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#### RESEARCH ARTICLE

# Site-specific fertilizer nitrogen management in Bt cotton using chlorophyll meter

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#### **Abstract**

Field experiments were conducted to standardize protocols for site-specific fertilizer nitrogen (N) management in Bt cotton using Soil Plant Analysis Development (SPAD) chlorophyll meter. Performance of different SPAD-based site-specific N management scenarios was evaluated vis-à-vis blanket fertilizer N recommendation. The N treatments comprised a no-N (control), four fixed-time and fixed N doses (60, 90, 120, and 150 kg N ha<sup>-1</sup>) including the recommended dose (150 kg ha<sup>-1</sup>), and eight fixed-time and adjustable N doses based on critical SPAD readings of 45 and 41 at first flowering and boll formation stages, respectively. The results revealed that by applying 45 or 60 kg N ha<sup>-1</sup> at thinning stage of the crop and critical SPAD valueguided dose of 45 or 30 kg N ha<sup>-1</sup> at first flowering stage resulted in yields similar to that recorded by applying the recommended dose of 150 kg N ha<sup>-1</sup>. However, significantly higher N use efficiency as well as 30-40% less total fertilizer N use was recorded with site-specific N management. Applying 30 kg N ha<sup>-1</sup> at thinning and SPAD meter-guided 45 kg N ha<sup>-1</sup> at first flowering were not enough and required additional SPAD meter-guided 45 kg N ha<sup>-1</sup> at boll formation for sustaining yield levels equivalent to those observed by following blanket recommendation but resulted in 20% less fertilizer N application. Our data revealed that SPAD meter-based site-specific N management in Bt cotton results in optimum yield with dynamic adjustment of fertilizer N doses at first flowering and boll formation stages. The total amount of N fertilizer following site-specific management strategies was substantially less than the blanket recommendation of 150 kg N ha<sup>-1</sup>, but the extent may vary in different fields.

Keywords: Bt cotton; Fertilizer nitrogen; Site-specific nitrogen management; SPAD meter

## Introduction

Cotton shows significant response to nitrogen (N) application (Patil *et al.*, 2013) as soils in the Indo-Gangetic plains in South Asia are inherently low in soil organic carbon. For several crops in South Asia, fertilizer N is generally supplied following blanket recommendations. These are formulated on the basis of crop response data averaged over large geographic areas having similar environmental conditions. Thus, blanket fertilizer recommendations do not take into account the spatial variability in indigenous N supplying capacity of soils among fields. As blanket fertilizer recommendations are designed to produce optimum yields in all the fields in the region, these may lead farmers to apply more N than the required amount. In the Indian state of Punjab, the recommendation for Bt cotton is to apply 150 kg N ha<sup>-1</sup> (50% at thinning stage and 50% at first flowering stage). As pre-set blanket recommendations do not account for large farm-to-farm variability in soil N supply, these generally lead to low N use efficiency in many fields. Also, farmers in the Indo-Gangetic plains apply more N than the blanket recommendation dose to avoid the risk of N deficiency, which leads to N use efficiency even lower than that observed by following blanket N recommendation (Varinderpal-Singh *et al.*, 2007). Excess fertilizer application leads to

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rank growth and delayed maturity, besides possible N loss from the soil–plant system *via* leaching, denitrification, and ammonia volatilization thereby posing threat to the environment. MacDonald *et al.* (2017) reported that losses of N in cotton production systems are significant to pose threats to the environment and can range from 12 to 65% of the applied N (Rochester, 2001).

Improved synchronization between N demand of the crop and the supply of N from all sources including fertilizers throughout the crop growing season can help to improve N use efficiency and optimize N supply (Cassman *et al.*, 2002, 2003). However, it can be achieved only on a site-specific or a field-specific basis. This approach explicitly recognizes the need to efficiently utilize indigenous N in the soil as well as spatial and temporal variability in crop responsiveness to N. In contrast to blanket fertilizer recommendations, site-specific N management strives to optimally account for the supply of soil N over time and space to match the crop requirements by applying the right amount of N at the right time during the crop growth season. Information in this regard is now available for cereals like rice, wheat, and maize (Bijay-Singh and Singh, 2017; Diacono *et al.*, 2013; Varinderpal-Singh *et al.*, 2010). Gadgets like chlorophyll meters, handheld optical sensors, or simple and inexpensive leaf color charts have been standardized to apply N as per need of different crops already growing in the field.

