

**SPECTRAL PROPERTIES OF LEAVES IN RELATION TO
YIELD, NITROGEN CONTENT, UPTAKE AND USE
EFFICIENCIES IN COTTON**

Thesis

**Submitted to the Punjab Agricultural University
In partial fulfillment of the requirements
for the degree of**

**MASTER OF SCIENCE
in
SOIL SCIENCE
(Minor Subject: Chemistry)**

By

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(L-2011-A-101-M)**

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2014

CERTIFICATE – I

This is to certify that the thesis entitled, “**SPECTRAL PROPERTIES OF LEAVES IN RELATION TO YIELD, NITROGEN CONTENT, UPTAKE AND USE EFFICIENCIES IN COTTON**” submitted for the degree of Master of Science in the subject of **Soil Science** (Minor subject: **Chemistry**) of the Punjab Agricultural University, Ludhiana, is a bonafide research work carried out by **Lakhwinder Singh (L-2011-A-101-M)** under my supervision and that no part of this thesis has been submitted for any other degree.

The assistance and help received during the course of investigation have been fully acknowledged.

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CERTIFICATE II

This is to certify that the thesis entitled “**Spectral properties of leaves in relation to yield, nitrogen content, uptake and use efficiencies in cotton**” submitted by **Lakhwinder Singh (L-2011-A-101-M)** to the Punjab Agricultural University, Ludhiana, in partial fulfillment of the requirements for the degree of M.Sc., in the subject of **Soil Science** (Minor subject: **Chemistry**) has been approved by the Student’s Advisory Committee along with Head of the Department after an oral examination on the same.

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ABSTRACT

The study of spectral properties of cotton using leaf colour chart, SPAD meter and GreenSeeker was conducted in two cotton cultivars *i.e.* MRC 7017 and Ankur 3028. The cultivars do not differ significantly in leaf colour chart, SPAD meter readings and NDVI values at different growth stages. The seed cotton yield, nitrogen uptake and leaf N content were significantly correlated to LCC, SPAD meter readings and NDVI values. The maximum coefficient of determination (R^2) between seed cotton yield and LCC (0.531) and SPAD meter readings (0.572) was observed at squaring stage. The NDVI values were correlated to seed cotton yield with maximum R^2 value of 0.613 at boll opening stage. The maximum R^2 value between total N uptake and LCC (0.582) and SPAD meter readings (0.576) was observed at early flowering stage, however NDVI values were correlated with maximum R^2 value of 0.655 at peak flowering stage. The maximum R^2 value between leaf N content and SPAD meter readings (0.635) and NDVI values (0.722) was observed at mid flowering stage, however LCC readings were correlated with maximum R^2 value of 0.583 at early flowering stage. The significant correlations of leaf N content at different growth stages, seed cotton yield and total nitrogen uptake with LCC, SPAD meter readings and NDVI values suggest that spectral properties of leaves can be used to establish need based fertilizer N management strategies using LCC, SPAD meter and GreenSeeker optical sensor during squaring and flowering growth stages in cotton. The significant correlation of LCC and SPAD readings at different growth stages revealed that LCC can be used as an economical substitute of SPAD meter to assess in-season plant N need in cotton. The application of 60 kg N ha⁻¹ produced statistically highest seed cotton yield with maximum nitrogen use efficiencies. Insignificant differences in N availability indices of no N control and fertilizer N treatments suggest that application of higher fertilizer N is neither helpful in improving inherent soil N nor seed cotton yield.

Keywords : Cotton, leaf colour chart, SPAD meter, GreenSeeker, Nitrogen.

Signature of Major Advisor

Signature of the Student

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ਕਪਾਹ ਦੇ ਪੱਤਿਆਂ ਦੀਆਂ ਸਤਰੰਗੀ ਵਿਸ਼ੇਸ਼ਤਾਵਾਂ ਦਾ ਅਧਿਐਨ ਦੋ ਵੱਖ-ਵੱਖ ਕਿਸਮਾਂ (MRC 7017, ANKUR 3028) ਵਿਚ ਪੱਤਾ ਰੰਗ ਚਾਰਟ, ਕਲੋਰੋਫਿਲ ਮੀਟਰ ਅਤੇ ਗਰੀਨ ਸੀਕਰ ਦੇ ਸਹਿਯੋਗ ਨਾਲ ਕੀਤਾ ਗਿਆ । ਖੋਜ ਨਤੀਜਿਆਂ ਮੁਤਾਬਿਕ ਦੋਨੋਂ ਕਿਸਮਾਂ ਦੇ ਪੱਤਿਆਂ ਦੇ ਰੰਗ ਵਿਚ ਫਸਲ ਦੇ ਵਿਕਾਸ ਦੀਆਂ ਵੱਖ-ਵੱਖ ਸਥਿਤੀਆਂ ਸਮੇਂ ਕੋਈ ਵਿਸ਼ੇਸ਼ ਅੰਤਰ ਦੇਖਣ ਨੂੰ ਨਹੀਂ ਮਿਲਿਆ । ਕਪਾਹ ਦਾ ਝਾੜ, ਨਾਈਟ੍ਰੋਜਨ ਦੀ ਉਪਯੋਗਤਾ ਅਤੇ ਪੱਤਿਆਂ ਵਿਚ ਨਾਈਟ੍ਰੋਜਨ ਦੀ ਮਾਤਰਾ ਦਾ ਪੱਤਾ ਰੰਗ ਚਾਰਟ, ਕਲੋਰੋਫਿਲ ਮੀਟਰ ਪੜ੍ਹਤਾਂ ਅਤੇ ਐਨ.ਡੀ.ਵੀ.ਆਈ. ਅੰਕੜਿਆਂ ਨਾਲ ਸੰਬੰਧ ਸੀ। ਅੰਕੜਾ ਗਿਆਨ ਮੁਤਾਬਿਕ ਕਪਾਹ ਦੇ ਝਾੜ ਦਾ ਇਹ ਵੱਧ ਤੋਂ ਵੱਧ ਨੇੜਲਾ ਸੰਬੰਧ (R^2) ਡੇਡੀਆਂ ਨਿਕਲਣ ਸਮੇਂ ਪੱਤਾ ਰੰਗ ਚਾਰਟ ਨਾਲ 0.531 ਅਤੇ ਕਲੋਰੋਫਿਲ ਮੀਟਰ ਪੜ੍ਹਤ ਨਾਲ 0.572 ਸੀ । ਗਰੀਨ ਸੀਕਰ ਦੀ ਵਰਤੋਂ ਨਾਲ ਪ੍ਰਾਪਤ ਕੀਤੇ ਐਨ.ਡੀ.ਵੀ.ਆਈ. ਅੰਕੜਿਆਂ ਦਾ ਕਪਾਹ ਦੇ ਝਾੜ ਨਾਲ ਵੱਧ ਤੋਂ ਵੱਧ ਸੰਬੰਧ ($R^2 = 0.613$) ਟੀਡੇ ਖੁੱਲ੍ਹਣ ਸਮੇਂ ਸੀ। ਅੰਕੜਾ ਗਿਆਨ ਮੁਤਾਬਿਕ ਪੌਦੇ ਵਿਚ ਨਾਈਟ੍ਰੋਜਨ ਦੀ ਵਰਤੋਂ ਦਾ ਪੱਤਾ ਰੰਗ ਚਾਰਟ ਅਤੇ ਕਲੋਰੋਫਿਲ ਮੀਟਰ ਨਾਲ ਨੇੜਲਾ ਸੰਬੰਧ (R^2) ਫੁੱਲ ਖਿੜਨ ਦੀ ਸ਼ੁਰੂਆਤ ਸਮੇਂ ਕਰਮਵਾਰ 0.582 ਅਤੇ 0.576 ਪ੍ਰਾਪਤ ਹੋਇਆ, ਜੋ ਕਿ ਐਨ.ਡੀ.ਵੀ.ਆਈ. ਅੰਕੜਿਆਂ ਨਾਲ ਪੂਰੇ ਫੁੱਲਾਂ ਦੇ ਖਿੜ ਜਾਣ ਸਮੇਂ 0.655 ਸੀ। ਪੱਤਿਆਂ ਵਿਚ ਨਾਈਟ੍ਰੋਜਨ ਦੀ ਮਾਤਰਾ ਕਲੋਰੋਫਿਲ ਮੀਟਰ ਦੀ ਪੜ੍ਹਤ ਅਤੇ ਐਨ.ਡੀ.ਵੀ.ਆਈ. ਅੰਕੜਿਆਂ ਨਾਲ ਅੱਧੇ ਫੁੱਲ ਖਿੜ ਜਾਣ ਸਮੇਂ $R^2 = 0.635$ ਅਤੇ $R^2 = 0.722$ ਨਾਲ ਕ੍ਰਮਵਾਰ ਸੰਬੰਧਿਤ ਸੀ, ਜਦਕਿ ਪੱਤਾ ਰੰਗ ਚਾਰਟ ਨਾਲ ਇਹ ਸੰਬੰਧ ਫੁੱਲ ਦੇ ਖਿੜਨ ਦੀ ਸ਼ੁਰੂਆਤ ਸਮੇਂ $R^2 = 0.583$ ਸੀ । ਕਪਾਹ ਦੇ ਵਿਕਾਸ ਦੀਆਂ ਵੱਖ-ਵੱਖ ਸਥਿਤੀਆਂ ਸਮੇਂ ਪੱਤਿਆਂ ਵਿਚ ਨਾਈਟ੍ਰੋਜਨ ਦੀ ਮਾਤਰਾ ਦੇ ਕਪਾਹ ਦੇ ਝਾੜ, ਪੱਤਾ ਰੰਗ ਚਾਰਟ, ਕਲੋਰੋਫਿਲ ਮੀਟਰ ਪੜ੍ਹਤਾਂ ਅਤੇ ਐਨ.ਡੀ.ਵੀ.ਆਈ. ਅੰਕੜਿਆਂ ਨਾਲ ਨੇੜਲੇ ਸੰਬੰਧ ਨੇ ਇਸ ਗੱਲ ਦੀ ਪੁਸ਼ਟੀ ਕੀਤੀ ਹੈ ਕਿ ਪੱਤਾ ਰੰਗ ਚਾਰਟ, ਕਲੋਰੋਫਿਲ ਮੀਟਰ ਅਤੇ ਗਰੀਨ ਸੀਕਰ ਨੂੰ ਡੇਡੀਆਂ ਲੱਗਣ ਤੋਂ ਫੁੱਲਾਂ ਦੇ ਖਿੜਨ ਤੱਕ ਕਪਾਹ ਵਿਚ ਨਾਈਟ੍ਰੋਜਨ ਦੀ ਲੋੜ ਨੂੰ ਜਾਨਣ ਲਈ ਵਰਤਿਆ ਜਾ ਸਕਦਾ ਹੈ । ਪੱਤਾ ਰੰਗ ਚਾਰਟ ਅਤੇ ਕਲੋਰੋਫਿਲ ਮੀਟਰ ਦੀਆਂ ਪੜ੍ਹਤਾਂ ਦੇ ਨੇੜਲੇ ਸੰਬੰਧ ਤੋਂ ਇਹ ਵੀ ਜਾਣਕਾਰੀ ਪ੍ਰਾਪਤ ਹੋਈ ਕਿ ਪੱਤਾ ਰੰਗ ਚਾਰਟ ਨੂੰ ਕਲੋਰੋਫਿਲ ਮੀਟਰ ਦੀ ਥਾਂ ਤੇ ਕਪਾਹ ਨੂੰ ਨਾਈਟ੍ਰੋਜਨ ਦੀ ਲੋੜ ਦੇ ਠੀਕ ਸਮੇਂ ਦਾ ਪਤਾ ਲਗਾਉਣ ਲਈ ਸਸਤੇ ਬਦਲ ਦੇ ਰੂਪ ਵਿਚ ਵਰਤਿਆ ਜਾ ਸਕਦਾ ਹੈ । ਕਪਾਹ ਵਿਚ 60 ਕਿਲੋ ਨਾਈਟ੍ਰੋਜਨ ਪ੍ਰਤੀ ਹੈਕਟੇਅਰ ਦੀ ਵਰਤੋਂ ਨਾਲ ਕਪਾਹ ਦੇ ਪੂਰੇ ਝਾੜ ਅਤੇ ਵੱਧ ਤੋਂ ਵੱਧ ਉਪਯੋਗਤਾ ਪ੍ਰਾਪਤ ਹੋਈ । ਨਾਈਟ੍ਰੋਜਨ ਦੀ ਵੱਧ ਖਾਦ ਪਾਉਣ ਨਾਲ ਨਾ ਤਾਂ ਝਾੜ ਵਿਚ ਵਾਧਾ ਹੋਇਆ ਅਤੇ ਨਾ ਹੀ ਭੂਮੀ ਦੀ ਉਪਜਾਊ ਸ਼ਕਤੀ ਵਧੀ । ਜਿਸ ਤੋਂ ਸੋਧ ਪ੍ਰਾਪਤ ਹੋਈ ਕਿ ਬੇਲੋੜੇ ਸਮੇਂ, ਬੇਲੋੜੀ ਮਾਤਰਾ ਵਿਚ ਪਾਈ ਹੋਈ ਨਾਈਟ੍ਰੋਜਨ ਖਾਦ ਦਾ ਫਸਲ ਜਾਂ ਭੂਮੀ ਨੂੰ ਕੋਈ ਲਾਭ ਪ੍ਰਾਪਤ ਨਹੀਂ ਹੋਇਆ ਅਤੇ ਇਹ ਵਾਧੂ ਨਾਈਟ੍ਰੋਜਨ ਅਜਾਈਂ ਖਰਾਬ ਹੋ ਗਈ ।

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CHAPTER I

INTRODUCTION

Cotton (*Gossypium* species) is the world's most important fibre crop plant. The commercially grown species of *Gossypium* include *G. hirsutum*, *G. barbedense*, *G. arboreum* and *G. herbaceum*. Cotton is the most prevalent natural fibre used in textile production and is one of the most important industrial crops in the world. It is cultivated in more than 70 countries in Asia, America, Europe, Australia and Africa. India is an important grower of cotton and ranks third in global cotton production after the United States and China. India accounts for approximately 25% of world's total cotton area and 16% of global cotton production (Benett *et al* 2004). Most of the cotton in India is grown under rainfed conditions, and about a third is grown under irrigated conditions (Sundaram *et al* 1999). Cotton production in India was 334 lakh bales (170 kg) from 116.14 lakh hectares (Anonymous 2012) in 2012. Cotton accounts for more than 75 per cent of the annual fibre consumption in spinning mills, about 58 per cent of the total fibre consumption in textile sector and 30-35 per cent of total export earnings in India (Gumber *et al* 2008). The Indian cotton cultivation is most diverse in the world in terms of botanical status and quality of the fibre. India is the only country where all the four cultivated species and their interspecific hybrids are grown.

Nitrogen (N) is the most important fertilizer nutrient in cotton and its consumption has increased substantially in the last decades (Anonymous 2012). In northwestern India, the blanket fertilizer N recommendation consists of applying 150 kg N ha⁻¹ for Bt and non-Bt hybrids and 75 kg N ha⁻¹ for Desi cotton at fixed growth stages of the crop. These recommendations do not consider spatial and temporal variability in soil N supply and are established for large tracts. Moreover, the measurement of soil N supply which can be taken up by crop plants has always remained a challenge because availability indices of soil N are not very reliable (Nayyar *et al* 2006). As soil N exists largely in organic forms, soil organic carbon status has been frequently used as an index for soil N supply (Pathak *et al* 2003). However, kinetics of N release from soil organic matter and crop residues is very dynamic and the time and quantity of N supply from the soil always remain uncertain. Thus, lack of synchronization in plant N demand and soil N supply leads to poor N use efficiency when blanket recommendations are followed.

Inadequate fertilizer N application reduces the number of fruiting sites and potential yield, thus to avoid N deficiency risks, farmers generally apply fertilizer in excess of the blanket recommendations which further decreases N use efficiency. Lower recovery of N is not only responsible for higher cost of crop production, increased insect-pest incidence but also for environmental degradation (Fageria and Baligar 2005). Increased application of N leads to the elongation of the vegetative period and thus reducing the reproductive phase causing decline in yield. The excessive nitrate in the soil may leach down and contaminate

ground water which poses a serious threat to the humans and animals.

In the mid eighties and nineties, the emphasis was shifted from reducing N losses to matching crop N demand with fertilizer N supply for achieving high N use efficiency (Buresh 2007) and the research since then has been oriented more towards finding means and ways to apply fertilizer N in real time using crop demand-driven and field-specific needs. Predicting the amount of supplementary fertilizer N needed by a crop is difficult because N can undergo quick transformations that not only influence its mobility and retention in soil, but also its availability to plants. Nitrogen leaching, denitrification, ammonia volatilization and mineralization/immobilization are processes that directly govern the availability of N in soil.

In-season crop N demand can be estimated by using conventional tissue testing procedures (Roth *et al* 1989, Yadava 1986). Monitoring N status of plant has advantages that plant integrates N supply over a period of time, and hence can reflect N supply as affected by weather, soil processing and fertilization. However, plant tissue analysis may require 1-2 weeks starting from tissue sampling to deciding a fertilizer recommendation and may not turn out to be a practically feasible proposition with large number of fields. The farmers generally use leaf color as a visual and subjective indicator of the need for N fertilizer (Furuya 1987). Since farmers generally prefer to keep leaves of the crop dark green, it leads to over application of fertilizers N resulting in low recovery efficiency. Thus, the spectral properties of leaves should be used in a more rational manner to guide need-based fertilizer N applications. Leaf color chart and SPAD meter can quickly measure greenness of leaf N status. These tools provided an excellent opportunity in terms of developing real time N management, but these tools do not make into-account photosynthetic rates or the biomass production and expected yields for working out N requirements. So for determination of these factors, optical sensors are used based on the reflectance in red and near infrared spectrum (Gupta 2006). Li *et al* (2001) reported that NDVI value and red vegetative index were positively correlated with cotton biomass, N accumulation and lint yield. The application of optical sensor in agriculture has advanced rapidly in recent years.

The proposed research has been planned to study spectral properties of cotton leaves in relation to yield, N content, uptake and use efficiencies using leaf color chart (LCC), chlorophyll (SPAD) meter and GreenSeeker optical sensor. The present study has been planned with the following objectives:

1. To study the relationship of leaf greenness and biomass as measured by LCC, SPAD meter and GreenSeeker optical sensor with leaf N content, plant N uptake and seed cotton yield
2. To study relationship between estimates of LCC, SPAD and NDVI for guiding need-based fertilizer N applications in cotton
3. To study the varietal differences in spectral properties of cotton leaves.

CHAPTER II

REVIEW OF LITERATURE

Nitrogen (N) is the most widely used fertilizer nutrient in cotton and its consumption has increased substantially in the past decades (Anonymous 2012). Nitrogenous fertilizers have remarkably contributed to increased crop production during the past 50 years, however, fertilizer N also accounts for 33 per cent of the total annual creation of reactive N (Smil 2001). High reactivity and mobility of nitrogenous fertilizers in the soil environment has remained always a challenge in the efficient management of fertilizer N. Recent advances in fertilizer N management research have given new dimension to synchronize N application with plant N demand instead of only plugging N losses. Emphasis is to develop fertilizer N management strategies that ensure the sufficient N supply throughout the growing season as well as reduces N losses and improve NUE.

The literature pertaining to optimization of fertilizer N use following need based N management strategies in cotton has been reviewed under the following heads:

- 2.1 Nitrogen use by cotton
- 2.2 Improving fertilizer N use efficiency
- 2.3 Need based N management
 - 2.3.1 Chlorophyll (SPAD meter)
 - 2.3.2 Leaf color chart (LCC)
 - 2.3.3 Optical sensors based N management

2.1 Nitrogen use by Cotton

Nitrogen is the important nutrient to which cotton shows a good response. As most of the soils are low in N, it is most often the major limiting factor in cotton production, after water. Nitrogen management is essential and critical in a dynamic crop like cotton. An excess of N supply would result in more of vegetative growth and delay maturity. Excessive N also leads to weakening of fibre and thereby affects quality. Over application of N cause excessive vegetative growth, increased susceptibility to aphids and boll rot, delayed crop maturity, delayed defoliation and may also decrease yield. An under application of N causes detrimental loss to yield proportional to the fertilizer shortfall, depletion of the soil N reserve and depletion of soil fertility (Nichols and Green 2009). The key to N management is, therefore, to provide adequate amounts rather than low or high amounts to the crop as needed by the crop (Mc Connell *et al* 1996). In the northwestern India, the blanket recommendation consists of applying 150 kg N ha⁻¹ for Bt and non- Bt hybrids and 75 kg N ha⁻¹ for Desi cotton. Among the macronutrients, N is the most susceptible to losses (Prasad and Power 1995) and the nitrogen use efficiency (NUE) is very low (Singh and Mannikar 1988). Excessive N application to cotton is an unnecessary cost and a potential cause of elevated

ground water N concentrations (Hunt *et al* 1997). Poor N management can cause detrimental effects on yield either due to over or under application of the nutrient. The most efficient and widely accepted method to supply N to any plant is only at the time when it was required (Arnall 2008). Boquet and Breitenbeck (2000) observed a maximum N uptake of 2.9 to 4.3 kg ha⁻¹ occurring during the period of 49 to 71 days after sowing (DAS). Maximum uptake was recorded between early square and early bloom in cotton by Fritschi *et al* (2004).

The cotton plant's N requirements are not constant throughout the season. Early vegetative growth requires only a small amount of N usually less than 25% of the seasonal total. With the initiation of boll filling the rate of N uptake increases dramatically. It had been reported that between 25% and 40% of the seasonal N accumulation may occur during the first two weeks of bloom. This translates into N uptake rate of approximately 4 lbs ac⁻¹ day⁻¹ at peak bloom (Guthrie *et al* 1994).

The implications of variation in N uptake at different stages are significant and nitrogen must be available to the plant to meet crop N need at all the growth stages. On the other hand balance soil N may meet another fate. Thus the challenge is to track and meet crop requirements within an economically and environmentally responsible framework. The variation in N demand through the season poses a burden to the plant's physiological systems. Competition for carbohydrates during boll filling results in decreased N uptake efficiency by the roots. Reduced N supply to the plant results in a decline in the total leaf area, which in effect sets an upper limit on the number of bolls those, can be filled. The plant sheds small bolls as a means of adjusting the fruit load to match its leaf area. Clearly, N must be readily available in the soil and sufficient levels must be stored in leaf tissue to meet the demands of the crop.

2.2 Improving fertilizer N use efficiency

Nitrogen use efficiency is defined as the ratio of biological yield or economic yield to the fertilizer N used. More commonly used measures of NUE are agronomic efficiency (AE) and recovery efficiency (RE) computed by different methods. The most efficient use of N fertilizer is achieved by applying the correct rate at a time when N loss will be minimum. The crop use less than half of the fertilizer N applied. Large quantities are lost from the system through either leaching or biological denitrification and there is need to improve N use efficiency. Various management options are available to enhance the NUE which can be done by either using coated urea fertilizers and nitrification inhibitors, split application, spot/band placement. Blanket fertilizer N management recommendations consisting of split applications of preset rates of total fertilizer N have well served the purpose to produce optimum yields, but these cannot help to increase N use efficiency beyond a limit. Large field-to-field variability of soil N supply, agro-climatic conditions and varietal differences restrict efficient use of fertilizer N when broad-based blanket recommendations are used.

The efficiency of urea can be improved by the use of controlled release systems, chemical additives (*e.g.* acidifying agents, soluble salts), by altering granule size or soil incorporation (Watson 2000). One of the most promising ways to improve the efficiency of urea is to use a urease inhibitor which slows the conversion of urea to NH_4^+ , and hence reduces the concentration of NH_4^+ present in the soil solution and the potential for NH_3 volatilization and seedling damage. Slowing the hydrolysis of urea allows more time for its dispersion from application site in the soil by means of rain or irrigation (Byrnes and Freney 1996). However, the efficiency of urea is decreased by losses of N as ammonia gas after the urea is hydrolyzed at the soil surface by reaction with the enzyme urease.

Freney *et al* (1993) in N balanced study on cotton showed that in the absence of nitrification inhibitors only 57% of applied N was recovered in the plants and soils at crop maturity, the recovery was increased ($p < 0.05$) up to 70% by addition of phenylacetylene, 74% by nitrapyrin, 78% by coated calcium carbide and 92% by 2-ethynylpyridine, in a large scale field experiment and it was reported that addition of wax coated calcium carbide significantly slowed the rate of NH_4^+ oxidation. The lint yield was significantly increased by addition of inhibitors. The inhibitors helped to conserve the indigenous as well as applied N. Yaseen *et al* (2006) suggested that with the combined application of CaC_2 and N fertilizer in cotton, N uptake was increased up to 31.5% over the application of N fertilizer alone.

Modification of sources also enhances the nutrient uptake by plants from fertilizers which can be achieved either chemically to reduce their solubility (by coating/encapsulation) and use of N stabilizers (nitrapyrin, dicyandiamide, n-butylthiophosphorictriamide) to inhibit nitrification or urease activity (Prasad and Power 1995, Havlin *et al* 2005) that will increase N use efficiency. Slow release fertilizers are excellent alternatives to soluble fertilizers. Nitrification inhibitors can increase fertilizer N recovery by delaying the nitrification of ammonical fertilizers and subsequently minimizing the losses of N through leaching and denitrification (Juma and Paul 1983). One of the common liquid N fertilizers, ammonium thiosulphate (ATS), has been reported to be a nitrification inhibitor (Goos and Johnson 1992). Nitrification inhibitor can limit the amount of inorganic N in the NO_3^- form and minimize denitrification. Using ATS with UAN (Urea ammonium nitrate) alone may reduce denitrification losses and improve N fertilizer use efficiency. Poor recovery of N fertilizer in furrow irrigated cotton due to N loss through denitrification can be improved by using nitrification inhibitors such as ethynyl pyridine, etridiazole and nitrapyrin (Rochester *et al* 1996).

