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EFFECT OF TIMING AND RATIO OF UREA FERTILIZATION ON ^{15}N RECOVERY AND YIELD OF COTTON

P.C. Li, A.Z. Liu, J.R. Liu, C.S. Zheng, M. Sun, G.P. Wang, Y,B. Li, X.H. Zhao and H.L. Dong*

State Key Laboratory of Cotton Biology, Institute of Cotton Research of Chinese Academy of Agricultural Sciences, Anyang, Henan 455000,China
*Corresponding author E-mail:donghl668@sina.com

ABSTRACT

Applications of urea before planting and at the initial flowering stage at ratios of 1:1 are the conventional nitrogen (N) fertilization method in the Yellow River Valley Cotton (*Gossypium hirsutum* L.) Area of China. This study was conducted to determine whether increasing urea fertilization ratios at the initial flowering stage or fertilization once at the budding or the initial flowering stage could improve seed cotton yield, plant ^{15}N recovery, and decrease fertilizerN loss (FNL). Pot experiments were conducted in 2013 and 2014, arranged in a randomized complete block design with two contemporary cultivars (CCRI 79 and CCRI 60), and four fertilization timing/ratios with the same total N application amount 3.5g N per 35 kg soil per pot over the entire growing stage, the conventional fertilization method (1:1), application of urea before planting and at the initial flowering stage at ratios of 1:2 and the application of urea all at the initial flowering stage and all at the budding stage.The results revealed that the application of urea all at the budding stage and all at the initial flowering stage could increase cotton plant ^{15}N recovery and decrease the FNL compared to the split application of urea, and achieved similar seed cotton yield of conventional fertilization in addition to application of urea all at the budding stagefor CCRI 60in 2013. Application of urea before planting and at the initial flowering stage at ratios of 1:2 improved the seed cotton yields although could not significantly increase the cotton plant^{15}N recovery and decreased the FNL comparing to the conventional fertilization method. It was feasible to decrease the N ratio of base N and increase the ratio of topdressing Nat the initial flowering stage based on conventional N fertilization method in cotton N management.

Keywords: urea; fertilization timing and ratio; seed cotton yield; ^{15}N recovery; cotton.

INTRODUCTION

Nitrogen (N) is the most limiting nutrient for crop production (Fageria and Baligar, 2005). More than 55% of the increase in crop production in developing countries is attributed to the use of chemical fertilizers, mainly N fertilizers. Crops often obtain up to 45%–70% of their total N from the soil (Li *et al*., 2009). Most plants can use N as $NO_3^-$and NH_4^+, which are the available inorganic forms of N absorbed by plant roots from the soil solution (Mokhele *et al*., 2012). Effective use of soil N supply and better synchronization between crop N demand and supply were main measures for the increase of N use efficiency (Cui *et al*., 2008), and N fertilization should be concentrated in the middle-late stage to match crop demand while farmers often applied the majority of N fertilizer either before sowing or during early growth stages (Meng *et al*., 2016). At the same time, because of the increase of labor costs in agricultural production, split application of fertilizer was more costly than one-time fertilization (Knowles *et al*., 1994). Optimizing N fertilizer application dose and time to achieve both high yield and high N use efficiency simultaneously has become a major challenge.

Proper management of N fertilization is particularly important for improving crop yields and N use efficiency (Wuest and Cassman, 1992; Wienhold *et al*., 1995; Meng *et al*., 2006). The N isotope tracer technique can be used to distinguish how much N is absorbed and used by crops from fertilizer N or soil N and to investigate the movement and distribution of N fertilizer in crop plants (Navarro-Ainza, 2007; Ju *et al*., 2007; Wei *et al*., 2012) to provide a theoretical basis for managing crop N fertilization. In a study, the ^{15}N urea recovered by a combine-sown crop of Calrose rice in South-east Australia ranged from 3.8% for N added at sowing to 41.3% for N added at panicle initiation (Humphreys *et al*., 1987). Similar study on wheat indicated that the mean fertilizer N uptake efficiency was 68% for N added at wheat anthesis and 41% for N applied before planting (Wuest and Cassman, 1992). Different views exist regarding the most suitable N application period for cotton cultivation (Polychronaki *et al*., 2012). Some scholars recommended that the best periods for N application for cotton are at the emergence and budding stages (McConnell and Mozaffari, 2005). Yang *et al*. (2013) suggested that the application of N at first bloom should be the most important for N absorption and yield. However, other researchers have concluded that the split application of half of the N before sowing and the other half N at the initial flowering stage is better in cotton (Bonner, 1995), which corresponds with the conventional N application periods in the Yellow River Valley in China. However, whether the conventional N application method is beneficial for cotton yield and N use efficiency has not been demonstrated. Tang *et al*. (2012) found that allocating N fertilizer from before planting to before peak bloom would result in faster fertilizer N uptake during boll setting and improve the recovery of fertilizer N by cotton plants. One-time fertilization by a compound fertilizer at the first bloom stage in the Yangtze River Valley of China reduces labor costs without reducing cotton yield (Yang *et al*., 2012). however, little is known of the effects of one-time urea fertilization at the first bloom and budding stages on plant N recovery and seed cotton yield. The objectives of this study were aimed at determining how ^{15}N recovery and seed cotton yield are affected by the timing and ratio of urea fertilization.

MATERIALS AND METHODS

Outdoor pot experiments were conducted at an experimental farm at the Institute of Cotton Research of the Chinese Academy of Agricultural Sciences in Anyang, China (36°06′N, 114°21′E, 76 m above sea level), in 2013 and 2014.The soil at the site was a clay loam containing 1.05% organic matter, 0.07% total N, 68.2 mg kg^{-1} alkaline N, 10.6 mg kg^{-1} P, and 99.4 mg kg^{-1} K. All treatments were arranged in a complete randomized block design with 8 treatments,10 replications, 1 pot per replication and 1 plant per pot, where 2 varieties (*Gossypium hirsutum* L. cv. CCRI79 and CCRI60) and 4 urea fertilization timing/ratios were designed, which were the split application of urea before planting and by topdressing at the initial flowering stage at ratios of 1:1 (N1), 1:2 (N2) and the application of urea all at the initial flowering stage (N3) and all at the budding stage (N4).

