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OPTIMIZATION OF NITROGENOUS FERTILIZER REQUIREMENT IN COTTON CROP TO ENHANCE THE SEED YIELD BY USING CHLOROPHYLL METER

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ABSTRACT

Field experiments were conducted to investigate the use of portable chlorophyll meter to improve nitrogen management during the growth period of cotton at Central Cotton Research Institute, Multan. The treatments consisted of six nitrogen levels i.e. 0, 50, 100, 150, 200, 250 kg ha^{-1}, arranged in RCB design with four replications, applied at planting, first flowering and peak flowering phases. Cotton cultivar CIM-534 was planted during second week of May at spacing 75 cm between rows and 30 cm between plants. Results indicated that applied nitrogen fertilizer showed highly significant relationship with chlorophyll meter readings. The SPAD values increased from 52.2 to 64.2 as nitrogen fertilizer was increased from 0 to 250 kg ha^{-1}. Results showed that seed cotton yield and its components differed significantly due to the application of different levels of nitrogen fertilizer with a range from 1731 to 2521 kgha^{-1} in various treatments. Main stem height, number of nodes on main stem and inter-nodal length increased significantly with the increasing dose of nitrogen fertilizer. The values of main stem height ranged from 79.5 to 119.7 cm and the nodes on main stem ranged from 23 to 31. The relationships between seed cotton yield and nitrogen doses showed that seed cotton yield increased with the increasing levels of nitrogen fertilizer. Regression analysis exhibited highly significant relationship of nitrogen doses with seed cotton yield and SPAD values. Chlorophyll meter is being used in different parts of the world to measure the chlorophyll contents (SPAD value) of leaves for N management during the cropping season. Keeping the above facts in view, there is a need to quantify chlorophyll meter readings for matching N levels during the cotton growing season.

Key-words: Nitrogenous fertilizers, cotton yield, Chorophyll content,

INTRODUCTION

Nitrogen is the most critical plant nutrient applied to cotton. Nitrogen is the most critical nutrient in cotton production. The under-fertilization of nitrogen results in reduced yields whereas over fertilization can promote excessive vegetative growth, boll rot, delayed maturity and may lead to environmental contamination. Due to being mobile in nature, it is subjected to greater loss from soil-plant-system. Even under optimum management practices, about 30-50 percent applied nitrogen is lost through different processes occurring in soil. Adequate levels of nitrogen only can ensure optimum cotton production as its excess promotes rank growth and the plant become succulent. Resultantly, the plants are more susceptible to disease and pest attack. Conversely, the plants show stunted growth due to nitrogen deficiency resulting in considerable yield losses.

In order to minimize nitrogen fertilization cost and its losses, nitrogen fertilizer needs to be applied only after making sure that it will be beneficial for the crop. Soil and tissue tests for assessing cotton nitrogen needs have been investigated (Sabbe and Zelinski, 1990; Bell *et al*., 1998 and Constable and Rochester, 1988). A non- destructive, instantaneous method for assessing cotton nitrogen status would be more efficient and possibly, avoid source of the inherent problems associated with soil and tissue testing (Boquet *et al.*, 1999).

Chlorophyll, a nitrogen-rich pigment molecule in leaves, has been correlated with leaf nitrogen content in corn (Schepers *et al.*, 1990), rice (Turner and Jund, 1991) and cotton (Edminsten and wood, 1992). The development of the SPAD-502 chlorophyll meter (Minolta Camera Co, Ltd., Japan) has increased interest in using chlorophyll contents as indicator of plant-nitrogen status. This hand- held device estimate the chlorophyll contents of leaves nondestructively by measuring the difference in light attenuation at 430 and 750 nm. The spectral transmittance peak for both chlorophyll a and b occurs at 430 nm, whereas the 750 nm wavelength is in the near-infrared spectral region where transmittance occurs.