Fashola *et al* (2002) found that slow release fertilizers markedly increased yields and NUE compared with urea. Polymer-coated products, which can be designed to release N in a controlled manner are promising slow release N sources (Shoji and Kanno 1995). Fertigation (N fertilizer such as urea-ammonium nitrate-UAN, applied through center pivot irrigation

systems) is a practice that is gaining in popularity in many areas due to reduced application costs. The preferred fertigation method is to apply 20% of the total N required in a preplant application, additional 50% at squaring stage, with the remaining 30% by early bloom (Hons *et al* 2001)

The placement of N fertilizer also helps to increase NUE in cotton. N fertilizer should be placed a short distance from where seedling roots will grow, especially when N is applied at sowing. Fertilizer N can be applied in different ways, but commonly used are surface or sub-surface applications before or after planting. With surface application, N can be lost via volatilization until it is incorporated or moves into the soil with precipitation (Mohanty *et al* 1999). In contrast, deep placement of urea or urea super granules (USG) results in improved N use efficiency by reducing losses through ammonia volatilization.

The environmental conditions have to be considered while making fertilizer use timing and rate decisions. The split applications have also been found to be beneficial in improving N use efficiency in cotton. Soormo *et al* (2001) revealed that the yield, biomass, number of bolls and N uptake was significantly affected by N fertilizer application at various timings and splits. It was also reported that the yield, biomass, number of bolls and N uptake were significantly higher when N was applied in four equal splits at different timings as compared with one or two splits. Mullins *et al* (2003) suggested that when leaching potentials are great in sandy soils of the Coastal Plain, N should be applied in a more splits.

The approaches based on reducing N losses by split application, deep placement of urea super granules, controlled release N fertilizers, nitrification inhibitors etc. have been successful in improving fertilizer N use efficiency but to a limited extent (Buresh 2007). The advances in plant nutrition management have shifted from reducing nutrient losses to synchronizing plant demand with the fertilizer supply. The recent research approaches are thus oriented more towards finding means and ways to apply fertilizer N in real-time using crop and field-specific needs. The truthful alternative is the plant tissue analysis at different growth stages. However, it takes 10-14 days to predict the plant N requirement by analyzing the plant tissue sample and does not seem to be a practical proposition. The potential alternative for making need based fertilizer N management decisions is to measure spectral properties of leaves using gadgets like SPAD meter or LCC (Varinderpal-Singh *et al* 2010). The farmers use leaf color as a visual and subjective indicator of the need for N fertilizer (Wells and Turner 1984, Furuya 1987). Since farmers generally prefer to keep leaves of the crop dark green, it leads to over application of fertilizers N resulting in low use efficiency. The relationship between fertilizer N uptake and total N uptake over the growing season depends on timing of the fertilizer N application (Guindo *et al* 1994) and understanding of spectral properties of leaves can help to decide best N application timings. Synchrony between plant-available N in the rooting zone and the crop uptake of N may minimize N

losses prior to plant uptake (Ladha *et al* 2005).

2.3 Need-based fertilizer N management

Nitrogen need to be applied in right amount at right time and through right method. Fertilizer N-recovery efficiency up to 50-70 % of total N has been achieved when it was applied at proper rate and at the proper time (Peng and Cassman 1998, Wang *et al* 2001). Determination of N requirement of the crop, available soil N and N application rate was also required for optimum yield (Bufogle *et al* 1997). The concept of using spectral reflectance ratio to quantify color of intact crop leaves was reported in the early sixties in Japan (Inada 1963). Later during 80's and early 90's the studies (Jund and Turner 1990, Peng *et al* 1993) focused on using gadgets such as LCC based on spectral properties of leaves / SPAD meter based on light transmittance through leaves for guiding real time need based fertilizer N management in rice and wheat. Based on color of leaf, SPAD meter and LCC have been successfully used to know when the crop needs N application (Balasubramanian *et al* 2003, Bijay-Singh *et al* 2002). These techniques have been evaluated in a wide range of farmer's fields in Asia and are now positioned for wider scale validation and farmer adaptation. These technologies provide instantaneous results and have been demonstrated as an effective tool to schedule N fertilization to crops (Turner and Jund 1994, Peng *et al* 1993). These gadgets helped efficient N management in rice (Varinderpal-Singh *et al* 2007, Yadvinder-Singh *et al* 2007), wheat (Varinderpal-Singh *et al* 2012, Bijay-Singh *et al* 2002, Shukla *et al* 2004, Alam *et al* 2005, Maiti and Das 2006) and maize (Varinderpal-Singh *et al* 2010) under situations encountering diversity in field, season and variety by ensuring high yields and providing economic benefits to the farmers. Bajwa *et al* (2004) observed the usefulness of canopy reflectance in identifying N stress in cotton fields. Vegetative indices derived from canopy reflectance have showed strong correlation with N rates. Optical sensors provide a rapid and non destructive in-season diagnosis of cotton N status and cotton biomass (Bronson *et al* 2005, Yabaji *et al* 2009). The gadgets (SPAD meter, LCC and GreenSeeker) are discussed below.

2.3.1 Chlorophyll (SPAD) meter

The SPAD meter is a compact, light weight meter which can be used to determine the amount of chlorophyll present in plant leaves (Takebe and Yoneyama 1989, Takabe *et al* 1990). The Minolta SPAD meter (Minolta, 1989) provides readings (SPAD units) which, within limits, are directly correlated with chlorophyll content (Peterson *et al* 1993, Nielsen *et al* 1995) and tissue N concentration. SPAD meter are reliable alternatives to traditional tissue analysis as plant N nutritional diagnostic tools. These instantly provide an estimate of leaf N status as chlorophyll content by clamping the un-plucked leafy tissue in the meter. It has two LEDs (light emitting diodes) which emit light with a peak wavelength of 650 nm and an infrared radiation peak wavelength of 940 nm. The red and infrared radiations are made to

pass through the leaf. A portion of light is absorbed and the remainder is transmitted through the leaf and silicon photodiode detector converts it into an electrical signal. The amount of light reaching the photodiode detector is inversely proportional to the amount of chlorophyll in the path of the light. Leaf chlorophyll content is displayed in arbitrary units (0-99.9). The SPAD meter readings are unitless and need to be calibrated with chlorophyll or N content and leaf greenness. The SPAD meter uses a silicon photodiode to derive the ratio of transmittance through the leaf tissue at 650 nm compared with transmittance at 940 nm, and a value was given based on that ratio (Schepers and Francis 1998).

It has been used successfully for synchronizing N application with N needs of rice (Balasubramanian *et al* 1999, Hussain *et al* 2000, Bijay-Singh *et al* 2002), maize (*Zea mays* L.) (Peterson *et al* 1993), wheat (Follett *et al* 1992) and cotton (Bronson *et al* 2005, Yabaji *et al* 2009). The SPAD meter was the tool that enables to determine the relative amount of chlorophyll content by measuring leaf greenness (Peterson *et al* 1993) and the linear relationship of SPAD readings and N status in crops varies depending on the growth stages and cultivars. Since chlorophyll content was usually strongly related to N concentration, SPAD meters can be used as indicator of need for N application (Schepers *et al* 1992, Blackmer and Schepers, 1995).

The SPAD meter readings do not depend on environmental brightness levels (Sibley *et al* 1994). The results of the application of the indirect SPAD meter have been found satisfactory for the assessment of the N nutritional status of crop (Fox *et al* 1994). Hence, the evaluation of N nutritional status of cotton could be done with the SPAD meter, using readings made in leaves of the middle third from top of the plant (Neves *et al* 2005). The hand-held chlorophyll meter has been used to rapidly determine greenness of cotton leaves, and indirectly N status (Wood *et al* 1992, Wu *et al* 1998, Bronson *et al* 2001). Both chlorophyll meter readings and petiole NO_3^- were positively affected by N fertilizer rate, but petiole NO_3^- was more variable (Bronson *et al* 2001). Leaf chlorophyll content estimated by chlorophyll meter readings correlated with corn yield just as well as leaf N content (Schepers *et al* 1992).

Malavolta *et al* (2004) found a positive correlation between chlorophyll concentration (SPAD readings) and cotton lint yields. He concluded that SPAD readings during the flowering period were effective in determining the nutritional status of the plant and were related with cotton yields. According to Malavolta (2006), cotton leaf samples for chlorophyll content analysis with the SPAD meter must be collected at full bloom and the meter must be placed on the side lobes of the 4th or 5th leaf from the apex. Rosolem and Mellis (2010) suggested that the chlorophyll meter can also be used to estimate the nutritional status of cotton from the third week of flowering. In this case the readings should be above 48 in well-nourished plants.

Bronson *et al* (2001) reported that in-season chlorophyll meter measurements of cotton correlated with petiole NO_3^- -N, leaf N, and lint yield. Wood *et al* (1992) observed that chlorophyll meter measurements correlated with cotton seed yield better than petiole NO_3^- -N concentrations. Chlorophyll meter as a guide to in-season N fertilization can also be used following sufficiency index approach that needs to maintain a well-fertilized reference plot (Varvel *et al* 1997, Hussain *et al* 2000). In-season N was applied when average SPAD meter readings of the test plot was below 95% of chlorophyll meter to be used in different environments and with different varieties, factors that affect chlorophyll meter readings (Peterson *et al* 1993).

Johnson and Saunders (2003) proved that Minolta SPAD meter as most reliable hand held meters for infield readouts when monitoring nitrogen in cotton and observed that there was no difference in the SPAD readings of plots having different N levels at first week of bloom. However, at second week of bloom the SPAD readings were lower for 30 lbs N ac^{-1} than the 60, 90, 120 and 150 lbs N ac^{-1} . There was no difference in the seed cotton yield above the 60 lbs N ac^{-1} . Rosolem and Mellis (2010) also reported usefulness of chlorophyll meter to estimate the N need of cotton.

Wang *et al* (1992) reported that additional fertilizer was usually applied at early flowering or flowering stage for short-season cotton, while at peak flowering stage for mid-season cultivars in Yangtze River delta and the middle Yangtze plain (Wu and Jia 1994). Later fertilizer applications following wet conditions could lead to severe production problems by further delaying maturity and by producing excessive vegetative growth (Wang *et al* 1992, Wu and Jia 1994, Wu *et al* 1986). Therefore, SPAD readings should be taken at the early flowering stage to allow sufficient time for applied N fertilizer to remedy any N deficiency determined by the test.

A field experiment was conducted in China by Wu *et al* (1998) at two locations using five N levels (0, 45, 90, 135, 180 kg N ha^{-1}) at ZAU farm and six N levels (0, 47.8, 92.8, 137.8, 182.8, 227.8 kg N ha^{-1}) at the Cixi site to study the relationship between SPAD readings and physiological or yield traits in short-season cotton. The results showed that there were highly significant ($P < 0.01$) linear relationships between SPAD readings and contents of both nitrogen and chlorophyll at each growth stage and as well as with the daily increase in plant height during early flowering. These data provided evidence that the SPAD meter could be used to determine side dress N requirements of short-season cotton before boll opening stage. It was also established that 24.2–25.0 kg ha^{-1} increase in N application should be necessary for each unit decrease in SPAD readings below the critical level.

Chua *et al* (2003) used chlorophyll meter measurements as in-season N decision aids for irrigated cotton in comparison with soil test based N management. Need-based fertilizer N applications produced lint yield similar to the soil test based fertilizer N applications with

the use of 34-101 kg ha⁻¹ less nitrogen. They observed that need-based N applications based on in-season monitoring of spectral properties can reduce N applications in low yielding seasons and match the high yield potential in high yielding seasons.

2.3.2 Leaf color charts (LCC)

The relatively high cost of the SPAD meter seems to have limited its acceptance by the farmers even in USA (Turner and Jund 1994). SPAD meter is highly useful for researches and crop consultants as research and training tool. A Simple, quick and non destructive tool for estimating leaf N status is LCC. First LCC was developed in Japan (Furuya 1987). LCC is a high quality plastic strip with different shades of green color ranging from light yellowish green to dark green. It consists of six color shades ranging from light yellowish green (No. 1) to dark green (No. 6) color strips fabricated with veins resembling those of leaves.

An improved version of six panel LCC was developed through collaboration of the International Rice Research Institute (IRRI) with agricultural research systems of several countries in Asia (IRRI 1996). Recently, researchers at IRRI have further refined the color panels of the IRRI-LCC to best match the spectral reflectance of plant leaves and a four panel IRRI-LCC (four green color shades from number 2 to number 5) has been promoted since 2003 (Fairhurst *et al* 2007). The lightest (shade 1) and the darkest (shade 6) shades of the six panel LCC have been removed and shades of panel number 2, 3, 4 and 5 have been standardized for modern high yielding rice varieties in Asia (Witt *et al* 2005).

The LCC technology have been established for real time N management in wheat (Varinderpal-Singh *et al* 2012), rice (Yadvinder-Singh *et al* 2007) and maize (Varinderpal-Singh *et al* 2010). The critical or threshold values of the LCC are defined as the intensity of green color that must be maintained in the uppermost fully opened leaf and fertilizer N recommendations are given whenever leaf greenness was below the critical LCC value. Leaf greenness or leaf N content was closely related to photosynthesis rate and biomass production and was a sensitive indicator of changes in crop N demand during the growing season. Thus, maintaining the leaf greenness just above the LCC critical value ensures high yields with need based N applications thereby leading to high fertilizer N use efficiency. At least 10 disease free healthy plants were selected at random for each plot and color of the index leaf (first fully exposed leaf) of selected plants was compared with the color strips of the chart. If six or more leaves read below the set critical value, fertilizer N was applied in the form of urea as per plant need. No work has been reported on the spectral properties of cotton using LCC. The use of LCC for scheduling N application may not be uniformly applicable to all varieties that differ in inherent leaf color, thereby necessitating individual or group standardization (Sheoran *et al* 2004).

Win (2003) reported that LCC values were closely related to SPAD readings in rice

($r=0.94$) and wheat ($r=0.97$). Similarly, Shukla *et al* (2004) correlated LCC and SPAD readings and reported a strong correlation ($r=0.84-0.91$) between both rice and wheat. Thus, the economical LCC can be used as an alternative to the costly SPAD meter for the purpose of guiding need based fertilizer N applications in crops.

2.3.3 Optical sensors based N management

The GreenSeeker Hand Held Data Collection and Mapping Unit is a crop research and consulting tool that provides useful data to determine Normalized Difference Vegetative Index (NDVI) and Red to Near Infrared Ratios

$$NDVI = (NIR_{ref} - RED_{ref}) / (NIR_{ref} + RED_{ref})$$

These data points can be used in conjunction with other agronomic references to index basic nutrient response, crop condition, yield potential, stress, pest and disease impact in a quantitative objective manner. The unit can be used to monitor changing field (crop, plant) conditions during the growing season and the effects of different levels of an input compared to a local standard. Recent advances in optical sensing allow the collection of information on crop growth and physiological parameters temporally and spatially as affected by environmental stresses. Reflection of light at leaf level was primarily dependent on the anatomy of the leaf (Asner 1998). GreenSeeker hand held optical sensor (N Tech industries, Inc. Ukiah, CA.) developed by Oklahoma State University, senses a 0.6×0.01 m area when held at a distance approximately 67 cm from the illuminated surface. The sensed dimensions remain approximately constant over the height range of sensor. The sensor unit has self-contained illumination in both red ($671 + 6$ nm) and near infra red (NIR) ($780 + 6$ nm) bands. The device measures the fraction of emitted light in the sensed area that was reflected back to the sensor (reflectance).

Chlorophyll contained in the palisade layer of the leaf controls much of the visible light (400-720 nm) reflectance. Chlorophyll absorbs between 70 to 90 percent of all incident light in the blue and red wavelength bands while reflecting light in the green band (Campbell 2002). The amount of light reflected in the visible region is defined by the chlorophyll content in the cell and the amount of light reflected in the near infra red (NIR) region is defined by living vegetation or biomass. The amount of blue and red light absorbed by the leaf is proportional to the chlorophyll density of the leaf. Reflectance of the NIR portion of the electromagnetic spectrum (720-1300 nm) is predominantly influenced by the mesophyll cells. The upper layers of the leaf are nearly transparent to NIR energy. Mesophyll tissue scatters and reflects as much as 60 % of all incident NIR radiation. The degree to which near infrared energy was reflected depends on the structure of the mesophyll cells and cavities between these cells (Campbell 2002).

Several researchers have assessed N status and other physiological parameters of field crops using optical sensors (Zhao *et al* 2003, Tarpley *et al* 2000, Read *et al* 2002).

Tarpley *et al* (2000) reported that using specific reflectance ratios (*i.e.* leaf reflectance values at 700 or 716 nm divided by reflectance values at 755 to 920 nm) could improve precision and accuracy in predicting cotton leaf N content. In maize plant, 50-70 % of plant N was contained in the chloroplast (Vleeshouwers and Jongschaap 2001). Ercoli *et al* (1993) reported a strong linear relationship between leaf chlorophyll and leaf N content. The relationship between plant reflectance and N concentration has also been established in field situations (Bausch and Duke 1996, Bausch *et al* 1996).

Saranga *et al* (1998) evaluated reflectance based and blanket fertilizer N recommendations and observed that seed cotton yield and number of bolls per meter square were same in both the treatments but in reflectance based treatments only 60 kg N ha⁻¹ was used as compared with blanket recommendation of 150 kg N ha⁻¹. Bronson *et al* (2011) observed that seed and lint yield were similar in soil test and reflectance based treatment but in reflectance based 50% less N was used. Read *et al* (2002) noted that as the N deficiency in cotton increases, chlorophyll content and the rate of leaf expansion and canopy development will decrease. Ma *et al* (1996) found that remotely sensed canopy reflectance measurements could provide an in-season indication of N deficiency in maize hybrids. Lough and Varco (2000) evaluated the relationship between N treatment level and relative leaf reflectance and found that the greatest separation between N treatments occurred at 550 nm (green) waveband in cotton. According to Buscaglia and Varco (2002), cotton leaf N content have a strong linear correlation with leaf reflectance at 550, 612, 700 and 728 nm.

Tarpley *et al* (2000) found that the reflectance ratios, which were obtained by dividing cotton reflectance at 700 nm or 760 nm by a higher reflectance at 755 to 920 nm, could provide good accuracy in predicting nitrogen concentration. Tucker (1979) proposed the normalized difference vegetative index or NDVI as $(RNIR - Rred)/(RNIR + Rred)$, where *RNIR* and *Rred* are reflectance in the NIR and in the red regions, respectively. The NDVI has been correlated with leaf area index, canopy cover, and chlorophyll concentration in cotton (Thomas and Gausman 1977, Maas 1998, Huete and Jackson 1988). Li *et al* (2001) measured spectral reflectance from 3 m above the ground and reported that NDVI and red vegetative index were positively correlated with cotton biomass, N accumulation, and lint yield. Leaf N was negatively correlated with blue reflectance in the Li *et al* (2001) study. Spectral reflectance therefore may help determine N fertilizer needs in cotton, regardless of soil test NO₃-N results. In addition to canopy measurements of spectral reflectance, recent studies have demonstrated the use of measuring spectral reflectance of harvested cotton leaves to estimate N content (Saranga *et al* 1998, Tarpley *et al* 2000).

A statistical correlation among the NDVI sensor readings, and number of days after planting versus the N rate was found in multiple studies. In a study conducted by Khalian *et al* (2008) it was found that the optimum dates of sensing falls somewhere within the time

frame of 39-67 days after emergence. Sensor readings collected before and after this range were poorly correlated with the actual yield and could not be used to accurately predict the in-season N requirements for the plant.

Taylor *et al* (2007) found similar results and stated there was no significant difference in NDVI values as affected by pre-plant N rate before 38 days after planting (DAP) and after 70 DAP. The correlations found in multiple studies of sensing date and pre-plant nitrogen rate have shown that there was a possibility to predict the in-season nitrogen requirements of the cotton plant using mid-season sensor readings, however these sensor readings must be collected within a specific range of DAP to accurately predict the N requirement. The optimum range of sensing discovered in these studies falls well within the optimum range of N uptake by the cotton plant discovered in the study performed by Boquet and Breitenbeck (2000).

Earnest and Varco (2005) observed that canopy reflectance determines leaf N content and plant height and could help in improving N use efficiency. In a variable N rate plot study conducted at Mississippi State University the results showed that NDVI correlated strongly with leaf N content at peak bloom and reflectance data acquired at or after peak bloom could be useful in predicting yield. The results of a study conducted by Emerine (2006) showed an average reduction of 50.4 kg fertilizer N ha⁻¹ without any reduction in yield. However, Arnall (2008) observed that NDVI N management is a new practice which still requires more research to better understand plant responses to sensor readings.

CHAPTER III

MATERIALS AND METHODS

An account of location, climate during crop season, materials used, methodology and techniques adopted in conducting the present investigation entitled, "**SPECTRAL PROPERTIES OF LEAVES IN RELATION TO YIELD, NITROGEN CONTENT, UPTAKE AND USE EFFICIENCIES IN COTTON**" are presented in this chapter.

3.1 Site

The field experiment was conducted during *kharif* 2012 at the experimental farm Department of soil science, Punjab Agricultural University, Ludhiana, Punjab, India.

3.2 Weather and Climate

Ludhiana is situated at 30°56' N latitude and 75°52' E longitude with a mean height of 247 meter above the mean sea level. The climate of the location is designated as sub-tropical and semi-arid with hot and dry summer (April to June), hot and humid monsoon period (July to September), mild winter (October to November) and cold winter (December to February). The mean maximum and minimum temperatures show considerable variations during different months of year. The maximum air temperature often exceeds 38°C during summer and sometimes touches 45°C with dry spells during May and June. Minimum temperature falls below 0.5°C with frosty spells during winter months of December and January. The average annual rainfall of Ludhiana is 650 mm, about three-fourth of which is contributed by the south-west monsoon during July to September. Scanty rainfall is received during winter months of December, January and February.

The meteorological data recorded at the meteorological observatory of the Punjab Agricultural University, Ludhiana located at a distance of 50 m from the experimental field during the crop growing season (May to Dec) is presented in Table 3.2.1 and Fig. 3.2.1.

3.3 Soil properties

Analysis of 5 composite soil samples taken from 0-0.15 m depth before starting the experiment revealed that soil was sandy loam in texture, non-saline, non-calcareous and neutral in reaction. The soil of the experimental site is classified as Typic Ustisamments. The soil characteristics before the start of experiment are given in Table 3.3.1

3.4 Experimental detail

Technical Programme of work

i) **Name of Experiment:** To study the spectral properties of leaves in relation to yield, nitrogen content, uptake and use efficiencies in cotton.

Table 3.2.1. Weekly mean meteorological data during crop season

Standard Meteorological Week (SMW)		Temperature (°C)			Relative humidity (%)			Rainfall (mm)	No. of rainy days	Total evaporation (mm)	Sunshine (hrs)
Week No.	Dates	Max.	Min.	Mean	Morning	Evening	Mean				
19	7-5-12 to 13-5-12	39.0	23.1	31.1	49.0	19.6	34.3	0.0	0.0	64.2	9.1
20	14-5-12 to 20-5-12	39.7	23.6	31.7	48.1	19.6	33.9	1.6	1.0	66.3	7.1
21	21-5-12 to 27-5-12	40.9	23.1	32.0	44.9	17.3	31.1	0.0	0.0	72.4	11.4
22	28-5-12 to 3-6-12	43.7	25.9	34.8	43.6	18.9	26.6	0.0	0.0	80.0	10.9
23	04-06-12 to 10-06-12	39.2	25.4	32.3	57.6	33.3	45.4	1.5	1.0	66.8	7.2
24	11-06-12 to 17-06-12	42.1	25.9	34.0	55.7	28.4	42.1	0.0	0.0	88.0	11.4
25	18-06-12 to 24-06-12	40.8	29.3	35.0	56.4	33.4	44.9	2.0	2.0	75.0	8.3
26	25-06-12 to 01-07-12	39.4	27.9	33.6	63.1	39.6	43.9	0.0	0.0	70.0	8.7
27	02-07-12 to 08-07-12	36.9	28.6	32.7	70.0	53.0	61.5	14.5	3.0	64.1	6.6
28	09-07-12 to 15-07-12	35.0	27.6	31.3	78.7	56.0	67.4	5.6	3.0	42.2	6.2
29	16-07-12 to 22-07-12	36.4	27.8	32.1	72.6	52.1	62.4	1.6	1.0	48.0	9.3
30	23-07-12 to 29-07-12	35.0	28.0	31.5	82.1	67.6	64.4	44.8	2.0	38.8	4.9
31	30-07-12 to 05-08-12	33.8	27.4	30.6	80.0	68.0	74.0	11.4	2.0	35.0	4.7
32	06-08-12 to 12-08-12	34.0	27.1	30.5	88.0	72.4	80.2	24.3	3.0	26.3	4.0
33	13-08-12 to 19-08-12	33.8	26.8	30.3	86.4	72.7	79.6	56.4	4.0	26.1	5.9
34	20-08-12 to 26-08-12	31.5	25.9	28.7	90.7	64.7	77.7	40.2	4.0	25.6	3.3
35	27-08-12 to 02-09-12	33.0	26.0	29.5	90.6	69.3	68.2	40.7	5.0	21.4	4.2
36	03-09-12 to 09-09-12	32.8	26.3	29.5	81.6	69.0	75.3	18.8	2.0	27.9	6.5

Standard Meteorological Week (SMW)		Temperature (°C)			Relative humidity (%)			Rainfall (mm)	No. of rainy days	Total evaporation (mm)	Sunshine (hrs)
Week No.	Dates	Max.	Min.	Mean	Morning	Evening	Mean				
37	10-09-12 to 16-09-12	33.9	25.5	29.7	89.0	63.7	76.4	93.4	4.0	22.8	7.1
38	17-09-12 to 23-09-12	31.0	22.5	26.7	93.0	68.1	80.6	27.3	2.0	22.8	7.9
39	24-09-12 to 30-09-12	33.0	20.7	26.6	93.7	48.4	60.7	0.0	0.0	28.6	10.3
40	01-10-12 to 07-10-12	34.0	19.8	26.9	89.7	44.1	66.9	0.0	0.0	28.0	9.9
41	08-10-12 to 14-10-12	33.4	17.4	25.4	90.6	41.3	65.9	0.0	0.0	27.2	9.2
42	15-10-12 to 21-10-12	30.8	15.9	23.4	90.0	43.7	66.9	0.0	0.0	24.0	7.4
43	22-10-12 to 28-10-12	29.1	12.7	21.5	90.1	44.9	67.5	0.1	0.0	20.8	8.7
44	29-10-12 to 4-11-12	29.6	13.6	21.6	92.0	42.4	57.2	0.0	0.0	18.4	7.0
45	5-11-12 to 11-11-12	28.7	12.4	20.6	91.1	39.4	65.3	0.0	0.0	13.8	5.5
46	12-11-12 to 18-11-12	26.2	10.5	18.4	95.0	44.7	69.9	0.0	0.0	11.4	6.2
47	19-11-12 to 25-11-12	25.8	9.0	17.1	89	36	63	0.0	0.0	14.0	6.9
48	26-11-12 to 2-12-12	23.7	7.5	15.6	89	39	64	0.0	0.0	15.9	8.1
49	3-12-12 to 9-12-12	24.1	5.9	15.0	91	37	64	0.0	0.0	14.0	8.1
50	10-12-12 to 16-12-12	20.1	9.7	14.9	91	65	78	17.4	2.0	9.7	4.4
51	17-12-12 to 23-12-12	19.4	6.9	13.1	93	59	76	0.0	0.0	12.4	7.6

Source: School of Climate Change and Agricultural Meteorology, Punjab Agricultural University, Ludhiana.