Quick and non-destructive quantification of leaf greenness as an indicator of N status using handheld Soil Plant Analysis Development (SPAD) chlorophyll meter (or simply the SPAD meter) has attracted the attention of several researchers to manage N fertilization in cotton on a site-specific basis. Wood *et al.* (1992) reported that SPAD readings on the topmost fully expanded leaf were significantly correlated with leaf-blade N concentration at the first-square, first-bloom, and mid-bloom stages of cotton. Recently, Brandão *et al.* (2015) found that SPAD value of fifth fully expanded leaf from the top was highly correlated with lint yield. While Wu *et al.* (1998) showed that there were highly significant linear relationships between SPAD values and contents of both N and chlorophyll at different growth stages of cotton, Bronson *et al.* (2003) demonstrated that indices based on SPAD readings could successfully predict N needs of irrigated cotton. Reference leaf and its threshold SPAD values for nitrogen management using chlorophyll meter and leaf color chart in Bt cotton have already been established by Arun Shankar *et al.* (2019). Herein, our aim is to develop protocols for fixed-time and adjustable-dose N management in Bt cotton using predetermined critical SPAD values at first flowering and boll formation stages of the crop.

## Material and Methods

# **Experimental site**

Field experiments were conducted during 2016 and 2017 at the research farm of the Department of Soil Science, Punjab Agricultural University, Ludhiana (30°56′N, 75°32′E, and 247 m a.s.l.). The field was under mono-cropping of wheat before start of cotton–wheat rotation in 2015. The soil was sandy loam in texture (76% sand, 13% silt, and 11% clay) (International pipette method as described by Day, 1965). The pH (1:2 soil to water ratio) was 7.3 and the electrical conductivity (1:2 soil to water ratio) was 0.30 dS m<sup>-1</sup>, which were determined using a pH meter (FE20 Five Easy; Mettler Toledo GmbH, Schwerzenbach, Switzerland) and a conductivity meter (FE30 Five Easy; Mettler Toledo GmbH), respectively (Jackson, 1973). The soil under study contained 0.31% organic carbon (Walkley, 1947), 12.0 kg P ha<sup>-1</sup> (Olsen *et al.*, 1954) and 215 kg K ha<sup>-1</sup> (ammonium acetate extractable using flame photometer, following Jackson (1973).

## Treatment description

The N treatments comprised a no-N (control), four fixed N doses of 60, 90, 120, and 150 kg N ha<sup>-1</sup> applied in two equal split doses at thinning and at first flowering stages, and eight fixed-time

Treatment no.	Fe	rtilizer nitrogen appl	lied (kg ha <sup>-1</sup> )		PAD values at flowering stage	Mean SPAD values at the boll formation stage	
	At thinning	At first flowering stage	At boll formation stage	2016	2017	2016	2017
T1	0	0	0	38.1	37.9	35.6	35.2
T2	30	30	0	42.2	42.8	38.1	38.3
T3	45	45	0	44.0	44.7	41.3	41.1
T4	60	60	0	45.2	45.2	41.6	41.5
T5	75	75	0	46.0	46.1	42.0	42.2
T6	30	$Ad_1$	0	42.5	42.1	39.6	39.9
T7	30	$\operatorname{Ad}_1$	$Ad_2$	42.3	42.2	39.7	39.5
T8	45	$Ad_1$	0	43.8	43.4	41.6	41.4
T9	45	$\operatorname{Ad}_1$	$Ad_2$	43.6	43.9	41.3	41.2
T10	60	$Ad_1$	Õ	45.3	45.2	41.5	41.4
T11	60	$Ad_1$	$Ad_2$	45.4	45.5	41.8	41.6
T12	75	$\operatorname{Ad}_1$	0	45.7	46.4	42.0	41.8
T13	75	$Ad_1$	$Ad_2$	45.9	46.0	42.3	41.8
LSD (0.05)		_	_	2.71	3.00	2.91	3.12