The chlorophyll meter has been used successfully to monitor nitrogen status of wheat (Murdock *et al.*, 1997), maize (Piekielek *et al.*, 1992) and rice (Peng *et al.*, 1993). The reports of Wood *et al.*, (1992), Feibo *et al.*, (1998) and Boquet *et al.*, (1999) indicate that leaf chlorophyll contents measured with the SPAD meter and leaf nitrogen contents were correlated for field- grown cotton. Feibo *et al.,* (1998) reported that chlorophyll meter reading of the upper-most fully expanded leaf (4th from terminal) were positively correlated with leaf-blade nitrogen and seed cotton yield. Keeping this view in mind, an experiment was conducted to determine that whether the chlorophyll meter readings of functional leaves were correlated with seed cotton yield, nitrogen concentration in leaf blade and would these be helpful in determining nitrogen fertilizer requirement in cotton.

MATERIALS AND METHODS

This experiment was conducted at Research Farm of Central Cotton Research Institute, Multan during the season (2009-2010). Composite soil samples were collected before imposition of treatments at the time of seedbed preparation for planting crop and were analyzed for physical and chemical characteristics. Soil particle size distribution was determined by hydrometer method (Moodie *et al.,* 1959) and textural class according to USDA system. The available nitrogen, phosphorus and potassium determination were made according to Black (1965) and other analyses were done according to methods described by U.S. Salinity Lab. Staff (1954). Physical and chemical characteristics of the experimental site are given in Table 1. The soil is medium in texture, calcareous in nature and alkaline in reaction. Fertility rating indicates low level of organic matter, poor supply of nitrogen, low to marginally adequate supply of phosphorus and sufficient level of potassium for cotton production. The soil belongs to Miani soil series and is classified as Calcaric Cambisols and fine loamy, mixed Hyperthermic Fluventic Haplocambids according to FAO and USDA Soil Classification Systems.

Cotton cv. CIM-534 (*Gossypium hirsutum* L.) was planted during the second week of May at a spacing 75cm between rows and 30 cm between the plants. The treatments consisted of six nitrogen doses of 0, 50, 100, 150, 200, 250 kg ha^{-1}. The layout of experiment was randomized complete block design with four replications. Crop also received 50 kg P_2O_5 and 50 kg K_2O ha^{-1}. The whole quantity of phosphorus and potassium and 1/3rd nitrogen fertilizer was applied at planting. The remaining quantity of nitrogen was applied at first flower and peak flowering stages. Standard production practices were followed during the season. Crop was kept free of insect-pests through regular sprays.

Chlorophyll meter (Minolta SPAD-502) was used to record SPAD values (chlorophyll meter readings) of the intact fully expanded functional leaves i.e. 4th from the apex at various growth stages (i.e. squaring, flowering, peak flowering, first boll split and maturity).At each stages, fully expanded fourth leaves from apex (one leaf per plant) were taken avoiding mid-rib of each plot. After the SPAD measurements, the 10 measured leaves were detached for the determination of total nitrogen content. Total nitrogen content was analyzed by kjeldahl digestion and distillation method (Bremner and Mulvaney, 1982). Leaf nitrogen concentration was expressed on dry weight basis. The seed cotton was hand picked in each plot and total yield calculated on the area basis. Data on number of bolls per plant and boll weight were recorded on 10 random plants at maturity stages from each treatment. Data were statistically analyzed according to methods described by Gomez and Gomez (1984).

RESULTS AND DISCUSSION

Seed cotton yield and its components differed significantly due to the application of different levels of nitrogen fertilizer. The values of seed cotton yield ranged from 1731 to 2521 kg.ha^{-1} in various treatments. The increase in yield occurred due to increase in number of bolls per plant and boll weight (Table 2). The relationships between seed cotton yield and nitrogen doses showed that seed cotton yield increased with the increasing levels of nitrogen fertilizer. Regression analysis showed a highly significant relationship (r= 0.85**) between nitrogen doses and seed cotton yield with the regression equation as: $y=-0.0139x^2 + 7.0072x + 1660.2$ (Fig.1). These results are in agreement with those of Malik *et al.* (2001).