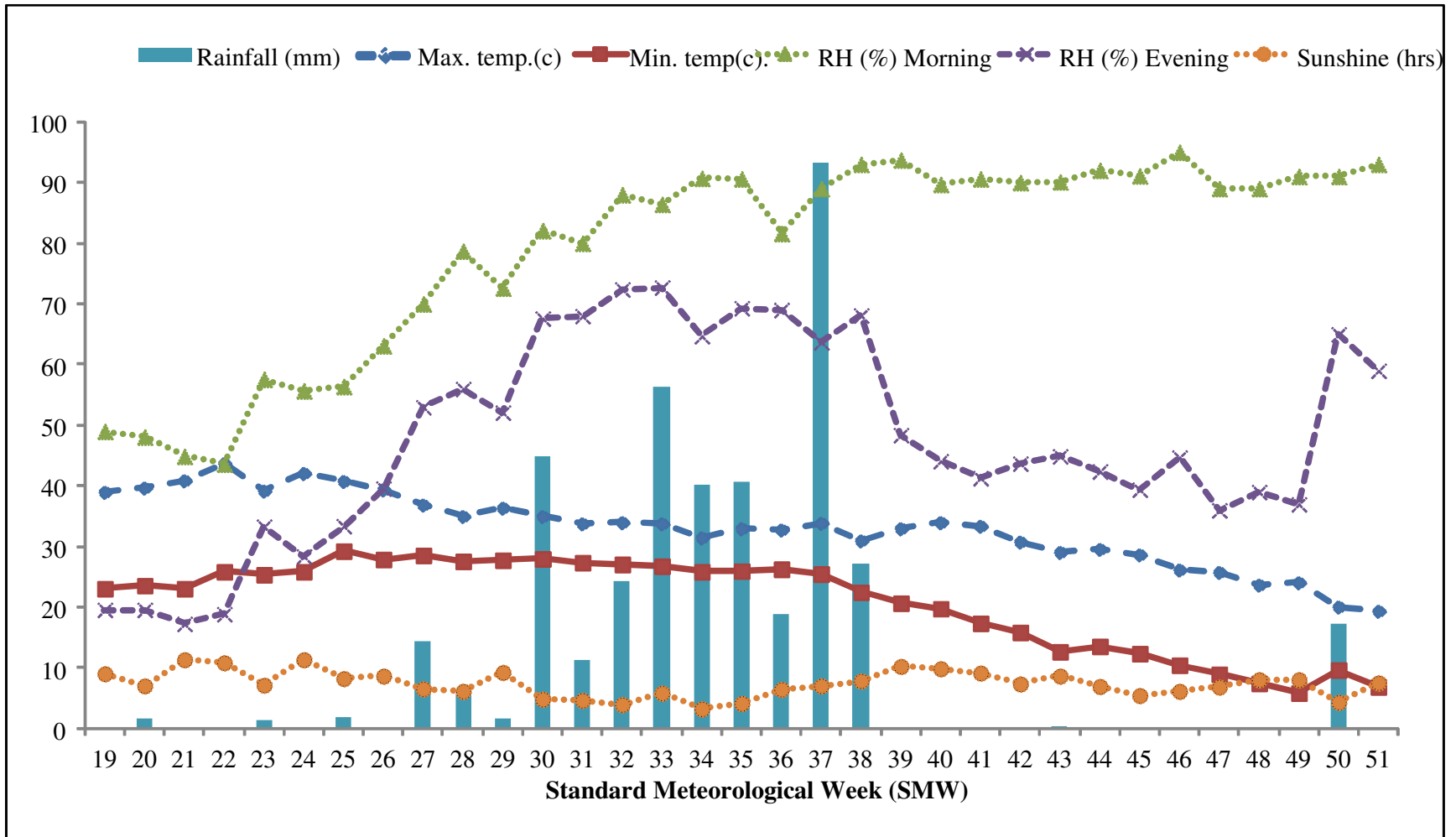


Fig. 3.2.1. Weekly mean meteorological data recorded during the crop season (2012) at School of Climate Change and Agricultural Meteorology, PAU, Ludhiana

Table 3.3.1. Physico-chemical characteristics of surface soil (0-0.15 m) sample at start of the experiment

Parameters	Values
Sand (%)	76.27
Silt (%)	18.33
Clay (%)	5.2
Available N (kg ha ⁻¹)	84.8
Ammonical-N (mg kg ⁻¹)	12.0
Nitrate-N (mg kg ⁻¹)	13.0
pH (1:2)	7.09
EC (dSm ⁻¹) 1:2	0.15
Available Fe (mg kg ⁻¹)	40.2
Available Mn (mg kg ⁻¹)	19.2
Soil organic carbon (%)	0.36
Olsen's P (kg ha ⁻¹)	25.2
Ammonium acetate extractable K (kg ha ⁻¹)	101.3

ii) Location: Research Farm, Department of Soil Science, Punjab Agricultural University, Ludhiana.

iii) Treatment

A. Main plots:

Two cotton cultivars

1. MRC 7017
2. ANKUR 3028

B. Sub-plot

Nitrogen levels (kg ha⁻¹)

1. No-N control
2. 60 N
3. 75 N
4. 90 N
5. 120 N
6. 180 N
7. 240 N

Layout details

Design : Split plot
 Number of treatment combinations : 7 x 2

Replications	: 3
Total No. of plots	: 42
Date of sowing	: 12-May-2012
Plot size	: 9 x 2.7 (24.3 m ²)

3.4 Crop management

3.4.1 Seedbed preparation

The pre sowing irrigation was applied in the first fortnight of May, 2012. After attaining field capacity, the field was tilled thrice with a tractor drawn cultivator followed by planking to obtain good seed bed.

3.4.2 Sowing

The crop was sown on May 12, 2012. The sowing was done after attaining field capacity with the hand drill. Cotton cultivars MRC 7017 and ANKUR 3028 were sown at row to row spacing of 60 cm and plant to plant spacing of 67.5 cm in different plots. The seed was sown at the rate of 1.875 kg ha⁻¹ as recommended in package of practices for crops of Punjab.

3.4.3 Fertilizer

At the time of planting phosphorus (@ 30 kg P₂O₅ ha⁻¹) and potassium (@ 30 kg K₂O ha⁻¹) were applied respectively as single super phosphate and muriate of potash. Zinc was applied through ZnSO₄ monohydrate @ 16 kg ha⁻¹. In addition to soil applied fertilizers, four sprays of 2% KNO₃ (13:0:45) were given at weekly intervals starting from flower initiation in all the treatments.

3.4.4 Weed control measures

The weeds were controlled as desired following recommended package of practices by Punjab Agricultural University, Ludhiana. Herbicide Stomp 30 EC (Pendimethalin) @ 2.5 liter ha⁻¹ was applied within 24 hours of sowing. Three hand hoeing were done to control the weeds after first, second and third irrigation respectively.

3.4.5 Irrigation

The field was irrigated three days before sowing with a pre-sowing irrigation and first irrigation was applied twenty days after sowing (DAS) and subsequent was given according to need of the crop.

3.4.6 Plant protection

Trizophos 40EC @ 1.5 litre ha⁻¹ and Ethion 50EC @ 2 litre ha⁻¹ was used to control whitefly, since Bt cotton does not provide effective control of sucking pests like whitefly, aphids, etc.

3.4.7 Picking and Stover yield

The crop was ready for picking in the fourth week of September. The picking was done after every 10 days interval to avoid losses. Five pickings were done and the yield of

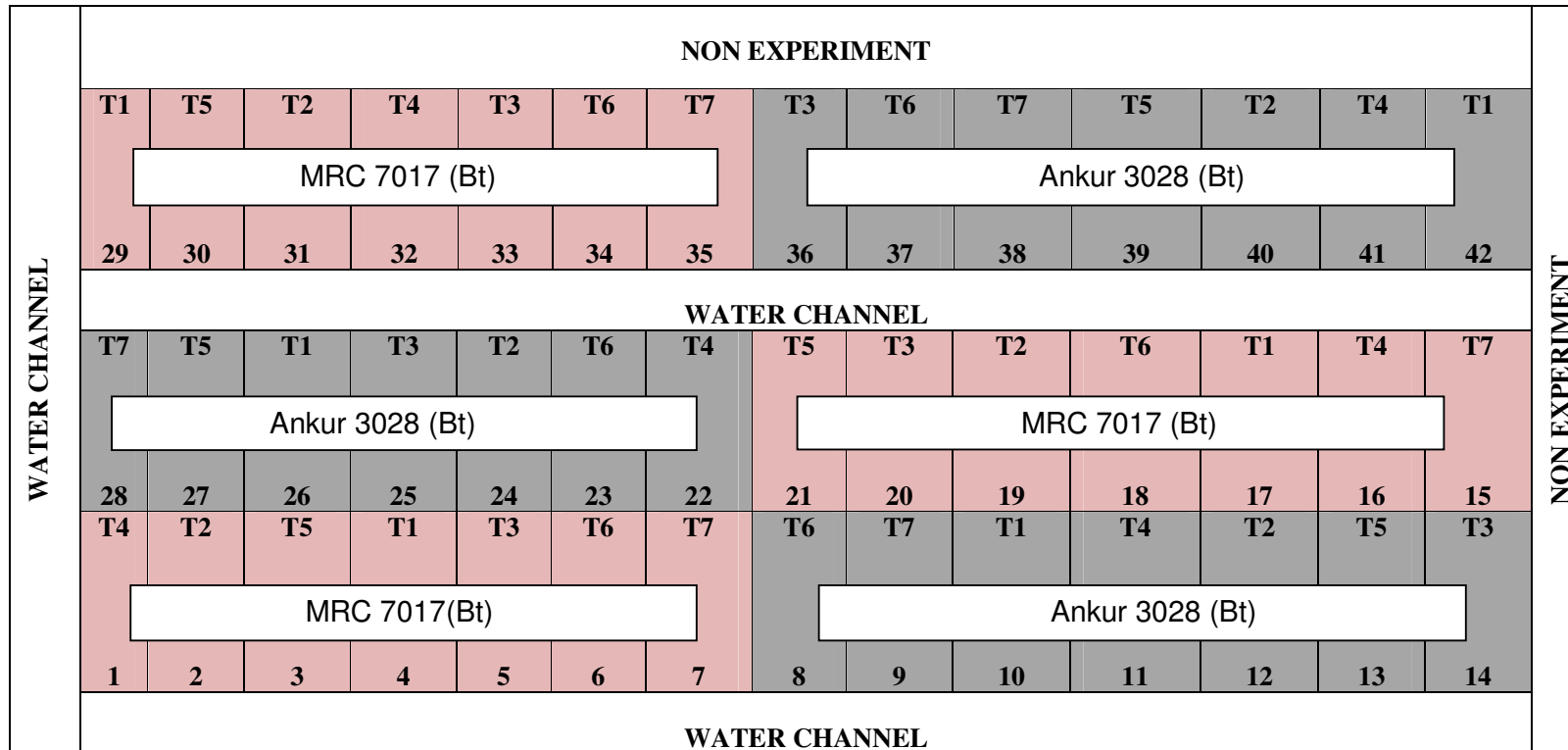


Fig. 3.4.1. Layout plan of the experiment

cotton lint was recorded after every picking separately from each plot. Bundle weight of the stover was recorded at time of harvesting.

3.5 Measurement of spectral properties of leaves

Spectral properties of cotton leaves were measured using LCC, SPAD 502 and GreenSeeker optical sensor. Spectral properties were measured at 10 days interval starting from node formation (21 DAS) stage to boll opening stage (101DAS). First fully exposed leaf from top of the plant was used as an index leaf to measure the spectral properties.

Table 3.5.1. Dates of LCC, SPAD meter and GreenSeeker optical sensor measurement

Date	Growth stages (DAS)
June 2, 2012	Node formation stage (21)
June 12, 2012	First Squaring stage (31)
June 22, 2012	Squaring stage (41)
July 2, 2012	Early flowering stage (51)
July 12, 2012	Mid flowering stage (61)
July 22, 2012	Peak flowering stage (71)
August 1, 2012	Boll formation stage (81)
August 11, 2012	Boll filling stage (91)
August 21, 2012	Boll opening stage (101)

3.5.2 LCC (leaf color chart) measurement

LCC is an economical and farmer's friendly gadget to measure green color intensity of leaf which is related to plant nitrogen contents. LCC is a high quality plastic strip with different shades of green color ranging from light yellowish green (No. 1) to dark green (No. 6). The LCC readings were taken at 10 day intervals starting from node formation stage to boll opening stage. The readings were taken at the same time of the day with the sun at the back to shade the leaf being measured. Ten disease free healthy cotton plants were selected at random for each plot and color of the first fully exposed top leaf of selected plants was compared with the color strips of the chart.

3.5.3 SPAD 502 chlorophyll meter measurement

SPAD meter is a simple, portable diagnostic tool that measures the greenness or relative chlorophyll content of leaves. Before taking readings the meter was calibrated by switching on the meter with no leaf in the sample slot, followed by completely closing the measuring head until a beep sound was heard. The meter was thus calibrated and ready for measurements. The receiving window of the SPAD meter was cleaned with a soft cloth before taking measurements. The moisture present on the sample leaves was avoided while recording the data. The selected leaf was inserted into the sample slot of measuring head of SPAD meter

to make sure that the sample was completely covered by the receiving window. The measuring head was kept completely closed until a beep sound was heard which indicated that measurement was over and the value appeared on the display. The chlorophyll reading was automatically saved in the meter.

The head was opened and the reading for next leaf was taken in the same way. The average of all the SPAD meter readings for a plot were recorded. After noting the average SPAD readings of the plot, the meter was cleared for the next set of reading by pressing the "all data clear" button. The extremely thick, disease affected and insect bitten leaves were avoided for measurement. For cotton, the SPAD meter reading were recorded from the fully expanded uppermost leaf of 10 randomly selected plants. The SPAD meter readings were taken at 10 days interval starting from node formation stage to boll opening stage.

3.5.4 GreenSeeker Optical sensor

The GreenSeeker™ hand held optical sensor unit Model 505 was used to measure NDVI from the crop canopy. Before taking the readings, iPAQ was inserted in the powered cradle and battery was charged properly. Then shoulder strap was put around the body and sensor angle was such adjusted that it was parallel to sensing area at a height of about 70 cm above the canopy. The trigger of GreenSeeker optical sensor was pressed continuously while moving in the two middle crop rows and trigger was released after completing one plot. A photodiode detector within the sensor measured the magnitude of the light reflected off the target and NDVI was computed. The data from the sensor was transmitted serially to HP iPAQ Personal Digital Assistant, which was later exported to a desktop computer for analysis. Normalized difference vegetative index (NDVI) an indicator of total biomass and greenness of leaves is computed with the following equation:

$$\text{NDVI} = (\text{NIR}_{\text{ref}} - \text{RED}_{\text{ref}}) / (\text{NIR}_{\text{ref}} + \text{RED}_{\text{ref}})$$

Where, NIR_{ref} or Red_{ref} represents reflectance in the near infrared and red bands. The NDVI values were taken at 10 days interval starting from node formation stage to boll opening stage of cotton.

3.6 Collection and analysis of plant samples

Leaf samples of ten fully exposed top leaves from different cotton plants were collected at the time of recording LCC and SPAD meter data at different growth stages. The samples were oven dried at 60°C to constant weight and dry weight was recorded. Dried samples were ground in grinder for estimating N content. The stover samples collected at harvesting were also dried at 60°C to constant weight and ground for N analysis. Cotton lint samples were also processed for N analysis. Nitrogen concentration in the leaf, lint and stover was determined by Kjeldhal method (Bremner and Mulvaney 1982). A 0.5 g portion of the samples was digested in concentrated sulfuric acid with digestion mixture (K_2SO_4 , CuSO_4 , Se, HgO and salicylic acid) in triplicate. The extract was diluted to 50 ml. A 5 ml

aliquot was taken in a distillation flask to which 10 ml of 40 per cent NaOH was added. About 30 ml of distillate was collected in 4% boric acid, which was then titrated with N/70 H₂SO₄ to know per cent N content. The readings were used to compute per cent N content in plant samples.

3.8 Methods used for soil analysis

3.8.1 Soil reaction (Jackson, 1973)

Soil pH was measured using Elico pH meter. Ten gram portion of soil was taken in 50 ml beaker and 20 ml of distilled water was added to it. The soil-water slurry was stirred for 30 minutes. The pH meter was calibrated with buffer solution and pH of the soil-water suspension was measured using glass electrode assembly attached to the pH meter.

3.8.2 Electrical conductivity (Chopra and Kanwar, 1976)

25 g portion of soil was taken in a beaker and 50 ml of distilled water was added to it. The soil-water suspension was stirred with a glass rod for about 30 minutes and then allowed to stand overnight in order to obtain clear supernatant solution. Conductivity cell attached to Elico EC Bridge was lowered into supernatant solution so that platinum electrodes were completely immersed in solution. Reading was noted on the scale and conductance was calculated by multiplying the reading on EC bridge with cell constant.

3.8.3 Organic carbon (Walkley and Black, 1934)

2 g portion of soil sample was placed in a 250 ml conical flask to which 10 ml of IN K₂Cr₂O₇ solution was added. The contents were mixed and 20 ml of concentrated H₂SO₄ was added while swirling the flask. The contents of the flask were allowed to cool for 30 minutes to complete the reaction. Three gram of NaF and 100 ml of distilled water was added and the contents were vigorously mixed. After adding 10 drops of diphenylamine indicator, contents of the flask were titrated against 0.5 N ferrous ammonium sulphate solution till the color changed from violet to bright green. A no-soil blank titration was also carried out.

3.8.4 Available phosphorus (Olsen and Sommers, 1982)

One gram portion of the soil sample was taken in a 100 ml conical flask. A pinch of Darco-G 60 and 20 ml of 0.5 N NaHCO₃ solutions were added. Contents were shaken for half an hour on a end-to-end shaker and then filtered through Whatman No. 1 filter paper. Five ml aliquot of the filtrate was taken in a 25 ml volumetric flask to which 5 ml of 1.5% ammonium molybdate solution was added. About 10 ml of distilled water washing the neck of the flask was added. Then 1 ml of dilute SnCl₂ (0.5 ml of 40% SnCl₂ solution in HCl diluted to 66 ml with distilled water) was added and volume was made up to the mark with distilled water. Intensity of the blue color developed was estimated using a colorimeter at a wavelength of 660 nm.

3.8.5 Available potassium (Jackson, 1973)

Available K was determined by extracting 5 g portion of soil sample with 25 ml

neutral normal ammonium acetate solution in a 150 ml conical flask. Contents were shaken on a shaker for 5 minutes and filtered through Whatman No. 1 filter paper. Potassium in the filtrate was estimated by measuring intensity of light emitted by the excitement of potassium ions in the digital flame photometer by comparing with standards containing known amounts of K.

3.8.6 Ammoniacal and Nitrate Nitrogen (Keeney 1982)

Mineral N ($\text{NH}_4^+ + \text{NO}_3^- - \text{N}$) extracted by shaking the soil sample with 2M KCl (1:10 soil: solution) for one hour and steam distillation of the extract using MgO powder and Deverda's alloy.

3.8.7 Available Fe and Mn (Lindsay and Norwell 1978)

Available Fe and Mn were measured by DTPA method.

3.8.8 Particle Size distribution

Soil texture was determined by International Pipette Method outlined by Piper (1966).

3.9 Nitrogen use efficiency

Different measures of nitrogen use efficiency-Recovery efficiency (RE_N) and Agronomic efficiency (AE_N) were calculated as defined by Baligar *et al* (2001)

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

where, N uptake is the total N uptake in cotton lint and cotton stover.

$$\text{AE}_\text{N} (\text{kg yield kg}^{-1} \text{ N}) = \frac{(\text{Seed cotton yield in N fertilized plot} - \text{Seed cotton yield in no N plot})}{(\text{Quantity of N fertilizer applied in fertilized plot})}$$

3.10 Statistical Analysis

Statistical analysis of data collected on different growth stages of the crop and at harvest was done as per Cochran and Cox (1967). The significance of differences among the treatments was judged by analysis of variance (ANOVA) in split plot design. The critical difference at 5 % level of significance was worked out whenever necessary.

CHAPTER IV

RESULTS AND DISCUSSION

The present study entitled 'SPECTRAL PROPERTIES OF LEAVES IN RELATION TO YIELD, NITROGEN CONTENT, UPTAKE AND USE EFFICIENCIES IN COTTON' was conducted at the experimental farm of the Department of Soil Science, Punjab Agricultural University, Ludhiana during *kharif* 2012. Data recorded on different parameters of crop are discussed under the following heads:

4.1 Temporal changes and N affect on LCC, SPAD readings and NDVI values

The SPAD meter and LCC readings of the first top fully exposed leaf of two cotton cultivars as recorded at various growth stages of cotton are presented in Table 4.1.1 and Table 4.1.2, respectively. The data revealed that the green colour intensity of the first top fully exposed leaf of the cotton plant as measured using SPAD meter and LCC do not differ across the two cultivars MRC 7017 and Ankur 3028 at different growth stages of cotton. The average SPAD meter and LCC readings of two cotton cultivars as affected by fertilizer N application were plotted against different growth stages in Fig. 4.1.1 and Fig. 4.1.2, respectively.

The SPAD meter readings of cotton leaves at node formation stage (21 DAS) were statistically at par irrespective of the level of fertilizer N application. The effect of fertilizer N application on SPAD meter readings was visible at first squaring stage (31 DAS) when SPAD meter readings of cotton leaves improved with fertilizer N application. However the differences among the rate of fertilizer N application remain insignificant. The SPAD meter readings of no-N control plot was significantly less than fertilizer N application treatments at all the growth stages, however there was no significant difference in the SPAD meter readings with the rate of fertilizer N application. The SPAD readings showed an increasing trend during initial growth stages followed by decline toward the maturity of the crop in all the fertilizer N application treatments. The maximum value was observed at squaring stage followed by steep decline during the flowering stages. The SPAD readings remain almost constant during boll formation, boll filling and boll opening stages of the cotton. The SPAD readings of the no N control treatment showed a decline at first squaring and remain almost constant during squaring stage followed by decline during flowering stages and again become constant during boll formation, boll filling and boll opening stages. Insignificant differences in SPAD meter readings of no N control and fertilizer N application treatments at node formation stage may be due to sufficient N supply in no N control treatment from the inherent soil N at early growth stages. Johnson and Saunders (2003) also observed insignificant difference in SPAD meter readings of no-N and fertilizer N plots during the initial growth stages of cotton.

The LCC readings of cotton leaves do not differ with the fertilizer N application at node formation, first squaring and boll opening stages and effect of fertilizer N application on LCC reading was visible only at squaring stage, early flowering, mid flowering, peak flowering, boll formation and boll filling stages of cotton when leaf colour greenness of no-N control plot was significantly lower than the fertilizer N application treatments. The differences in the leaf colour greenness of the cotton plants were not significant among the levels of fertilizer N in fertilizer N application treatments at all the growth stages of cotton. The maximum LCC reading in fertilizer N application treatments were observed at early flowering stage those were closer to leaf colour greenness at squaring and mid flowering stages followed by decline at later growth stages. The leaf colour greenness in no N control treatment remain almost constant till peak flowering stage followed by slight decline during boll formation, boll filling and boll opening stages of cotton. The differences in LCC readings of cotton at different growth stages were not as prominent as in SPAD meter readings. The SPAD meter being more sensitive and accurate was able to differentiate the variation in leaf greenness at different growth stages more precisely than LCC.

The NDVI values of two cotton cultivars measured using GreenSeeker optical sensor are provided in the Table 4.1.3. The average NDVI values for the two cultivars as affected by fertilizer N application are plotted against the different growth stages in Fig. 4.1.3. The data revealed that cotton cultivars do not differ in NDVI values at different growth stages of cotton. The effect of fertilizer N application on NDVI values was not significant at node formation, first squaring and squaring stages of cotton. The NDVI values of no-N control and fertilizer N treatments differ significantly at early flowering, mid flowering, peak flowering, boll formation, boll filling and boll opening stages, however the NDVI values do not differ among the levels of fertilizer N in N application treatments at all the growth stages of cotton. The NDVI values showed an increasing trend with the growth of the crop at different levels of fertilizer N application and maximum value was observed at boll opening stage of cotton. The cotton showed steep increase in NDVI values after early flowering till boll filling stage at all the levels of fertilizer N application. The increasing NDVI values with the growth of the crop indicate increase in biomass of the crop with time. Reflectance in the visible range and red edge was affected by the N rate (Zhao *et al* 2005). Fridgen and Varco 2004 reported that the cotton treatment with 0 N ha⁻¹ had higher reflectance than the other fertilizer N treatments near 550 nm at early flower and peak bloom.

