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Genotypic variation in root morphology, cotton subtending leaf physiology and fiber quality against nitrogen

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Abstract

Background: Nitrogen (N) is important for improving various morphological and physiological processes of cotton but their contribution to fiber quality is still lacking.

Aims: The current study aimed to explore the relationship between root morphology, subtending leaf physiology, and fiber quality of contrasting N-efficient cotton genotypes in response to N.

Methods: We analyzed the above parameters of CCRI 69 (N-efficient) and Xinluzao-30 (XLZ-30, N-inefficient) under control (2.5 mmol·L^{-1}) and high N (5 mmol·L^{-1}) conditions.

Results: The results showed that root morphological traits were increased in CCRI-69 under control conditions than high N. Subtending leaf morphology, chlorophyll and carotenoid contents, free amino acids, and soluble proteins were higher under high N as compared with the control. However, soluble sugars, fructose, sucrose contents, and sucrose phosphate synthase were higher under control conditions than high N across the growth stages. Irrespective of the N conditions, all morphological and physiological traits of cotton subtending leaf were higher in CCRI-69 than XLZ-30. Except for fiber uniformity, fiber quality traits like fiber length, strength, micronaire, and elongation were improved under control conditions than high N. Between the genotypes, CCRI-69 had significantly higher fiber length, strength, micronaire, and elongation as compared with XLZ-30. Strong positive correlations were found between root morphology, soluble sugars, sucrose content, and sucrose phosphate synthase activity with fiber quality traits, respectively.

Conclusions: These findings suggest that CCRI-69 performed better in terms of growth and fiber quality under relatively low N condition, which will help to reduce fertilizer use, the cost of production, and environmental pollution.

Keywords: Cotton, Fiber quality, Nitrogen, Root morphology, Subtending leaf

Introduction

Cotton (*Gossypium hirsutum* L.) is the most popular natural fiber for clothing and textile products accounting for approximately 25% of total world fiber use (Iqbal et al. 2020a). It has been associated with ancient civilizations, which have contributed greatly to the industrial and economic development in many countries (Saleem et al. 2010). Globally, cotton is planted in more than 75 countries, occupying more than 30 million hectares (Ali 2015). China is one of the leading cotton producers in the world with an average lint yield of 1 438 kg·hm^{-2}. However, cotton production is stagnant in terms of both quality and quantity in the past decade (Yang and Zhou

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2010). There are many field management aspects responsible for this stagnancy in China, such as inappropriate sowing method and time, unbalanced fertilization, inappropriate irrigation, poor pest management, and others. Of all these aspects, the unbalanced application of fertilizer, especially nitrogen (N) is certainly a major factor (Nasim et al. 2011).

Both the yield and fiber quality of cotton are important factors in determining a producer's profit. Cotton fiber quality is mainly influenced by the genotype of the cultivars, but agronomic practices and environmental conditions are the secondary factors influencing fiber quality (Subhan et al. 2001). Producing high-quality cotton requires careful management in every production stage, including field management practices and fertilization. In general, N is a major constraint limiting the yield and quality of cotton (Girma et al. 2007). Either underuse or overuse of N fertilizer can create a negative effect on the desired growth pattern of cotton plants and cause a decrease in the yield and fiber quality (Gerik et al. 1998; Sui et al. 2017). N deficiency can reduce plant vegetative growth and fruiting, and induce premature senescence resulting in low yield and poor fiber quality (Gerik et al. 1994; Sui et al. 2017). Excess nitrogen can cause excessive vegetative growth, delay maturity, and create difficulty in defoliation, increase pest problems, and ultimately reduce the crop yield and fiber quality (Cisneros and Godfrey 2001; Howard et al. 2001). The increase in N rate reduces the lint percentage, increases boll weight, mineral uptake, photosynthetic assimilation, and accumulation in sinks (Iqbal et al. 2020a). Excess application of N than required for optimum crop performance can reduce yield or fiber quality (Gerik et al. 1998). Many researches on N effect on cotton yield and fiber quality used N fertilization rate as the independent variable. There have been limited studies on the genotypic difference for fiber quality under normal and high N conditions.

In cotton, the subtending leaf is the basic source of photosynthates for boll development and is the major contributor to cotton yield, especially boll weight (Liu et al. 2013). During boll development, it provides about 60%∼87% of the photosynthates (Liu et al. 2013; Wullschleger and Oosterhuis 1990). Bolls and their subtending leaves are the sinks and source for photosynthates and their relationships reflect the coordination between vegetative and reproductive growths and affect yield (Xie et al. 2003). Carbohydrates like sucrose and starch are the end products of photosynthesis and are translocated into the sink to provide carbon and energy for growth and development (Lunn and Hatch 1995). Regarding plant physiology, it was speculated that the reason for poor fiber quality in cotton might be due to insufficient carbohydrates and flavonoid biosynthesis (Yuan et al. 2012). Carbohydrates and energy, which are required for fiber development, are mainly supplied by the subtending leaves. Carbohydrates synthesized in the subtending leaf, are first transferred into the developing boll wall and then into the developing fiber (Zhang et al. 2019). Each step in the pathway could affect the transformation and utilization of the carbohydrates, and abundant enzymes and substances play an important role in this process (Yuan et al. 2012). In cotton, sucrose is the main photosynthetic product that translocates mainly from subtending leaves to sink tissues (Hu et al. 2007), and its accumulation improves fiber strength (Haigler et al. 2001). Fiber strength is one of the important fiber quality traits which mainly depends on sucrose accumulation (Shu et al. 2007) as initially secondary wall cellulose synthesis required sucrose. In cotton subtending leaf, sucrose synthesis and its translocation to fiber are regulated by sucrose synthase and sucrose phosphate synthase (SPS) (Ma et al. 2008). SPS contributes to more sucrose production and cellulose synthesis with the help of UDP-glucose by providing the resources (Babb and Haigler 2001; Haigler et al. 2001).

After a series of experiments, we have identified the high N-efficient cotton genotype (CCRI-69) that can produce high economic yield at a reduced N fertilizer. The genotype CCRI-69 has a flourishing root system, high photosynthetic efficiency, more biomass production, N metabolism, and high N use efficiency (NUE) (Iqbal et al. 2020a, 2020b). However, the relationship between root morphology, fiber quality, and physiological processes, such as carbohydrate metabolism of the subtending leaf of contrasting N-efficient cotton genotypes has not been clarified. Subsequently, the objective of our study was to explore the relative performance of cotton genotypes for root morphology, subtending leaf morphology, and physiology and their relationship with fiber quality in contrasting N-efficient cotton genotypes under control and high N conditions.

