolls opened. In this experiment, a genetic standard for many breeding and molecular studies of upland cotton (*Gossypium hirsutum* L.) cultivar Texas Marker (TM)-1 (Stelly et al., 2005) was seeded on June 16, 2009 in the SPAR units utilizing fine sand as the rooting medium.

## Measurements

### Leaf nitrogen

Three uppermost fully expanded leaves on mainstem from each N treatment were excised every 4 days starting the day treatments, four leaves from four different plants, were imposed to physiological maturity. Samples were dried at 70  $^{\circ}$ C for 72 hours and ground to pass a 40 mesh screen. Leaf N

#### 196 S.B. Lokhande & K. Raja Reddy / International Journal of Plant Production (2015) 9(2): 191-210

was determined by standard micro-Kjeldahl method (Nelson and Sommers, 1972) and expressed in %N as well as g N kg<sup>-1</sup> on a dry matter basis. As leaves were excised prior to analysis, the number of observations on given sampling dates were equivalent to the number of treatments. The main focus of leaf N analysis was to determine reproductive performance and quality of lint produced in different fruiting zones, based on period of first flower as a function of temporal changes in leaf N under the two N levels.

#### Growth and biomass

Mainstem height to the newest unfolded mainstem leaf and number of main stem nodes were recorded every four days from seedling emergence to 21 days after N stress treatment. Flowers and open bolls were tagged daily to record day of flowering, based on cotton flowers that are creamy-white in color on the day of anthesis and turning to purple on the day after anthesis. Based on flowering and open boll dates, boll maturation period (BMP) for each boll was estimated for each boll in both N fertility treatments (Reddy et al., 1999). At final harvest, total number of bolls produced and matured (opened) per plant were recorded. Also, stems, leaves and reproductive structures were separated from each plant and total biomass per plant calculated by adding dry weight of different plant parts. Each boll was separated into burr, seed and lint and weights were recorded. Seedcotton and seed weight for each plant was calculated by adding the boll component's weight for given plant.

## Photosynthesis and chlorophyll measurements

Net photosynthetic rate and stomatal conductance of the uppermost, fully expanded mainstem leaves, which were third or fourth from main axis terminal, from three plants in each treatment were measured between 10:00 and 13:00 h using LI-6400 (LI-COR Inc., Lincoln, Nebraska, USA) with an integrated fluorescence chamber head (LI-6400-40 leaf chamber fluorometer). The measurements were taken at 1500  $\mu$ moles of photon m<sup>-1</sup> s<sup>-1</sup> photosynthetically active radiation, cuvette temperature set to daytime temperature of 30 °C and carbon dioxide concentration was maintained at 400  $\mu$ mol mol<sup>-1</sup> and relative humidity was adjusted to ambient level (50%). Measurements were taken weekly from day of imposed treatments to physiological maturity.

Leaf pigment content and chlorophyll stability index (CSI) were measured by taking two sets of leaf samples collected from five fullyexpanded leaves for each treatment during the same period. Five leaf discs, each 2.0 cm<sup>2</sup>, from each sample were collected randomly and placed in vials containing 5 ml of dimethyl sulphoxide for chlorophyll (Chl) extraction. Absorbance of the extract was measured using a Bio-Rad ultraviolet/VIS spectrophotometer (Bio-Rad Laboratories, Hercules, CA, USA) at 470, 648, and 662 nm to calculate concentrations of Chla, Chlb and carotenoid content (Chapple et al., 1992). The chlorophyll stability index (CSI) was determined according to Sairam et al. (1997). Accordingly, another set of leaf discs, each 2.0 cm<sup>2</sup>, was collected similarly from each cultivar and incubated at 56 °C in a temperature-controlled water bath for 30 min. The set of tubes was brought to 25 °C and the Chl content was measured from the heat-treated samples as described previously. The CSI was estimated as the ratio of Chl content in heated leaf (56 °C) to that in fresh leaf expressed as a percentage.