Main stem height, number of nodes on main stem and inter-nodal length increased significantly with the increasing dose of nitrogen fertilizer. The values of main stem height ranged from 79.5 to 119.7 cm and the nodes on main stem ranged from 23 to 31. Main stem height increased mainly due to increase in inter-nodal length (Table 3). The fruiting positions and intact fruit per unit land area increased significantly with the increasing levels of nitrogen fertilizers. Fruiting positions ranged from 405 to 460 m^{-2} and intact fruits from 108 to 142 m^{-2} in different treatments. Fruit shedding percentage decreased with the addition of nitrogen fertilizer. Fruit shedding ranged from 69 to 73 % in different treatments (Table 4).

Table 1. Physical and Chemical Characteristics of the Experimental Site at Pre-Plant (0-30 cm depth).

Characteristics	**Values**
pHs	8.5
EC_e (dS m^{-1})	1.77
$CaCO_3$ (%)	4.12
Organic matter (%)	0.55
NO_3-N (mg kg^{-1})	8.25
Available $NaHCO_3$ Extracted-P (mg kg^{-1})	8.5
Available NH_4OAc Extracted-K (mg kh^{-1})	120
Textural Class	Silt loam

Table 2. Effect of different doses of nitrogen fertilizer application on seed cotton yield and its components.

Nitrogen levels (kg ha^{-1})	**Cotton seed yield (Kg ha^{-1})**	**No. of bolls $plant^{-1}$**	**Boll weight (g)**
0	1731	17	2.71
50	1840	18	2.80
100	2231	22	2.87
150	2485	25	2.98
200	2500	25	3.03
250	2521	25	3.07
LSD ($p<0.01$)	479.6	4.16	0.15

Table 3. Effect of nitrogen fertilizer application on plant structure at maturity.

Nitrogen levels (kg ha^{-1})	**Main stem height (cm)**	**Number of nodes on main stem**	**Inter-nodal length (cm)**
0	79.5	23	3.46
50	86.7	25	3.47
100	92.6	26	3.56
150	101.2	29	3.81
200	115.0	30	3.84
250	119.7	31	3.85
LSD ($p<0.01$)	6.90	2.22	0.04

Table 4. Effect of nitrogen fertilizer application on fruit production at maturity.

Nitrogen levels (kg ha^{-1})	**Fruits position/plant Fruiting positions m^{-2}**	**No. of Fruits / plant Intact fruits m^{-2}**	**Fruit shedding percentage**
0	405 91	108 24.3	73
50	415 93.4	115 25.9	72
100	426 95.8	123 27.7	71
150	436 98.1	130 29.2	70
200	448 100.8	139 31.3	69
250	460 103.8	142 31.9	69
LSD ($p<0.01$)	42.84 ??	11.57 ??	2.68

Please ckeck red marks which seem better b/c it is quite un-natural to present position on per m^2 basis.

Plants were harvested at maturity and partitioned into leaves, stalk and fruit portions. Material was oven dried. Results indicated that dry matter yield of leaves, stalk and fruit increased with the application of nitrogen fertilizer. Values of total dry matter yield ranged from 705 to 919 g m-2 in different treatments (Table 5). Data on Cotton Leaf

Curl Virus infestation were recorded at day 30, 45, 60, 75 and 90 after planting. The disease infestation varied in different fertilizer doses. Disease incidence was slightly lower where nitrogen fertilizer was applied (Table 6).

Table 5. Effect of nitrogen fertilizer application on dry matter production (gm-2) at maturity.

Nitrogen levels (kg ha^{-1})	Leaves	Stalk	Fruit	Total
0	155	190	360	705
50	170	206	379	755
100	195	220	395	810
150	215	240	408	863
200	229	265	416	910
250	232	267	420	919
LSD (p<0.01)	9.43	9.10	10.11	14.40

Table 6. Effect of nitrogen fertilizer application on virus infestation % in cotton.