Materials and methods

Plant materials and growth conditions

The experiment was conducted at Institute of Cotton Research, Chinese Academy of Agricultural Sciences (Anyang, China). Based on the previous experiments, two contrasting N-efficient cotton genotypes, CCRI-69 (N-efficient) and XLZ-30 (N-inefficient) selected based on NUE and biomass potential, were grown in the field and hydroponic culture as described in our earlier studies (Iqbal et al. 2019a, b, 2020a; Niu et al. 2021). The seeds of both cotton genotypes were placed in a mixture of sands and vermiculite for 7 days in a growth chamber. After germination, the uniform seedlings were selected and transplanted into black color plastic boxes

(10 L) in the growth chamber as mentioned in our earlier studies (Iqbal et al. 2019a, 2020a, c). During the first week, plants were supplied with half-strength Hoagland solutions followed by full-strength Hoagland solution (2 mmol·L $^{-1}$ KH_2PO_4, 2 mmol·L $^{-1}$ KCL, 2 mmol·L $^{-1}$ $MgSO_4$, 0.1 mmol·L $^{-1}$ EDTA·Fe·Na, 46.2 µmol·L $^{-1}$ H_3BO_3, 9.1 µmol·L $^{-1}$ $MnCl_2{\cdot}4H_2O$, 0.8 µmol·L $^{-1}$ $ZnSO_4{\cdot}7H_2O$, 0.3 µmol·L $^{-1}$ $CuSO_4{\cdot}5H_2O$, 1.0 µmol·L $^{-1}$ $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$) till the end of the experiment (Iqbal et al. 2019a, 2020a). At two true leaves stage, seedlings of both cotton genotypes were exposed to high (5 mmol·L^{-1}), and control (2.5 mmol·L^{-1}) N conditions. The solutions were changed every week and the position of boxes was changed to avoid the position effect.

Sampling and processing

Plants attaining white flowers were selected and the subtending leaves at the first position in the fruiting branches were tagged. About 8∼10 tagged leaves from each treatment were collected from 9:00 to 10: 00 a.m., at 10, 25, and 40 days post-anthesis (DPA). The collected leaves were divided into two parts, one was directly frozen in the liquid nitrogen and then put in a freezer (− 80 °C) for the determination of chlorophyll a, chlorophyll b, carotenoids, fructose contents, sucrose contents, sucrose phosphate synthase activity, free amino acids, soluble protein, and soluble sugar. The rest of the samples were used to determine subtending leaf area and biomass.

Quantification of subtending leaf morphology and N concentration

Before harvesting, the total number of leaves per plant in both cotton genotypes under control and high N conditions were counted as the number of leaves per plant. The lengths and widths of the subtending leaves were measured and the mean subtending leaf area was obtained from the product of length, width, and correction factor (0.75) (Iqbal et al. 2019a). After harvesting, the subtending leaves of both cotton genotypes under control and high N conditions were collected and placed in the oven for one hour at 105 °C, followed by 80 °C for the next 48 h. Finally, the subtending leaf biomass was measured on an electronic balance. The oven-dried subtending leaf samples were ground and 0.1 g of the samples was taken in digestion tubes for the digestion using sulfuric acid (H_2SO_4) and hydrogen peroxide (H_2O_2). A total of 2 mL of H_2SO_4 was added to digestion tubes and incubated overnight at room temperature. The next day, H_2SO_4 was added in tubes again and the tubes were rotated. The tubes were placed on a hot plate and heated up to 350 °C for 30 min until the fumes were produced. Then 1 mL of hydrogen peroxide was added and heated for 20 min. After that, the tubes were removed from the hot plate. These steps were repeated until the material became colorless. The extract was filtered and a 50 mL volume was made using distilled water. The digested leaf sample (5 mL) was then used for the determination of N concentration using the Bran + Luebbe Continuous-Flow AutoAnalyzer III (AA3) (Iqbal et al. 2020a, c).

Determination of chlorophyll and carotenoid contents

The subtending leaf chlorophyll and carotenoid contents were measured by using 50 mg of leaf samples from each treatment. The collected leaves were first cut into small pieces and then put in an equal concentration (1:1) of ethanol and acetone in dark condition for 48 h at 25 °C. The final values for chlorophyll and carotenoid contents were measured according to our developed protocol (Iqbal et al. 2020d).

Measurement of free amino acids, soluble protein, and soluble sugars

The total free amino acids were determined by using the ninhydrin method (Yokoyama and Hiramatsu 2003) with some modifications (Sun et al. 2007). Acetic acid/sodium acetate was used as a buffer and the final values were detected through a spectrophotometer at 580 nm (Iqbal et al. 2020a).

Total soluble protein in the cotton subtending leaf was measured according to the standard protocol using Coomassie Brilliant Blue (G-250) as a dye and albumin as a standard (Theymoli and Sadasivam 1987). About 0.5 g of subtending leaf sample was grounded and homogenized in phosphate buffer (5 mL) followed by 100 °C water bath for 10 min and centrifugation at 3 000 ×*g* for 5 min at 22∼25 °C. Bradford reagent (0.5 mL), enzyme extract (20 µL), and dH_2O were used as reaction mixture. The final value was detected at 595 nm using the distilled water as the control and bovine albumin as standard with the help spectrophotometer (Iqbal et al. 2020a).

The total soluble sugar in the cotton subtending leaf was determined according to the standard protocol with some modifications (Shields and Burnett 1960). The subtending leaf samples (0.5 g) were grounded in a mortar and pestle using liquid nitrogen. The samples were homogenized in ethanol (90%) followed by incubation for 1 h at 60∼70 °C. Post incubation, ethanol was added to the extract again and then 1 mL sample was mixed in sulphuric acid and anthrone solution each with 5 mL. The final value was detected at 585 nm using glucose as a standard (Iqbal et al. 2020a).

Measurements of fructose, sucrose, and sucrose phosphate synthase activity

Sucrose and fructose contents were determined by using the commercial chemical kit following the manufacturer's

instructions (Suzhou Comin Biotechnology, Suzhou, China). About 0.1 g dried leaf tissues or 0.3 g samples were extracted with 5 mL of 80% ethanol. The alcoholic extract was centrifuged for 10 min (3 500×*g*) and then the supernatant was stored at 4 °C for further analysis. The determination of the sucrose and fructose was assessed according to the protocol provided by the manufacturer. The pellet obtained after centrifugation was dried and then incubated for 48 h at 37 °C in buffer acetate (4.5 mmol·L $^{-1}$) and α-glucoamylase (0.5%, w/v) and water for further determination. The absorption values of sucrose and fructose were recorded at 480 nm. The concentration of sucrose and fructose was expressed as mg·g^{-1} FW.

For SPS, approximately 1 g of frozen material was ground to a fine powder in an ice bath with 5 mL of 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid-NaOH buffer (50 mmol·L $^{-1}$, pH 7.5) containing 50 mmol·L $^{-1}$ $MgCl_2$, 2 mmol·L $^{-1}$ EDTA, 0.2% (w/v) bovine serum albumin, and 2% polyvinyl pyrrolidone (PVP). After centrifugation (10 000×*g*, 10 min), 50 µL of supernatant was mixed with 50 µL of HEPES–NaOH buffer, 20 µL of 50 mmol·L $^{-1}$ $MgCl_2$, 20 µL of 100 mmol·L $^{-1}$ uridine diphosphoglucose (UDPG), and 20 µL of 100 mmol·L $^{-1}$ fructose. The mixture was incubated at 30 °C for 30 min, and the reaction was stopped by the addition of 200 µL of 2 mmol·L $^{-1}$ NaOH and the mixture was boiled for 10 min. The solution was then cooled to room temperature. Next, 1.5 mL of 30% HCl and 0.5 mL of 0.1% resorcin were added and mixed thoroughly. Then, the mixture was incubated at 80 °C for 10 min. The solution was cooled to room temperature again and light absorption was measured spectrophotometrically at 480 nm.

Fiber quality traits

Fiber quality traits like fiber strength (cN·tex^{-1}), length (mm), micronaire, uniformity, and elongation were measured according to the international standard in the Laboratory of Quality and Safety Risk Assessment for Cotton Products, Ministry of Agriculture and Rural Affairs, Peoples' Republic of China, Anyang.