#### Fiber quality measurement

All of the open bolls were divided into different 'age' groups, based on the flowering date with bolls developed from the flowers produced in the first three days of flower constituting the first group and the remaining groups classified similarly by a successive interval of three days in each treatment. Overall, from both nitrogen stress treatments, 22 groups were obtained. Average leaf N concentration for each group was estimated by regression equations as days after N treatment and by running average of leaf N over boll maturation period for each group. Lint samples from each boll group (days after treatment equivalence) were subjected for fiber quality assessment using High Volume Instrumentation (HVI) by the Fiber and Biopolymer Research Institute at Texas Tech University, Lubbock, TX as described by Davidonis and Hinojosa (1994). The HVI provides reports on five important quality characteristics describing the fiber length, strength, fineness, elongation and uniformity.

## Data analysis

The SPAR chambers were designed to be identical to provide even growth conditions and the treatments under study were finely controlled. All the measurements on 20 plants in each treatment were used as replicates for testing the significance of treatments and standard errors of the mean are provided in the tables and figures. To test the significance of N stress on growth and boll parameters were analyzed using general linear model PROC GLM in SAS and Fisher protected LSD tests at P=0.05 (SAS Institute Inc., 2011). Regressions were fitted for leaf nitrogen content and fiber quality parameters from both treatments and 22 groups using SAS (SAS Institute Inc., 2011) and SigmaPlot 11.0 (Systat Software Inc., San Jose, CA).

#### **Results and Discussion**

#### Leaf nitrogen status

Leaf N declined in both N treatments during the treatment period due to plant growth over time and availability of N due to N treatments (Figure 1). The decline in N deficient treatment was steeper (slope= -0.045 g N kg<sup>-1</sup>;  $r^2$ =0.91) than for N-sufficient treatment (slope= -0.24 g N kg<sup>-1</sup> d<sup>-1</sup>;  $r^2$ =0.92). At 72 days after treatment, when the plants were harvested for biomass, leaf N contents were 35.9 g kg<sup>-1</sup> and 16.1 g kg<sup>-1</sup> in N-sufficient and N-deficient treatments, respectively. Under optimum conditions cotton plants accumulated 49 g kg<sup>-1</sup> of leaf N which is important indicator of plant nitrogen status (Bell et al., 2003).

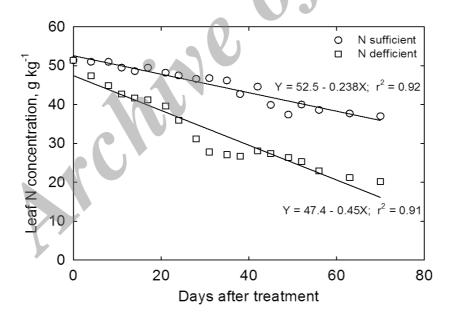


Figure 1. Daily average leaf nitrogen concentration plotted for two different nitrogen stress treatments (%). Each nitrogen level was represented by lines in curves. Plants were harvested as they reached 80% open bolls.

#### Leaf chlorophyll and gas exchange processes

Leaf N content altered cotton chlorophyll content and gas exchange processes. Photosynthesis was linearly decreased ( $r^2=0.92$ ; Figure 2) with decrease in leaf N content. Maximum photosynthesis of 32.7 µmol m<sup>-2</sup> s<sup>-1</sup> was observed at N content of 52 g kg<sup>-1</sup>, whereas, at 25.2 g kg<sup>-1</sup> it was reduced by 41% (19.2 µmol m<sup>-2</sup> s<sup>-1</sup>; Figure 2). Photosynthesis decreased 0.48 µmol m<sup>-2</sup> s<sup>-1</sup> per unit decrease in leaf N content. The reduction in photosynthesis was due to decreased N content which is key component of photosynthetic enzymes and chlorophyll content (Chapin, 1980) which significantly declined in N-deficient plants (P=0.01; Figure 3). Also the membrane stability of chlorophyll which was expressed as Chlorophyll Stability Index (CSI) was also significantly decreased (P=0.03) in N-deficient condition (Figure 3).