Nitrogen levels (Kg ha^{-1})	Days after planting				
	30	45	60	75	90
0	30.5	48.5	65.4	72.6	79.3
50	32.8	48.2	63.3	70.1	77.8
100	29.8	47.7	61.5	68.5	76.4
150	26.3	46.4	58.4	67.2	75.3
200	26.5	46.7	59.3	67.8	76.5
250	27.2	47.4	60.2	68.3	76.8

Table 7. Effect of different doses of nitrogen fertilizer on Chlorophyll meter readings (SPAD) values during the season

Nitrogen Doses (kg ha^{-1})	Stages of Growth					Mean
	Squaring	Flowering	Peak Flowering	First boll Split	Maturity	
0	52.9	54.8	55.2	55.6	56.2	54.94
50	53.3	55.5	56.4	57.8	58.4	56.28
100	55.8	58.3	59.6	60.2	60.8	58.94
150	57.9	59.0	61.3	62.0	63.0	60.64
200	59.6	61.0	62.0	62.5	63.5	61.72
250	59.7	61.3	62.5	63.1	64.2	62.16

Table 8. Effect of different doses of nitrogen fertilizer on nitrogen concentrations (%) in leaf blades.

Nitrogen Doses (kg ha^{-1})	Stages of Growth					Mean
	Squaring	Flowering	Peak Flowering	First boll Split	Maturity	
0	2.34	2.25	2.12	2.02	1.86	2.12
50	2.47	2.75	2.84	3.05	1.90	2.60
100	2.53	2.85	2.94	3.08	2.04	2.68
150	2.60	2.96	3.18	3.16	2.10	2.80
200	2.67	3.03	3.24	3.23	2.18	2.87
250	2.72	3.16	3.40	3.46	2.28	3.01
Mean	2.56	2.83	2.95	3.0	2.06	

Chlorophyll meter (Minolta SPAD-502) was used to record SPAD values (chlorophyll meter readings) of the intact fully expanded functional leaves i.e. 4th from the apex at various growth stages (i.e., squaring, flowering, peak flowering, first boll split and maturity). A minimum of about thirty readings per treatment were taken avoiding mid-rib of each leaf blade. The chlorophyll content (SPAD values) increased with the increasing doses of nitrogen fertilizers with concurrent advancement in the age of crop. The highest SPAD values were observed at 90 days after planting (Table 7).

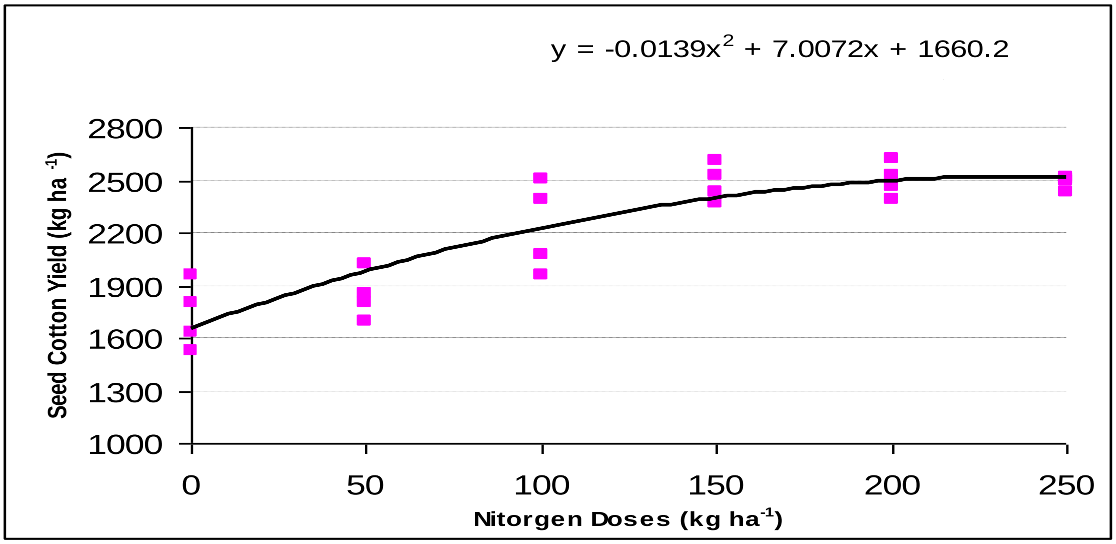

Fig. 1. Relationships between nitrogen doses and seed cotton yield.