Statistical analysis

The collected data were analyzed using Statistix 10 software (Analytical Software, Tallahassee, FL, USA). The least significant difference (LSD) was used for mean separation at a 5% level of significance. Correlation analysis between the root morphology, subtending leaf morphology and physiology, and fiber quality traits were performed according to the suggested method (Iqbal et al. 2020a).

Table 1 Changes in root morphological characteristics of contrasting N-efficient cotton genotypes under control (2.5 mmol·L^{-1}), and high (5 mmol·L^{-1}) N conditions

N condition	RL /m	RSA /cm^2	RD /mm	RV /cm^3
Control	3.168 a	307.0 a	0.837 a	5.82 a
High N	2.861 b	278.1 b	0.886 7 a	5.47 b
LSD for N	0.007	5.7	0.004	0.08
Genotypes (G)				
CCRI-69	3.181 a	308.5 a	0.936 a	5.91 a
XLZ-30	2.848 b	276.6 b	0.788 b	5.38 b
LSD for G	0.004	3.4	0.006	0.15
Interactions (N × G)	***	*	*	ns

Means not sharing a letter in common with in a column differ significantly at 5% probability level. ns = Non-significant, * Significant at $P \leq 0.05$, and *** Significant at $P \leq 0.001$. RL: Root length, RSA: Root surface area, RD: Root diameter, and RV: Root volume

Results

Changes in morphological traits and N content in the cotton subtending leaf

In both cotton genotypes, a significant difference was found in root morphological traits under control and high N concentrations (Table 1). Except for root diameter, root length, root surface area, and root volume were increased by 9.7%, 9.4% and 6.1% under the control as compared with high N treatment, respectively (Table 1). Between the genotypes, CCRI-69 had a higher root length (10.5%), root surface area (9.4%), root diameter (15.9%), and root volume (9.1%) as compared with XLZ-30 (Table 1).

The leaf morphological traits of both cotton genotypes reduced with DPA increasing (Fig. 1A–C). Leaf morphological traits like the total number of leaf per plant, subtending leaf area, subtending leaf biomass, and N content in the subtending leaf drastically reduced from 10 to 40 DPA (Fig. 1). Compared with the control, the number of leaves increased by 17.8% at 10 DPA, 25.9% at 25 DPA, and 30.3% at 40 DPA (Fig. 1A). At each growth stage, the number of leaves per plant in CCRI-69 was significantly higher by 12.5% at 10 DPA, 13.2% at 25 DPA, and 14.5% at 40 DPA as compared with XLZ-30 (Fig. 1A). Under high N level, the subtending leaf area and biomass were increased by 5.3%∼13.7%, 14.2%∼6.2% and 34.4%∼4.4% at 10, 25 and 40 DPA as compared with low N level, respectively (Fig. 1B,C). In comparison with XLZ-30, the subtending leaf area and biomass of CCRI-69 was higher by 3.7%∼6.4% at 10 DPA, 7.5%∼4.6% at 25 DPA, and 12.9%∼2.5% at 40 DPA on average of both control and high N (Fig. 1B, C).

The N content in the subtending leaf of both cotton genotypes reduced with DPA increasing (Fig. 1D). In the current study, the difference between the control

and high N for N content in the subtending was negligible. In comparison with the control, N content in the subtending leaf was 1.3%, 3.1%, and 2.4% higher under high N at 10, 25, and 40 DPA, respectively (Fig. 1D). The N content in the subtending leaf of CCRI-69 was higher by 5.9% at 10 DPA, 6.6% at 25 DPA, and 5.2% at 40 DPA than XLZ-30 (Fig. 1D).

Changes in the chlorophyll and carotenoids contents in the subtending leaf

Chlorophyll a, chlorophyll b, and carotenoid contents in the cotton subtending leaves were declined from 10 to 40 DPA in both cotton genotypes, and the lowest values were recorded at 40 DPA (Fig. 2). The difference between the control and high N was not significant for chlorophyll a and carotenoid contents, especially at 25 and 40 DPA (Fig. 2A–C). Compared with the control, chlorophyll a and chlorophyll b contents were increased by 5.6%∼20.1% at 10 DPA, 1.0%∼15.4% 25 DPA, and 3.8%∼11.9% at 40 DPA (Fig. 2A, B). The chlorophyll a and chlorophyll b contents in the subtending leaf of CCRI-69 were 3.1%∼8.6%, 1.5%∼5.6%, and 2.6%∼7.0% at 10, 25, and 40 DPA as compared with XLZ-30, respectively (Fig. 2A, B). There was no significant difference between the control and high N for carotenoid contents (Fig. 2C), however, genotype CCRI-69 has 3.1%, 2.3%, and 3.5% higher carotenoid contents than XLZ-30 (Fig. 2C).

Free amino acid, soluble protein, and soluble sugar contents in the subtending leaf

Free amino acids were dramatically decreased in subtending leaves of both cotton genotypes from 10 to 40 DPA and a great reduction was found at 40 DPA (Fig. 3A). In comparison with the control, free amino acid content in the cotton subtending leaf increased by 5.6%, 8.0% and 6.3% under high N, respectively (Fig. 3A). Comparatively, free amino acid content in CCRI-69 leaf substantially increased by 9.6% at 10 DPA, 14.3% at 25 DPA, and 16.4% at 40 DPA (Fig. 3A). Soluble protein content in the subtending leaf of both cotton genotypes decreased gradually with DPA increased but was enhanced significantly by high N (Fig. 3B). Under high N level, the soluble protein content in both cotton genotypes increased by 19.1%, 7.6% and 8.6% at 10, 25, and 40 DPA, respectively (Fig. 3B). A clear increase in soluble protein content was noted for CCRI-69 leaf with an increase of 17.2% at 10 DPA, 12.9% at 25 DPA, and 16.3% at 40 DPA as compared with XLZ-30 (Fig. 3B). Soluble sugar content in the subtending leaves of both cotton genotypes decreased from 10 to 40 DPA, however, the reduction was found more at 40 DPA (Fig. 3C). Compared with high N treatments, soluble sugar content under the control increased by 16.5%, 2.7%, and 20.1% at 10, 25 ,and 40 DPA, respectively (Fig. 3C). Average soluble sugar content in CCRI-69 leaf increased by 12.8% at 10 DPA, 9.3% at 25 DPA, and 10.8% at 40 DPA relative to XLZ-30 (Fig. 3C).

Fructose and sucrose content, and SPS activity in the subtending leaf

Fructose and sucrose content, and sucrose phosphate synthase (SPS) activity in the cotton subtending leaf were declined from 10 to 40 DPA in both cotton genotypes, and the lowest values were recorded at 40 DPA (Fig. 4). In the present study, fructose content in cotton subtending leaf was increased by 30.9%, 29.6%, and 34.8% under the control as compared with high N treatment, respectively (Fig. 4A). A significant increase of 8.6%, 7.8%, and 16.2% in the leaf fructose content of CCRI-69 was observed at 10, 25, and 40 DPA, respectively mas compared with XLZ-30 (Fig. 4A). In comparison with high N, the sucrose content in the leaves of both cotton genotypes were higher under control, however, the increase greatly varied among the growth stages, where 30.1% at 10 DPA, 32.7% at 25 DPA, and 41.8% at 40 DPA were recorded (Fig. 4B). Meanwhile, the increase in leaf sucrose content of CCRI-69 was 9.7% at 10 DPA, 10.4% at 25 DPA, and 12.2% at 40 DPA as compared with XLZ-30 (Fig. 4B). SPS is an important enzyme for sucrose metabolism. As expected, the SPS activity under the control was increased by 10.2%, 15.5%, and 11.9% at 10, 25, and 40 DPA as compared with high N treatment, respectively (Fig. 4C). Irrespective of the N levels, the increase of SPS activity in the subtending leaf of CCRI-69 was 9.1% at 10 DPA, 12.2% at 25 DPA, and 17.8% at 40 DPA as compared with XLZ-30. The higher SPS activity in the subtending leaf of CCRI-69 under the control indicates its high potential for sucrose metabolism and their partitioning to sink during boll development.