**Table 1.** Treatment description and mean SPAD values recorded at the first flowering and boll formation stages. The adjustable N doses were based on SPAD values at the first flowering (Ad1\*) and at the boll formation (Ad2<sup>†</sup>)

adjustable N doses. The blanket N recommendation is 150 kg N ha<sup>-1</sup> in this region (Bhatti, 2018). The fixed-time adjustable-dose treatments consisted of applying 30, 45, or 60 kg N ha<sup>-1</sup> depending on SPAD value >45, 41–45, and less than 41 at first flowering stage (Ad<sub>1</sub>) and 30, 45, or 60 kg N ha<sup>-1</sup> depending on SPAD value >41, 37–41, and <37 at boll formation stage (Ad<sub>2</sub>), respectively. While Ad<sub>1</sub> was applied in all the eight fixed-time adjustable-dose treatments, Ad<sub>2</sub> was applied only in treatments T7, T9, T11, and T13 (Table 1). The critical SPAD values of the fourth fully expanded leaf from the top of main stem for the first flowering (45) and boll formation (41) were determined from an early experiment conducted in 2015 with three Bt cotton varieties in which the yield predictability of SPAD was found to be homoscedastic with respect to varieties (Arun Shankar *et al.*, 2019)

### Soil and crop management

The field was plowed, leveled, and given a pre-sowing irrigation (100 mm). When the soil moisture content reached the field capacity (23%), the field was plowed and leveled again and divided into 39 plots of  $8.25 \times 5.40$  m. The experiment was laid out in a randomized block design with 13 fertilizer treatments (Table 1) arranged in three replications. Seeds of Bt cotton (*Gossypium hirsutum*) cv. RCH 650 were planted on 3 May 2016 and 5 May 2017, at a spacing of 75  $\times$  67.5 cm by dibbling two seeds per hill. After 40 days, the plants were thinned to one plant per hill. Basal fertilizer dose consisting of 13 kg P ha<sup>-1</sup> and 25 kg K ha<sup>-1</sup> was applied as single super phosphate and muriate of potash, respectively. Nitrogen was applied as per treatments as urea. To supplement the rainfall, three flood irrigations (75 mm depth each) were applied in each year. Weeds, pests, and diseases were controlled when required, following standard procedures.

## SPAD measurement, plant sampling, and analysis

Handheld Minolta SPAD-502 was used for SPAD readings of 10 uniform plants per plot, taking into account the fourth fully expanded leaf from the top of the main stem at the first flowering (about 55 days after sowing) and at the boll formation (about 85 days after sowing) stages. Leaves used for SPAD reading were sampled for determination of N concentration. Seed cotton was

LSD: least significant difference.

<sup>\*</sup>Ad<sub>1</sub>: Adjustable dose of 30, 45, or 60 kg N ha<sup>-1</sup> depending on SPAD value >45, 41–45, and less than 41, respectively.

<sup>†</sup>Ad<sub>2</sub>: Adjustable dose of 30, 45, or 60 kg N ha<sup>-1</sup>depending on SPAD value >41, 37-41, and <37, respectively.

harvested by manual pickings in October–November each year and yields were obtained on plot basis. Five uniform stovers per plot left after harvest were oven dried at 65°C and weighed to evaluate stover dry weight. Nitrogen concentration of finely ground oven dried stovers, leaves, and seeds was determined by digesting the samples in H<sub>2</sub>SO<sub>4</sub>, followed by analysis for total N by a micro-Kjeldahl method (Yoshida *et al.*, 1976). Nitrogen concentration in stovers and seed cotton was used to estimate total N uptake at maturity.

#### Statistical analysis and calculations

Analysis of variance for yield and N use efficiency was performed using CPCS I software (Punjab Agricultural University, Ludhiana, India), considering a randomized block design. Regression analysis was carried out using Microsoft Excel. Point of maxima can be obtained by differentiating polynomial equations, following further calculation for theoretical maximum yield. Sustainable yield index (SYI) was estimated using

$$SYI = \frac{(A-Y)}{Y_{\text{max}}} \times 100, \tag{1}$$

where A and Y represent the mean and standard deviation of a particular treatment, respectively, and  $Y_{\text{max}}$  is the maximum potential yield in different years and treatments.