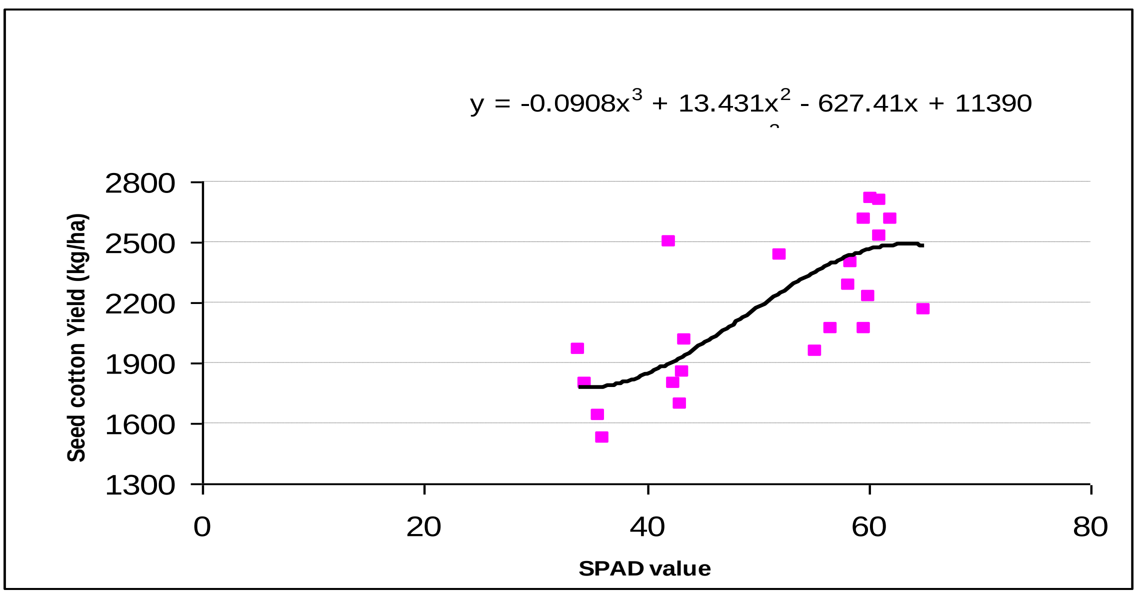

Fig. 2. Relationships between seed cotton yield and SPAD value.

Data on chlorophyll meter readings recorded at different stages of growth differed significantly due to various rates of nitrogen fertilizer (Table 7). The chlorophyll readings increased with increasing doses of nitrogen fertilizers with concurrent advancement in the age of crop. Crop maintained higher SPAD values at first boll split stages and then it declined at maturity stages. The increase in chlorophyll meter readings resulted due to higher nitrogen nutrient concentration in leaf tissue and sufficient availability of nitrogen fertilizer. The results corroborate with those of Boquet *et al.* (1999) who reported that there was a strong association between nitrogen rate and SPAD readings. They reported increase in SPAD values with the advancement of growth at higher N rate and decreased at lower N rate. At maturity the SPAD values decreased in all the treatments due to decreased in photosynthetic activity. Total nitrogen concentration in leaf tissue differed significantly due to nitrogen fertilization and at various stages of growth (Table 8). Nitrogen concentration increased with the increasing doses of nitrogen fertilizer, with concurrent advancement in age of the crop. However, its concentration decreased at maturity stage. The increased nutrient concentration in leaf tissue resulted due to sufficient availability of nitrogen supply in the soil during the season. Sabbe *et al.*, (1990) and Bell *et al.*,(1998) also reported that leaf-blade nitrogen content of the upper-most fully expanded leaf could be used as a criterion to determined nitrogen nutrient requirement of cotton.