Fiber quality attributes

Except fiber uniformity, fiber quality attributes such as fiber length, strength, micronaire, and elongation of both cotton genotypes were significantly different between the control and high N treatments (Table 2). In comparison with high N treament, fiber length, strength, micronaire, and elongation were increased by 3.0%, 4.9%, 4.4%, and 1.0% under control condition, respectively (Table 2). Genotype

(See figure on next page.)

Fig. 1 **A** The number of leaves per plant, **B** subtending leaf area (cm^{-2}), **C** subtending leaf biomass (g), and **D** N content in the subtending leaf (%) of CCRI-69 and XLZ-30 in under control (2.5 $mmol{\cdot}L^{-1}$), and high (5 $mmol{\cdot}L^{-1}$) N conditions at 10, 25 and 40 days post-anthesis (DPA). Error bars with different small letters show a significant difference between genotypes under control and high N condition and capital letters in the brackets show significant difference among growth stages (10, 25, and 40 DPA) at $P < 0.05$

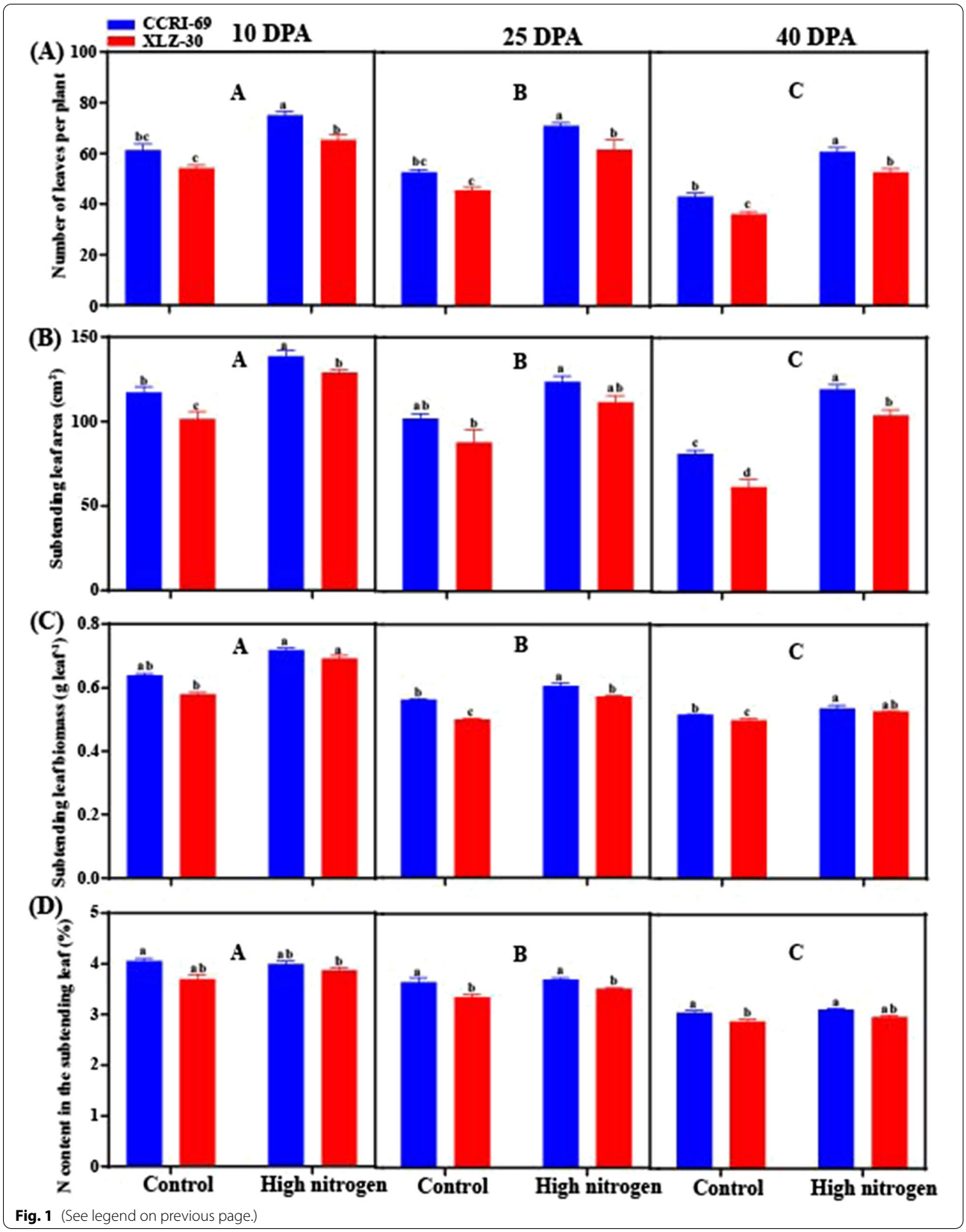

Fig. 1 (See legend on previous page.)