The agronomic efficiency (AEN) and recovery efficiency (REN) of applied N were calculated as described by Baligar *et al.* (2001):

$$AEN(kg grain/kg N applied) = \frac{\text{(Grain yield in N fertilized plot - Grain yield in no N plot)}}{\text{(Quantity of N fertilizer applied in N fertilized plot)}},$$
(2)

REN (%) = 
$$\frac{\text{(Total N uptake in N fertilized plot - Total N uptake in no N plot)}}{\text{(Quantity of N fertilizer applied in N fertilized plot)}} \times 100. (3)$$

#### Results

## SPAD values as affected by fixed-time and adjustable N dose

The cotton plants showed typical N deficiency symptoms (chlorosis of old leaves) at the first flowering when no N was applied and the symptoms worsened at the boll formation. The mean SPAD values of cotton leaves decreased from the first flowering to the boll formation stage (Table 1), irrespective of N application at both the growth stages and in both years. At the first flowering stage, the mean SPAD values over the years ranged from 37.9 to 46.1 in the control treatment (no-N) and the blanket recommended dose treatments, respectively. The corresponding N concentration over the years ranged from 3.67 to 4.50% (Table 2). Among the fixed N doses, SPAD value above the critical level of 45 was maintained in treatments receiving 60 (T4) and 75 kg N  $\mathrm{ha}^{-1}$ (T5) at the thinning stage, respectively. Although the SPAD values in T3 (90 kg N ha<sup>-1</sup> in two equal split doses at thinning and at first flowering stage) were below the critical level of 45 in 2016 and 2017, these were statistically similar to the SPAD values recorded in T4 and T5 treatments (Table 1). Likewise the N concentration at the first flowering stage in T4 and T5 treatments (with critical SPAD above 45) was also high and statistically significant as compared to the leaf N concentrations in plots receiving no nitrogen. Among the adjustable-dose treatments, the SPAD values were maintained above the critical level at the first flowering stage in treatments receiving 60 or 75 kg N ha<sup>-1</sup> at the thinning stage. Similar trend in leaf N concentration was exhibited in adjustable-dose treatments and was on par with the N concentration found in T5 receiving recommended dose of nitrogen (Table 2). Although the SPAD values recorded in the adjustable-dose

Table 2. Effect	of different	fixed-time	fixed-dose	and	fixed-time	adjustable	N dose
treatments on le	af N concen	tration (%)	at the first	flowe	ring and b	oll formatio	n stages
of Bt cotton in 20	016 and 2017	7					

	First flowe	ering stage	Boll formation stage		
Treatment	2016	2017	2016	2017	
T1	3.83	3.67	2.90	2.93	
T2	4.20	4.10	3.30	3.13	
T3	4.43	4.33	3.40	3.63	
T4	4.37	4.50	3.70	3.73	
T5	4.50	4.40	3.77	3.83	
T6	4.17	4.03	3.50	3.30	
T7	4.37	4.00	3.47	3.40	
T8	4.13	4.43	3.70	3.47	
T9	4.17	4.40	3.63	3.57	
T10	4.40	4.53	3.67	3.67	
T11	4.40	4.57	3.67	3.70	
T12	4.53	4.47	3.77	3.77	
T13	4.40	4.57	3.67	3.80	
LSD (0.05)	0.317	0.299	0.347	0.424	

LSD: least significant difference.

Table 3. Effect of different fixed-time fixed-dose and fixed-time adjustable-dose fertilizer N treatments on the yield of seed cotton, sustainable yield index, and pooled (2016/2017) AEN and REN of fertilizer N in Bt cotton fields in 2016 and 2017