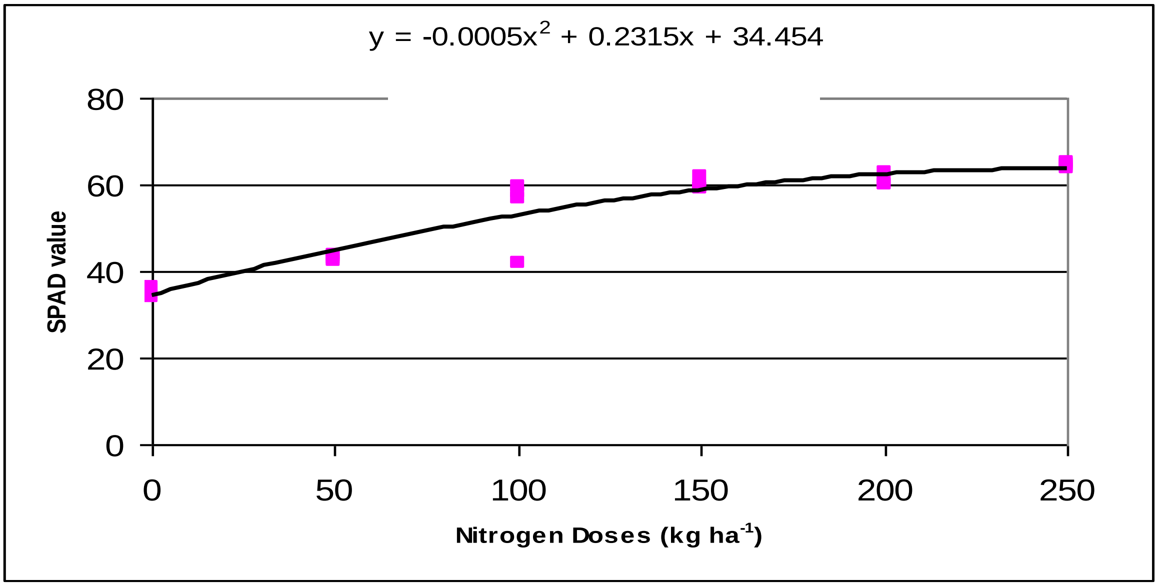

Fig. 3. Relationships between nitrogen does and SPAD value.