Leaf stomatal conductance decreased as leaf N decreased ( $r^2=0.86$ ; Figure 2). Gas exchange processes trends those were found in this study are in agreement with prior reports of a close relationship between leaf chlorophyll and nitrogen content and decline in leaf chlorophyll content and photosynthesis rate (Reddy et al., 1996; Lu et al., 2001) under N-deficient conditions.

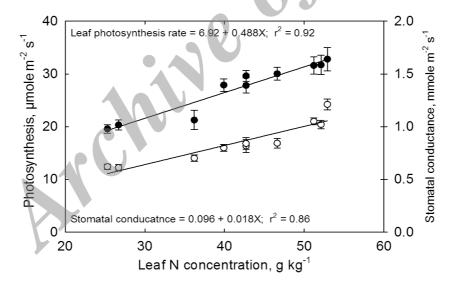


Figure 2. Relationship between leaf nitrogen concentration and leaf photosynthesis rate and stomatal conductance in topmost fully expanded leaf (from 0 to 56 days after treatment at interval of seven days) with three samples per treatment by using Li-Cor-6400 measurement system calibrated at ambient  $CO_2$  concentration (380 µmol mol<sup>-1</sup>), 30 °C temperature and light level of 1500 µmole m<sup>-2</sup> s<sup>-1</sup>. Measurements were taken between 10:00 and 13:30 hours on sunny days.

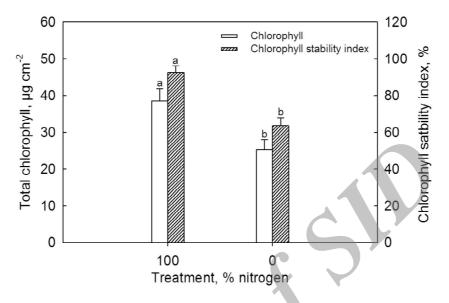


Figure 3. Nitrogen stress effects on total chlorophyll content and chlorophyll stability index. Measurements were taken at 56 days after treatment on topmost fully expanded leaves from three plants in nitrogen fertility treatment. Values are mean  $\pm$  standard errors.

## Plant growth and yield attributes

Nitrogen deficient condition did not significantly affect the mainstem length, but mainstem node numbers were significantly decreased in with N stress (P=0.031; Table 2). By the time the N-treatment has any significant effects, the cotton plants in this study achieved enough fruit load which competed with vegetative growth. Therefore, no significant differences were observed in this study between the N treatments as compared to many studies conducted during early stages of cotton development (Reddy et al., 1997b). Mainstem length was accounted by intermodal in elongation differences rather than mainstem node numbers (Gardner and Tucker, 1967). Plants grown under nitrogen deficient conditions produced significantly lower biomass (P<0.001) per plant. The 100N treatment plants produced 241 g plant<sup>-1</sup> of biomass; whereas in 0N treatment, biomass was reduced by 23% (Table 2). Reduction in biomass is due to insufficient N supply has been related to reduction in leaf area (Fernáandez et al., 1996) and CO<sub>2</sub> assimilation rate (Ciompi et al., 1996; Reddy et al., 1997a) that in turn restrict reproductive growth.

Boll number was least (P=0.002) in plants under N-deficient condition, with 100N treatment having about 20 bolls plant<sup>-1</sup> and 0N treatment having only 14 bolls plant<sup>-1</sup> (Table 2). There was no significant decrease in open (matured) bolls in N-deficient treatment; but boll weight per plant in the 3<sup>rd</sup> and 4<sup>th</sup> week of first flower significantly declined (P=0.021) in 0N treatment (Figure 4). By imposing the treatments a few days before flowering, leaf N started depleting gradually in the cotton plants. Therefore, in later stages of flowering, there was a significant reduction in boll number and individual boll weight (Gerik et al., 1994). This is due to reduction in leaf area and canopy photosynthesis (Bondada et al., 1996; Bondada and Oosterhuis, 2001) under N deficient conditions.