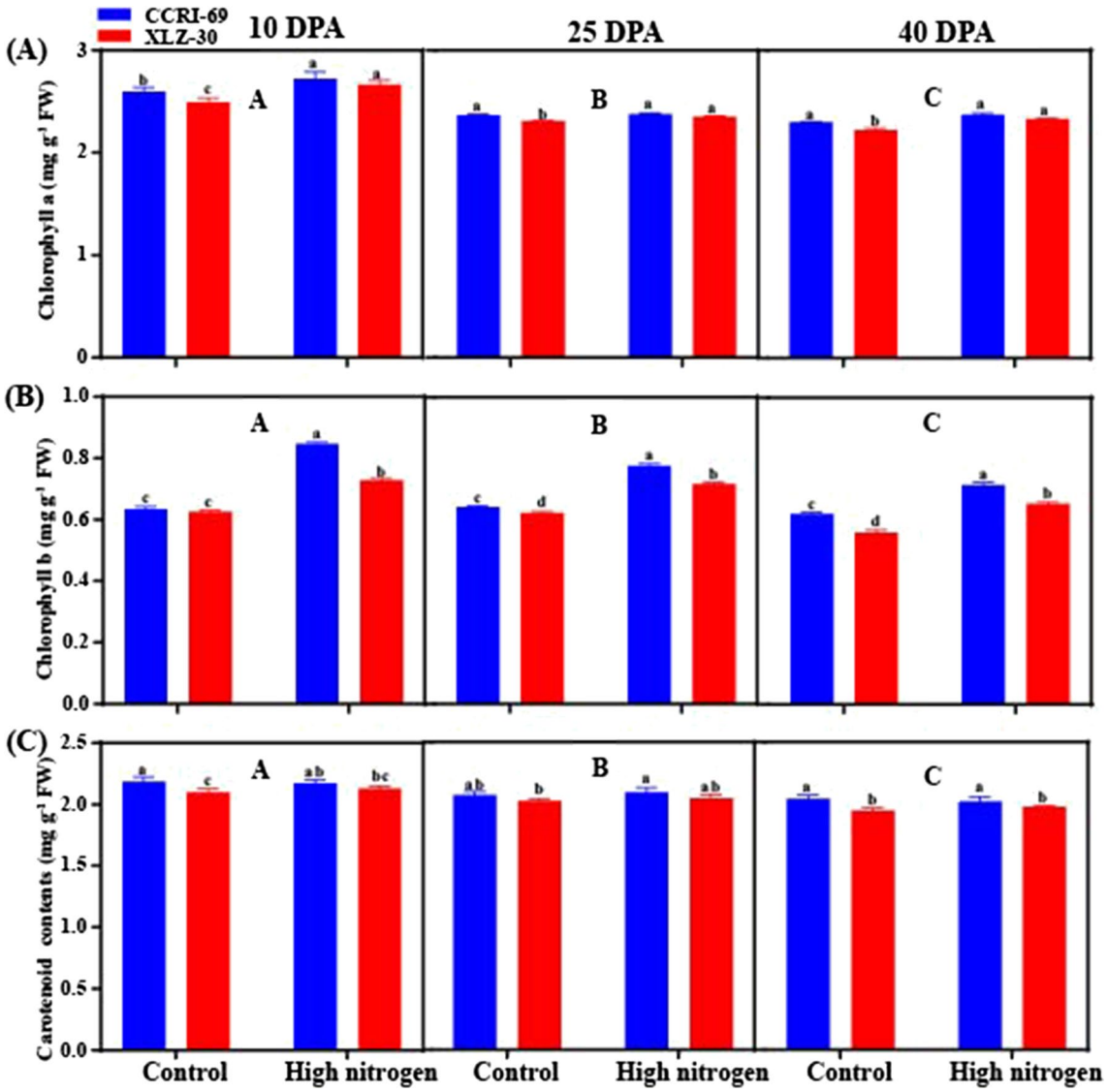

Fig. 2 **A** Chlorophyll a (mg·g^{-1}), **B** chlorophyll b (mg·g^{-1}), and **C** carotenoid (mg·g^{-1}) contents in fresh weight in cotton subtending leaves of CCRI-69 and XLZ-30 under control (2.5 mmol·L^{-1}), and high (5 mmol·L^{-1}) N conditions at 10, 25 and 40 days post-anthesis (DPA). Error bars with different lower-case letters show a significant difference between genotypes under control and high N condition, those with different capital letters in the brackets show significant difference among growth stages (10, 25 and 40 DPA) at $P < 0.05$

CCRI-69 has significantly higher fiber length (3.0%), fiber uniformity (1%), fiber strength (3.9%), fiber micronaire (4.2%), and fiber elongation (1%) as compared with XLZ-30 (Table 2).

Multivariate analysis

To understand the relationship among root morphology, subtending leaf morphology, and physiology with fiber quality, correlation analysis was performed (Fig. 5 and Additional file 1: Table S1). The result showed that 22 nodes (traits) were connected with 231 edges in the correlation network (Fig. 5). Among the total direct correlation, 151 pairs trait showed a positive correlation, and 80 pairs were negatively correlated (Fig. 5). Among the fiber quality traits, fiber length has a strong positive correlation with fiber strength (r = 0.99) and micronaire (r = 0.97). Root morphological traits have a strong positive correlation (r = 0.97–0.99) with fiber quality traits (Fig. 5 and Additional file 1: Table S1). Among the subtending leaf traits, SPS, soluble sugars, and sucrose contents have a strong positive correlation (r = 0.97–0.98) with fiber quality traits except for fiber uniformity (Fig. 5 and Additional file 1: Table S1). The

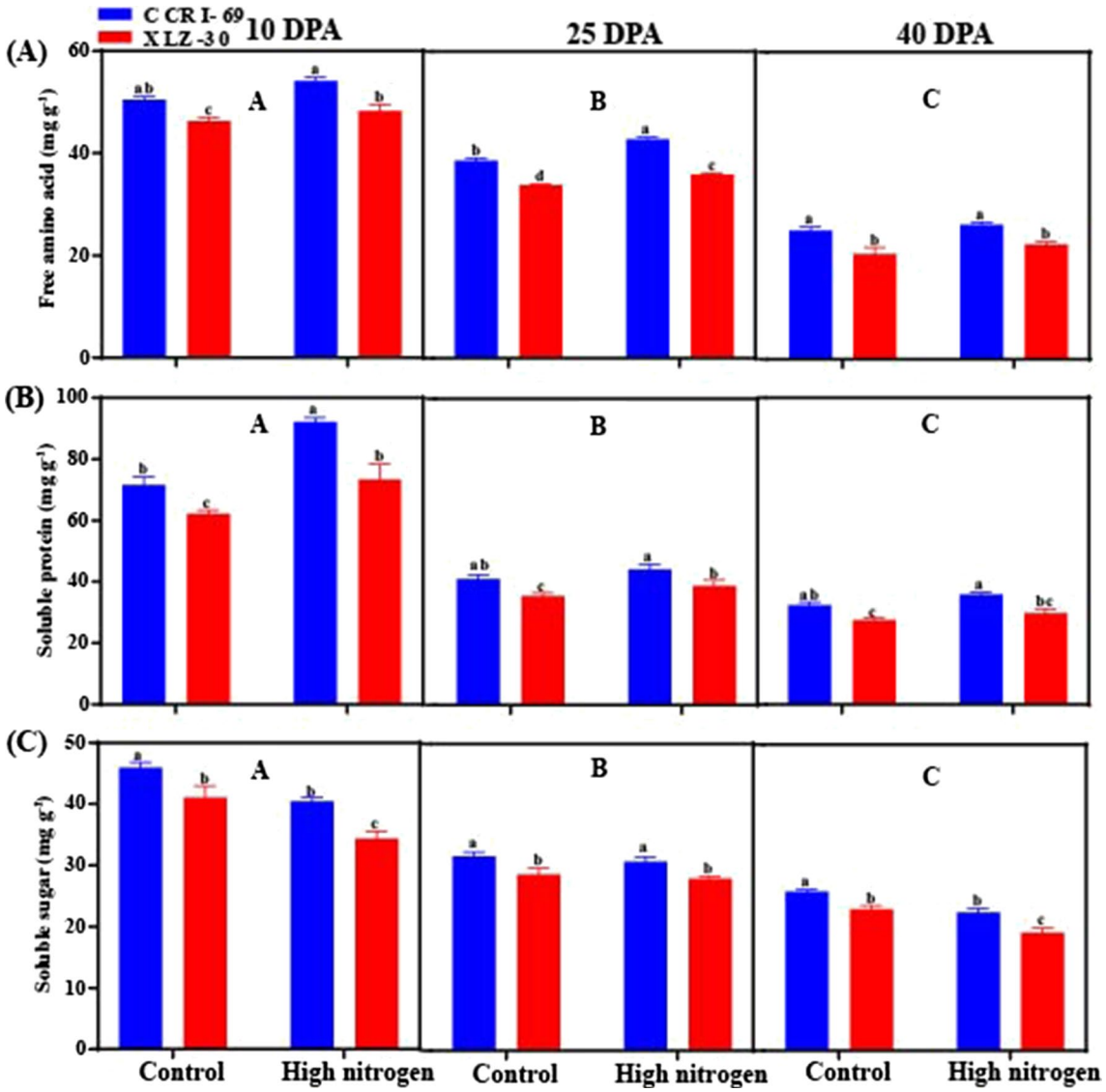

Fig. 3 **A** Free amino acid (mg·g^{-1}), **B** soluble protein (mg·g^{-1}), and **C** soluble sugar (mg·g^{-1}) in cotton subtending leaves of CCRI-69 and XLZ-30 under control (2.5 mmol·L^{-1}), and high (5 mmol·L^{-1}) N conditions at 10, 25, and 40 days post-anthesis (DPA). Error bars with different lower case letters show a significant difference between genotypes under control and high N condition and capital letters in the brackets show significant difference among growth stages (10, 25, and 40 DPA) at $P < 0.05$