Each PVC pot (39 cm tall, 35 cm bottom diameter, 30 cm top diameter) contained 35 kg of soil with two holes (5 mm diameter) drilled in the bottom to allow for leaching. The total N application rate in each pot was 3.5 g. ^{15}N labeled urea (10.08% atom enrichment, 46.3% N) was used for tracing the N in the cotton plants and soil in treatments. Additional 8.33 g triple superphosphate (44% P_2O_5) and 6.86 g potassium sulfate (51% K_2O) were applied to each pot before sowing. Fertilizers were mixed and blended evenly with soil 2 d before sowing. Each treatment consisted of 10 pots, and 5 pots for sampling at first bloom and 5 pots for sampling at plant withdrawal. For topdressing, fertilizer was applied around the plant 10 cm from the plant roots after dissolving in water to obtain a 0.4% (w/w) urea solution for the highest urea dosage.

Three cotton were sown in each pot on April 25, 2013, and April 29, 2014, and seedlings were thinned to one per pot at the two-leaf stage. At the squaring stage, plants were tied to bamboo sticks placed 5 cm away from the plant stems with plastic belts to prevent lodging in case of wind. The belts were moved upward as the plants grew. In the evenings, 2.0 L of water was added when the upper leaves wilted before 11 a.m. that day. Pots were set under rainproof shelters with rolling plastic film to prevent water lodging and overflowing. Buds of vegetative branches and main stems were completely topped by hand at the peak squaring(June 10, 2013; June 12, 2014) and boll-setting stages(July 25, 2013; July 27, 2014). Insect and weed control managements were conducted according to local practices.

Plant samples were collected at first blooming (July 8, 2013; July 10, 2014) and plant removal (October 15, 2013; October 20, 2014). At each sampling, 5 plants were sampled from each treatment by carefully uprooting them and separating into root, stem, leaf, and bud and boll fractions. The individual samples were placed in envelopes before placing in an electric fan-assisted oven at 105°C for 30 min to stop biomass consumption due to respiration. Next, the envelopes containing the samples were heated at 80°C for 48 h before weighing, grinding, and passing the sample through a 0.2 mm sieve. The grinder was thoroughly cleaned between samples to prevent cross-contamination of the ^{15}N plant material.

Three soil cores measuring 35 cm in length and 3 cm in diameter were collected from each pot soon after plants were sampled. The soil cores were air dried, plant debris were removed, and a subsample was finely ground using a mortar and pestle before passing the samples through a 0.2 mm sieve.

Calculations and Analyses: The cotton plant and soil samples were analyzed using a modified ZHT-30 isotope ratio mass spectrometer to determine the ^{15}N abundance, and a K-05 automatic N tester was used to determine the total amount of N. The percentage of plant ^{15}N derived from the fertilizer (Ndff) was calculated as follows (Wienhold *et al*., 1995):

Ndff (%)=(a-b)/(c-d)×100, where, a=atom% ^{15}N abundance in the plant; b=atom% ^{15}N abundance in the control plant; c=atom% ^{15}N abundance of urea; and d=natural atom% ^{15}N abundance.

The amount of plant ^{15}N absorbed was calculated as follows: ^{15}N(mg)=Wg×N%×Ndff(%)×1000, where Wg=sample weight; N%=N concentration; and Ndff%=% N derived from fertilizer.

Plant ^{15}N recovery was calculated as follows: Plant ^{15}N recovery(%)=Wn×Ndff(%)/R×100, where Wn=g N/plant=Wg×N% and R=g N applied/pot.

The soil ^{15}N recovery was determined as follows: Soil ^{15}N recovery(%)=(a-c)/(b-c)×Ns/Nf×100, where a=atom%^{15}N abundance in the fertilized soil material; b=atom% ^{15}N abundance in the labeled N fertilizer; c=atom% ^{15}N abundance in the non-fertilized soil (average background level); Ns=total N in the soil sample (g); and Nf=total amount of ^{15}N applied to the soil as labeled fertilizer (g).

The fertilizer N loss (FNL) (%) was calculated as follows: FNL(%)=100-Plant ^{15}N recovery-Soil ^{15}N recovery.

Statistical Analysis: Monthly mean temperature, precipitation and sunshine duration from May through October, which were different in 2013 and 2014 (Fig.1), and were recorded by a weather station located in the experimental field. Due to these weather differences, each year was analyzed separately. The experimental design used was a complete randomized block with 8 treatments including 2 cultivars and 4 fertilization timing/ratios. Data analysis was conducted using Proc GLM (ANOVA) (SAS, Ver. 9.2, 2004), and the mean difference was compared by the LSD test at 5% levels of probability.

RESULTS AND DISCUSSION

Dry Matter and Seed Cotton Yield: At the initial flowering stage, N1 for CCRI 79 and N1 for CCRI 60 produced the greatest dry matter weight of cotton plant in 2013 and 2014, respectively, and N1 for CCRI 60 in 2013 and N3 for CCRI 79 in 2014 produced the least dry matter weight. Dry matter weight for the same treatment in 2014 were relatively greater than that in 2013 in addition to N3 for CCRI 79 (Table 1). At the harvest stage, N1 for CCRI 79 produced the greatest dry matter weight of cotton plant in the two years, and N4 for CCRI 60 in 2013 and N4 for CCRI 79 in 2014 produced the least dry matter weight. Dry matter weigh for N1 were significantly greater than that for N4 in 2013 and the same result appeared only in N1 for CCRI 79 in 2014, but there were no significant differences between N1, N2, and N3 for the same variety in 2013, and for CCRI 60 in 2014. Dry matter weight for the same treatment in 2013 were greater than that in 2014 in addition to N4 for CCRI 60, which were attributed to much greater dry matter of vegetative organs in 2013 than that in 2014. Yang etal.(2011) reported that cotton plant biomass accumulated following a normal organism growth curve by day after emergence, prior to the point, cotton plant biomass was increasing with the increase of base N ratio, and the final biomass increased as the N ratio for peak bloom application increased. In this study, the result at initial flowering stage was in accordance with Yang (2011), but result at harvest was different, and it maybe was because of different N split application ratio and ecological climate.