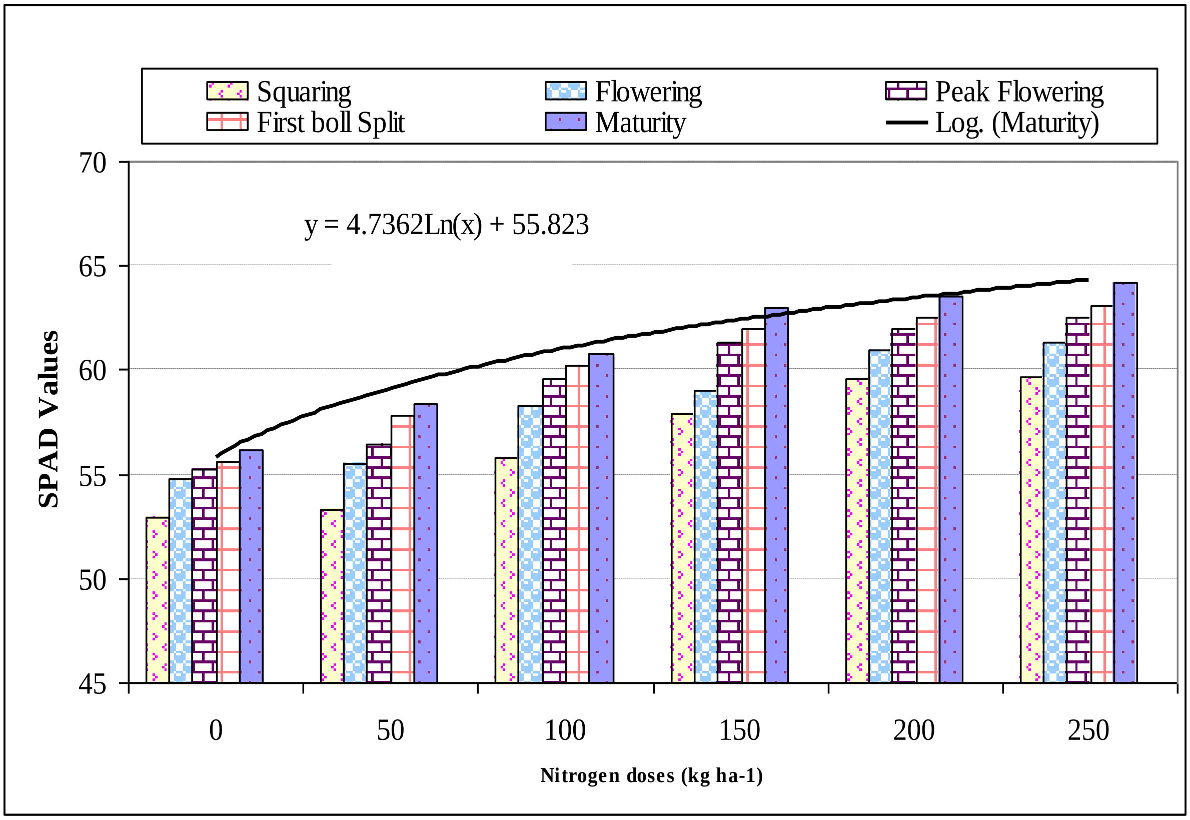

Fig. 4. Relationships between different doses of nitrogen fertilizer on Chlorophyll meter readings (SPAD) values during the season.

The relationships between SPAD values and seed cotton yield are illustrated in Fig. 2. Seed cotton yield increased significantly with the increase in SPAD values by up to 59.7. Regression between SPAD values and seed cotton yield showed a significant correlation coefficient with the regression equation as $y = -0.0908x^3 + 13.431x^2 - 627.41x + 11390$. According to this equation, diagnosis index or critical SPAD value was 59.7 to obtain the maximum seed cotton yields. Boquet *el al.* (1998) reported that SPAD values that indicated N sufficiency level were between 38 to 40. In present study SPAD values of 59.7 was best correlated with seed cotton yield, nitrogen doses and leaf concentration tissues.

Increasing levels of nitrogen fertilizers caused an increase in SPAD values. Regression analysis indicated that there was a highly significant relationship (r=0.92**) between nitrogen doses and SPAD values with the regression equation as $y =-0.0005x^2 + 0.2315x + 34.454$ (Fig. 3). Increasing levels of nitrogen fertilizers caused an increase in SPAD values. Regression analysis indicated that there was a highly significant relationship between nitrogen doses and SPAD values (Fig. 3). Similarly studies by Feibo *et al.* (1998) revealed that chlorophyll meter readings are best correlated with seed cotton yield and could be used directly as a threshold for top-dressing of nitrogen fertilizer during the crop season.

CONCLUSIONS

The use of SPAD values to diagnose the nitrogen status of cotton plants may provide useful information for managing research experiments and varietal trails where adequate nitrogen supply must be maintained in diverse environments and on different soils. The optimum SPAD values may, however, vary slightly within the varieties depending upon the genetic make-up and crop physiology. The establishment of diagnostic criteria for different for different varieties would provide precision in nitrogen management during the cropping season. Therefore, the use of chlorophyll meter for determining the nitrogen status of different breeding lines as it relates to nitrogen-absorbing ability or rate of senescence could be used as a hand, reliable rapid research tool.

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