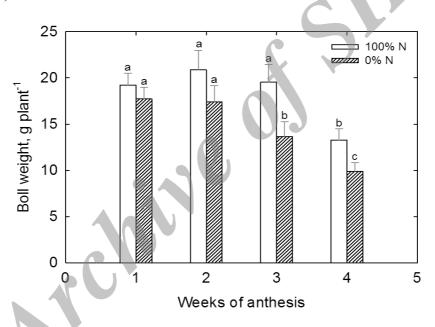


Figure 4. Nitrogen stress effects on cotton total boll weight per plant over week of first flower. Measurements were taken at final harvest carried out at 80% of boll opening in each treatment (20 plants per treatment). Values are mean  $\pm$  standard errors.

Seed cotton and seed weights per plant significantly decreased in N-deficient treatment compared to N-sufficient treatment (Table 2). At 100N, plants produced 64 g of seed cotton and 43 g of seed per plant<sup>-1</sup>, whereas a reduction of 10% (seed cotton) and 12% (seed weight) were recorded in 0N treatment. No significant differences in boll weights and

seed cotton weight per boll were observed between the N treatments, however, N-deficient condition significantly (P=0.021) increased the lint weight per boll whereas seed weight per boll significantly decreased (P=0.03) in N-deficient treatment (Table 2). The decrease in seed cotton weight was due to reduction in seed weight per boll and retained boll numbers. The reduction in lint yield is due to fewer bolls retained whereas, increase in lint weight per boll under N stress condition was likely a result of better light distribution within canopy due to low leaf area index and lower photosynthesis in the canopy (Wullschleger and Oosterhuis, 1990; Reddy et al., 2004).

Table 2. Treatment means and least significant difference (LSD) for all plant and boll biomass attributes studied. Final harvest was carried out at 80% of boll opening in each N fertility treatment (20 plants per treatment).

Plant noromator	Nitrogen treatment (% of control)		
Plant parameter	100	0	
Plant height, cm plant <sup>-1</sup>	222 <sup>a†</sup>	216 <sup>a</sup>	
Mainstem nodes, no. plant <sup>-1</sup>	21.2 <sup>a</sup>	19.3 <sup>b</sup>	
Total biomass, g plant <sup>-1</sup>	241 <sup>a</sup>	184 <sup>b</sup>	
Total bolls, no. plant <sup>-1</sup>	20.9 <sup>a</sup>	14.5 <sup>b</sup>	
Open bolls, no. plant <sup>-1</sup>	13.2 <sup>a</sup>	12.5 <sup>a</sup>	
Seed cotton weight, g plant <sup>-1</sup>	63.9 <sup>a</sup>	57.2 <sup>b</sup>	
Seed weight, g plant <sup>-1</sup>	40.4 <sup>a</sup>	35.3 <sup>b</sup>	
Boll components			
Boll weight, g boll <sup>-1</sup>	6.31 <sup>a</sup>	6.38 <sup>a</sup>	
Seed cotton weight, g boll <sup>-1</sup>	4.69 <sup>a</sup>	4.65 <sup>a</sup>	
Lint weight, g boll <sup>-1</sup>	1.43 <sup>a</sup>	1.59 <sup>b</sup>	
Seed weight, g boll <sup>-1</sup>	3.22 <sup>a</sup>	3.06 <sup>b</sup>	
*			

<sup>†</sup> Values in each row followed by same letter are not significantly different (P<0.05) according to Fisher's LSD. ns, not significant.