The highest seed cotton yield was observed in the N2 treatment in 2013 and was 4.7%, 10.3% and 22.3% higher than the seed cotton yields in the N1, N3 and N4 treatments, respectively (Table 2). Seed cotton yield inN2 for CCRI 60 was 8.0%, 13.9% and 3.3% higher than that in the N1, N3 and N4 for CCRI 60 in 2014, respectively, and seed cotton yield inN2 for CCRI 79 was 4.9%, 14.4% higher than that in the N1 and N4 treatments in 2014, respectively, but and no significant differences were observed between N2 and N3 for CCRI 79 in 2014 though N3 for CCRI 79 produced the highest seed cotton yield in 2014. The result was in accordance to Ma *et al*.(2008) who reported that ratios of base and topdressing N fertilizer at 1:2 harvested 3.66% more lint yield than ratios of base and topdressing N fertilizer at 1:1 using equal amounts of N. When compared with the N1 treatment, the average seed cotton yields in the N3 and N4 treatments in 2013 were 5.9% and 18.4% lower, respectively, and average seed cotton yields in the N4 treatments in 2014 were 2.7% lower, but average seed cotton yields in the N3 treatments in 2014 were 0.1% higher. These results show that the seed cotton yield could be improved by decreasing the urea fertilization ratio before planting and increasing the urea fertilization ratio at the initial flowering stage. During the seedling stage, a cotton plant has a small root system and has a low demand for N, and inorganic N in the soil could meet demand for N. After flowering stage, cotton plant needs more N(Yang etal.,2011), increasing the urea fertilization ratio at the initial flowering stage could better satisfy N demand, to extend the boll-setting period (Yeates *et al.*, 2010), speed up the biomass of boll accumulation (Ma *et al.*, 2008) and to obtain higher yield.

Dry matter of cotton plant in 2013 was greater than that in 2014 for the same treatment but seed cotton yield was contrary, which was attributed to higher monthly mean temperature from July to September, and longer sunshine hours from August to September in 2013 than those in 2014 lead to greater vegetative growth of cotton plant and less harvest index in 2013 than that in 2014.

Amount of ^{15}N Absorbed by the Plants: In 2013, the amounts of ^{15}N accumulated in the entire plants at the initial flowering stage in the N1 treatment were significantly greater than the amounts of ^{15}N accumulated in the plants in the N2 and N4 treatments, respectively. However, different results were shown in 2014. The accumulation of ^{15}N in the N4 treatments was significantly greater than the accumulation of ^{15}N in the N1 and N2 treatments (Table 3), due to the earlier urea fertilization time at budding in 2014 (9 June) than in 2013 (28 June).

In 2013, the amounts of ^{15}N accumulation in the entire plants at the harvest stage were significantly affected by the fertilization treatment (Table 4). When compared with the N1 treatment, the accumulation of ^{15}N in the entire plants at the harvest stage in the N2, N3 and

N4 treatments increased by 17.8%, 80.4% and 69.1% on average, respectively. The amounts of ^{15}N accumulated in the plants at harvest in the N3 treatment were significantly greater than the amounts of ^{15}N in N1 and N2. In 2014, the amounts of ^{15}N that accumulated throughout the CCRI 60 plants at the harvest stage in the N4 treatment were the highest. When compared with the N1 treatment, the average amounts of ^{15}N that accumulated in the plants at the harvest stage in treatments N3 and N4 increased by 2.0% and 1.9%, respectively. However, the average amount of ^{15}N accumulation in the N2 treatment decreased by 2.2%. The result showed that one-time urea fertilization at the budding stage and the initial flowering stage could improve ^{15}N absorption by the cotton plants compared with the N application before the sowing and initial flowering stages. Yang *et al.* (2013) suggested that cotton plant uptake fertilizer N intensively during flowering, while uptake soil N in a longer period but intensively during seedling. One-time urea fertilization at the budding stage and the initial flowering stage meet the maximum accumulation period of total N for cotton (Boquet and Breitenbeck 2000) should benefit improvement in fertilizer N uptake.

Ndff and Plant ^{15}N Recovery: The Ndffs in the cotton plants at the initial flowering stage varied from 25.88 to 45.44, except for CCRI 60 in the N4 treatment in 2014. Thus, the N absorbed in the cotton plants at the initial flowering stage was mainly derived from the soil. In 2013, the Ndffs of the cotton plants in the N1 treatment at the initial flowering stage was significantly greater than the Ndffs in the N2 and N4 treatments, respectively. However, in 2014, the amounts of ^{15}N that accumulated in the N4 treatment were significantly greater than the amounts of ^{15}N that accumulated in the N1 and N2 treatments (Table 5). In addition, these results were the same as the ^{15}N accumulation results observed for the entire plants at the initial flowering stage because urea was applied earlier in 2014 (9 June) than in 2013 (28 June).

In 2013, the average Ndffs of the cotton plants in the N2, N3 and N4 treatments at harvest were 8.9, 25.9 and 24.8 percentage points higher, respectively, than the Ndff in the N1 treatment (Table 6). The same trend was observed in 2014, and the Ndffs in the cotton plants in the N2, N3 and N4 treatments at the harvest stage were 3.0, 5.4 and 4.7 percentage points higher than those in the N1 treatment, respectively. These results suggest that increasing the amount of N applied at the initial flowering stage in split N application treatments or applying fertilizer only once at the budding stage or initial flowering stage could improve the absorption of ^{15}N by cotton plants compared with the application of half of the N before sowing and half of the N at the initial flowering stages.