Table 4.1.1. The SPAD meter readings of different cotton cultivars as affected by fertilizer N application rate at various growth stages of cotton

Cultivars	N application rate (kg ha ⁻¹)							
	0 N	60 N	75 N	90 N	120 N	180 N	240 N	Mean
Node formation stage (21 DAS)								
MRC 7017	42.3	44.7	46.0	45.7	44.3	44.3	46.0	44.8
Ankur 3028	42.3	43.7	43.0	44.7	44.0	44.3	45.7	44.0
Mean	42.3	44.2	44.5	45.2	44.2	44.3	45.8	
LSD (p=0.05)	Variety = NS; Nitrogen = NS; Variety x Nitrogen = NS							
First squaring stage (31 DAS)								
MRC 7017	40.3	46.4	46.6	47.5	47.2	45.6	46.8	45.8
Ankur 3028	39.3	46.1	46.0	47.1	47.0	46.9	45.7	45.4
Mean	39.8	46.2	46.3	47.3	47.1	46.3	46.2	
LSD (p=0.05)	Variety = NS; Nitrogen = 2.10; Variety x Nitrogen = NS							
Squaring stage (41 DAS)								
MRC 7017	41.7	50.0	49.9	48.4	49.4	51.0	48.2	48.4
Ankur 3028	41.1	49.7	50.5	50.3	50.9	48.9	49.3	48.7
Mean	41.4	49.8	50.2	49.4	50.1	49.9	48.7	
LSD (p=0.05)	Variety = NS; Nitrogen = 2.00; Variety x Nitrogen = NS							
Early flowering stage (51 DAS)								
MRC 7017	38.6	47.3	47.0	48.0	48.7	49.0	48.3	46.7
Ankur 3028	37.9	46.0	46.3	48.0	46.7	45.0	45.7	45.1
Mean	38.2	46.7	46.7	48.0	47.7	47.0	47.0	
LSD (p=0.05)	Variety = 1.19; Nitrogen = 1.60; Variety x Nitrogen = NS							
Mid flowering stage (61 DAS)								
MRC 7017	34.7	44.2	44.6	43.2	43.4	42.6	44.4	42.4
Ankur 3028	32.7	44.5	44.4	44.5	43.1	43.4	43.0	42.2
Mean	33.7	44.4	44.5	43.8	43.3	43.0	43.7	
LSD (p=0.05)	Variety = NS; Nitrogen = 1.90; Variety x Nitrogen = NS							
Peak flowering stage (71 DAS)								
MRC 7017	33.8	36.2	36.9	37.4	36.1	37.1	38.1	36.5
Ankur 3028	31.8	35.3	35.3	36.7	36.2	36.6	35.9	35.4
Mean	32.8	35.7	36.1	37.0	36.1	36.9	37.0	
LSD (p=0.05)	Variety = NS; Nitrogen = 1.66; Variety x Nitrogen = NS							

Cultivars	N application rate (kg ha ⁻¹)							
	0 N	60 N	75 N	90 N	120 N	180 N	240 N	Mean
Boll formation stage (81 DAS)								
MRC 7017	31.6	36.4	36.1	36.3	36.5	37.0	36.3	35.7
Ankur 3028	29.8	33.4	33.4	33.3	34.7	35.6	35.7	33.7
Mean	30.7	34.9	34.8	34.8	35.6	36.3	36.0	
LSD(p=0.05)	Variety = NS; Nitrogen = 2.23; Variety x Nitrogen = NS							
Boll filling stage (91 DAS)								
MRC 7017	31.3	36.5	36.2	36.4	36.7	37.4	36.6	35.9
Ankur 3028	31.7	36.4	35.8	35.2	35.6	36.2	35.2	35.1
Mean	31.5	36.4	36.0	35.8	36.1	36.8	35.9	
LSD(p=0.05)	Variety = NS; Nitrogen = 1.72; Variety x Nitrogen = NS							
Boll opening stage (101 DAS)								
MRC 7017	30.7	33.8	33.7	34.7	34.1	35.3	36.4	34.1
Ankur 3028	31.8	34.2	34.2	34.5	35.9	33.6	35.0	34.2
Mean	31.2	34.0	33.9	34.6	35.0	34.4	35.7	
LSD(p=0.05)	Variety = NS; Nitrogen = 1.49; Variety x Nitrogen = NS							

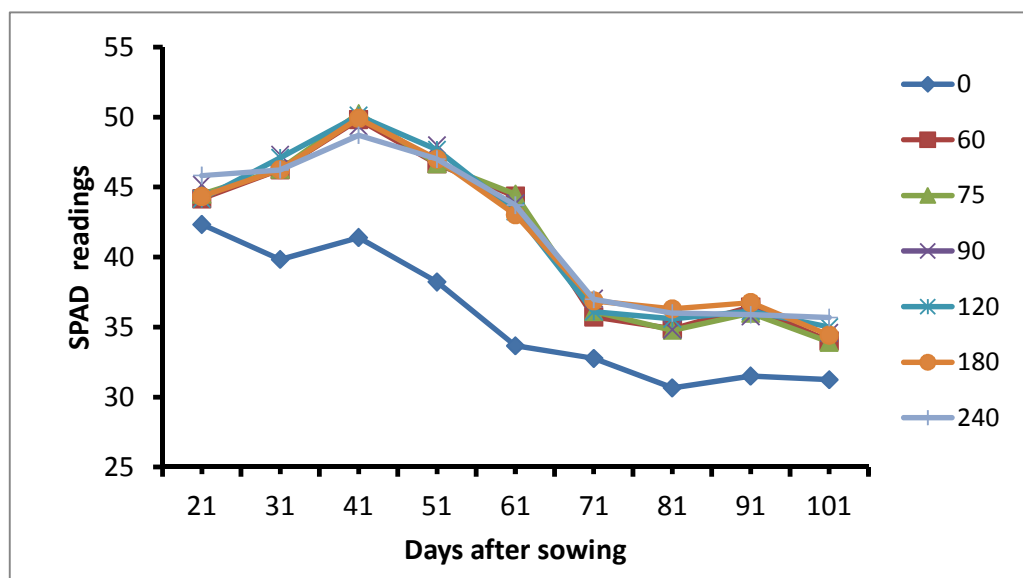


Fig. 4.1.1. The effect of fertilizer N application rate on average SPAD meter readings of two cotton cultivars at different growth stages of cotton

Table 4.1.2. The LCC readings of different cotton cultivars as affected by fertilizer N application rate at various growth stages of cotton

Cultivars	N application rate (kg ha ⁻¹)							Mean
	0 N	60 N	75 N	90 N	120 N	180 N	240 N	
Node formation stage (21 DAS)								
MRC 7017	4.12	4.20	4.27	4.22	4.28	4.17	4.28	4.22
Ankur 3028	4.17	4.22	4.25	4.20	4.17	4.22	4.28	4.22
Mean	4.14	4.21	4.26	4.21	4.23	4.19	4.28	
LSD (p=0.05)	Variety = NS; Nitrogen = NS; Variety x Nitrogen = NS							
First squaring stage (31 DAS)								
MRC 7017	4.00	4.15	4.13	4.25	4.28	4.15	4.15	4.16
Ankur 3028	4.00	4.13	4.15	4.18	4.13	4.13	4.18	4.13
Mean	4.00	4.14	4.14	4.22	4.21	4.14	4.17	
LSD (p=0.05)	Variety = NS; Nitrogen = NS; Variety x Nitrogen = NS							
Squaring stage (41 DAS)								
MRC 7017	4.00	4.35	4.32	4.38	4.38	4.30	4.37	4.30
Ankur 3028	4.00	4.35	4.35	4.32	4.40	4.35	4.32	4.30
Mean	4.00	4.35	4.33	4.35	4.39	4.33	4.34	
LSD (p=0.05)	Variety = NS; Nitrogen = 0.09; Variety x Nitrogen = NS							
Early flowering stage (51 DAS)								
MRC 7017	4.00	4.33	4.30	4.37	4.38	4.42	4.35	4.31
Ankur 3028	4.00	4.47	4.35	4.37	4.47	4.43	4.40	4.35
Mean	4.00	4.40	4.33	4.37	4.43	4.43	4.38	
LSD (p=0.05)	Variety = NS; Nitrogen = 0.09; Variety x Nitrogen = NS							
Mid flowering stage (61 DAS)								
MRC 7017	3.92	4.32	4.32	4.33	4.38	4.32	4.40	4.28
Ankur 3028	4.07	4.25	4.30	4.23	4.25	4.32	4.37	4.25
Mean	3.99	4.28	4.31	4.28	4.32	4.32	4.38	
LSD (p=0.05)	Variety = NS; Nitrogen = 0.09; Variety x Nitrogen = NS							
Peak flowering stage (71 DAS)								
MRC 7017	4.03	4.20	4.17	4.17	4.13	4.17	4.23	4.16
Ankur 3028	4.03	4.30	4.27	4.23	4.27	4.20	4.33	4.23
Mean	4.03	4.25	4.22	4.20	4.20	4.18	4.28	
LSD (p=0.05)	Variety = NS; Nitrogen = 0.12; Variety x Nitrogen = NS							

Cultivars	N application rate (kg ha ⁻¹)							
	0 N	60 N	75 N	90 N	120 N	180 N	240 N	Mean
Boll formation stage (81 DAS)								
MRC 7017	3.90	4.20	4.20	4.20	4.17	4.28	4.23	4.17
Ankur 3028	3.93	4.12	4.10	4.09	4.05	4.13	4.10	4.08
Mean	3.92	4.16	4.15	4.15	4.11	4.21	4.17	
LSD (p=0.05)	Variety = NS; Nitrogen = 0.10; Variety x Nitrogen = NS							
Boll filling stage (91 DAS)								
MRC 7017	3.83	4.10	4.10	4.03	4.13	4.03	3.98	4.03
Ankur 3028	3.80	4.07	4.20	4.10	4.18	4.08	4.22	4.09
Mean	3.82	4.08	4.15	4.07	4.16	4.06	4.10	
LSD (p=0.05)	Variety = NS; Nitrogen = 0.15; Variety x Nitrogen = NS							
Boll opening stage (101 DAS)								
MRC 7017	3.80	3.80	3.80	3.82	3.80	3.80	3.83	3.81
Ankur 3028	3.80	3.80	3.80	3.83	3.87	3.87	3.83	3.83
3.3.1. Mean	3.80	3.80	3.80	3.83	3.83	3.83	3.83	
LSD (p=0.05)	Variety = NS; Nitrogen = NS; Variety x Nitrogen = NS							

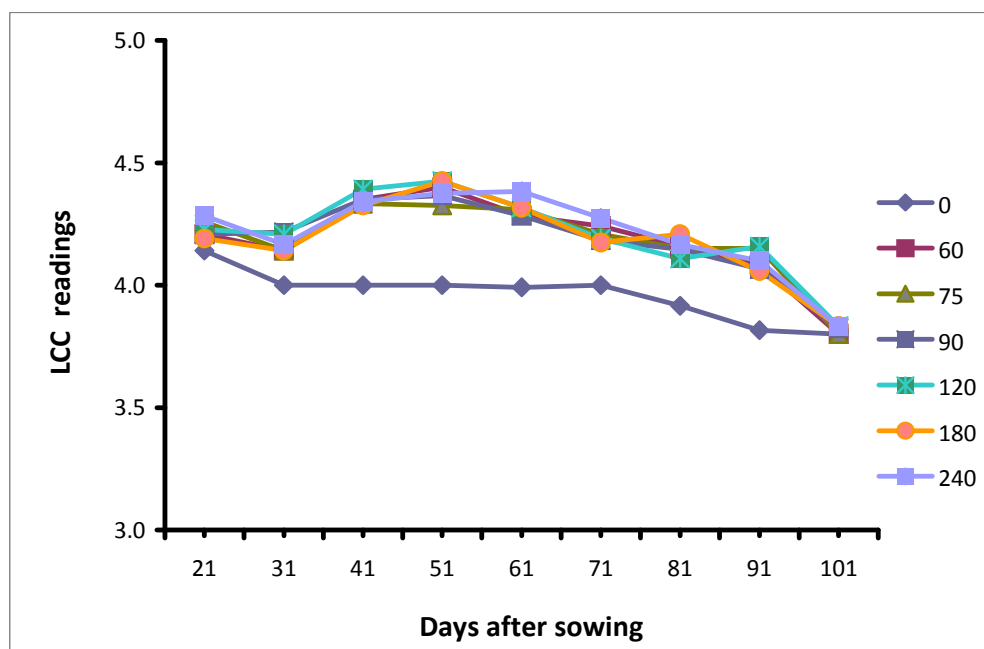


Fig. 4.1.2. The effect of fertilizer N application rate on average LCC readings of two cotton cultivars at different growth stages of cotton

Table 4.1.3. The NDVI values of different cotton cultivars as affected by fertilizer N application rate at various growth stages of cotton

Cultivars	N application rate (kg ha ⁻¹)							
	0 N	60 N	75 N	90 N	120 N	180 N	240N	Mean
Node formation stage (21 DAS)								
MRC 7017	0.13	0.12	0.15	0.13	0.13	0.13	0.14	0.13
Ankur 3028	0.13	0.14	0.12	0.13	0.12	0.14	0.12	0.13
Mean	0.13	0.13	0.14	0.13	0.13	0.13	0.13	
LSD (p=0.05)	Variety = NS; Nitrogen = NS; Variety x Nitrogen = NS							
First squaring stage (31 DAS)								
MRC 7017	0.18	0.20	0.20	0.19	0.20	0.20	0.20	0.20
Ankur 3028	0.19	0.20	0.20	0.20	0.21	0.20	0.22	0.20
Mean	0.18	0.20	0.20	0.20	0.21	0.20	0.21	
LSD (p=0.05)	Variety = NS; Nitrogen = NS; Variety x Nitrogen = NS							
Squaring stage (41 DAS)								
MRC 7017	0.15	0.21	0.21	0.21	0.21	0.20	0.22	0.20
Ankur 3028	0.16	0.20	0.23	0.20	0.20	0.21	0.20	0.20
Mean	0.16	0.20	0.22	0.21	0.20	0.20	0.21	
LSD (p=0.05)	Variety = NS; Nitrogen = NS; Variety x Nitrogen = NS							
Early flowering stage (51 DAS)								
MRC 7017	0.20	0.26	0.26	0.26	0.25	0.26	0.26	0.25
Ankur 3028	0.19	0.25	0.24	0.25	0.25	0.26	0.26	0.24
Mean	0.20	0.25	0.25	0.26	0.25	0.26	0.26	
LSD (p=0.05)	Variety = NS; Nitrogen = 0.03; Variety x Nitrogen = NS							
Mid flowering stage (61 DAS)								
MRC 7017	0.33	0.49	0.48	0.49	0.49	0.48	0.47	0.46
Ankur 3028	0.35	0.46	0.45	0.43	0.44	0.43	0.43	0.43
Mean	0.34	0.47	0.46	0.46	0.46	0.46	0.45	
LSD (p=0.05)	Variety = NS; Nitrogen = 0.03; Variety x Nitrogen = NS							
Peak flowering stage (71 DAS)								
MRC 7017	0.41	0.63	0.64	0.63	0.64	0.65	0.64	0.61
Ankur 3028	0.43	0.58	0.58	0.59	0.58	0.59	0.60	0.57
Mean	0.42	0.61	0.61	0.61	0.61	0.62	0.62	
LSD (p=0.05)	Variety = NS; Nitrogen = 0.03; Variety x Nitrogen = NS							

Cultivars	N application rate (kg ha ⁻¹)							
	0 N	60 N	75 N	90 N	120 N	180 N	240 N	Mean
Boll formation stage (81 DAS)								
MRC 7017	0.56	0.78	0.77	0.77	0.74	0.76	0.76	0.73
Ankur 3028	0.55	0.76	0.74	0.72	0.73	0.77	0.75	0.72
Mean	0.56	0.77	0.76	0.74	0.74	0.76	0.75	
LSD (p=0.05)	Variety = NS; Nitrogen = 0.06; Variety x Nitrogen = NS							
Boll filling stage (91 DAS)								
MRC 7017	0.68	0.80	0.82	0.81	0.82	0.81	0.83	0.80
Ankur 3028	0.68	0.83	0.82	0.82	0.84	0.83	0.84	0.81
Mean	0.68	0.82	0.82	0.82	0.83	0.82	0.83	
LSD (p=0.05)	Variety = NS; Nitrogen = 0.04; Variety x Nitrogen = NS							
Boll opening stage (101 DAS)								
MRC 7017	0.56	0.78	0.77	0.77	0.74	0.76	0.76	0.73
Ankur 3028	0.55	0.76	0.74	0.72	0.73	0.77	0.75	0.72
Mean	0.56	0.77	0.76	0.74	0.74	0.76	0.75	
LSD (p=0.05)	Variety = NS; Nitrogen = 0.04; Variety x Nitrogen = NS							

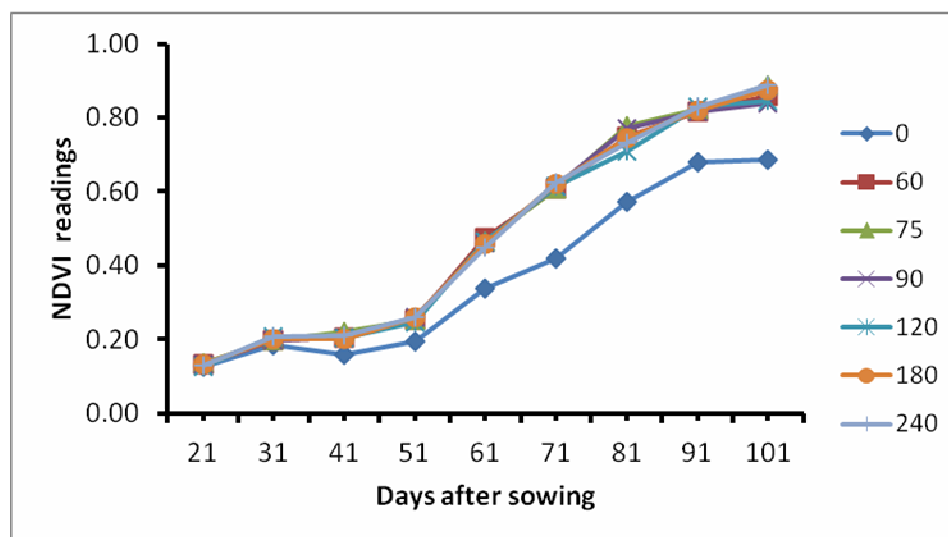


Fig. 4.1.3. The effect of fertilizer N application rate on average NDVI values of two cotton cultivars at different growth stages of cotton

4.2 Fertilizer N effect on leaf N content of cotton at different growth stages

The N content of leaves on dry weight basis of two different cultivars at various stages of growth is presented in Table 4.2.1. There was no significant difference in leaf N content between the cultivars at various growth stages of cotton except early flowering and mid flowering stages of cotton. The effect of fertilizer N level on leaf N content was visible at first squaring, squaring, early flowering, mid flowering, peak flowering, boll formation and boll filling stages of cotton. There was no difference in the leaf N content of cotton at node formation even among the no N control and fertilizer N application treatments. Insignificant differences in leaf N content at this stage indicates that inherent soil N supply was sufficient to support N assimilation from soil and thus maintained leaf N content equivalent to the N application treatment at node formation stage. The SPAD meter, LCC readings and NDVI values (Tables 4.1.1, 4.1.2, 4.1.3) also support the observation. The results suggest that cotton can sustain its growth till node formation stage even without application of fertilizer N at planting.

The leaf N content differences among no N control and fertilizer N application treatment become significant as crop growth promotes, however, leaf N content within fertilizer N treatment plots do not differ at various growth stages. Insignificant differences in leaf N content, SPAD meter, LCC readings and NDVI values of these plots suggest that 60 kg N ha⁻¹ may be sufficient to produce potential cotton seed yield. The leaf N content increased with growth stage and was found maximum at squaring stage of cotton which remains almost constant during flowering stages followed by decline towards the maturity of the crop (Fig. 4.2.1). Decrease in leaf N content at boll filling stage was attributed to translocation of leaf nitrogen to the reproductive organs for boll filling of the cotton (Yabaji 2006, Mullins and Burmester 2010).

4.3 Fertilizer N effect on leaf dry weight

The dry weight of ten index leaves of cotton collected at different growth stage of two different cultivars is provided in Table 4.3.1. There was no significant difference in leaf dry weight of two cultivars at different growth stages of cotton except boll filling stage. The effect of fertilizer N application on dry weight of leaf was not visible at node formation stage. The leaf dry weight of no N control was equivalent to different levels of fertilizer N application. The data support the observations recorded in SPAD meter, LCC readings, NDVI values and leaf N content attributed to sufficient inherent N supply to sustain plant growth till node formation stage of cotton without basal N application.

The leaf dry weight differences among no N control and fertilizer N application treatments were significant at first squaring, squaring, early flowering, mid flowering, peak flowering, boll formation, boll filling and boll opening stages, however leaf dry weight within

Table 4.2.1. The affect of N application on leaf N content of different cotton cultivars at various growth stages of cotton

Cultivars	N application rate (kg ha ⁻¹)							
	0 N	60 N	75 N	90 N	120 N	180 N	240 N	Mean
Node formation stage (21 DAS)								
MRC 7017	4.24	4.43	4.56	4.48	4.47	4.40	4.52	4.44
Ankur 3028	4.26	4.54	4.54	4.51	4.53	4.51	4.53	4.49
Mean	4.25	4.48	4.55	4.50	4.50	4.46	4.52	
LSD (p=0.05)	Variety = NS; Nitrogen = NS; Variety x Nitrogen = NS							
First squaring stage (31 DAS)								
MRC 7017	4.27	4.72	4.97	4.84	4.79	4.72	4.71	4.72
Ankur 3028	4.23	4.83	4.67	4.64	4.84	4.81	4.72	4.68
Mean	4.25	4.77	4.82	4.74	4.81	4.77	4.71	
LSD (p=0.05)	Variety = NS; Nitrogen = 0.31; Variety x Nitrogen = NS							
Squaring stage (41 DAS)								
MRC 7017	4.32	5.46	5.59	5.35	5.44	5.45	5.40	5.29
Ankur 3028	4.56	5.41	5.32	5.34	5.58	5.45	5.33	5.28
Mean	4.44	5.43	5.45	5.35	5.51	5.45	5.37	
LSD (p=0.05)	Variety = NS; Nitrogen = 0.24; Variety x Nitrogen = NS							
Early flowering stage (51 DAS)								
MRC 7017	4.33	5.31	5.20	5.37	5.30	5.33	5.47	5.19
Ankur 3028	4.38	5.50	5.47	5.50	5.33	5.40	5.30	5.27
Mean	4.36	5.41	5.33	5.44	5.32	5.37	5.38	
LSD (p=0.05)	Variety = 0.03; Nitrogen = 0.24; Variety x Nitrogen = NS							
Mid flowering stage (61 DAS)								
MRC 7017	4.15	5.15	5.30	5.27	5.33	5.43	5.28	5.13
Ankur 3028	4.30	5.35	5.30	5.40	5.49	5.51	5.49	5.26
Mean	4.22	5.25	5.30	5.34	5.41	5.47	5.39	
LSD (p=0.05)	Variety = 0.06; Nitrogen = 0.17; Variety x Nitrogen = NS							
Peak flowering stage (71 DAS)								
MRC 7017	4.41	5.33	5.35	5.27	5.43	5.37	5.43	5.23
Ankur 3028	4.30	5.43	5.63	5.37	5.61	5.43	5.45	5.32
Mean	4.36	5.38	5.49	5.32	5.52	5.40	5.44	
LSD (p=0.05)	Variety = NS; Nitrogen = 0.19; Variety x Nitrogen = NS							

Cultivars	N application rate (kg ha ⁻¹)							
	0 N	60 N	75 N	90 N	120 N	180 N	240 N	Mean
Boll formation stage (81 DAS)								
MRC 7017	4.29	5.21	5.24	5.20	5.23	5.20	5.30	5.10
Ankur 3028	4.27	5.17	5.27	5.20	5.37	5.27	5.37	5.13
Mean	4.28	5.19	5.26	5.20	5.30	5.24	5.33	
LSD (p=0.05)	Variety = NS; Nitrogen = 0.21; Variety x Nitrogen = NS							
Boll filling stage (91 DAS)								
MRC 7017	4.10	4.84	4.61	4.69	4.73	4.66	4.32	4.56
Ankur 3028	4.31	4.81	4.77	4.76	4.71	4.76	4.77	4.70
Mean	4.21	4.83	4.69	4.73	4.72	4.71	4.55	
LSD (p=0.05)	Variety = 0.03; Nitrogen = 0.23; Variety x Nitrogen = NS							
Boll opening stage (101 DAS)								
MRC 7017	4.48	4.72	4.81	4.69	4.76	4.65	4.68	4.69
Ankur 3028	4.49	4.69	4.73	4.80	4.76	4.73	4.75	4.71
Mean	4.49	4.71	4.77	4.75	4.76	4.69	4.71	
LSD (p=0.05)	Variety = NS; Nitrogen = NS; Variety x Nitrogen = NS							

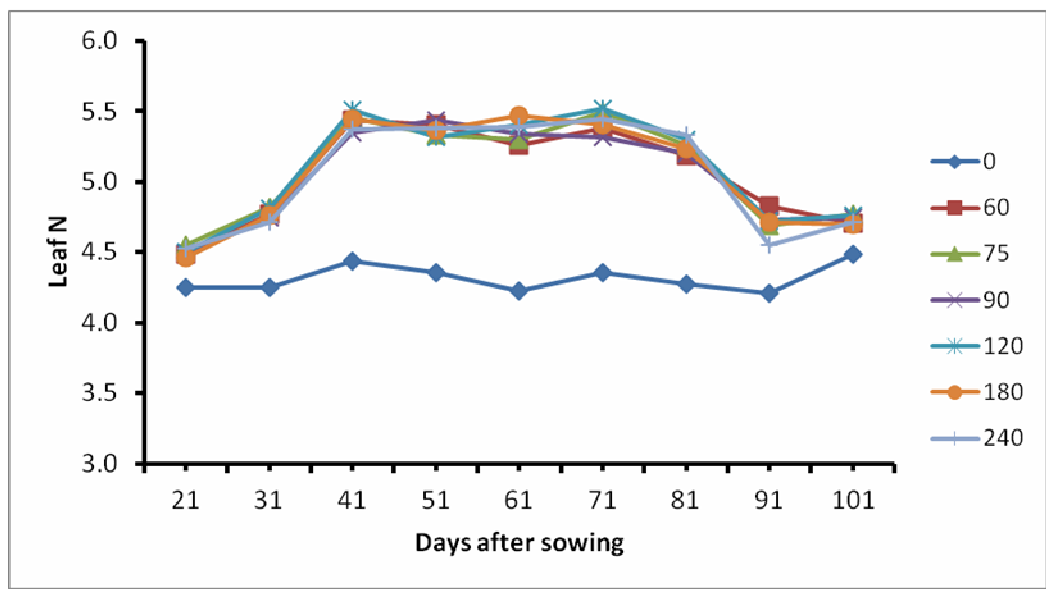


Fig. 4.2.1. The effect of fertilizer N application rate on average leaf N content of two cotton cultivars at different growth stages of cotton