## Fiber properties

Because fiber quality is mainly determined by fiber cell elongation and by both primary and secondary cell wall deposition during cell maturation, it is reasonable to evaluate fiber quality formation as a function of changes in leaf N concentration (Wang et al., 2012). Fiber length decreased linearly as leaf N decreased ( $r^2=0.81$ , Figure 5a), with the longest fibers (30.4 mm) recorded at optimum N (45 g kg<sup>-1</sup>). The decline in fiber length was 0.41 mm

per 10 g kg<sup>-1</sup> decline of leaf N concentration. At leaf N of 25 g kg<sup>-1</sup>, fiber length was reduced to 29.1 mm. However, despite the decrease, fiber length was still in the range of longer fibers (29-34 mm) that is acceptable for the mills across the flowering groups (Bradow and Davidonis, 2000). Although, fiber uniformity increased linearly ( $r^2=0.65$ , Figure 5c) as leaf N decreased, treatment difference in uniformity was not significant and values were within the range that is not being penalized by mill industry (83 to 85%) (Schleth and Peter, 2005). In a given single seed, fiber length varies as longer fiber occurs at chalazal end of the seed whereas, short fiber occurs at the micropyle end. This variation was converted into percent of total number of fiber by HVI fiber length data and expressed in terms of mean, upper half mean length and uniformity ratio (Behery, 1993). The elongation period in fiber development process is the critical formation period for fiber length (Thaker et al., 1989). Trends obtained for fiber length and uniformity in this study agree with Reddy et al. (2004) of positive correlation between length and leaf N during boll maturation and with Gerik (1998) of shortened fiber in plants under N stress having leaf N of approximately 25 g kg<sup>-1</sup>. Fiber strength decreased ( $r^2=0.79$ , Figure 5b) with decrease in leaf N. Fiber strength was recorded at 32.3 g tex<sup>-1</sup> when produced under optimum N conditions with leaf N of approximately 45 g kg<sup>-1</sup> and 30.5 g tex<sup>-1</sup> when produced under N stress with leaf N of 25 g kg<sup>-1</sup>. In spite of this N-induced decrease in fiber strength, values remained in the range of strong fiber (29 g tex<sup>-1</sup> and above) (Schleth and Peter, 2005). In the cotton boll, approximately 24 days after anthesis, a stage shift occurs for sucrose metabolism in cotton fiber that is regulated by N and therefore, N deficient condition during 20-40 days post-anthesis would affect fiber strength (Bradow and Davidonis, 2000). In a Mississippi study conducted outdoors in large pots, weighted-sum fiber strength and fiber strength in three of five flowering groups (bolls grouped by first flowering date) across a 35-day flowering period were lower in cotton plants grown with 0% N from first flower stage onward than with half-strength nutrient solution from emergence to maturity (Read et al., 2006).

Fiber micronaire readings measured with HVI instrument exhibited linear increase ( $r^2=0.77$ , Figure 5d) with decrease in leaf nitrogen concentration. The micronaire reading of 4.3 (base range) was reported at leaf N concentration of 25 g kg<sup>-1</sup>. The acceptable upland micronaire premium range is 3.7 to 4.2 while base range is 4.3 to 4.9. Any values below 3.5 and above 4.9 will suffer a price penalty (Bradow and Davidonis, 2000). Micronaire, a measure of fiber maturity and fineness is an indirect

#### 204 S.B. Lokhande & K. Raja Reddy / International Journal of Plant Production (2015) 9(2): 191-210

measurement of air permeability and a very important fiber quality parameter (Moore, 1996). Result trends similar to this study were recorded by studies conducted by Reddy et al. (2004). Leaf N is mostly related to the translocation capacity of photosynthate and carbohydrate to boll (Sun et al., 2007). The reduction in micronaire and maturity may be related lowered photosynthesis under N-stressed conditions (Bauer et al., 2000). In studies on yield and fiber quality under N stress conditions, which reduced leaf photosynthesis by 20% or more, Read et al. (2006) found that flowering groups with low quality fiber also comprised a large fraction of total lint yield (20 g plants<sup>-1</sup>) and suggested this condition placed a heavy demand on plant N and carbohydrate reserves under N stress. A number of studies have revealed a linear correlation between micronaire and canopy photosynthesis during boll developmental stages (Bauer et al., 2000).