The ^{15}N recovered by the cotton plants during the initial flowering stage was significantly affected by the fertilization treatments (Fig. 2). The averaged cotton ^{15}N recovery at the initial flowering stage in two years in the N1 treatment varied from 22.8% to 27.3%, with an average of 25.4%, and the average recovery of ^{15}N by the cotton in the N2 and N4 treatments ranged from 26.3% to 35.2% and 7.1% to 20.9%, with averages of 30.3% and 12.6%, respectively. These results showed that the recovery of ^{15}N by the cotton plants at the initial flowering stage increased as the base concentration of N increased.

The recoveries of ^{15}N by cotton plants at the harvest stage in the N3 and N4 treatments were significantly higher than the recoveries of ^{15}N in the N1 and N2 treatments in 2013andCCRI60 in 2014, however there were no differences between N2 and N4 treatment in 2014 (Fig. 3). In addition, the average recoveries of ^{15}N by cotton plants in the N2, N3 and N4 treatments during two years were 3.7, 17.7 and 14.6 percentage points higher than the recoveries in the N1 treatment, respectively.

Several studies have analyzed the recovery of ^{15}N by cotton under drip irrigation. However, these studies reported various results, potentially due to differences in soil texture and climatic conditions. In studies conducted by Hou *et al*. (2007, 2009), the recovery of ^{15}N ranged from 23% to 31% in a pot experiment and from 28% to 49% (with an average of 38%) in a field experiment. These results were similar to those reported by Rochester *et al*. (1994). Chua *et al*. (2003) reported that the plant ^{15}N recoveries ranged from 19% to 38% during a 3-year study at three sites in the western United States. In this study, the average recovery of ^{15}N by cotton at the harvest stage ranged from 27.4% to 56.3%, with an average of 45.0%,in different treatments. This result was similar to the results (43.0% to 49.0%) reported by Fritschi *et al*. (2004).

In this study, the averaged recoveries of ^{15}N by the cotton in the N1, N2, N3 and N4 treatments ranged from 27.4% to 42.3%, 32.1% to 46.6%, 52.4% to 56.3% and 47.0% to 54.4%, with averages of 36.2%, 39.4%, 53.8% and 50.7%, respectively. Overall, the results showed that the recovery of ^{15}N by the cotton plants at the harvest stage increased as the ratio of topdressing N at the budding stage or initial flowering stage increased.

Soil ^{15}N Recovery and FNL: The soil ^{15}N recovery and FNLs are summarized in Fig.4. The recovery of ^{15}N in the soil ranged from 15.7% to 20.7% in the different treatments, which was consistent with the ^{15}N recoveries of 12% to 21%in the soil that were reported by Hou *et al*. (2009). These results were similar to the soil ^{15}N recoveries of 12% to 35% reported by others in soils planted with cotton (Allen *et al*., 2004; Freney *et al*., 1993; Karlen *et al*., 1996; Rochester *et al*., 1993, 1994;

Torbert and Reeves, 1994). In 2013, the recoveries of soil ^{15}N recovery in the N3 and N4 treatments were 1.7 and 3.3 percentage points higher than the recoveries of ^{15}N in the soil in the N1 treatment, respectively. In 2014, the differences in the soil ^{15}N recoveries between the N fertilization treatments were not significant. This result was related to the greater biomass and total N accumulation in the cotton plants in 2013 than in 2014.

The FNLs ranged from 25.4% to 52.8% for the different treatments, which was consistent with the loss of 40% to 60% of the applied ^{15}N from the plant–soil system reported by Torbert and Reeves (1994) and Karlen *et al*. (1996). The FNLs in the N3 and N4 treatments were significantly lower than those in the N1 and N2 treatment in 2013 and 2014. When compared with the N1 treatment, the FNLs in the N3 and N4 treatments were 24.1 and 22.4 percentage points lower in 2013 and 13.1 and 10.2 percentage points lower in 2014, respectively. Relative less cotton plant and soil recovery of ^{15}N lead to higher FNLs in the N1 treatment, and the main loss way were perhaps by waterlodging and volatilization, because pots were set above ground under rainproof shelters, soil temperature in pot was similar to air temperature, and water in pot was easy to loss, and cotton plant were watered with an interval of two or three days. These results revealed that urea fertilization once at the budding stage or once at the initial flowering stage could increase the recovery of ^{15}N in the soil and decrease the FNL compared with the application of half of the N before sowing and half during the initial flowering stage.