Table 4.3.1. The affect of fertilizer N application on dry weight (g) of ten leaves collected from different cultivars at various growth stages of cotton

Cultivars	N application rate (kg ha ⁻¹)							
	0 N	60 N	75 N	90 N	120 N	180 N	240 N	Mean
Node formation stage (21 DAS)								
MRC 7017	0.47	0.48	0.46	0.47	0.46	0.47	0.48	0.47
Ankur 3028	0.46	0.47	0.48	0.46	0.45	0.46	0.45	0.46
Mean	0.46	0.48	0.47	0.47	0.45	0.47	0.46	
LSD (p=0.05)	Variety = NS; Nitrogen = NS; Variety x Nitrogen = NS							
First squaring stage (31 DAS)								
MRC 7017	1.13	1.53	1.52	1.53	1.54	1.62	1.72	1.51
Ankur 3028	1.14	1.53	1.50	1.50	1.50	1.58	1.62	1.48
Mean	1.14	1.53	1.51	1.52	1.52	1.60	1.67	
LSD (p=0.05)	Variety = NS; Nitrogen = 0.18; Variety x Nitrogen = NS							
Squaring stage (41 DAS)								
MRC 7017	2.20	2.76	2.71	2.79	2.83	2.77	2.82	2.70
Ankur 3028	2.14	2.76	2.80	2.70	2.66	2.70	2.74	2.64
Mean	2.17	2.76	2.76	2.74	2.75	2.74	2.78	
LSD (p=0.05)	Variety = NS; Nitrogen = 0.23; Variety x Nitrogen = NS							
Early flowering stage (51 DAS)								
MRC 7017	2.68	3.22	3.29	3.39	3.30	3.36	3.37	3.23
Ankur 3028	2.73	3.28	3.27	3.24	3.30	3.24	3.30	3.19
Mean	2.70	3.25	3.28	3.32	3.30	3.30	3.33	
LSD (p=0.05)	Variety = NS; Nitrogen = 0.26; Variety x Nitrogen = NS							
Mid flowering stage (61 DAS)								
MRC 7017	4.57	6.07	6.02	6.23	6.31	6.31	6.47	5.99
Ankur 3028	4.51	6.13	6.24	6.29	6.41	6.37	6.34	6.04
Mean	4.54	6.10	6.13	6.26	6.36	6.34	6.40	
LSD (p=0.05)	Variety = NS; Nitrogen = 0.53; Variety x Nitrogen = NS							
Peak flowering stage (71 DAS)								
MRC 7017	2.58	3.57	3.47	3.62	3.35	3.60	3.57	3.39
Ankur 3028	2.60	3.49	3.62	3.57	3.41	3.45	3.44	3.37
Mean	2.59	3.53	3.55	3.60	3.38	3.52	3.50	
LSD (p=0.05)	Variety = NS; Nitrogen = 0.35; Variety x Nitrogen = NS							

Cultivars	N application rate (kg ha ⁻¹)							
	0 N	60 N	75 N	90 N	120 N	180 N	240 N	Mean
Boll formation stage (81 DAS)								
MRC 7017	2.47	3.45	3.43	3.36	3.33	3.37	3.34	3.25
Ankur 3028	2.48	3.32	3.37	3.31	3.32	3.42	3.42	3.23
Mean	2.48	3.39	3.40	3.33	3.32	3.40	3.38	
LSD (p=0.05)	Variety = NS; Nitrogen = 0.27; Variety x Nitrogen = NS							
Boll filling stage (91 DAS)								
MRC 7017	2.43	3.43	3.44	3.38	3.40	3.54	3.50	3.30
Ankur 3028	2.51	3.48	3.36	3.53	3.60	3.53	3.60	3.37
Mean	2.47	3.46	3.40	3.45	3.50	3.53	3.55	
LSD (p=0.05)	Variety = NS; Nitrogen = 0.19; Variety x Nitrogen = NS							
Boll opening stage (101 DAS)								
MRC 7017	2.50	3.42	3.47	3.42	3.45	3.50	3.35	3.30
Ankur 3028	2.43	3.35	3.39	3.42	3.35	3.52	3.45	3.27
Mean	2.47	3.38	3.43	3.42	3.40	3.51	3.40	
LSD (p=0.05)	Variety = NS; Nitrogen = 0.32; Variety x Nitrogen = NS							

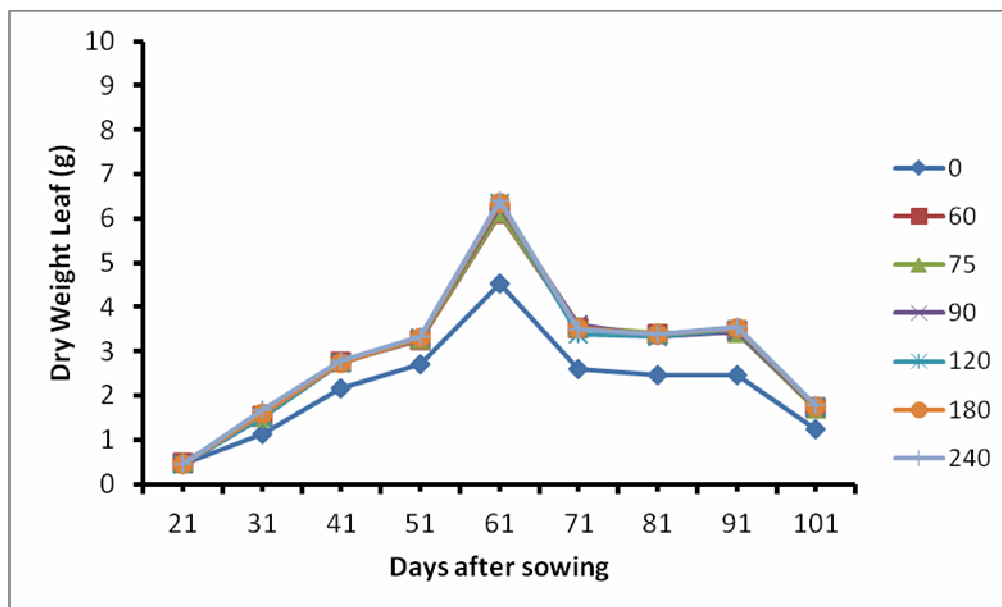


Fig. 4.3.1. The effect of fertilizer N application rate on average dry weight of ten leaves of two cotton cultivars at different growth stages of cotton

fertilizer N treatment plots do not differ at various growth stages. Insignificant differences in leaf dry weight, leaf N content, SPAD meter, LCC readings and NDVI values of these plots suggest that 60 kg N ha⁻¹ may be sufficient to produce potential cotton seed yield in this soil. The leaf dry weight increased with growth stage and was found maximum at mid flowering stage of cotton followed by decline towards the maturity of the crop (Fig. 4.3.1).

4.4 LCC, SPAD readings, NDVI values and leaf N content at different growth stages in cotton

The coefficient of determination (R^2) of LCC, SPAD meter readings and NDVI values in relation to leaf N content at different growth stages is presented in Fig. 4.4.1 to Fig. 4.4.9. The data revealed that LCC readings were correlated to leaf N content at $p=0.05$ level of significance at squaring, early flowering, mid flowering, peak flowering, boll formation and boll filling stage and maximum R^2 value (0.583) was observed at early flowering stage (Fig. 4.4.4). The SPAD meter being more sensitive than LCC is more accurate and gave better coefficient of determination for leaf N content than LCC at all the growth stages. The SPAD meter readings were correlated to leaf N content at $p=0.05$ level of significance at squaring, early flowering, mid flowering, peak flowering and boll formation stage and maximum R^2 value (0.635) was observed at mid flowering stage (Fig. 4.4.5). The NDVI values recorded with GreenSeeker optical sensor were correlated to leaf N content at $p=0.01$ level of significance at mid flowering, peak flowering and boll formation and maximum R^2 value (0.722) was observed at mid flowering stage (Fig. 4.4.5). The coefficient of determination obtained with NDVI values at mid flowering stage gave better understanding of leaf N content than SPAD meter and LCC readings; however NDVI values at early growth stages are not better indicator of leaf N content attributed to poor growth and interference of uncovered soil surface in data recording.

Buscaglia and Varco (2002) observed linear relationship between SPAD readings and leaf N content. They reported higher coefficient of determination and level of significance between the relative chlorophyll content and leaf N content at second week of flowering than at second week of squaring. Baker *et al* (1992) and Wood *et al* (1992) also found SPAD to be well correlated with leaf N content. Edmisten *et al* (1992) observed SPAD to be correlated with leaf N within sample date by site combinations, but variation in SPAD between samples dates was much greater than variation due to leaf N within sample dates. Bronsen *et al* (2003) reported SPAD reading correlated with leaf N but not with biomass production. They also found moderate correlations of NDVI values with leaf N content at early squaring but there was a general increase in correlations between the reflectance and leaf N status as the growing season progressed. Read *et al* (2002) indicated that in green region, the reflectance around 550 or 585 nm was closely correlated with cotton leaf N content. Motomiya *et al* (2007) also found a good correlation between N concentrations in cotton leaves and SPAD reading.

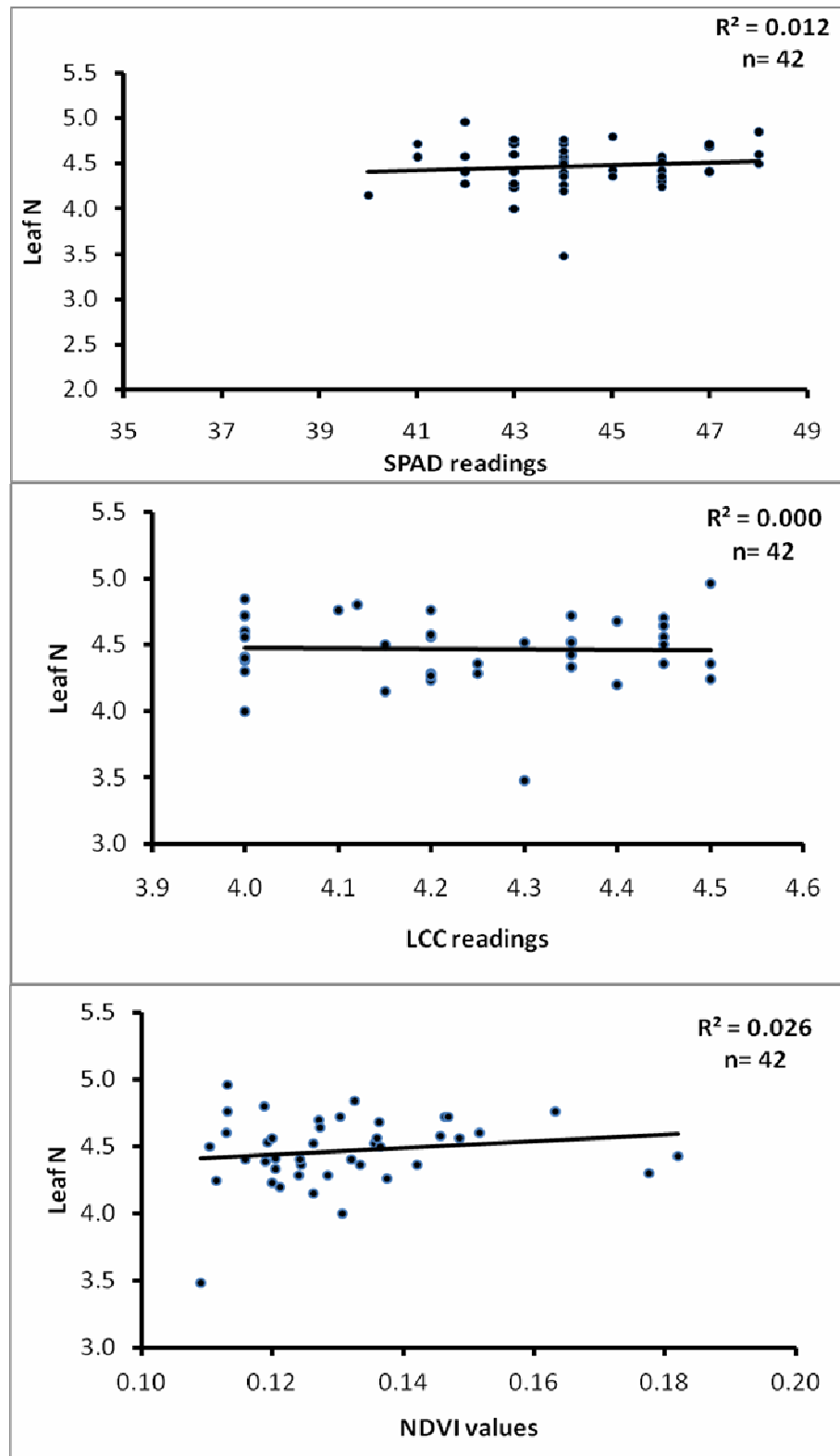


Fig. 4.4.1. Relationship of LCC, SPAD meter readings and NDVI values with leaf N at node formation stage

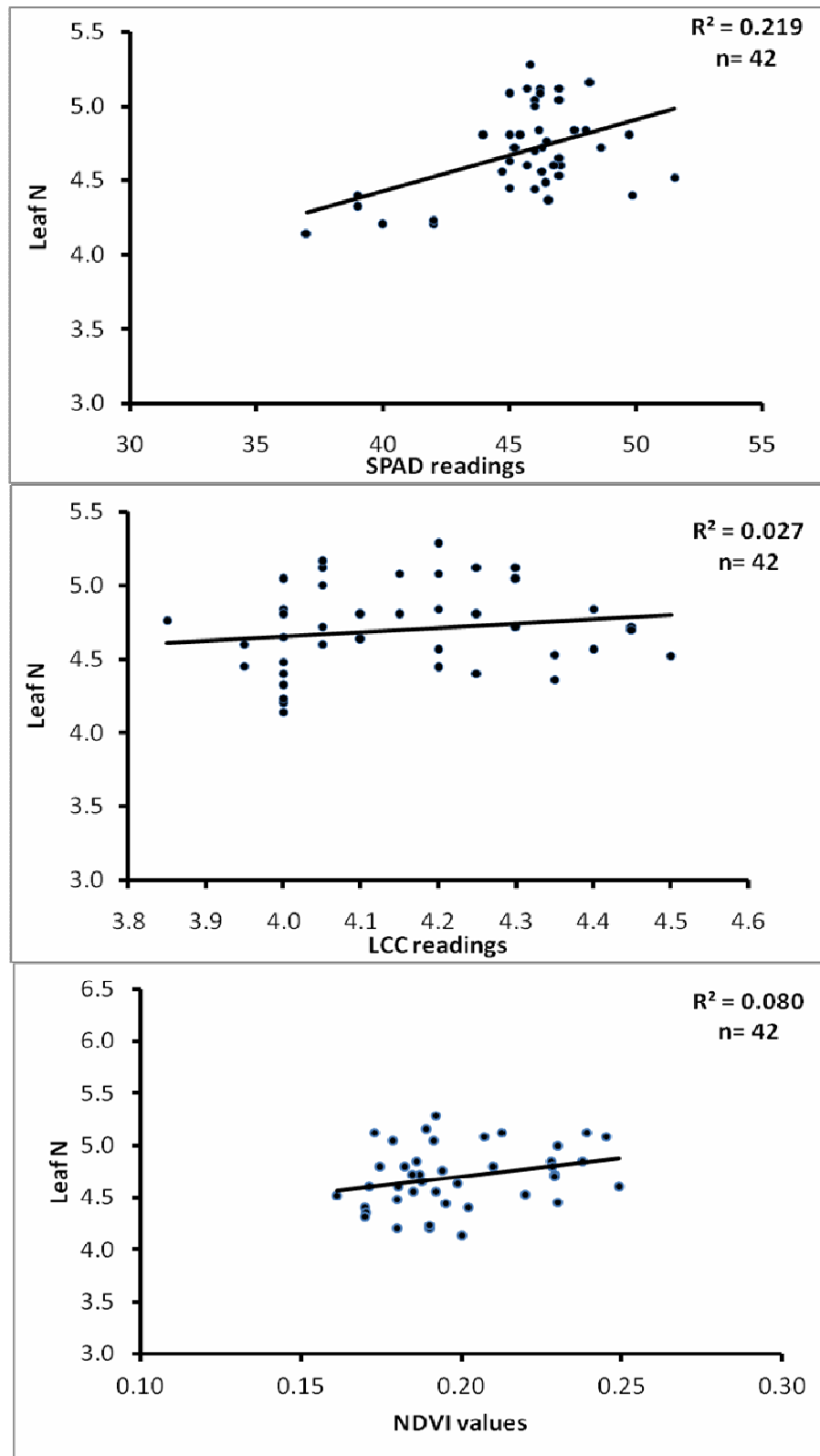


Fig. 4.4.2. Relationship of LCC, SPAD meter readings and NDVI values with leaf N at first squaring stage

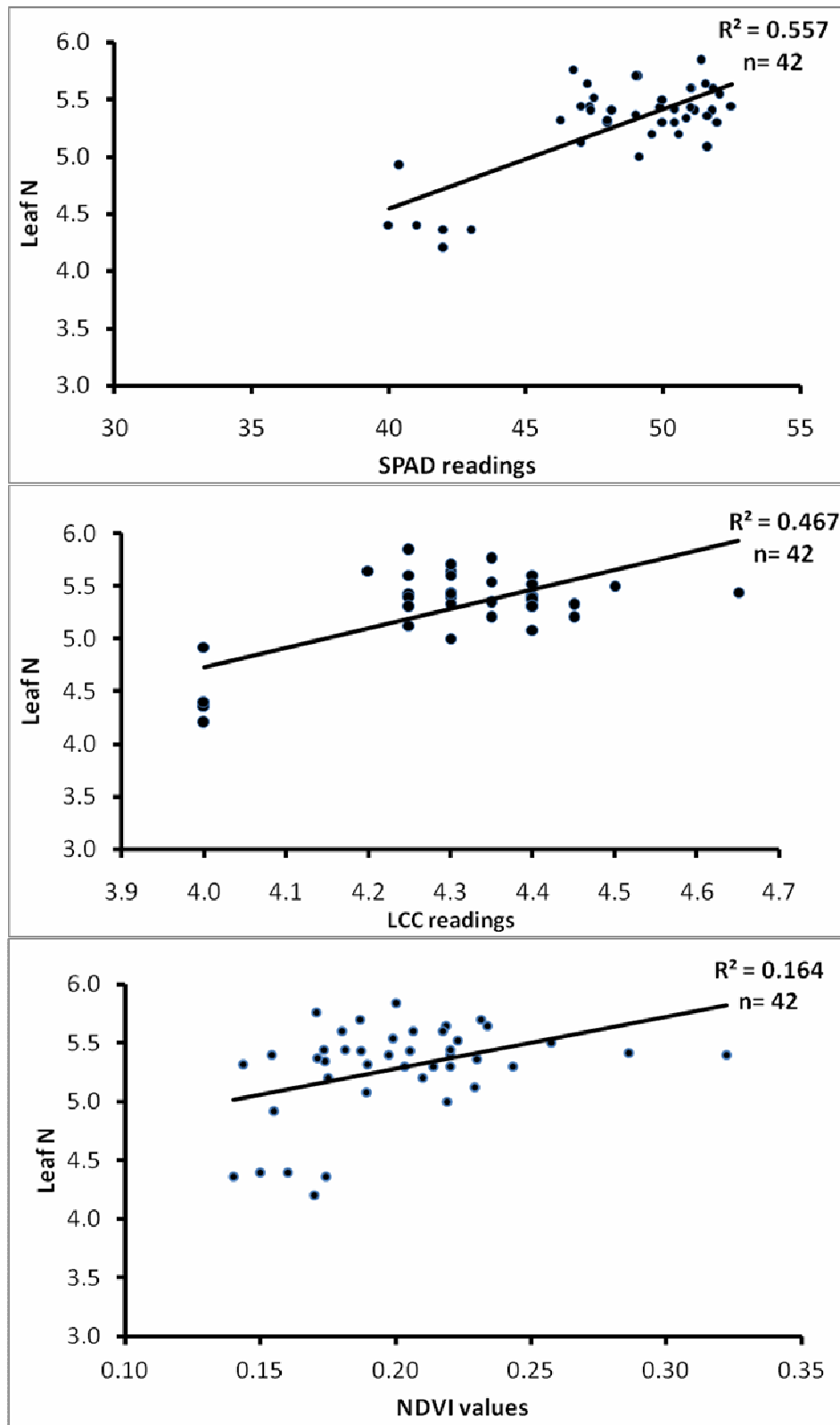


Fig. 4.4.3. Relationship of LCC, SPAD meter readings and NDVI values with leaf N at squaring stage

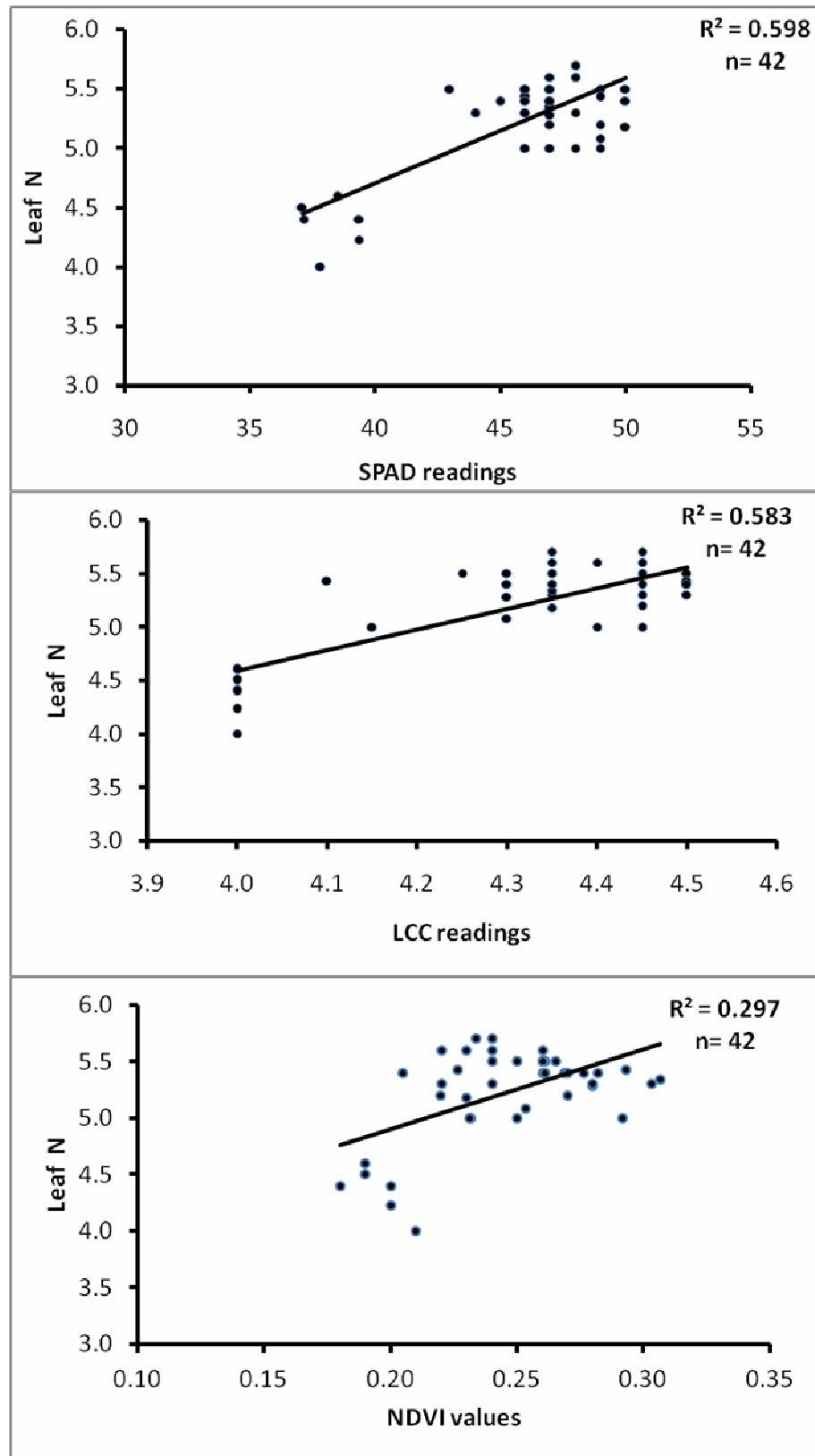


Fig.4.4.4. Relationship of LCC, SPAD meter readings and NDVI values with leaf N at early flowering stage