	Tot	al N	N applied (kg ha-1)						Seed cotton yield (t ha <sup>-1</sup> )			Pooled AEN (kg grain/kg nitrogen	Pooled REN
applie (kg ha-		lied	Thinning		First flowering		Boll formation				Sustainable yield index		
no.	2016	2017	2016	2017	2016	2017	2016	2017	2016	2017	(%)	applied)	(%)
T1	0	0	0	0	0	0	0	0	1.28	1.21	41		
T2	60	60	30	30	30	30	0	0	2.06	2.01	74	13.1	62
T3	90	90	45	45	45	45	0	0	2.30	2.34	87	11.4	59
T4	120	120	60	60	60	60	0	0	2.37	2.41	91	9.1	48
T5	150	150	75	75	75	75	0	0	2.46	2.45	95	7.9	49
T6	75	75	30	30	45	45	0	0	2.13	2.10	77	11.4	56
T7	120	120	30	30	45	45	45	45	2.38	2.39	91	9.2	49
T8	90	90	45	45	45	45	0	0	2.32	2.37	90	11.6	61
T9	120	120	45	45	45	45	30	30	2.43	2.39	91	9.6	51
T10	90	90	60	60	30	30	0	0	2.40	2.36	88	12.4	61
T11	120	120	60	60	30	30	30	30	2.45	2.40	91	9.8	52
T12	105	105	75	75	30	30	0	0	2.38	2.38	87	10.5	55
T13	135	135	75	75	30	30	30	30	2.44	2.42	93	8.6	48
LSD (0.05)									0.22	0.20		1.98	10.9

LSD: least significant difference.

treatments receiving 30 or 45 kg N ha $^{-1}$  were similar to the values recorded in the treatments receiving 60 or 75 kg N ha $^{-1}$  at the thinning stage, application of in-season N dose at the first flowering stage ensured good crop growth and also the maintenance of SPAD value above 41 at the boll formation stage in treatments T8-T13 in both years. The fixed-dose treatments T3, T4, and the blanket recommended dose treatment (T5) also maintained SPAD value above the critical level at the boll formation stage.

# Crop response to fixed-time and adjustable N dose

The N deficient plots exhibited 45–50% reduction in seed cotton yield and 50% reduction in SYI compared to the blanket recommended dose of 150 kg N ha<sup>-1</sup> (Table 3). Although the recommended dose

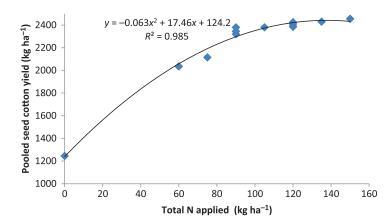


Figure 1. Pooled (2016/2017) seed cotton yield as function of total fertilizer N application.

is 150 kg N ha<sup>-1</sup> for Bt cotton in Punjab, significant crop response to N was observed only up to 90 kg ha<sup>-1</sup> (Table 3) in both years. Similar results were found by Arun Shankar *et al.* (2019).

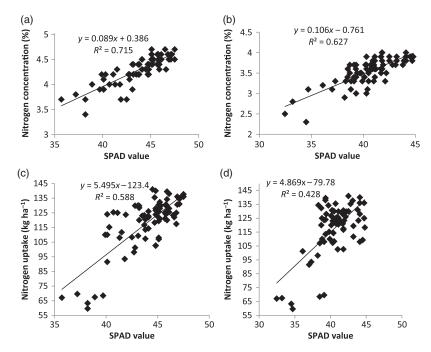
Application of critical SPAD value-guided 45 kg N ha<sup>-1</sup> at the boll formation stage (T7) significantly improved the yield as compared to T6, in which N was applied only up to the first flowering stage. The yield found in T7 was at par with the yield in the blanket recommendation treatment (T5) receiving 150 kg N ha<sup>-1</sup> but with less fertilizer. Applying 45 kg N ha<sup>-1</sup> at the thinning and SPAD meter-guided 45 kg N ha<sup>-1</sup> at the first flowering stage (T8) also recorded yield levels similar to those recorded by the blanket recommended dose, thereby saving 60 kg N ha<sup>-1</sup>. Similar results were obtained by applying 90 kg N ha<sup>-1</sup> (T3) in two split doses, thereby strengthening the above results. However, additional application of N fertilizer at the boll formation stage (as in treatment T9) did not significantly improve the yield as compared to T8 in both years. Applying 60 and 75 kg N ha<sup>-1</sup> at the thinning stage and application of SPAD meterguided dose of 30 kg N ha<sup>-1</sup> at the first flowering (T10 and T12) also resulted in yields comparable with those observed with the blanket recommended dose but saving 60 and 45 kg N ha<sup>-1</sup>, respectively.