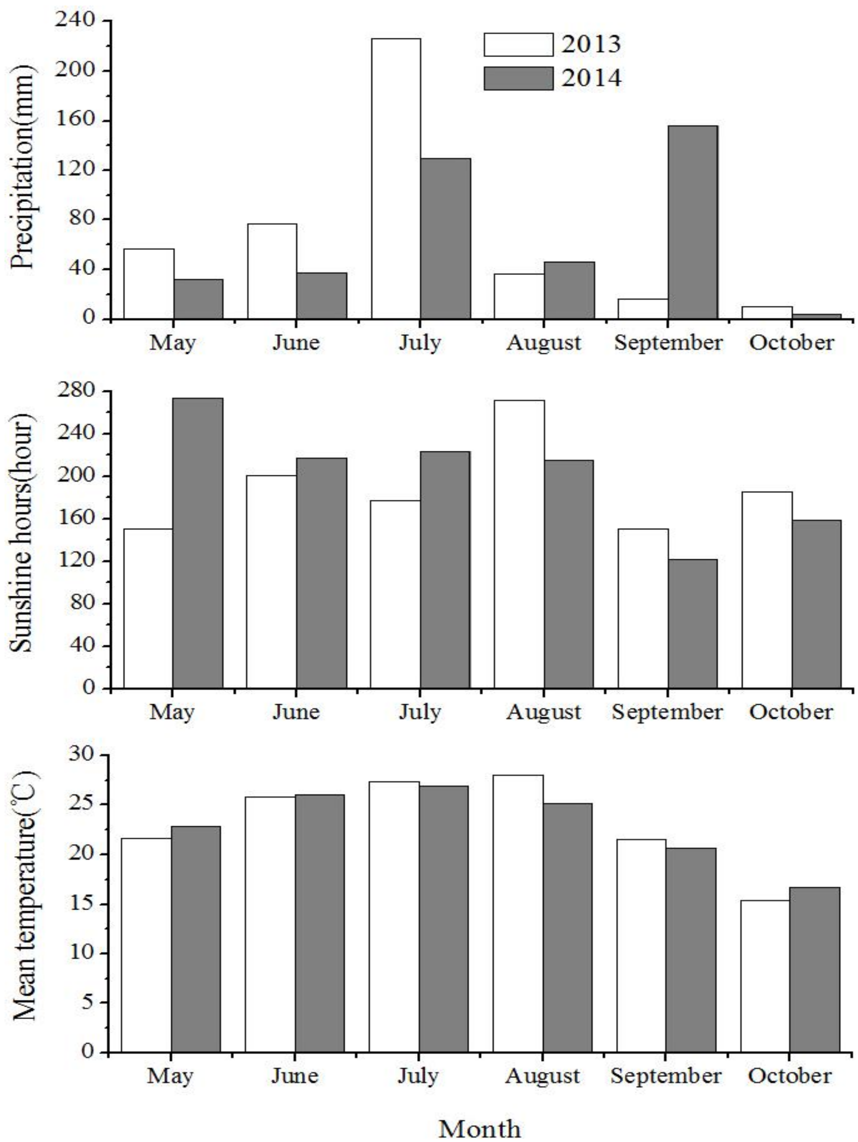

Fig.1 Mean temperature, sunshine hours and precipitation during growing stage in 2013 and 201

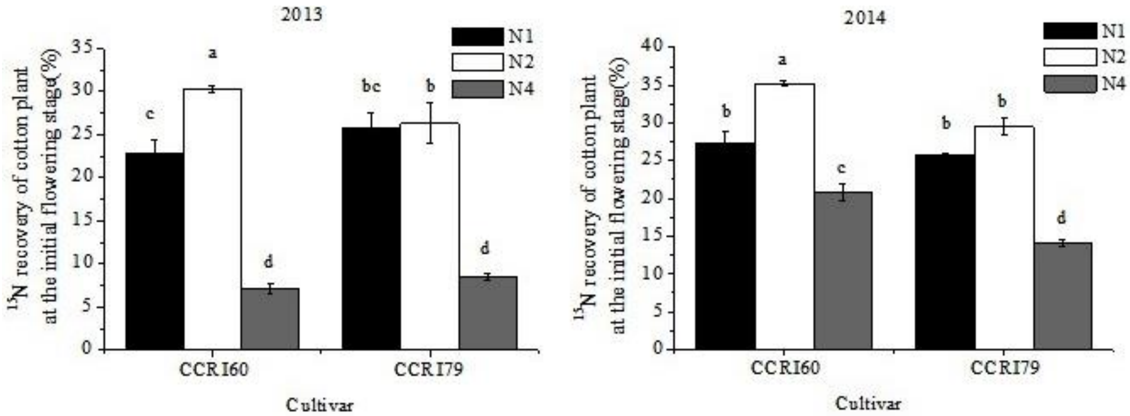

Fig.2 ^{15}N recovery of cotton plant at the initial flowering stage in 2013 and 2014

Note: N1,N2 represent N application rario of base N and topdressing N at the initial flowering stage are 1:1,1:2 respectively. N4 represents N all application at budding stage.Total N application amount for all fertization treatments is 3.5g N per 35 kg soil per pot. Means with same letter are not significantly different at p=0.05 level.

Fig. 2 ^{15}N recovery of cotton plants at the initial flowering stage in 2013 and 2014

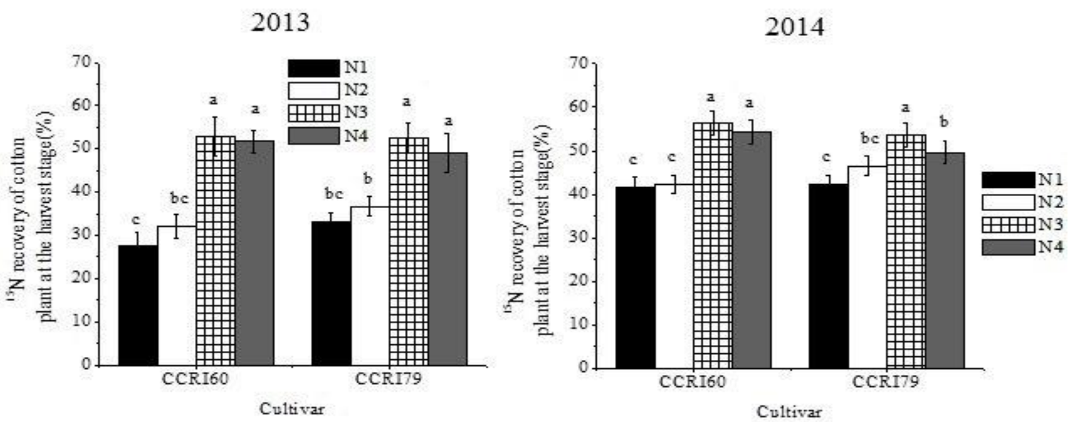

Fig.3 ^{15}N recovery of cotton plant at the harvesr stage in 2013 and 2014

Note: N1,N2 represent N application rario of base N and topdressing N at the initial flowering stage are 1:1,1:2 respectively. N3 and N4 represent N all application at the initial flowering stage and budding stage of cotton.Total N application amount for all fertization treatments is 3.5g N per 35 kg soil per pot. Means with same letter are not significantly different at p=0.05 level.