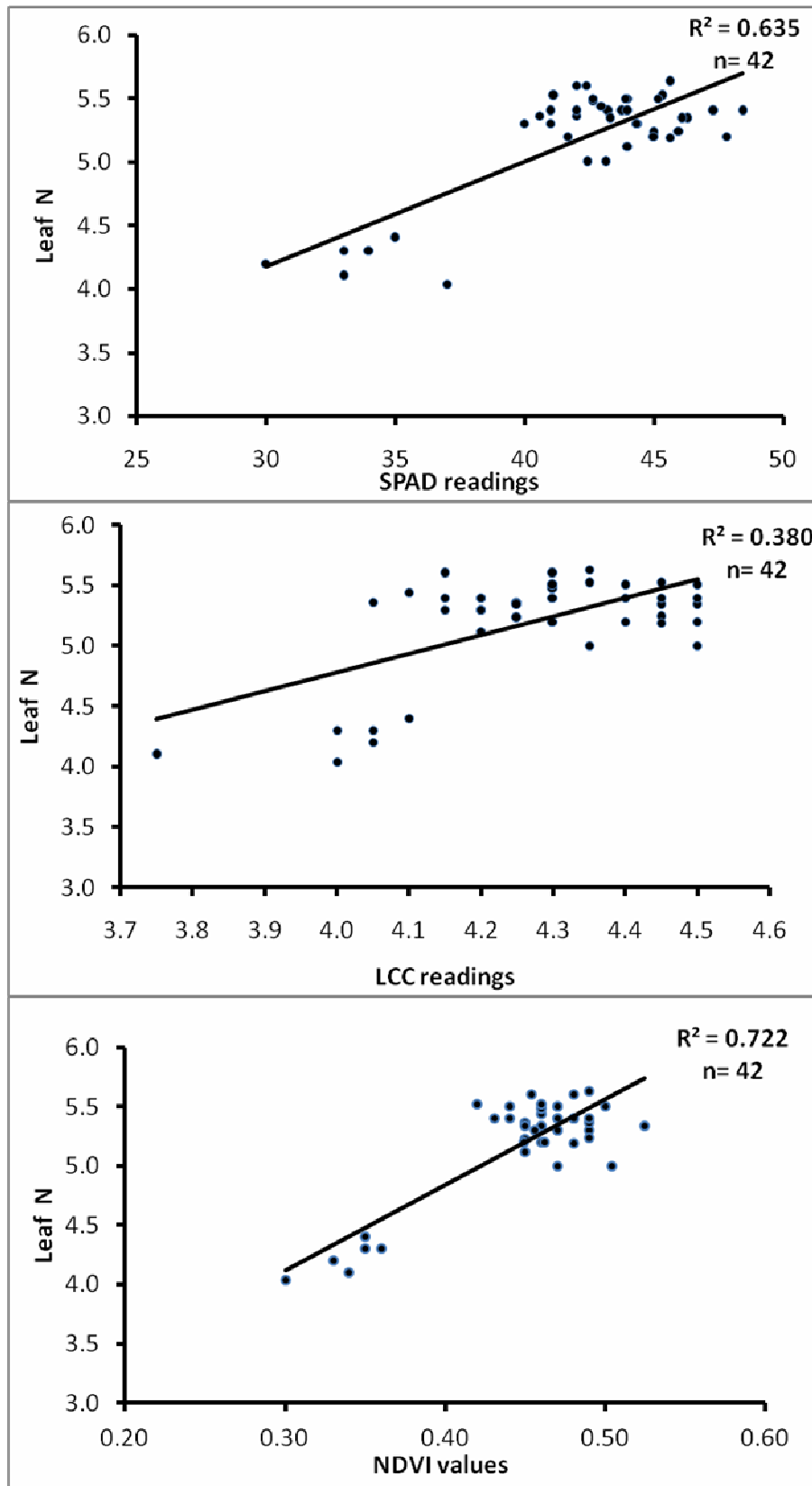


Fig. 4.4.5. Relationship of LCC, SPAD meter readings and NDVI values with leaf N at mid flowering stage

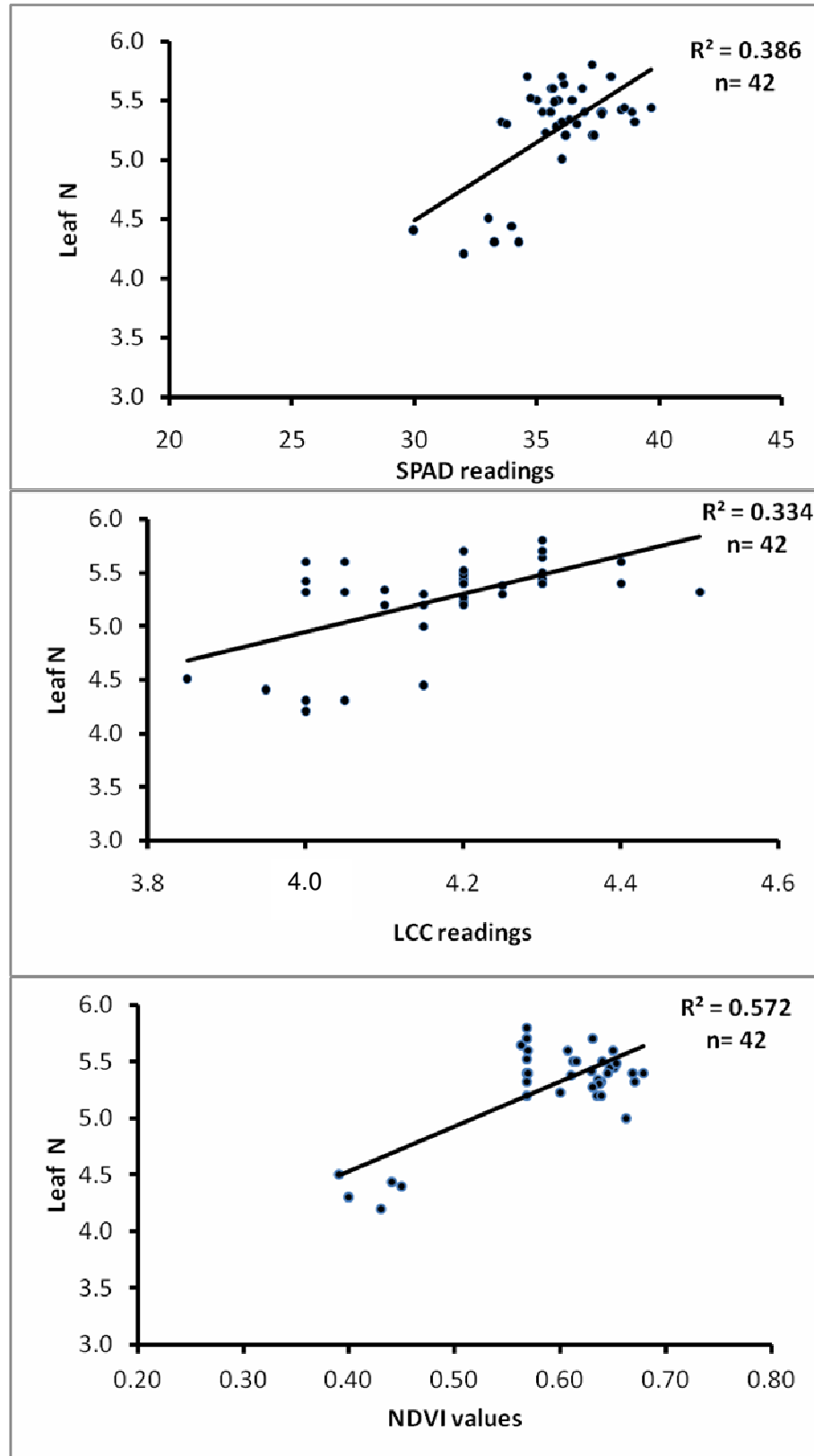


Fig. 4.4.6. Relationship of LCC, SPAD meter readings and NDVI values with leaf N at peak flowering stage

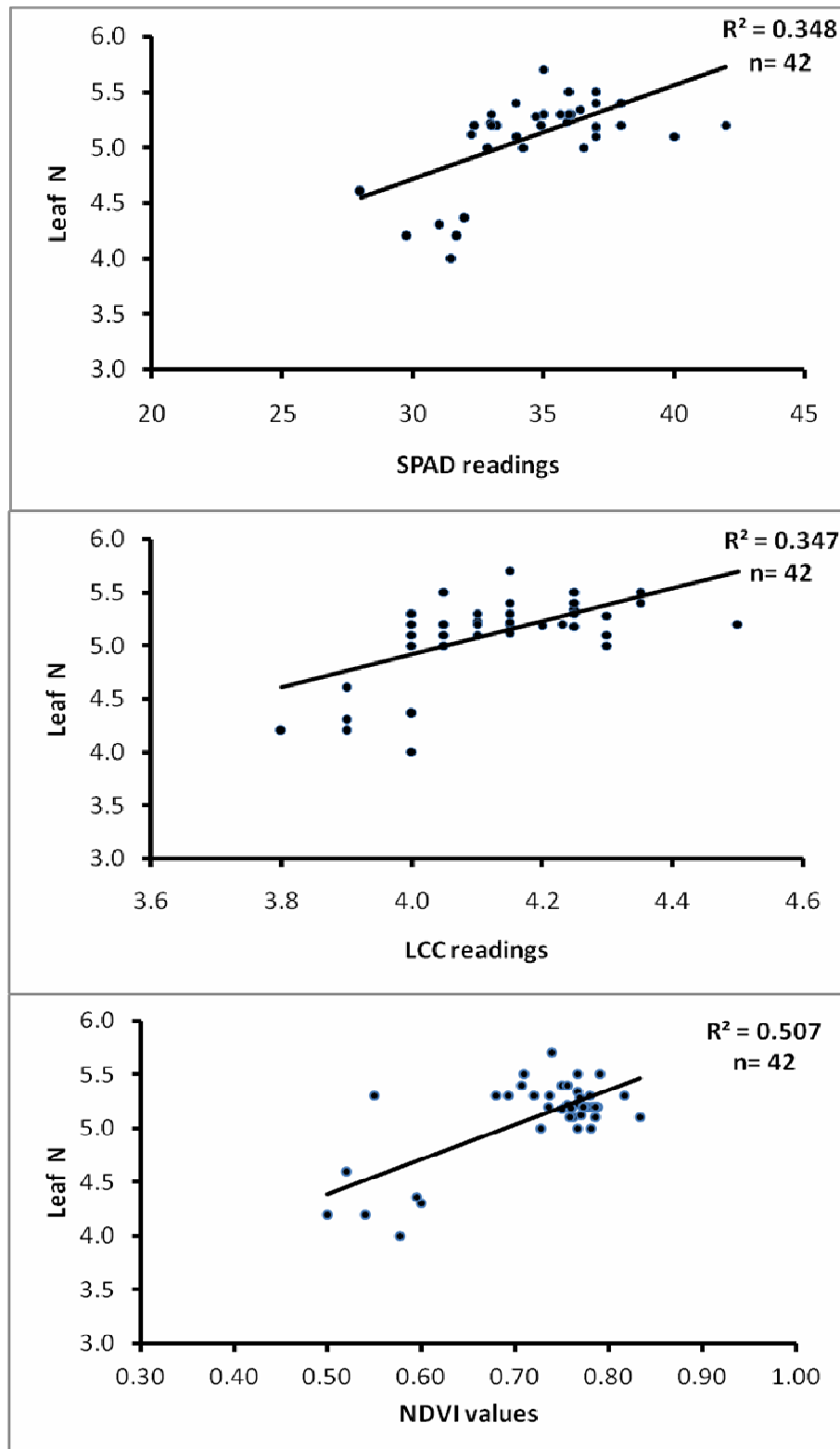


Fig. 4.4.7. Relationship of LCC, SPAD meter readings and NDVI values with leaf N at boll formation stage

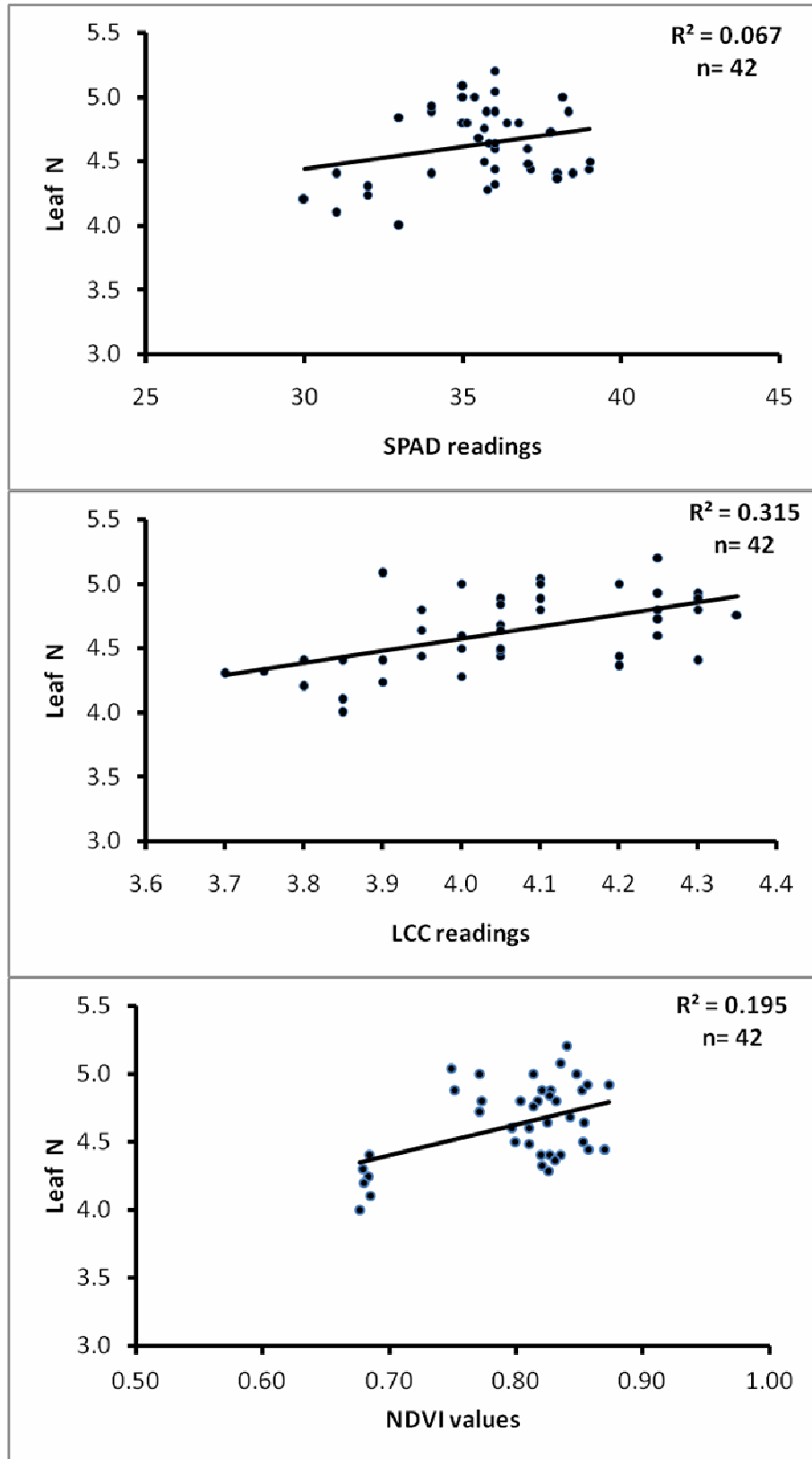


Fig. 4.4.8. Relationship of LCC, SPAD meter readings and NDVI values with leaf N at boll filling stage

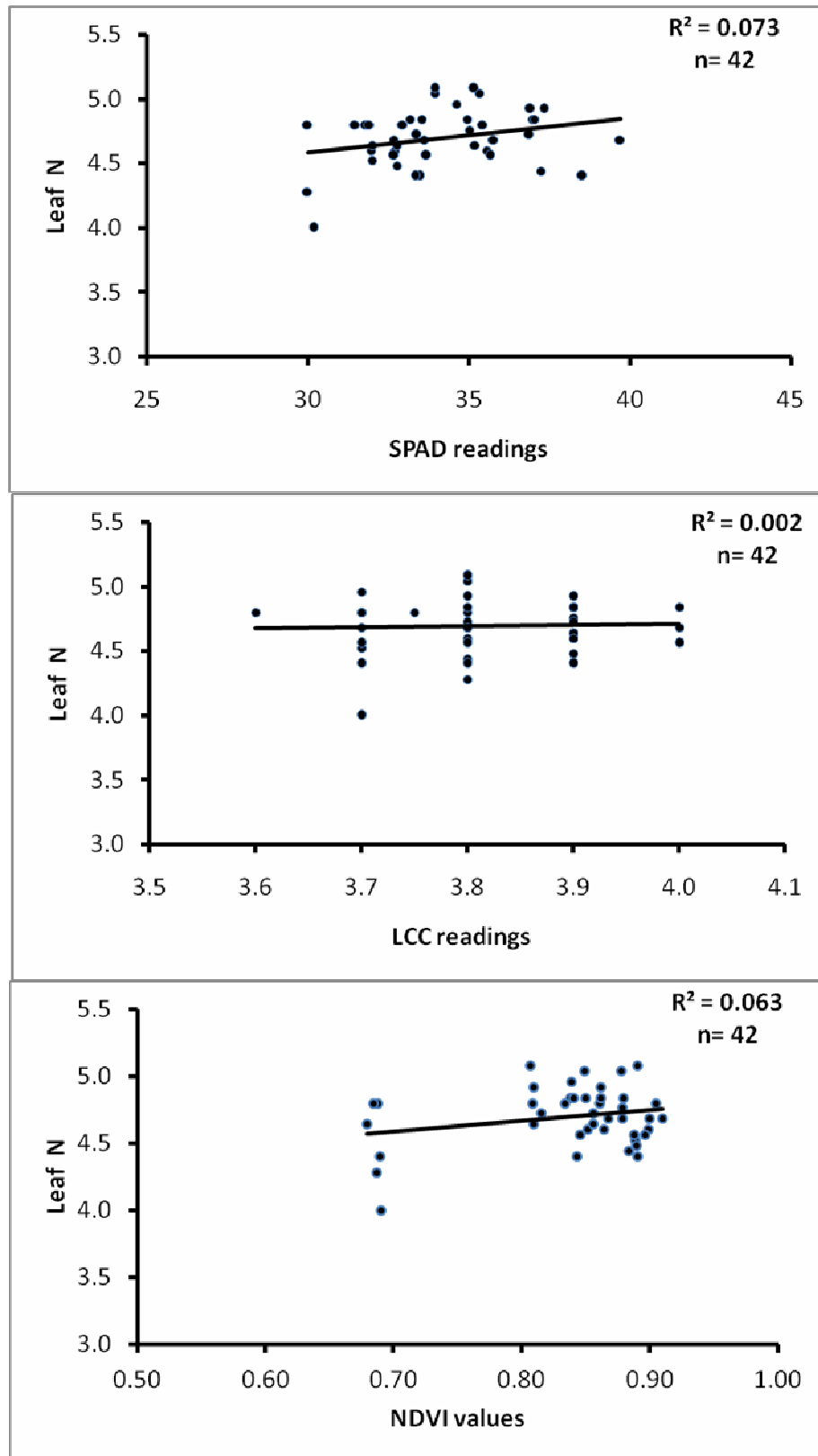


Fig. 4.4.9. Relationship of LCC, SPAD meter readings and NDVI values with leaf N at boll opening stage

4.5 LCC, SPAD readings, NDVI values at different growth stages and seed cotton yield

The coefficient of determination (R^2) of SPAD meter, LCC and NDVI values at different growth stages in relation to seed cotton yield is presented in Fig. 4.5.1 to Fig. 4.5.9. The data revealed that LCC readings were correlated to seed cotton yield at $p=0.01$ level of significance at squaring and early flowering growth stages and maximum R^2 value (0.531) was observed at squaring stage (Fig.4.5.3). The SPAD meter readings provide a better measure of seed cotton yield than LCC readings at all the growth stages. The SPAD meter readings were correlated to seed cotton yield at $p=0.01$ level of significance at first squaring, squaring, early flowering, mid flowering and boll filling growth stages and maximum R^2 value (0.572) was observed at squaring stage (Fig.4.5.3). The NDVI values recorded with GreenSeeker optical sensor were correlated to seed cotton yield at $p=0.01$ level of significance at mid flowering, peak flowering, boll formation, boll filling and boll opening stages and maximum R^2 value (0.613) was observed at boll opening stage (Fig.4.5.9). The coefficient of determination obtained with NDVI values after early flowering stage gave better understanding of seed cotton yield than SPAD meter and LCC readings; however NDVI values at early growth stages are not better indicator of seed cotton yield. The uncovered soil surface may create error in yield prediction using NDVI data recorded at early growth stages. The SPAD meter, LCC readings and NDVI values recorded at node formation stage were independent of seed cotton yield and cannot be used to predict seed cotton yield at maturity. The data revealed that SPAD meter and LCC may help guide to predict yield and manage fertilizer N as per need of the plant from squaring to mid flowering stage of cotton; however NDVI values can be used to develop need based fertilizer N management practices from mid flowering to boll formation stage in cotton. Boggs *et al* (2003) documented the importance of measuring leaf chlorophyll content to predict cotton yield. Li *et al* (2001) reported that NDVI calculated from reflectance at 2m above the cotton canopy was positively correlated with biomass and lint yield. Chua *et al* (2003) found spectral reflectance and chlorophyll meter measurements as in-season N decision aids for improved N use efficiency in comparison with soil test based N management in irrigated cotton. Need-based fertilizer N applications produced lint yield similar to the soil test based fertilizer N applications with the use of 34-101 kg ha⁻¹ less N. Yabaji (2006) also reported fertilizer N saving and improved N use efficiency with the reflectance based N management in comparison to the other N fertilizer management practices.

4.6 LCC, SPAD readings, NDVI values at different growth stages and total N uptake at maturity

The coefficient of determination (R^2) of SPAD meter, LCC readings and NDVI values at different growth stages in relation to total N uptake at maturity is presented in Fig.

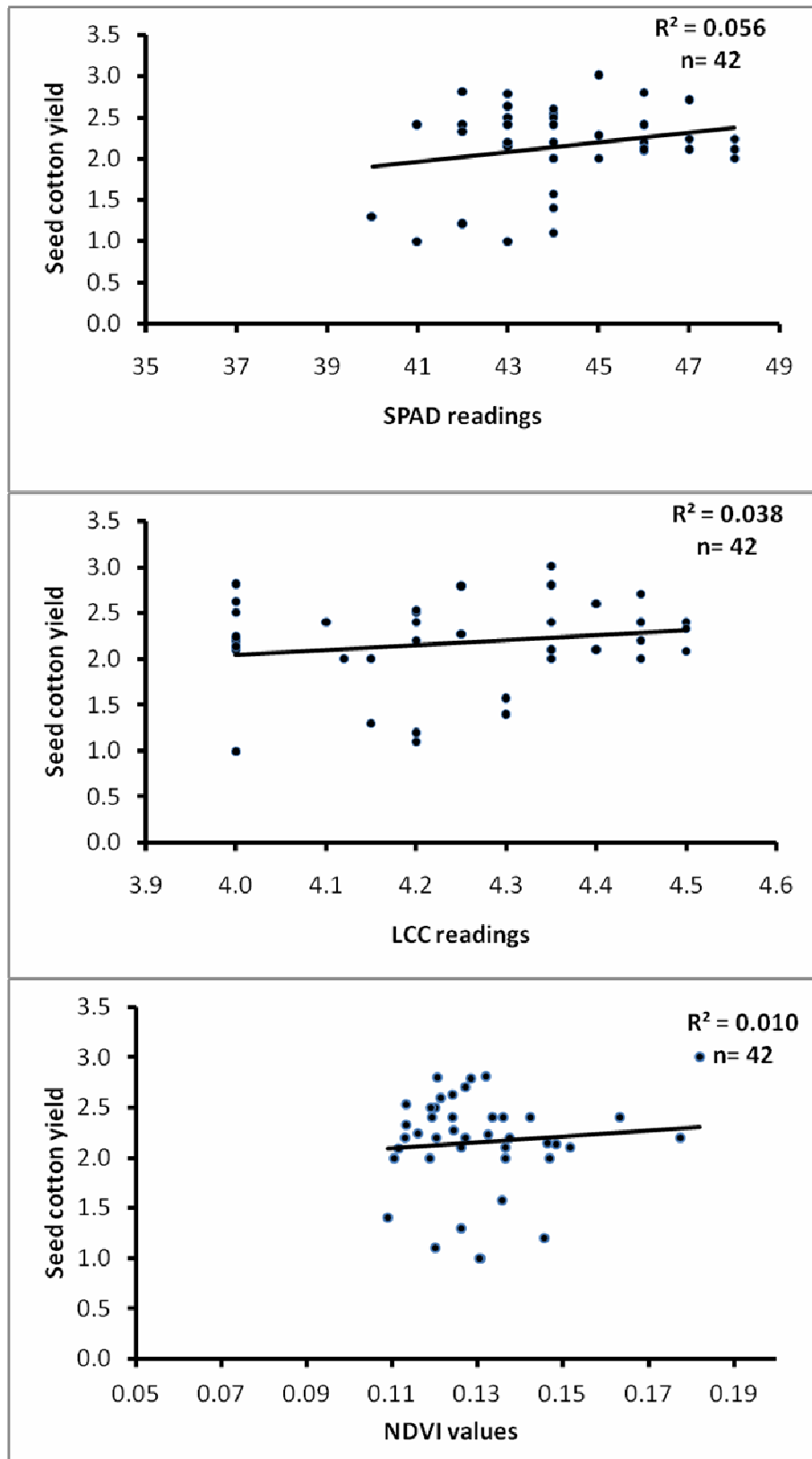


Fig. 4.5.1. Relationship of LCC, SPAD meter readings and NDVI values with seed cotton yield at node formation stage