chlorophyll a, chlorophyll b, carotenoids, subtending leaf area, and biomass has a negative correlation (r = −0.40 to 0.44) with fiber strength and elongation (Fig. 5 and Additional file 1: Table S1).

The principal component analysis (PCA) was also performed to know the response patterns of various traits of cotton genotypes under the control and high N (Fig. 6). The loading plots of PC1 and PC2 consisted of 22 traits obtained from the average of both cotton genotypes under the control and high N conditions. The loading plot of PC1 was associated with N conditions and contributed to 55.51% of the total variation. The loading plot of PC2 was associated with cotton genotypes and accounted for 31.51% of the total variation (Fig. 6 and Additional file 1: Table S2). The number of leaves per plant, subtending leaf biomass, chlorophyll a, chlorophyll b, and carotenoid contents were the main contributors of PC1 while root volume, SPS activity, fiber micronaire, and fiber length were the main contributors to PC2 (Fig. 6 and Additional file 1: Table S2). The larger distance between the control and high N indicated that cotton genotypes were highly responsive,

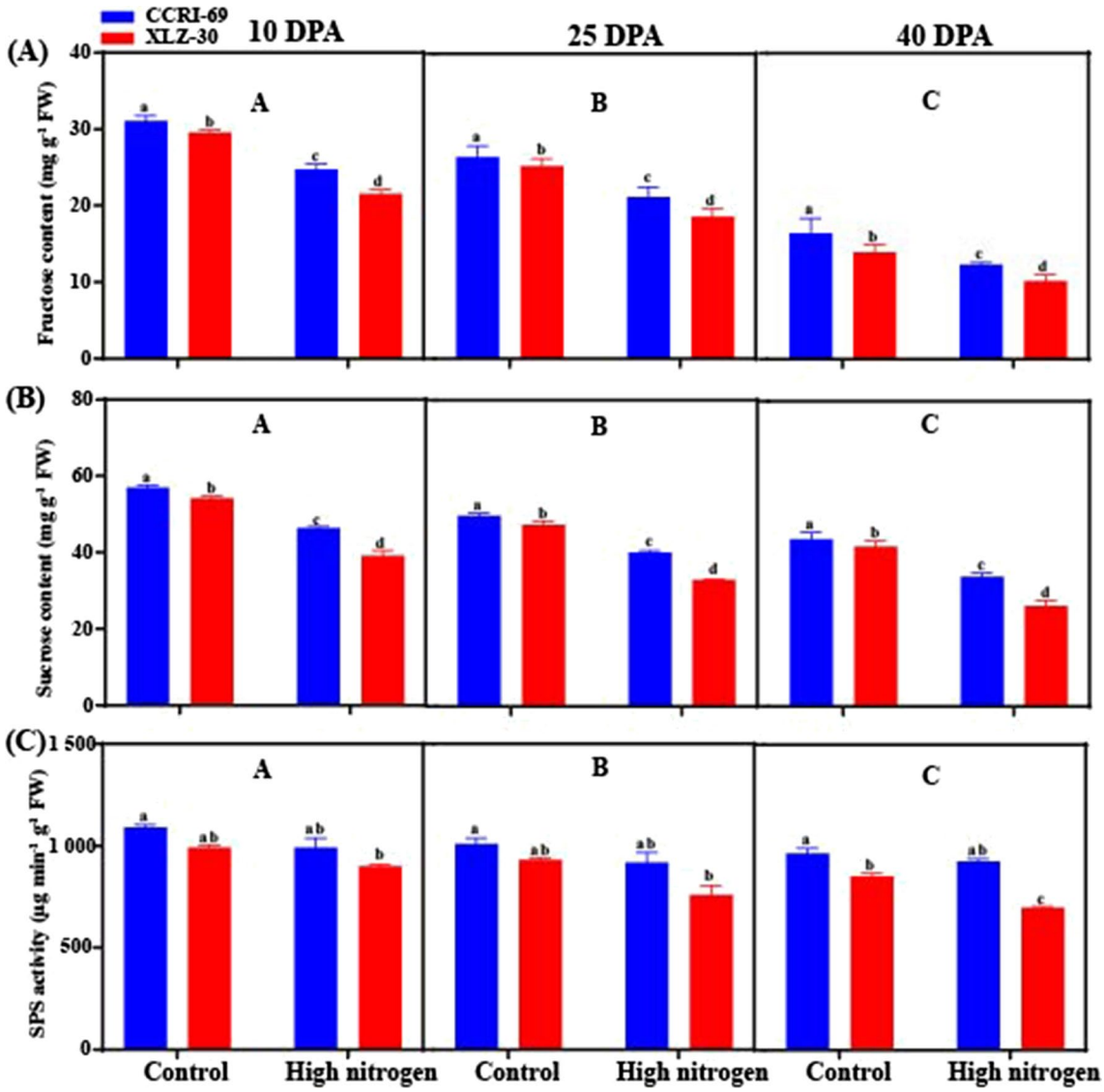

Fig. 4 **A** Fructose content (mg·g^{-1} FW), **B** sucrose content (mg·g^{-1} FW), and **C** sucrose phosphate synthase (SPS) activity (μg·min^{-1}·g^{-1} FW) in cotton subtending leaves of CCRI-69 and XLZ-30 under the control (2.5 mmol·L^{-1}), and high (5 mmol·L^{-1}) N conditions at 10, 25 and, 40 days post-anthesis (DPA). Error bars with different lower case letters show a significant difference between genotypes under the control and high N condition and capital letters in the brackets show significant difference among growth stages (10, 25, and, 40 DPA) at $P < 0.05$

especially N-efficient cotton genotype (CCRI-69) under both control and high N (Fig. 6).