Fig. 3 ^{15}N recovery of cotton plants at the harvest stage in 2013 and 2014

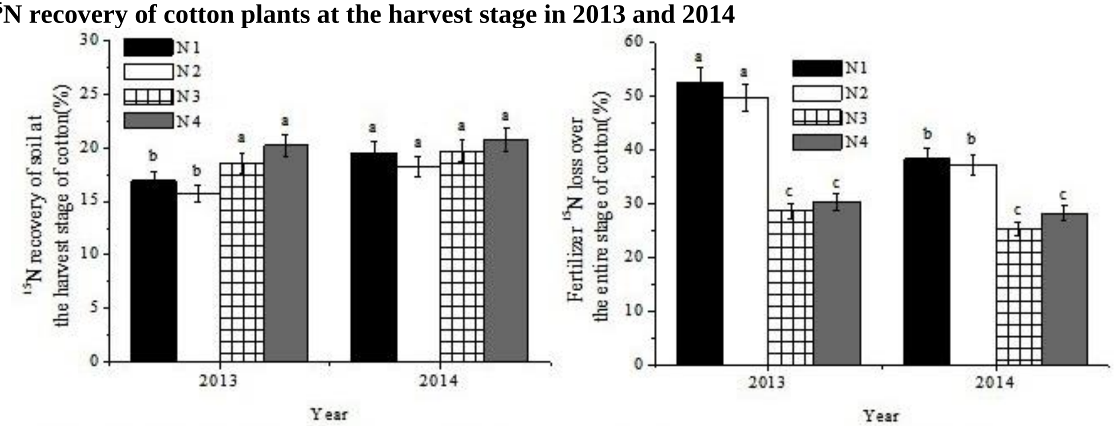

Fig.4 ^{15}N recovery of sil at the harvesr stage of cotton and fertilizer ^{15}N loss over the entire growing stage of cotton in 2013 and 2014

Note: N1,N2 represent N application rario of base N and topdressing N at the initial flowering stage are 1:1,1:2 respectively. N3 and N4 represent N all application at the initial flowering stage and budding stage of cotton.Total N application amount for all fertization treatments is 3.5g N per 35 kg soil per pot. Means with same letter are not significantly different at p=0.05 level.

Fig. 4 ^{15}N recovery of soil at the harvest stage and fertilizer ^{15}N loss over the entire growing stage of cotton in 2013 and 2014.

Table 1 Biomasses of the different organs of the cotton plants at the initial flowering stage(g/plant).

Cultivar	Treatment †	Root		Stem		Leaf		Flower and bud		Whole plant	
		2013	2014	2013	2014	2013	2014	2013	2014	2013	2014
CCRI 79	N1	7.87^{d‡}	9.34^{a}	20.82^{a}	27.09^{b}	23.65^{a}	21.53^{b}	3.13^{d}	4.66^{a}	55.48^{a}	62.61^{a}
	N2	7.64^{d}	9.58^{a}	16.86^{b}	27.18^{b}	19.67^{c}	22.17^{b}	3.60^{c}	2.07^{c}	47.76^{c}	61.00^{a}
	N3	7.85^{d}	7.52^{d}	16.74^{b}	19.9^{d}	19.79^{c}	17.12^{e}	2.75^{e}	2.23^{b}	47.13^{c}	46.77^{d}
	N4	8.36^{c}	7.30^{d}	20.28^{a}	18.54^{d}	21.65^{b}	16.30^{e}	3.82^{ab}	1.62^{d}	54.11^{a}	43.77^{e}
CCRI 60	N1	7.87^{d}	8.21^{c}	15.63^{c}	25.80^{c}	19.79^{c}	20.35^{c}	3.66^{bc}	2.27^{b}	46.94^{c}	56.62^{b}
	N2	8.63^{bc}	8.80^{b}	16.67^{b}	29.16^{a}	19.93^{c}	23.35^{a}	3.65^{bc}	1.52^{e}	48.87^{bc}	63.43^{a}
	N3	8.98b	9.37^{a}	17.19^{b}	25.77^{c}	20.70^{bc}	20.65^{c}	3.63^{c}	1.29^{f}	50.50^{b}	57.07^{b}
	N4	9.44a	8.32^{c}	17.19^{b}	22.16^{d}	19.69^{c}	18.61^{d}	3.94^{a}	1.65^{d}	50.27^{b}	50.74^{c}

† N1 and N2 represent the split application of urea as base fertilizer and top-dressed fertilizer at the initial flowering stage at the ratios of 1:1 and 1:2, respectively. N3 and N4 represent urea all topdressing at the initial flowering stage and the budding stage, respectively. The total amount of N applied in all fertilization treatments was 3.5 g N per 35 kg soil per pot.
‡ Different lowercase letters denote the differences between fertilization treatments at 5% level.

Table 2. Biomasses of the different organs of the cotton plants at the harvest stage(g/plant).

Cultivar	Treatment†	Root		Stem		Leaf		Boll shell		Seed cotton		Whole plant	
		2013	2014	2013	2014	2013	2014	2013	2014	2013	2014	2013	2014
CCRI 79	N1	17.33^{bc‡}	15.31^{a}	64.61^{a}	48.57^{b}	48.09^{a}	24.91^{b}	36.16^{cd}	32.46^{b}	73.66^{c}	91.33^{b}	239.85^{a}	212.59^{AAW}
	N2	17.16^{c}	10.89^{f}	51.53^{b}	39.48^{e}	45.63^{b}	15.53^{e}	40.77^{b}	33.53^{ab}	77.74^{b}	96.25^{a}	232.83^{a}	195.68^{b}
	N3	20.31^{a}	11.51^{e}	64.75^{a}	40.34^{de}	44.99^{b}	21.17^{c}	43.53^{a}	30.24^{c}	64.20^{e}	97.45^{a}	237.78^{a}	200.71^{b}
	N4	16.52^{c}	13.06^{d}	52.93^{b}	41.96^{d}	38.67^{d}	16.92^{d}	36.77^{c}	30.08^{c}	68.85^{d}	81.81^{c}	213.74^{b}	183.82^{c}
CCRI 60	N1	19.51^{a}	14.15^{b}	48.28^{c}	52.54^{a}	32.84^{e}	25.25^{b}	41.^{52ab}	34.43^{a}	79.^{30ab}	83.29^{c}	221.45^{b}	209.66^{ab}
	N2	18.13^{b}	13.24^{cd}	52.58^{b}	45.93^{c}	36.90^{d}	21.43^{c}	40.66^{b}	33.64^{ab}	82.75^{a}	91.27^{b}	231.^{01ab}	205.52^{ab}
	N3	16.69^{c}	14.24^{b}	43.59^{d}	47.34^{b}	41.07^{c}	27.00^{a}	34.77^{d}	34.41^{a}	79.74^{ab}	77.33^{d}	215.85^{b}	200.32^{b}
	N4	16.11^{a}	13.86^{bc}	39.50^{e}	47.12^{b}	41.18^{c}	24.04^{b}	29.67^{e}	29.65^{c}	55.90^{f}	87.95^{b}	182.35^{c}	202.62^{ab}