The fixed-time and SPAD meter-guided adjustable-dose fertilizer management in Bt cotton is site-specific and based on N demand of the crop. However, application of adjustable dose at the boll formation in T11 and T13 did not result in significant increase in yield compared to T10 and T12, respectively. Nevertheless, fertilizer N management in T13 also produced cotton seed yield equivalent to that recorded with application of the blanket recommended dose of 150 kg N ha<sup>-1</sup> but with 10% less fertilizer. Mean seed cotton yield was highly correlated with total N applied and was best described by a quadratic function (Figure 1).

Pooled AEN of the three best treatments T8, T10 (receiving 90 kg N ha<sup>-1</sup>), and T12 (receiving 105 kg N ha<sup>-1</sup>) was 11.6-, 12.4-, and 10.5-kg seed cotton yield per kg N applied, while T5 (receiving 150 kg N ha<sup>-1</sup>) yielded 7.9-kg seed cotton per kg N applied. T8 and T10 recorded significantly higher REN than the treatment receiving recommended dose of nitrogen. In addition, the SYI was highest in T8 and T10.

#### Relationship between SPAD values and N concentration

SPAD values were highly and linearly correlated with leaf N concentration at the first flowering and at the boll formation stages (Figure 2a, b). Leaf nitrogen concentration ranged between 3.67 and 4.57% at the first flowering stage and between 2.9 and 3.83% at the boll formation stage (Table 2). SPAD values recorded at both phenological stages were positively correlated to N uptake (Figure 2c, d).



**Figure 2.** Relationship between SPAD value and leaf N concentration (a, b) and total N uptake (c, d) at the first flowering stage (a, c) and at the boll formation stage (b, d) of Bt cotton grown under varying N management.

# Discussion

Higher nutrient use efficiency by crops can decrease fertilizer inputs and nutrient losses, while increasing crop yield (Baligar *et al.*, 2001). Significantly lower AEN when plants received the recommended dose of N (T5) and in plants receiving 135 kg N ha<sup>-1</sup> (T13) was associated with nitrogen partitioning to vegetative parts of cotton and rank growth as compared to plants of treatments T8 (45 kg N ha<sup>-1</sup> at thinning stage and SPAD meter-guided 45 kg N ha<sup>-1</sup> at first flowering stage) and T10 (60 kg N ha<sup>-1</sup> at thinning stage and SPAD meter-guided dose of 30 kg N ha<sup>-1</sup> at first flowering stage) (Table 3). This suggests that N application at the boll formation can be avoided if more than 60 kg N ha<sup>-1</sup> is applied at the thinning stage. As treatments T8 and T10 showed higher N use efficiency and yield sustainability as compared to the recommended dose treatment (T5), these can be regarded as the best fixed-time adjustable-dose N treatments.

Critical SPAD readings for the Bt cotton leaves were used to apply fertilizer as per need of the crop. Fertilizer application based on SPAD readings at the first flowering and the boll formation stages cannot only lead to optimum yields but also avoid application of excessive fertilizer. In fact, site-specific N management resulted in higher N use efficiency than that recorded by applying the blanket recommended dose of 150 kg N ha<sup>-1</sup> (Table 3).

Decline in SPAD values from the first flowering to the boll formation stages (Table 1) can be attributed to the nitrogen partitioning during boll development. Decrease in SPAD value of cotton leaves from early flowering toward mid-flowering was also reported by Wood *et al.* (1992). Zhu and Oosterhuis (1992) observed that most of the N accumulated in the bolls while leaf N content decreased, indicating mobilization of N from leaves toward the developing bolls. As treatments T1, T2, T6, and T7 were not able to maintain SPAD values above the critical level of 41 at the boll formation stage (Table 1), application of N at this stage was expected to improve the seed cotton yield and N use efficiency. Application of fixed-time and SPAD-guided adjustable-dose of N at the

first flowering could ensure optimum N acquisition by leaves with further N remobilization to the developing bolls at the same time, avoiding rank growth.