Discussion

Nitrogen (N) is one of the most important nutrients needed in large amounts for better cotton production and quality (Iqbal et al. 2019b, 2020a, b). Application of optimum N improves various metabolic activities, plant growth, and productivity. However, overuse of N increases vegetative growth and reduces fiber quality. The present study focused on the relationship of root morphology, subtending leaf morphology, and physiology with cotton fiber quality of CCRI-69 (N-efficient) and XLZ-30 (N-inefficient) under the control and high N conditions. Plants showed adaptive responses to changes in the external N availability (Sakakibara et al. 2006). In this regard, roots play a vital role in adaptive responses by altering the architecture (Rellán-Álvarez et al. 2016). Previous studies have shown that root morphological traits were greatly affected by N supply (Meng et al. 2016). However, there are still contradictory reports on the effect of N on root morphology and biomass, some

Table 2 Changes in fiber quality characteristics of contrasting N-efficient cotton genotypes under control (2.5 mmol·L^{-1}), and high (5 mmol·L^{-1}) N conditions

N condition	FL /mm	FU /%	FS /(cN·tex^{-1})	Micronaire	FE /%
Control	29.80 a	87.12 a	31.62 a	4.37 a	6.90 a
High N	28.93 b	87.37 a	30.15 b	4.18 b	6.84 b
LSD for N	0.74	ns	1.04	0.16	0.03
Genotypes (G)					
CCRI-69	29.82 a	87.48 a	31.50 a	4.37 a	6.89 a
XLZ-30	28.92 b	87.00 a	30.27 b	4.18 b	6.85 b
LSD for G	0.74	ns	1.04	0.16	0.03
Interactions (N × G)	ns	ns	ns	ns	*

Means not sharing a letter in common with in a column differ significantly at 5% probability level. ns = Non-significant, and * Significant at $P \leq 0.05$. FL = Fiber length (mm), FU = Fiber uniformity (%), FS = Fiber strength (cN·tex^{-1}), and FE = Fiber elongation (%)

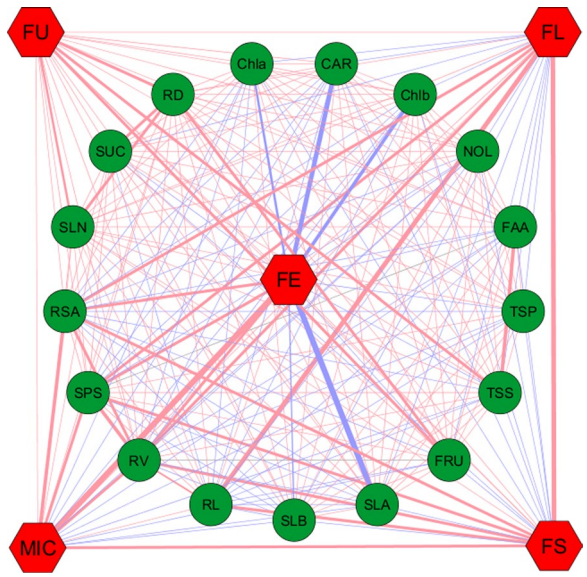

Fig. 5 Relationships among various morphological and physiological traits (green) and fiber quality traits (red). Nodes and edges represent traits and correlation, respectively. Red and blue color edges show positive and negative correlations, respectively. Edges thickness represents the strength of the correlation, where strong positive correlations were highlighted by red ($r = 0.99$) color and strong negative by green ($r = -0.95$ to -0.98) color. Root length (RL), root surface area (RSA), root diameter (RD), root volume (RV), the number of leaves per plant (NOL), subtending leaf area per plant (SLA), subtending leaf biomass (SLB), subtending leaf N content (SLN), chlorophyll a (Chla), chlorophyll b (Chlb), carotenoid (CAR), free amino acid (FAA), total soluble protein (TSP), total soluble sugar (TSS), fructose (FRU), sucrose (SUC), sucrose phosphate synthase (SPS), fiber length (FL), fiber uniformity (FU), fiber strength (FS), micronaire (MIC), and fiber elongation (FE)

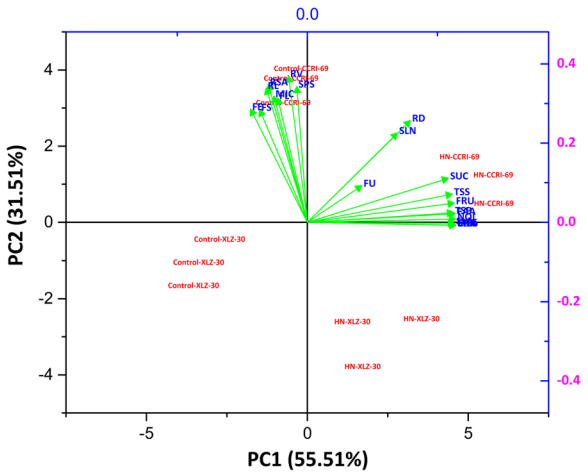

Fig. 6 PCA of the studied traits of CCRI-69 and XLZ-30 under the control (2.5 mmol·L^{-1}) and high N conditions (5 mmol·L^{-1}). The eigenvectors for PC1 and PC2 are shown in supplementary table 2

researchers reported that high N supply increased root development and biomass (Kraiser et al. 2011), while others reported no effect on root growth (Guo et al. 2010). However, in cotton, high N greatly decreased root morphological traits and the reduction was found more in XLZ-30 (Table 1). Similarly, a significant change in root morphological traits of *Arabidopsis* was noted under various N levels (Kellermeier et al. 2013). In the current study, a significant improvement in root morphological traits was observed in CCRI-69 under control condition (Table 1), suggesting that the roots of CCRI-69 showed a great response and can improve root architecture for better nutrient uptake that can increase cotton biomass production and fiber quality. Moreover, a strong positive correlation was observed between root morphological traits and fiber quality traits (Fig. 5). In addition, PCA analysis showed that root-related traits and fiber quality traits contributed more to the genotypic effect and were associated with the loading plot PC2 (Fig. 6). Thus, we can consider root morphological traits as potential indicators for improving fiber quality of cotton under normal N conditions.

Cotton subtending leaves are the major carbohydrates source for boll development and contribute more to boll weight and eventually the cotton yield and quality (Liu et al. 2013). It was reported that 60%–87% of the assimilates required for boll development are supplied from cotton subtending leaves (Liu et al. 2013; Wullschleger and Oosterhuis 1990). It has a unique characteristic of retaining limited assimilates and partitioning most of them to reproductive organs followed by high fiber quality (Hafeez et al. 2019). A better sink (reproductive organs) development has a close relationship with leaf morphology and physiology (Hu et al. 2015). A well-established leaf area is the guarantee of high photosynthate production in the plants. In the current study, more number of leaves per plant, improved subtending leaf area, and biomass is the basis for the production of higher sucrose contents. In our previous studies, an increase in the cotton leaf area was observed with the application of high N as compared with low N supply (Iqbal et al. 2020a, b). Thus, leaf morphology is important for the development of boll and fiber quality. In addition to leaf morphology, the leaf chlorophyll content is also a very important indicator that is sensitive to changes in N supply (Qin et al. 2018). The reason behind this is about 57% N of the leaf is located in the chloroplast and enzymes that are involved in photosynthesis (Ziadi et al. 2008). In the current study, the chlorophyll a, chlorophyll b, and carotenoid contents in the subtending leaf of CCRI-69 were higher than those in XLZ-30 across the growth stages (Fig. 2). Comparatively high chlorophyll content and photosynthetic activity in CCRI-69 under various N levels were also found in our previous studies (Iqbal et al. 2020a, b), and these results are supported by the one obtained in the poplar species (Luo et al. 2015). Thus, improved subtending leaf morphology and high chlorophyll content of CCRI-69 might have contributed to high fiber quality.