† N1 and N2 represent the split application of urea as base fertilizer and top-dressed fertilizer at the initial flowering stage at the ratios of 1:1 and 1:2, respectively. The N3 and N4 represent urea all topdressing at the initial flowering stage and the budding stage, respectively. The total amount of N applied in all fertilization treatments was 3.5 g N per 35 kg soil per pot.
‡ Different lowercase letters denote the differences between fertilization treatments at 5% level.

Table 3 Amounts of ^{15}N accumulation in the different organs of cotton at the initial flowering stage(mg/plant)

Cultivar	Treatment †	Root		Stem		Leaf		Flower and bud		Whole plant	
		2013	2014	2013	2014	2013	2014	2013	2014	2013	2014
CCRI 79	N1	32.12^{a‡}	33.13^{d}	111.38^{a}	152.23^{d}	258.40^{a}	221.53^{c}	49.73^{b}	44.94^{a}	451.63^{a}	451.83^{d}
	N2	22.30^{e}	29.12^{e}	68.74^{e}	110.40^{e}	159.11^{d}	187.08^{e}	40.88^{d}	17.57^{d}	291.02^{d}	344.17^{e}
	N4	25.55^{d}	45.91^{b}	73.73^{d}	177.10^{b}	150.38^{e}	243.12^{b}	47.78^{b}	26.52^{c}	297.44^{d}	492.65^{c}
CCRI 60	N1	28.01^{c}	38.83^{c}	94.37^{b}	169.30^{c}	241.39^{b}	243.57^{b}	53.32^{a}	26.65^{c}	417.08^{b}	478.35^{c}
	N2	26.37^{d}	29.67^{e}	77.99^{c}	285.90^{a}	201.52^{c}	209.72^{d}	44.58^{c}	13.42^{e}	350.46^{c}	538.71^{b}
	N4	30.19^{b}	63.58^{a}	59.10^{f}	158.07^{d}	108.78^{f}	348.57^{a}	48.87^{b}	31.93^{b}	246.94^{e}	602.15^{a}

† N1 and N2 represent the split application of urea as base fertilizer and top-dressed fertilizer at the initial flowering stage at the ratios of 1:1 and 1:2, respectively. N4 represents urea all topdressing at the budding stage, respectively. The total amount of N applied in all fertilization treatments was 3.5 g N per 35 kg soil per pot.

‡ Different lowercase letters denote the differences between fertilization treatments at 5% level.

Table 4. Amounts of ^{15}N accumulation in the different organs of cotton at the harvest stage(mg/plant).

Cultivar	Treatment†	Root		Stem		Leaf		Boll shell		Seed cotton		Whole plant	
		2013	2014	2013	2014	2013	2014	2013	2014	2013	2014	2013	2014
CCRI 79	N1	27.47 ^{h‡}	54.50^{c}	155.92^{e}	211.33^{c}	237.81^{e}	244.17^{c}	122.73 ^{g}	102.93^{f}	540.82^{d}	1216.40^{c}	1084.75^{d}	1829.33^{b}
	N2	37.42 ^{g}	22.63^{f}	188.57^{d}	116.43^{f}	301.04^{d}	165.13^{e}	275.64^{d}	128.37^{e}	482.54^{e}	1296.57^{b}	1285.22^{c}	1729.13^{c}
	N3	83.98^{b}	26.13^{e}	338.39^{a}	128.93^{e}	465.51^{a}	161.83^{e}	423.50^{a}	132.63^{e}	529.66^{d}	1428.93^{a}	1841.05^{a}	1878.47^{ab}
	N4	63.98^{d}	51.23^{c}	265.30^{c}	212.07^{c}	410.24^{b}	169.37^{e}	331.69^{b}	152.97^{d}	574.98^{c}	1152.30^{de}	1646.19^{b}	1737.93^{c}
CCRI 60	N1	45.01^{f}	47.73^{d}	161.17^{e}	224.50^{b}	186.92^{f}	239.60^{c}	302.16^{c}	166.27^{c}	264.94 ^{g}	1132.30e	960.20^{e}	1810.40^{bc}
	N2	53.45^{e}	49.83^{cd}	190.02^{d}	196.43^{d}	284.30^{d}	203.73^{d}	245.80^{e}	187.50^{b}	351.08^{f}	1191.60^{cd}	1124.64^{d}	1829.10^{b}
	N3	93.45^{a}	59.23^{b}	345.34^{a}	225.07^{b}	327.82^{c}	283.43^{a}	207.34^{f}	272.20^{a}	874.09^{a}	995.13^{f}	1848.05^{a}	1835.07^{b}
	N4	73.05^{c}	63.07^{a}	291.50^{b}	273.40^{a}	458.49^{a}	258.30^{b}	263.48^{d}	130.93^{e}	725.73^{b}	1244.63^{bc}	1812.26^{a}	1970.33^{a}

† N1 and N2 represent the split application of urea as base fertilizer and top-dressed fertilizer at the initial flowering stage at the ratios of 1:1 and 1:2, respectively. N3 and N4 represent urea all topdressing at the initial flowering stage and the budding stage, respectively. The total amount of N applied in all fertilization treatments was 3.5 g N per 35 kg soil per pot.