Critical N concentrations pertaining to pre-established critical SPAD values of 45 and 41 at the first flowering and the boll formation stages as estimated by using Cate and Nelson (1971) method were 4.5 and 3.7%, respectively (Figure 2a, b). Temporal dynamics of leaf N concentrations depends on the phenological stages of cotton crop (Oosterhuis *et al.*, 1983), and Bell *et al.* (2003) reported that the threshold leaf-blade N concentrations associated with seed cotton yield loss were 4.3% at the early flowering and 4.1% at 3 weeks after flowering.

In a 2-year experiment conducted at New Delhi in a soil similar to that in the current study, Gangaiah *et al.* (2013) reported that pooled seed cotton yield of Bt cotton hybrids increased by applying N up to 120 kg N ha<sup>-1</sup>. Nitrogen application beyond the optimum level in cotton may reduce the yield (Wood *et al.*, 1992) due to rank growth and decreases in the harvest index (Bouquet *et al.*, 1994). On the other hand, application of adequate amount of N can promote the cotton growth and development (Schils *et al.* 2006) and increase yield (Liu *et al.* 2013). Zhang *et al.* (2012) reported that application of 120 kg N ha<sup>-1</sup> caused similar lint yield and higher N use efficiency when compared to 300 kg N ha<sup>-1</sup> under high salinity stress. Then, successful N management requires application of adequate amounts of fertilizer at right time so that control of N deficiency and enhancement in N use efficiency can be achieved simultaneously (Baligar *et al.*, 2001). To decide the best N management, comparisons of seed cotton yield and N use efficiency were made between the recommended dose and fixed-time and adjustable N doses. Low AEN and low REN were recorded under the recommended N dose (Table 3), indicating poor synchronization between crop N demand and soil supply in such fertilizer management.

Application of 30 kg N ha<sup>-1</sup> at the thinning stage and SPAD meter-guided 45 kg N ha<sup>-1</sup> at the first flowering stage (T6) produced significantly lower yields in both years, thereby indicating need for application of N at the boll formation stage. A comparison of the mean AEN in treatments T7, T8, T10, and T12 with that observed by applying blanket recommended dose revealed that T8, T10, and T12 were the top three treatments (Table 3). REN further narrowed the options among these best treatments, and T8 and T10 recorded significantly higher REN than that observed with the recommended dose (Table 3). Significantly lower AEN in the recommended dose and T13 receiving a total of 150 and 135 kg N ha<sup>-1</sup>, respectively, depicts higher N partitioning to vegetative parts rather than bolls and rank growth compared to T8 and T10. Then, our data suggest avoidance of N application at the boll formation if more than 60 kg N is applied as prescriptive dose in order to increase N use efficiency. SYI for treatments T8 and T10 was more than 90% relative to the value recorded with the blanket recommended dose.

Due to higher N use efficiencies and yield sustainability, treatments T8 (receiving 45 kg N ha<sup>-1</sup> at thinning stage and SPAD meter-guided 45 kg N ha<sup>-1</sup> at first flowering stage) and T10 (receiving 60 kg N ha<sup>-1</sup> at thinning stage and SPAD meter-guided dose of 30 kg N ha<sup>-1</sup> at first flowering stage) can be regarded as the best fixed-time and adjustable-dose N treatments. Although the critical values used in this study have been found consistent for three varieties through the standard residual plot of regression between yield and SPAD values (Supplementary Material Figure S1), it might be argued that other nutritional deficiencies, phenological and morphological differences among varieties, variations in soil type, and darkness might affect SPAD values. Therefore, further studies are required to investigate significant differences in SPAD values with respect to these factors and fine tune the N nutrition of Bt cotton.

#### Conclusion

The results of this study convincingly show that SPAD meter can effectively guide site-specific N management in Bt cotton at the first flowering and boll formation stages. The fixed-time and SPAD meter-based adjustable N doses caused seed cotton yield similar to that observed with the existing blanket recommendation but with 10–40% less fertilizer. Application of 45 or 60 kg N ha<sup>-1</sup> at the

thinning stage and critical SPAD value-based adjustable dose of 45 or 30 kg N ha<sup>-1</sup> at the first flowering stage leads to cotton seed yield at par with that observed with blanket recommended dose of 150 kg N ha<sup>-1</sup> but with significantly higher AEN and REN of N and with 30–40% less fertilizer.

Supplementary material. To view supplementary material for this article, please visit https://doi.org/10.1017/S0014479720000046

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