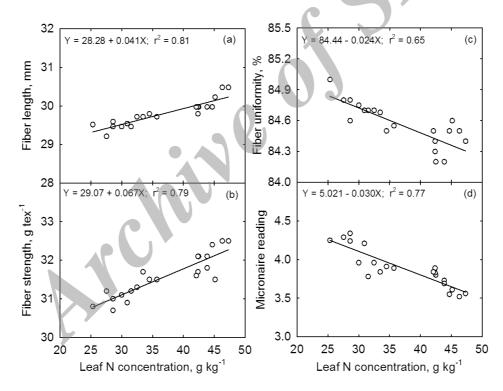


Figure 5. Nitrogen stress effects on (a) fiber length (b) fiber strength (c) micronaire reading and (d) fiber uniformity as a function of leaf nitrogen. The leaf nitrogen concentration was averaged from flowering to open bolls. Lint samples were collected at the final harvest carried out at 80 % of boll opening.

#### Nitrogen deficiency indices for cotton fiber properties

Using the protocols developed by Reddy et al. (2008) and Lokhande and Reddy (2014), N deficiency effects on fiber properties can be quantified and modeled through the functional relationship of leaf nitrogen-specific reduction indices (Figure 6) Corresponding regression parameters and coefficients are presented in Table 3. The resulting indices have values that ranged from 0, when a given stress factor is completely limiting to 1, when it does not limit the given fiber traits. Therefore, without any interference of other biotic or environmental factors, the effects of N stress on fiber properties can be quantified and incorporated into a mechanistic model as sub-model. At leaf N concentration of about 25 g kg<sup>-1</sup>, there was a 6% reduction in fiber strength estimates and a 4% reduction in fiber length estimates. Conversely, fiber micronaire was inversely proportional to leaf N status and was approximately 4.2 at high N stress condition (Figure 5d). The small amount of increase (2%) in fiber uniformity indicates less dependence on leaf N status.

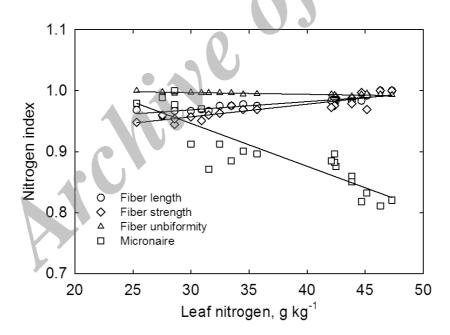


Figure 6. Nitrogen stress reduction indices for various cotton fiber quality parameters. Potential fiber quality values were estimated by dividing estimated maximum values by all the values to derive reduction factor and expressed in the fraction between 0 and 1.

Table 3. Regression parameters and regression coefficient  $(r^2)$  for the relationship between fiber quality parameters environmental productivity indices of cotton and leaf N concentration.

Fiber Parameters	Regression Parameter		Determination
	y-intercept	slope	coefficient, r <sup>2</sup>
Fiber length	0.928	0.001	0.81
Fiber strength	0.895	0.021	0.79
Fiber uniformity	1.005	-0.003	0.65
Fiber micronaire	1.157	-0.07	0.77

## Conclusions

This study evaluated cotton reproductive performance and fiber properties in relation to changes in leaf nitrogen. Variability in leaf N, which was achieved through two N treatments imposed during the flowering stage of development, enabled the derivation of its relationship to cotton reproductive and fiber parameters. Our results show that nitrogen deficiency reduced the node numbers and plant biomass. Retained bolls and boll components were substantially decreased in plants grown under limited N condition. The primary gas exchange processes such as leaf photosynthesis and stomatal conductance were also affected significantly under low nitrogen regime. Photosynthesis was more responsive to changes in leaf N compared to stomatal conductance. Fiber length and strength decreased as leaf N decreased; whereas, fiber micronaire values fell in the base range of 4.3or better under N-limiting condition. Fiber uniformity was not affected by changes in leaf N. The identified leaf N status-specific indices for fiber properties should be useful and can be incorporated in cotton simulation models to improve management practice for ensuring good N fertility.

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