‡ Different lowercase letters denote the differences between fertilization treatments at 5% level.

Table 5 Ndffs of the different organs of cotton at the initial flowering stage.

Cultivar	Treatment †	Root		Stem		Leaf		Flower and bud		Whole plant	
		2013	2014	2013	2014	2013	2014	2013	2014	2013	2014
CCRI 79	N1	40.76^{a‡}	30.70^{d}	43.19^{a}	30.82^{d}	42.36^{a}	31.05^{d}	42.37^{a}	30.59^{d}	42.45^{a}	30.92^{d}
	N2	33.45^{b}	25.^{69f}	34.41^{b}	24.97^{f}	33.87^{b}	24.83^{f}	33.02^{d}	24.82^{e}	33.84^{b}	24.94^{f}
	N4	29.78^{c}	40.16^{b}	29.25^{c}	45.68^{b}	23.97^{c}	46.08^{b}	38.03^{b}	48.04^{b}	27.27^{c}	45.44^{b}
CCRI 60	N1	39.45^{a}	35.59^{c}	41.47^{a}	35.07^{c}	41.42^{a}	34.80^{c}	40.53^{a}	34.51^{c}	41.18^{a}	34.93^{c}
	N2	33.43^{b}	27.25^{e}	33.23^{b}	26.69^{e}	33.18^{b}	26.63^{e}	33.31^{d}	25.84^{e}	33.22^{b}	26.68^{e}
	N4	29.39^{c}	49.21^{a}	27.43^{d}	55.42^{a}	21.80^{d}	54.79^{a}	35.70^{c}	56.41^{a}	25.88^{d}	54.53^{a}

† N1 and N2 represent the split application of urea as base fertilizer and top-dressed fertilizer at the initial flowering stage at the ratios of 1:1 and 1:2, respectively. N4 represents urea all topdressing at the budding stage. The total amount of N applied in all fertilization treatments was 3.5 g N per 35 kg soil per pot.

‡ Different lowercase letters denote the differences between fertilization treatments at 5% level.

Table 6. Ndffs of the different organs in cotton at the harvest stage in 2013 and 2014.

Cultivar	Treatment†	Root		Stem		Leaf		Boll shell		Seed cotton		Whole plant	
		2013	2014	2013	2014	2013	2014	2013	2014	2013	2014	2013	2014
CCRI 79	N1	23.59^{f‡}	43.70^{c}	24.54^{e}	43.07^{d}	22.64^{h}	42.80^{cd}	28.66^{d}	46.20^{d}	30.75^{d}	50.80^{b}	26.92^{c}	24.40^{e}
	N2	30.87^{d}	41.33^{d}	33.75^{c}	33.50^{e}	33.89^{f}	41.10^{d}	37.05^{c}	46.40^{d}	38.32^{c}	50.60^{b}	36.06^{b}	28.30^{d}
	N3	48.35^{a}	39.10^{e}	52.82^{a}	41.03^{d}	55.34^{a}	42.13^{d}	54.74^{ab}	49.30^{c}	55.20^{b}	56.03^{a}	54.14^{a}	29.30^{d}
	N4	45.73^{b}	45.47^{bc}	50.17^{b}	47.90^{ab}	50.34^{b}	45.77^{ab}	52.63^{b}	49.93^{bc}	55.25^{b}	51.30^{b}	52.13^{a}	31.53^{bc}
CCRI 60	N1	25.61^{e}	45.57^{bc}	27.54^{d}	45.80^{bc}	29.75^{g}	43.10^{bcd}	28.32^{d}	49.40^{c}	27.67^{e}	51.43^{b}	28.07^{c}	30.70^{cd}
	N2	33.59^{c}	44.83^{bc}	35.00^{c}	45.50^{c}	35.80^{e}	44.67^{bc}	35.89^{c}	50.13^{b}	40.09^{c}	50.17^{b}	36.63^{b}	32.80^{b}
	N3	49.31^{a}	46.03^{ab}	47.83^{b}	46.03^{bc}	41.74^{d}	47.10^{a}	55.89^{a}	52.93^{a}	59.87^{a}	50.93^{b}	52.73^{a}	36.57^{a}
	N4	50.11^{a}	48.13^{a}	50.20^{b}	50.03^{a}	45.03^{c}	47.63^{a}	56.67^{a}	52.07^{ab}	58.63^{a}	52.37^{b}	52.60^{a}	32.87^{b}

† N1 and N2 represent the split application of urea as base fertilizer and top-dressed fertilizer at the initial flowering stage at the ratios of 1:1 and 1:2, respectively. N3 and N4 represent urea all topdressing at the initial flowering stage and the budding stage, respectively. The total amount of N applied in all fertilization treatments was 3.5 g N per 35 kg soil per pot.

‡ Different lowercase letters denote the differences between fertilization treatments at 5% level.

Conclusions: Pot experiments were conducted to study the effects of the timing and ratio of urea fertilization by using the same total amount of N and determining the ^{15}N absorption, biomass accumulation, plant and soil ^{15}N recovery, FNL and seed cotton yield. When compared with conventional N application method (the application of half of the N before sowing and half during the initial flowering stage),applying fertilizer only once at the budding stage or initial flowering stage could increase amounts of ^{15}N accumulation, Ndff, ^{15}N recovery of cotton plant and decrease FNL, and obtain comparable seed cotton yield, while increasing the amount of N applied at the initial flowering stage and decreasing the base N ratio in split N application treatments could increase amounts of ^{15}N accumulation, Ndff and achieve higher cotton yield without increasing FNL.

Increasing the N ratio at initial flowering stage of cotton and decreasing the base N ratio could better satisfy the demand for less N supply for the vegetative growth at seedling stage and enough N supply for the reproductivegrowth at flowering and boll-setting stage, and it would be a better N management measure for cotton producerto improve cotton yield and decrease fertilizer N loss.

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