Carbohydrates metabolism of cotton is closely associated with N availability (data unpublished). Comparatively, fructose, sucrose contents, soluble sugars, and SPS was higher in the subtending leaf of CCRI-69 under control condition. Similarly, fiber quality traits were significantly improved under control condition than high N in both cotton genotypes with a more increase in CCRI-69 (Table 2). All the earlier reports agreed that leaf starch accumulation increased under N deprivation (Boussadia et al. 2010; Hendrix 2010). Under comparatively low N, the olive leaves had significantly higher sugar contents (Boussadia et al. 2010), which is in accordance with our results with cotton leaves. The leaf sucrose level was predominantly controlled by the availability of N, and the rate of sucrose export was linearly associated with SPS activity (Hendrix and Huber 1986). Interestingly, leaves in high N conditions exhibited consistently lower concentrations of fructose and sucrose than the control during boll development (Fig. 4). It seems likely that the redundant N in leaves in high N coditions could consume more sucrose to synthesize amino acids and proteins. In our study, not only sucrose but also fructose and leaf N concentrations were significantly greater in control (normal N) than in high N condition (Figs. 1D and 4B, C). Thus, we assumed more sucrose translocation under control condition than high N based on the higher SPS activity and sucrose contents (Fig. 4). The increase in sucrose translocation from the subtending leaf to reproductive parts may facilitate cotton fiber quality. Therefore, the higher fiber quality of CCRI-69 might be due to high carbohydrates translocation from subtending leaf to cotton fiber. In a similar pattern, the variation in root/shoot carbohydrates content were observed with the changes in N in cotton genotypes (data unpublished) as well as in mulberry (Yamashita and Hikasa 1988), soybean (Huber 1984), and cotton (Hu et al. 2015; Zahoor et al. 2017) with different K application. The limited translocation of sucrose and fructose in high N conditions might be due to low SPS activity and poor phloem loading (Pettigrew and Gerik 2007) which resulted in a poor fiber quality (Fig. 4 and Table 2). Furthermore, this poor translocation of sucrose and fructose to the reproductive parts indicated retention of carbohydrates in the leaf (Ali et al. 2018), which might be the reason for low fiber quality under high N condition, especially in XLZ-30. In line with our results, changes in the level of non-structural carbohydrates in cotton subtending leaf was observed under different K applications (Zahoor et al. 2017). In the current study, the changes in the SPS activity across the growth stages (Fig. 4C) might be due to more tendency of growth in the subtending leaves to bolls (Hu et al. 2015). We assumed that the increase in total carbohydrates in the subtending leaf stimulated the activity of SPS in CCRI-69 under control condition, indicating that sucrose metabolism depends on the availability of sufficient N in the subtending leaf. Previous studies have shown that an increase in protein synthesis results in greater demands for carbohydrates (Schlüter et al. 2012; Zhao et al. 2015). The high SPS activity in the subtending leaf of CCRI-69 might be due to enhanced glycogen metabolism and respiration in response to N application. Another reason for high sucrose metabolism in CCRI-69 might be due to an increase in the transcript levels of genes responsible for carbon metabolism as shown in our previous study (Iqbal et al. 2020e). In addition, the increased SPS activity might be attributed to the increased level of sucrose and fructose content in the leaves of both cotton genotypes, especially CCRI-69. Similarly, the high enzymatic activities under K application were attributed to more substrate

(sucrose) availability to active sites and thus increase the synthesis of free glucose and fructose in the tissues (Ali et al. 2018).

These pieces of evidence indicate clearly that assimilates exported from subtending leaves are preferentially partitioned to the fiber. The limited carbohydrate export from the subtending leaf led to decreased starch levels in fibers, which may be caused by reduced carbon influx and enhanced requirement for starch-degraded hexose under high N condition. In this study, most of the variables failed to respond to high N including carbohydrates contents and fiber quality traits. A possible reason is associated with cotton's ability to rapidly enhance vegetative growth under high N condition (Boquet et al. 1993). Furthermore, other nutrients can be remobilized into carbohydrates including starch within ovules (Tang et al. 2014). Fructose and sucrose are considered as the major osmotically active solutes in the fibers to promote fiber elongation. Sucrose metabolism is very important for fiber cellulose accumulation (Hu et al. 2007). In our study, the response of sucrose content and sucrose depredation to N is consistent with the enzymatic analysis results related to sucrose metabolism. High enzymatic activity for a long duration was observed under control conditions, which is important for sucrose degradation and cellulose synthesis. The maximum speed of cellulose accumulation in the fiber is attained when cellulose percentage exceeds 80% and the cellulose accumulated fast but not the cellulose content (Shu et al. 2007; Zhang et al. 2009). Under high N conditions, the N concentration in the subtending leaf was high in the early fiber development stage leading to a reduction in the photosynthetic rate and therefore the mature fiber strength significantly decreased (Ma et al. 2008). Subsequently, high N decreased the enzymatic activities related to sucrose metabolism as well as the maximum speed of cellulose accumulation at the early stage of fiber development. The reason behind this might be the restricted translocation and accumulation of carbohydrates in the subtending leaf due to high N concentration and N metabolism (Hikosaka 2005) followed by vigorous vegetative growth and poor fiber quality of cotton (Sun et al. 2007). Early study had also found that the capability of synthesis and translocation of subtending leaf reduced after 25 DPA or early fiber development stage (Hikosaka 2005). Thus, the duration of speedy cellulose accumulation shrinks and the fiber strength is greatly reduced after 25 DPA. In contrast, the duration of speedy cellulose accumulation must be prolonged to achieve high fiber strength (Ma et al. 2008). Generally, 25 days-old boll is considered a transition point of sucrose metabolism and fiber strength regulated by N (Ma et al. 2008). Following these results, the fiber quality-related traits were the highest under control condition and the difference of N concentration in the subtending leaf was significant between the genotypes.

Conclusions

N plays a critical role in the root morphology, subtending leaf morphology and physiology as well as in cotton fiber quality. This role is complex and varied in cotton genotypes, as it involved several physiological and metabolic processes. Root morphology (root length, root surface area, and root volume) and non-structural carbohydrate contents like fructose, sucrose contents, and SPS activity were considerably increased under control conditions in CCRI-69 as compared with XLZ-30 across the growth stages. In contrast, high N application increased subtending leaf morphology, chlorophyll, and carotenoid contents as well as N concentration in the subtending leaf of CCRI-69. The fiber quality traits like fiber length, strength, micronaire, and elongation were significantly higher in CCRI-69 under the control as compared with high N conditions. A strong positive correlation was observed between root morphology, soluble sugars, sucrose contents, and SPS activity with fiber quality traits. Thus, normal N application proved as a key to improve root morphology, sucrose metabolism in the cotton subtending leaf to achieve higher fiber quality in N-efficient cotton genotype. Further research is required to determine the molecular mechanism involved in source-sink anatomy and sucrose metabolism in the cotton subtending leaf of CCRI-69 to know the exact mechanism of improving cotton fiber quality.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s42397-021-00107-2.

Additional file 1. **Table S1**. List of source and target traits used for correlation network based on correlation coefficient. **Table S2**. Principal component analysis (PCA) of various morphological, physiological and fiber quality traits of CCRI-69 and XLZ-30 under control (2.5 mmol·L^{-1}) and high (5 mmol·$^{-1}$) N conditions.

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Authors' contributions
Asif I conducted the main experiment and drafted the manuscript and Dong Q, Wang XR, and Gui HP participated in data collection and analysis and modified the language. Asif I, Zhang HH, and Pang NC performed part of the statistical analysis and revised the manuscript. Gui HP, Dong Q, Pang NC, and Wang XR helped to collect data. Song MZ and Zhang XL designed and funded the study. All authors have read and approved the final manuscript.

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Availability of data and materials
The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no conflict of interest.

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