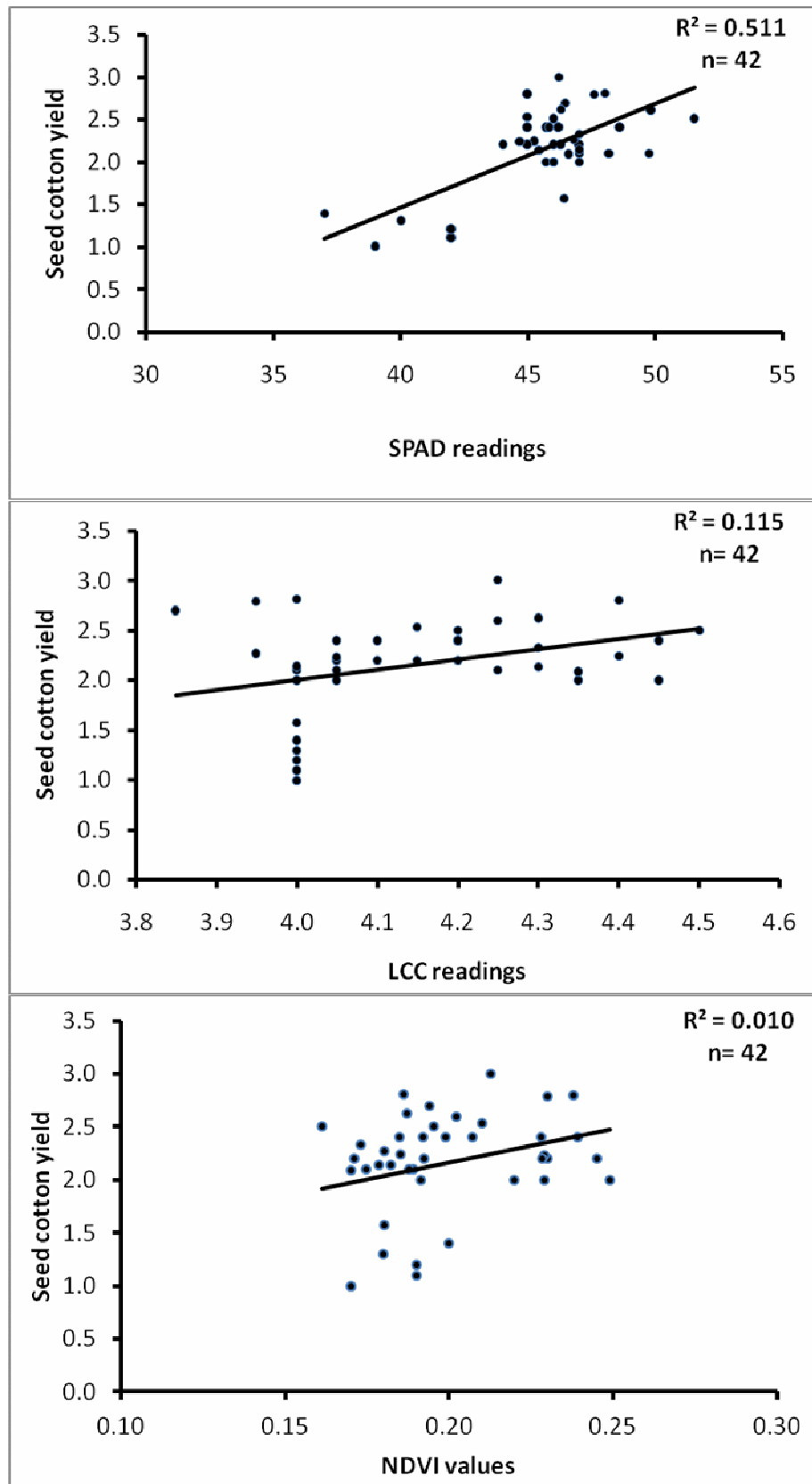


Fig. 4.5.2. Relationship of LCC, SPAD meter readings and NDVI values with seed cotton yield at first squaring stage

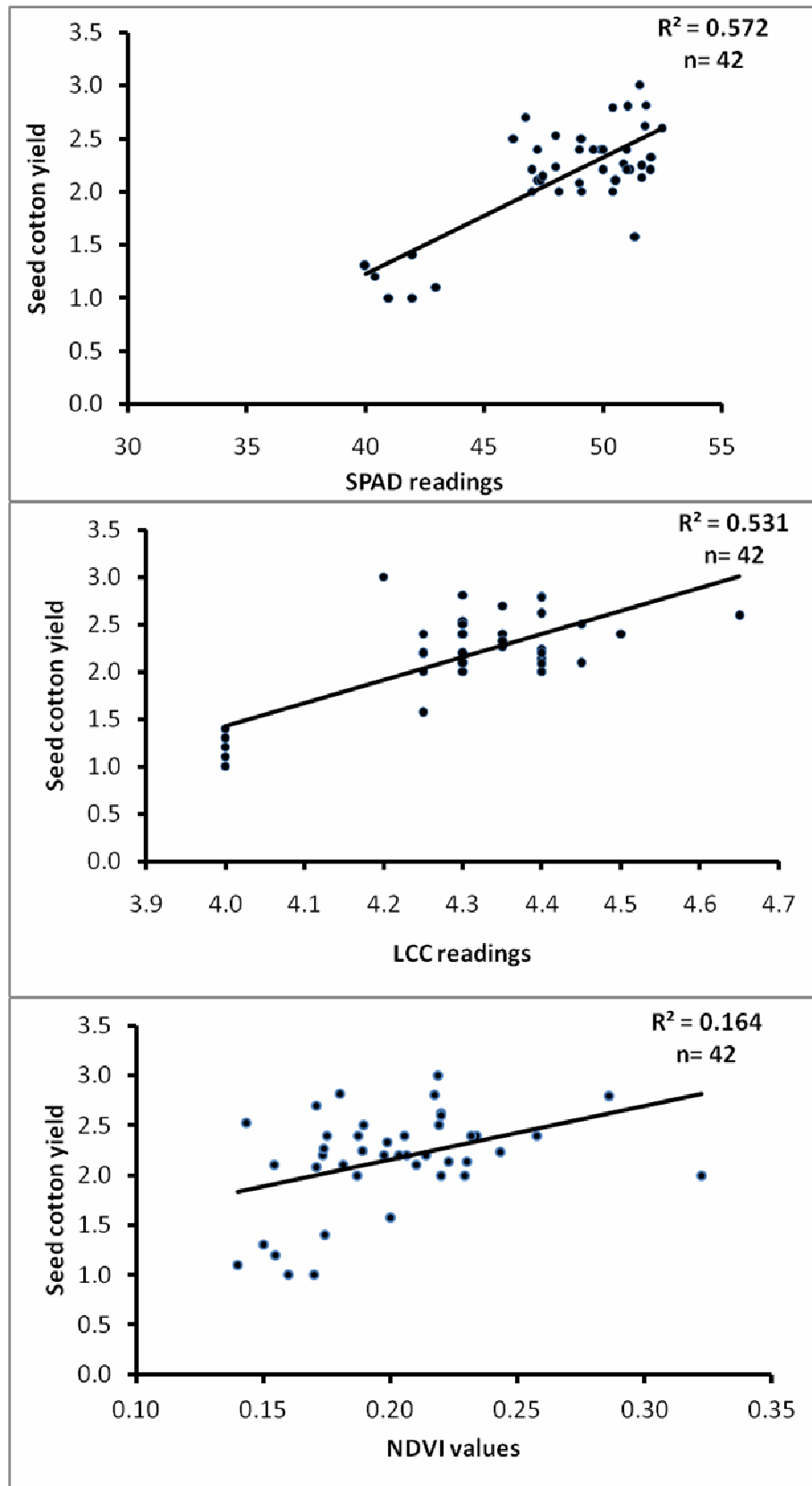


Fig. 4.5.3. Relationship of LCC, SPAD meter readings and NDVI values with seed cotton yield at Squaring stage

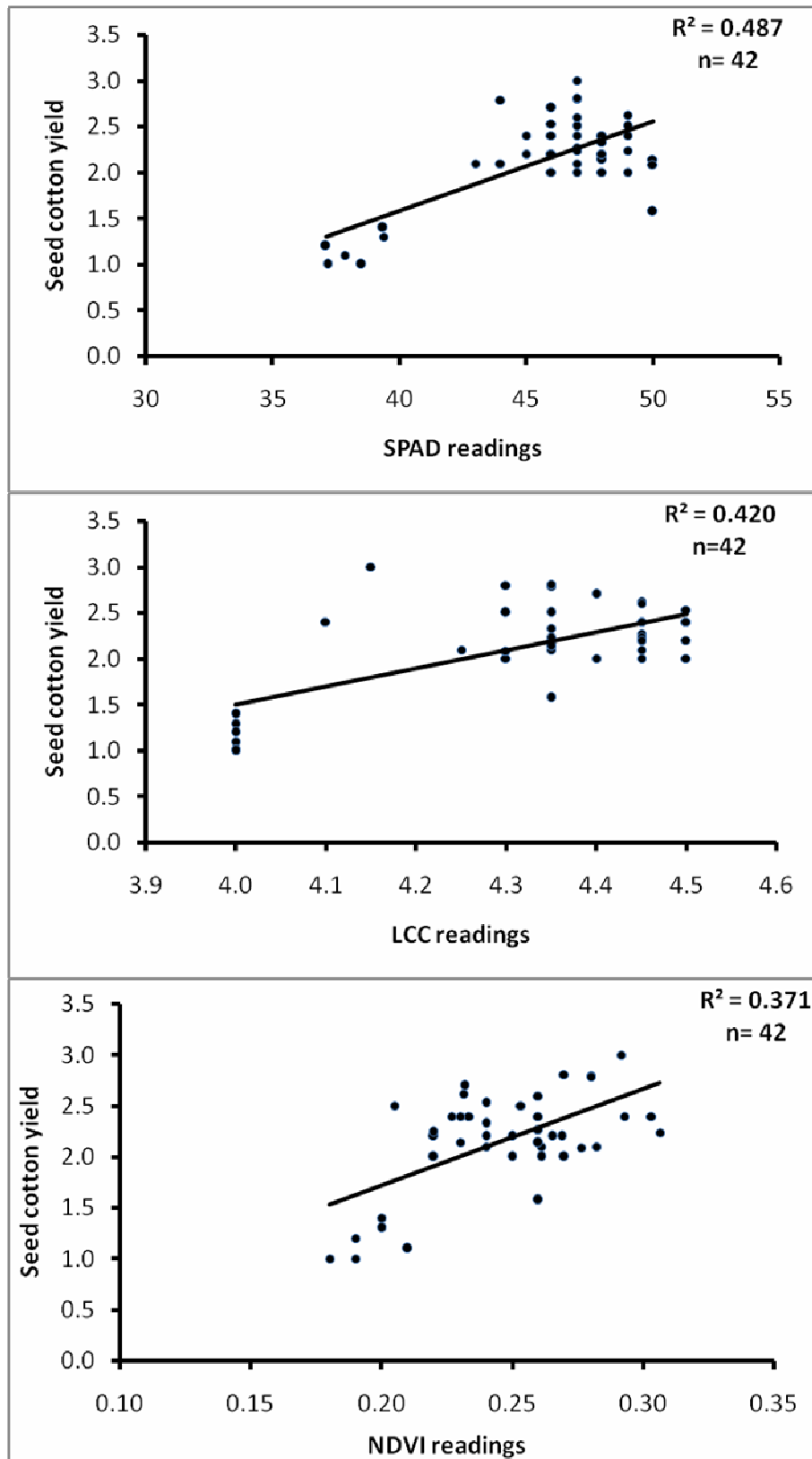


Fig. 4.5.4. Relationship of LCC, SPAD meter readings and NDVI values with seed cotton yield at early flowering stage

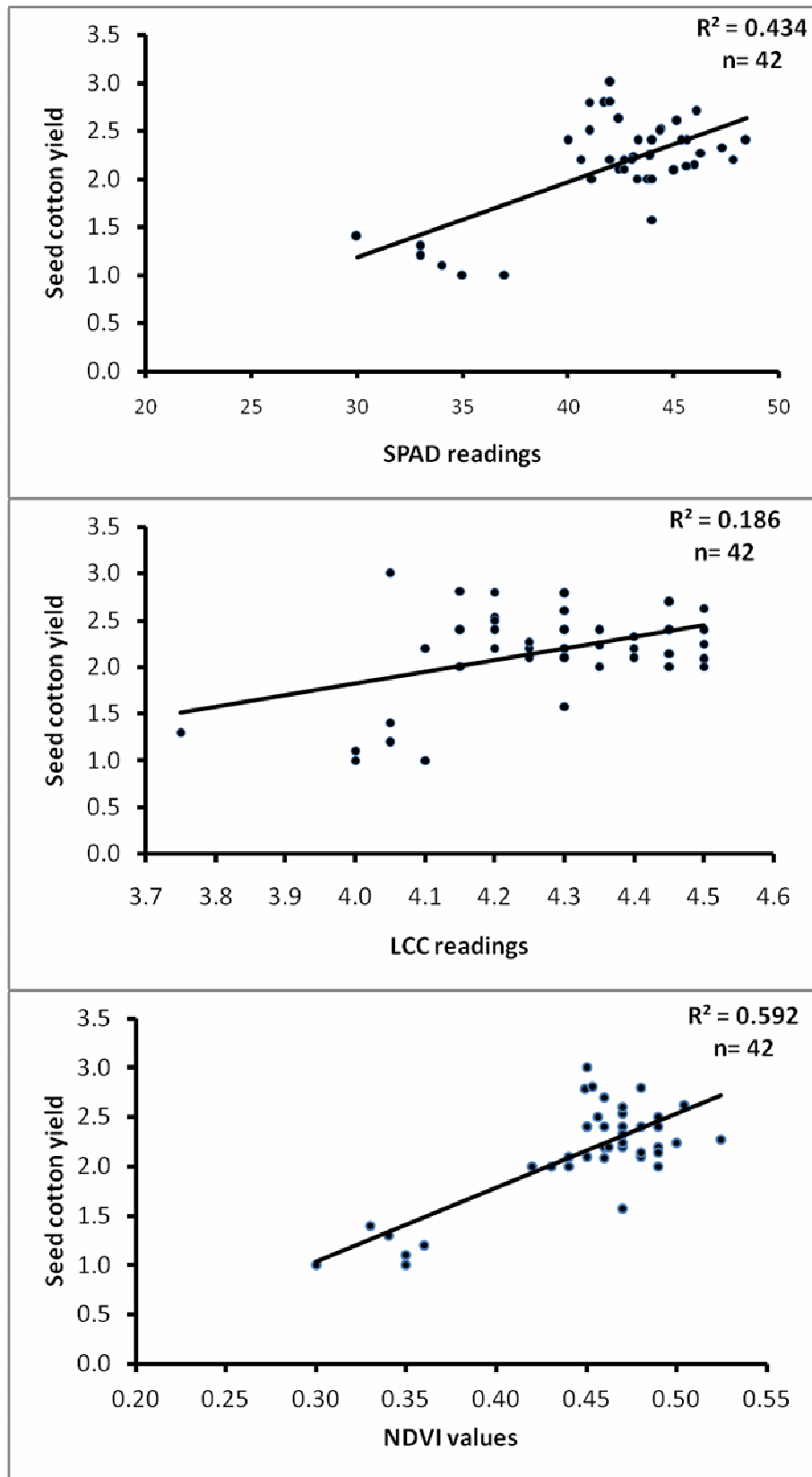


Fig. 4.5.5. Relationship of LCC, SPAD meter readings and NDVI values with seed cotton yield at mid flowering stage

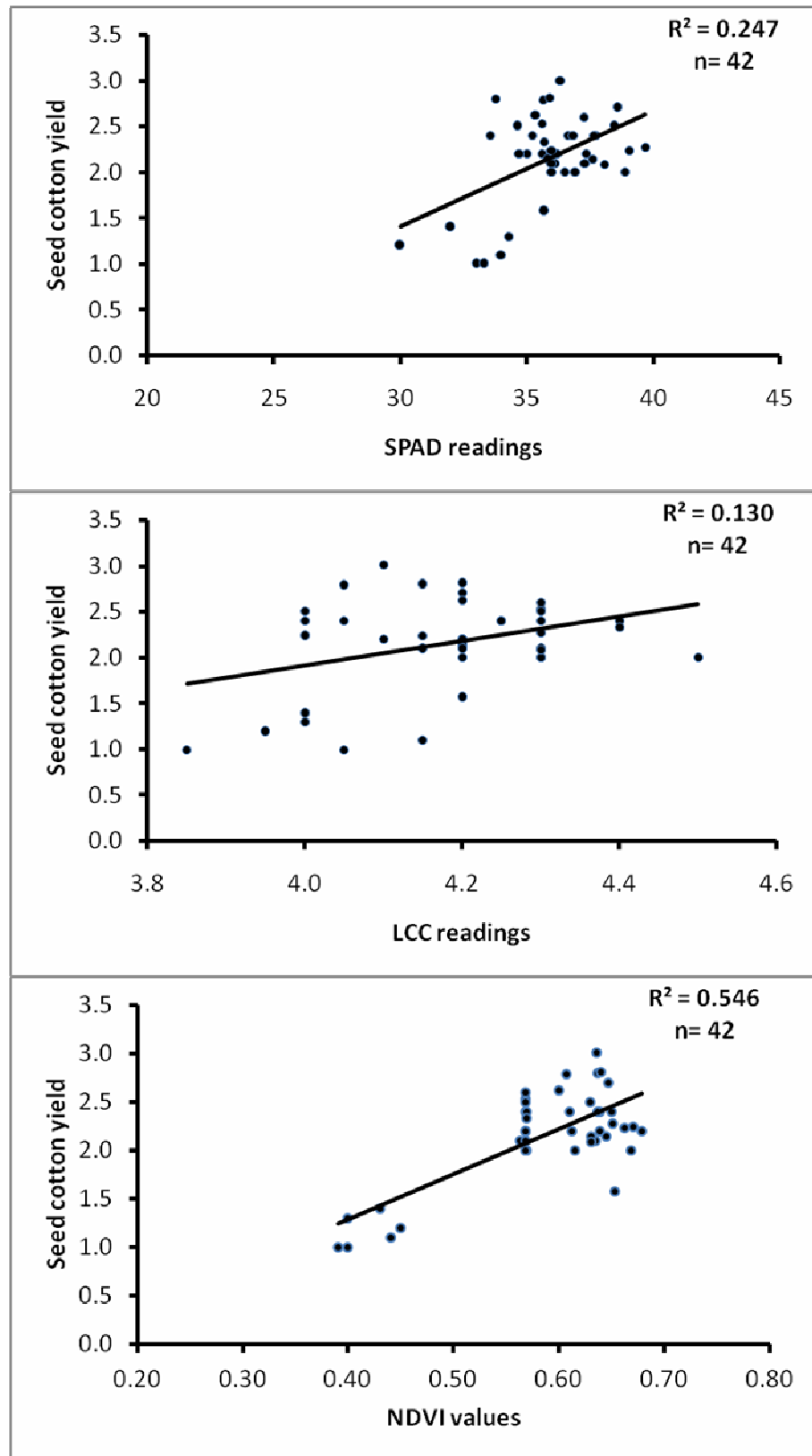


Fig. 4.5.6. Relationship of LCC, SPAD meter readings and NDVI values with seed cotton yield at peak flowering stage

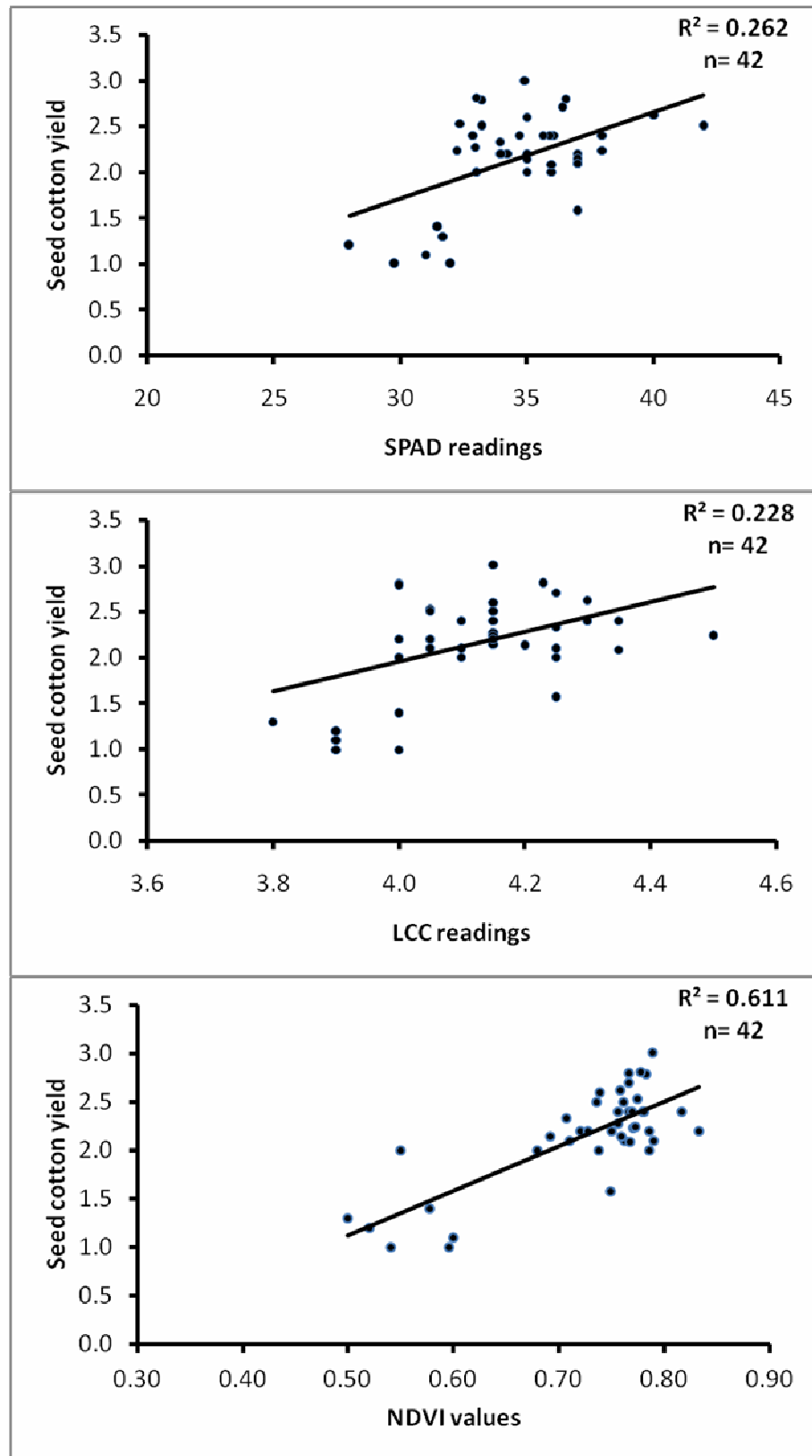


Fig. 4.5.7. Relationship of LCC, SPAD meter readings and NDVI values with seed cotton yield at boll formation stage

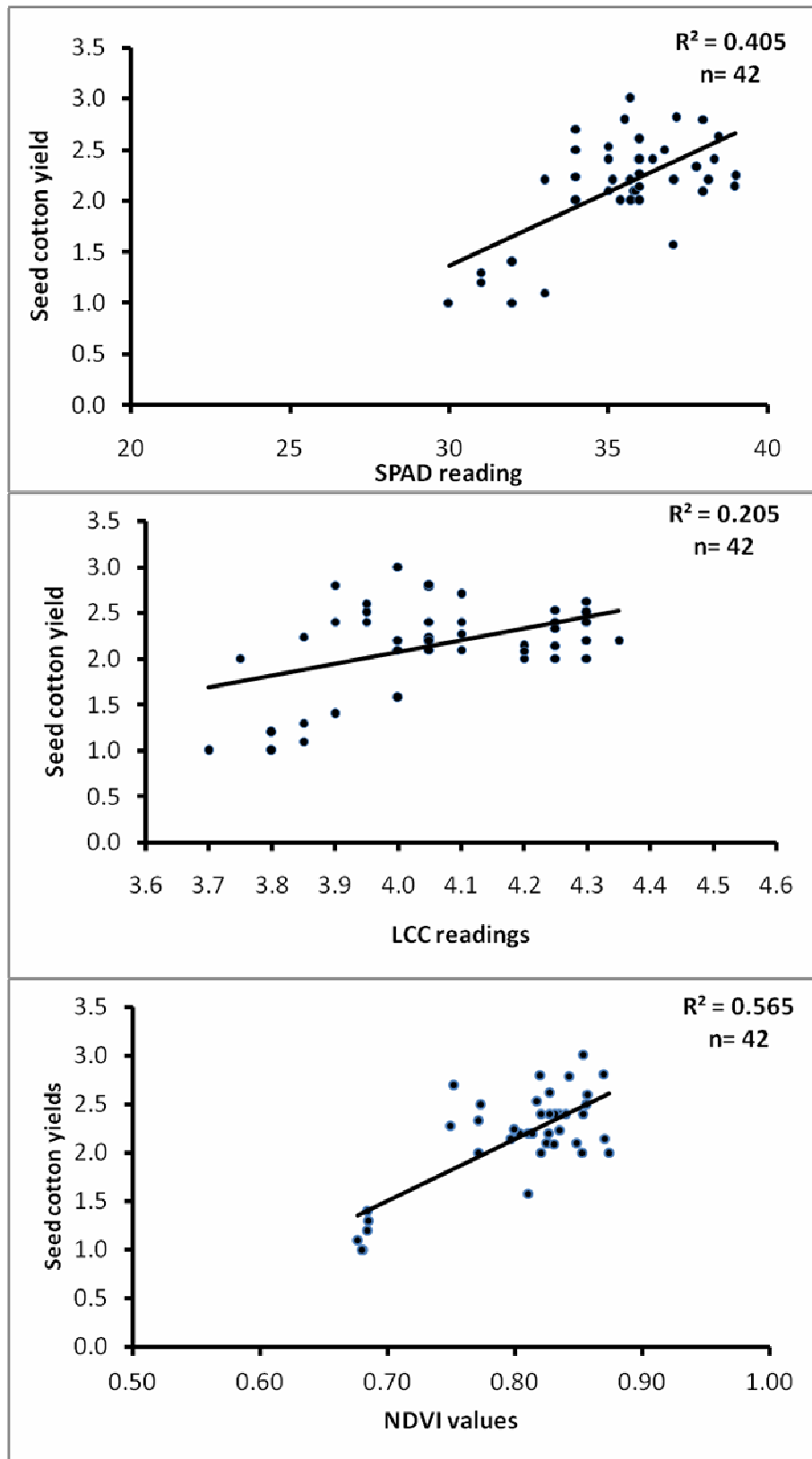


Fig. 4.5.8. Relationship of LCC, SPAD meter readings and NDVI values with seed cotton yield at boll filling stage

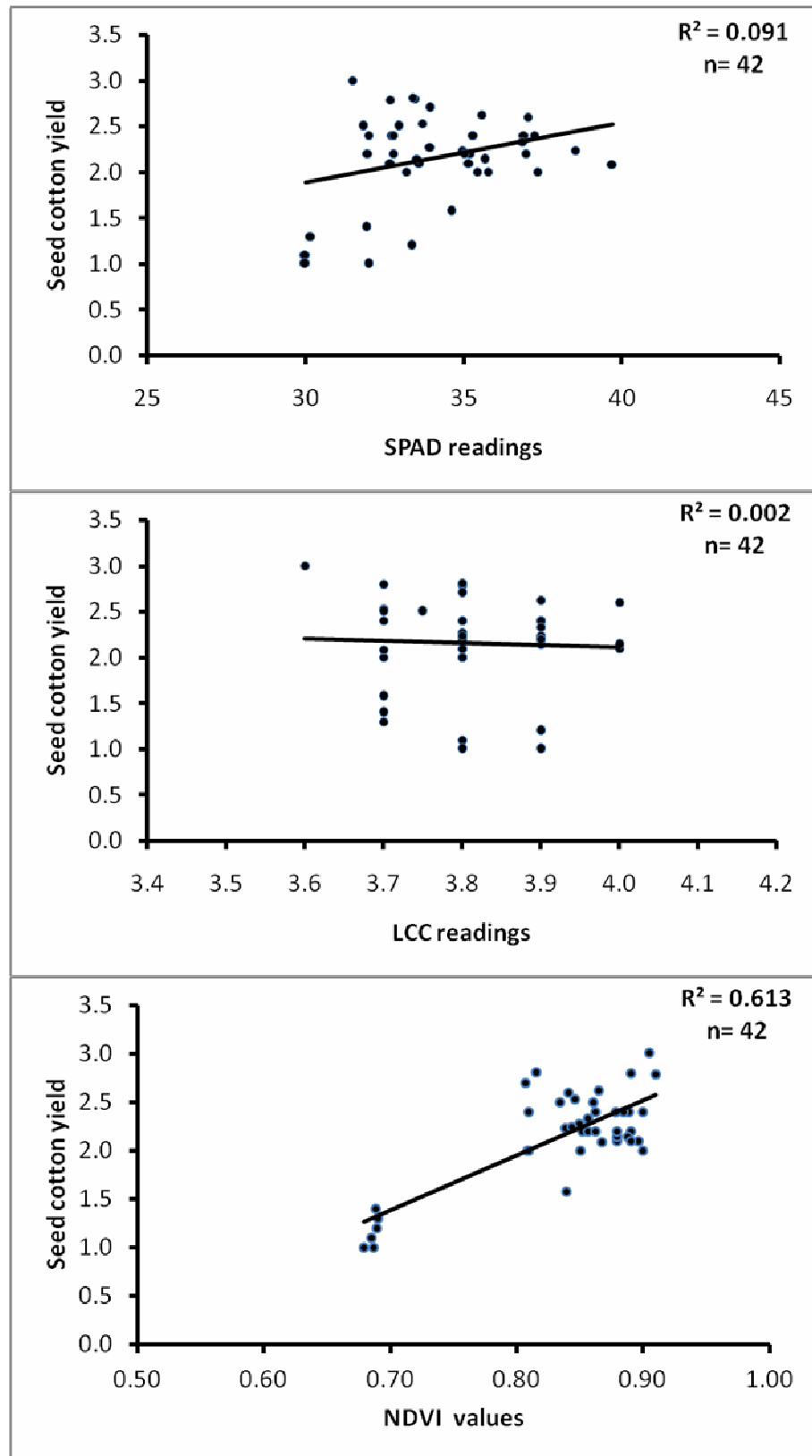


Fig.4.5.9. Relationship of LCC, SPAD meter readings and NDVI values with seed cotton yield at boll opening stage

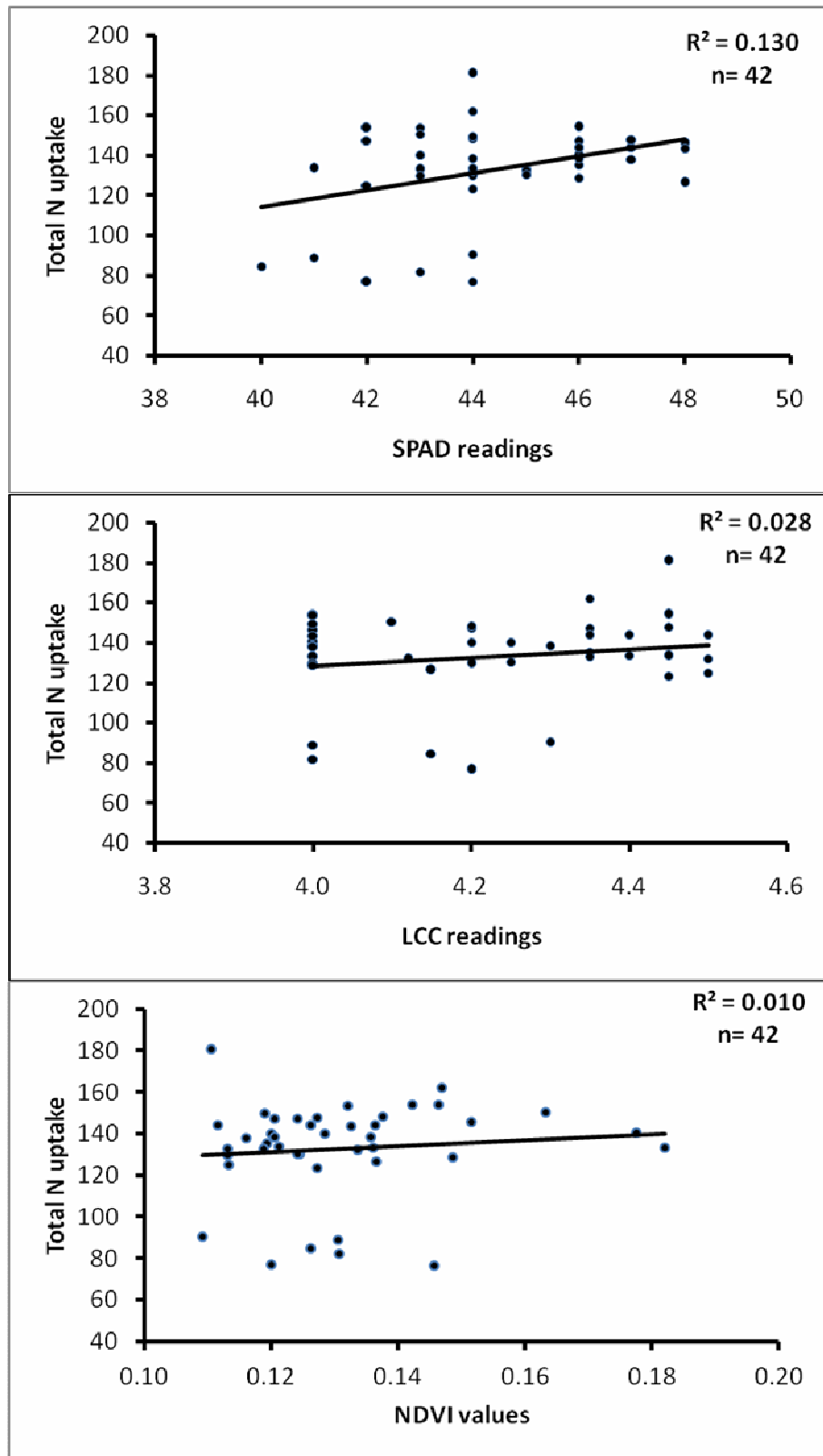


Fig. 4.6.1. Relationship of LCC, SPAD meter readings and NDVI values with total N uptake at node formation stage

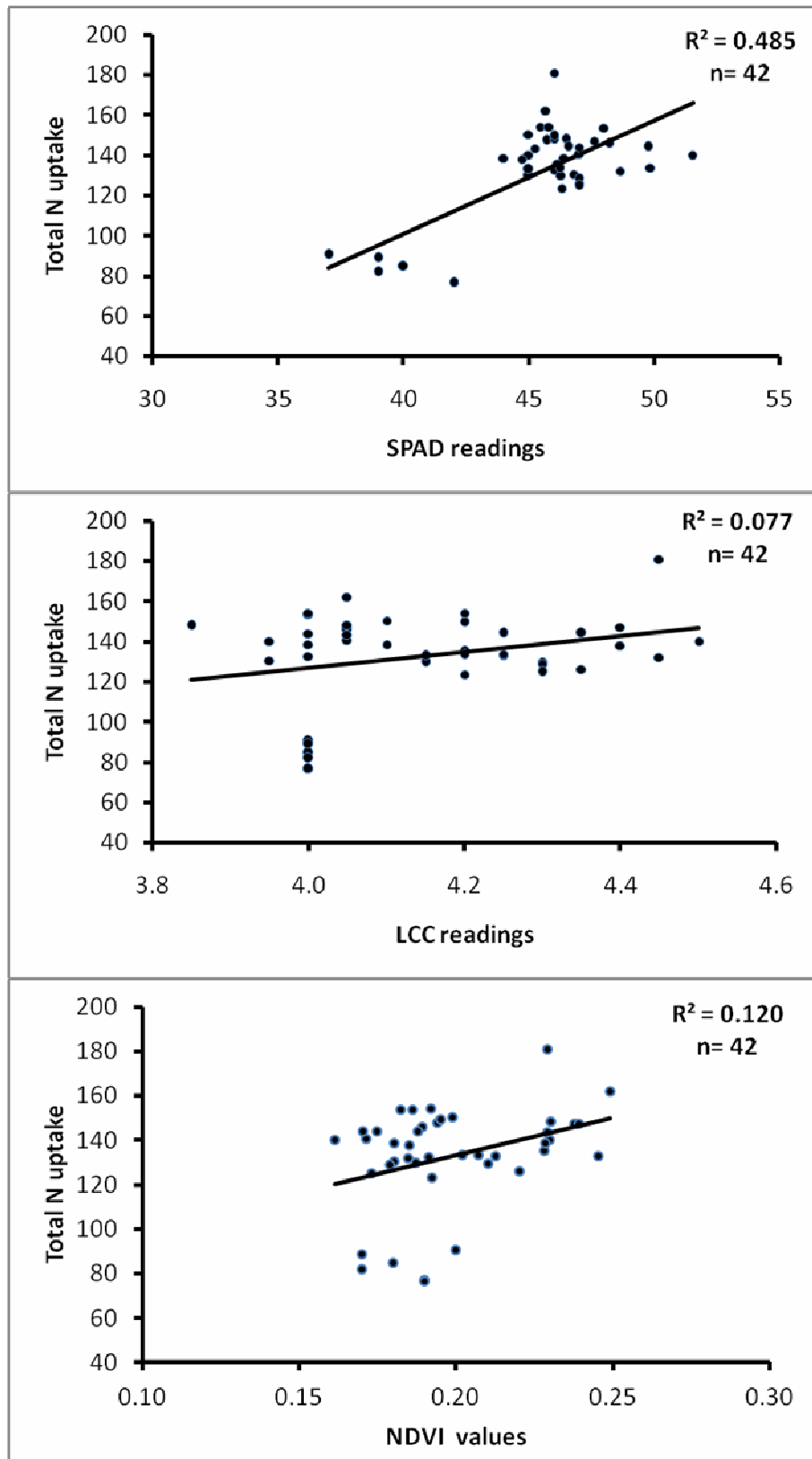


Fig. 4.6.2. Relationship of LCC, SPAD readings and NDVI values with total N uptake at first squaring stage

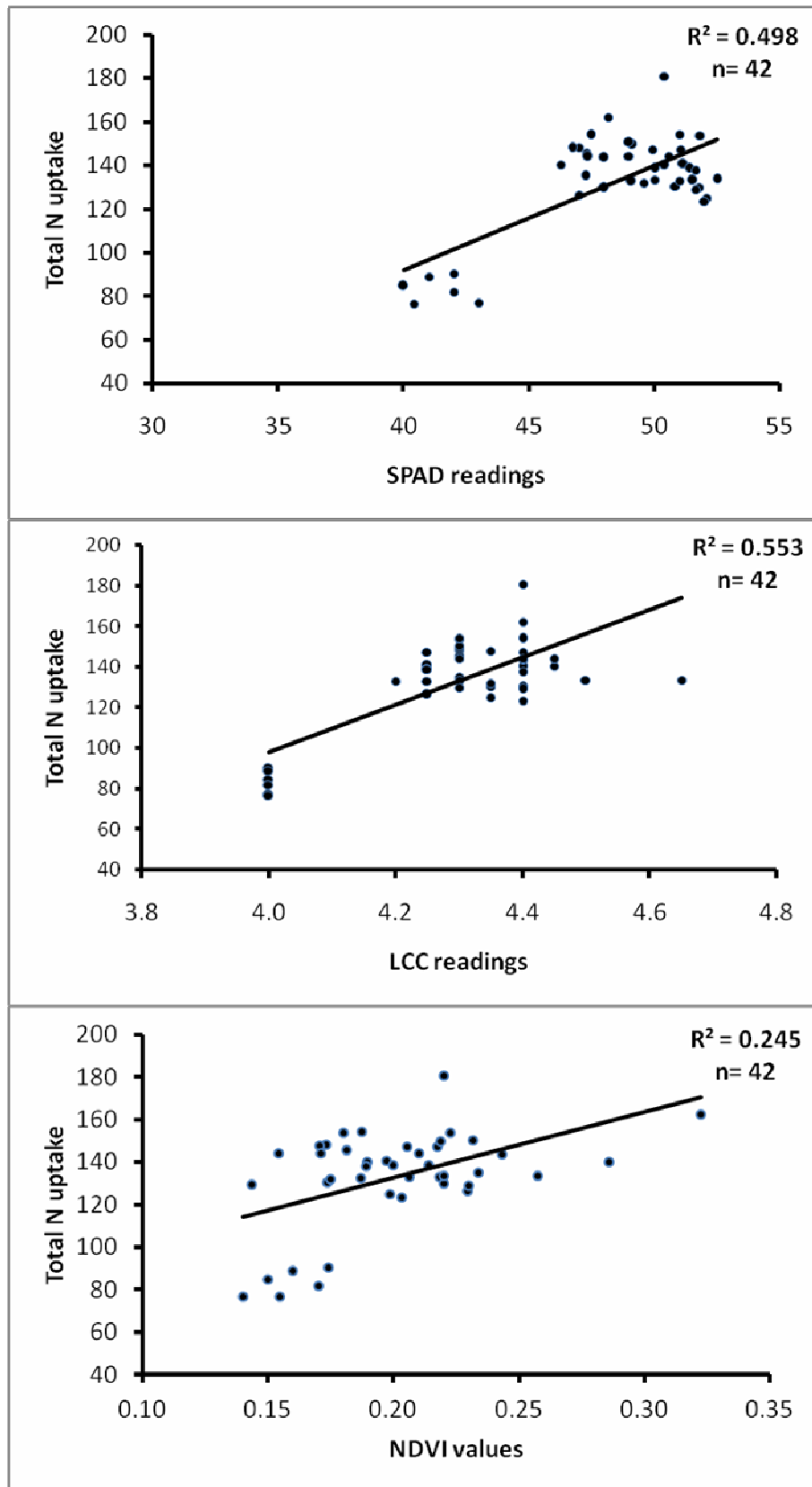


Fig. 4.6.3. Relationship of LCC, SPAD meter readings and NDVI values with total N uptake at squaring stage

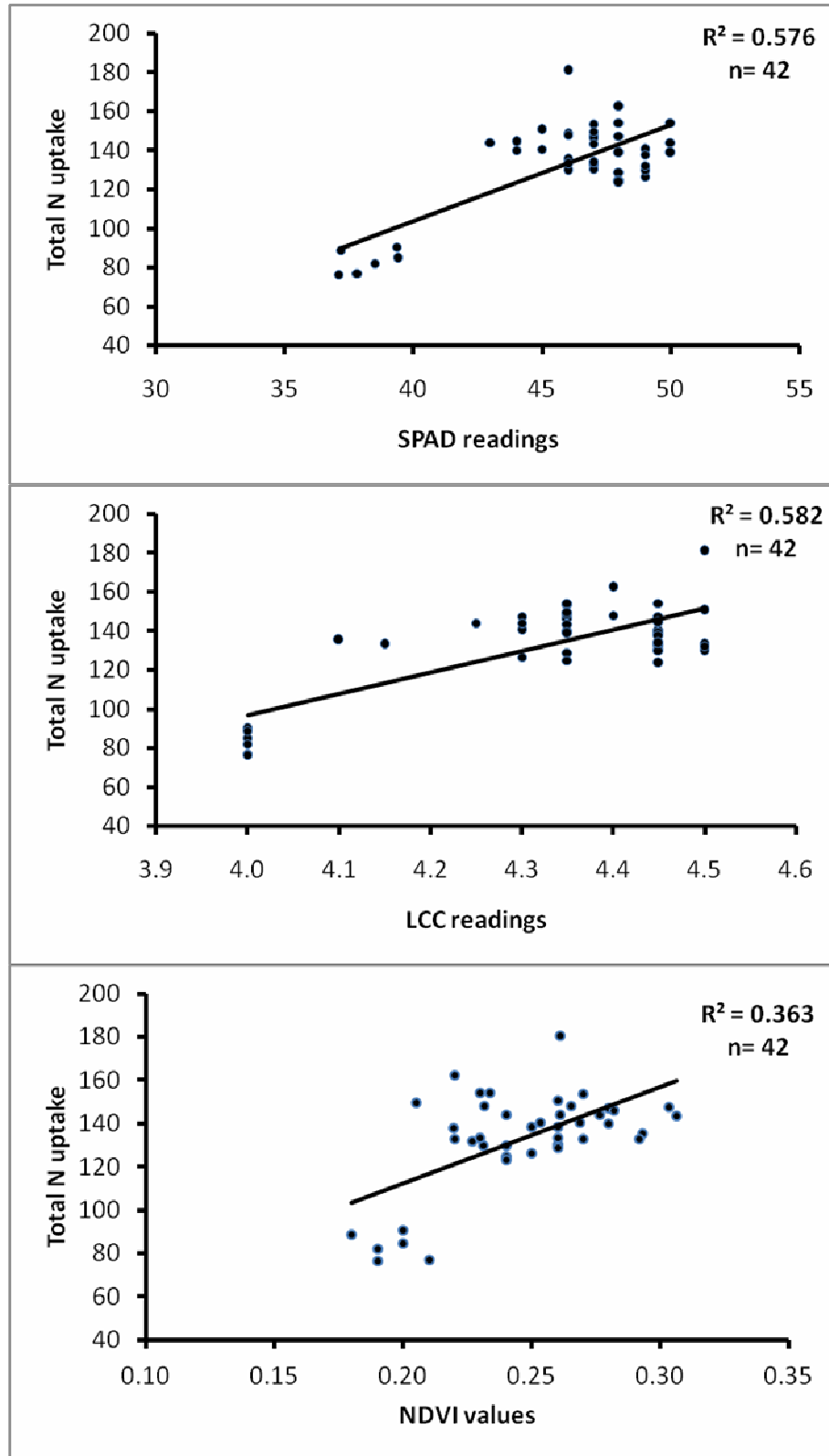


Fig. 4.6.4. Relationship of LCC, SPAD meter readings and NDVI values with total N uptake at early flowering stage

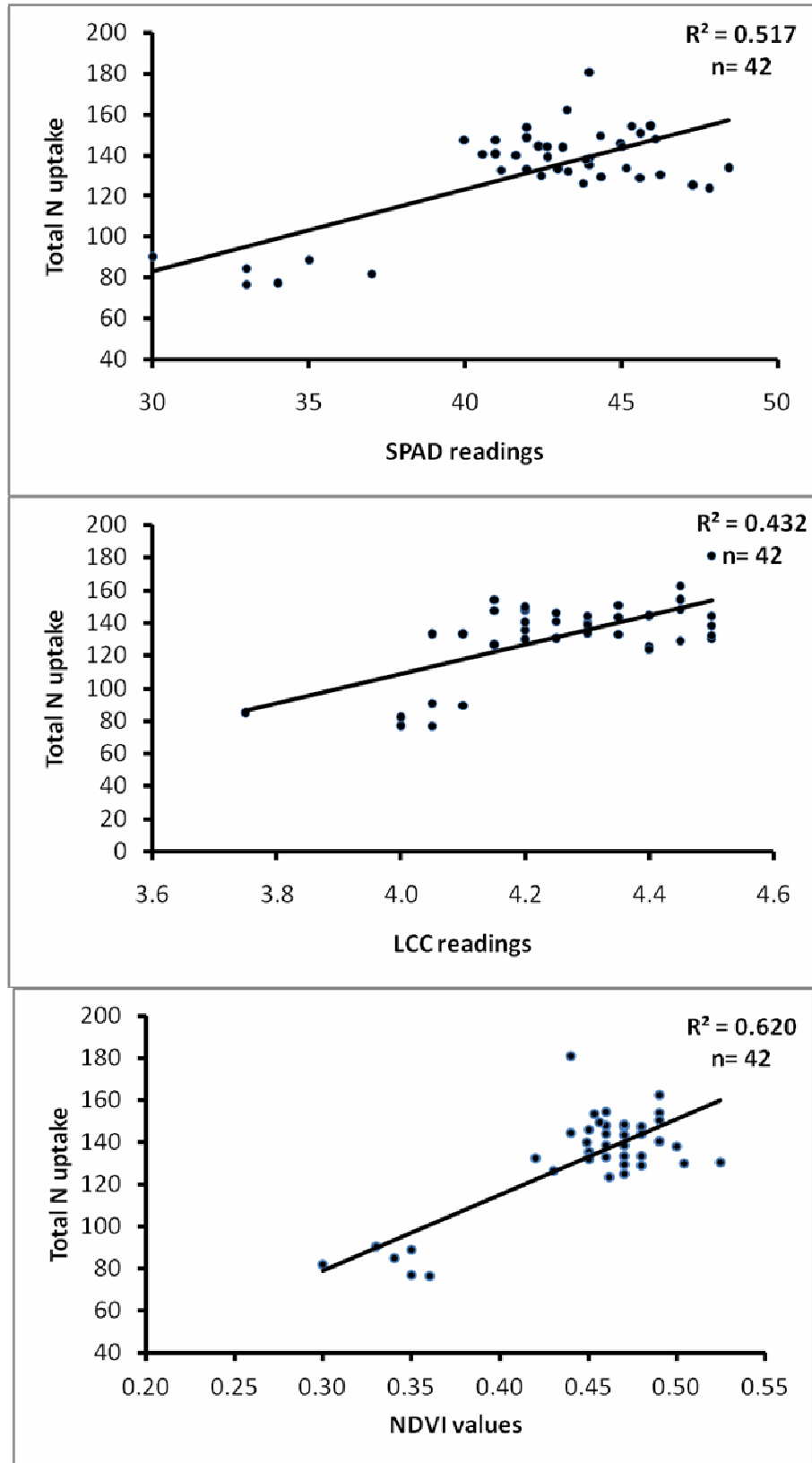


Fig. 4.6.5. Relationship of LCC, SPAD meter readings and NDVI values with total N uptake at mid flowering stage

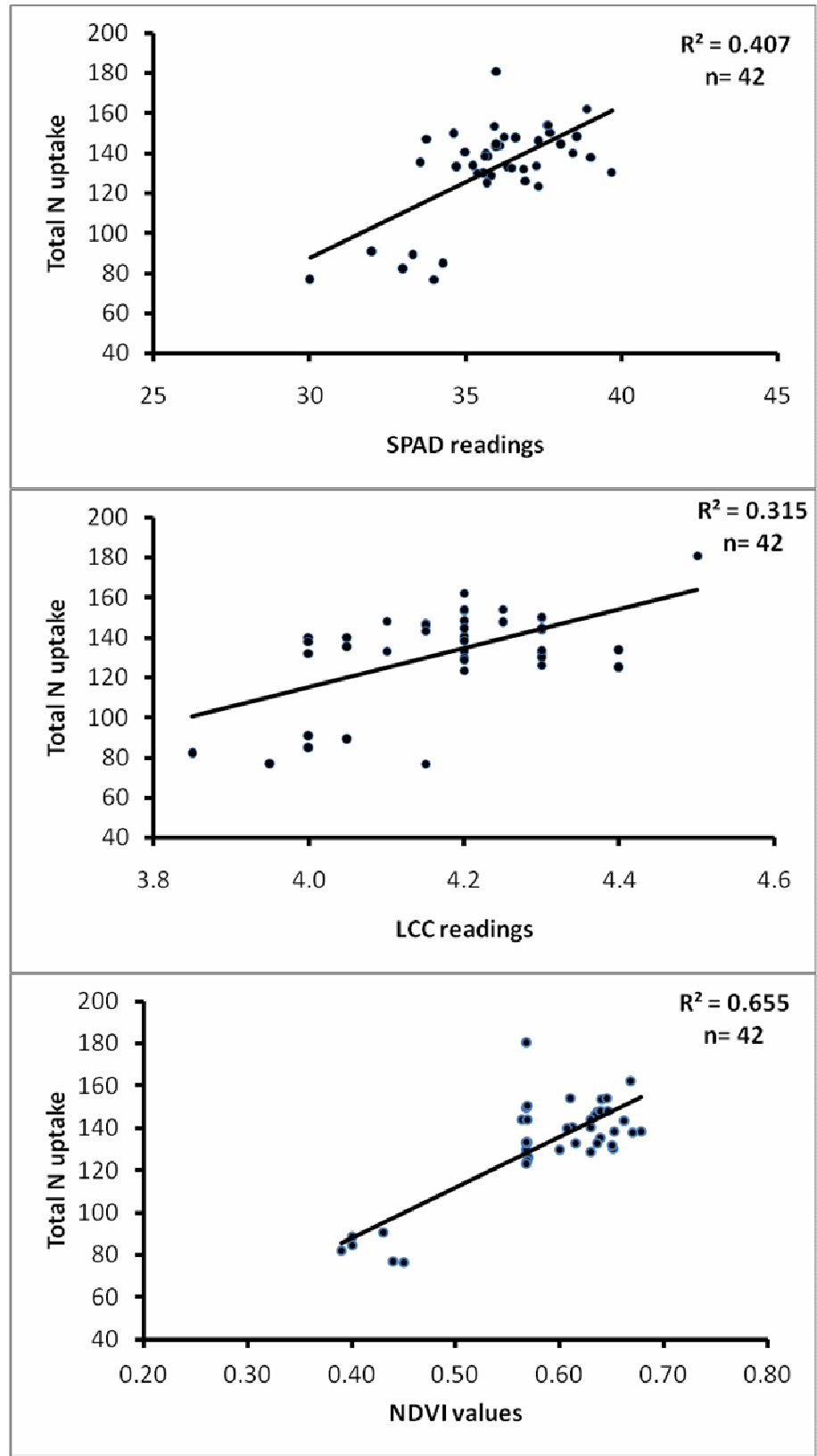


Fig. 4.6.6. Relationship of LCC, SPAD meter readings and NDVI values with total N uptake at peak flowering stage

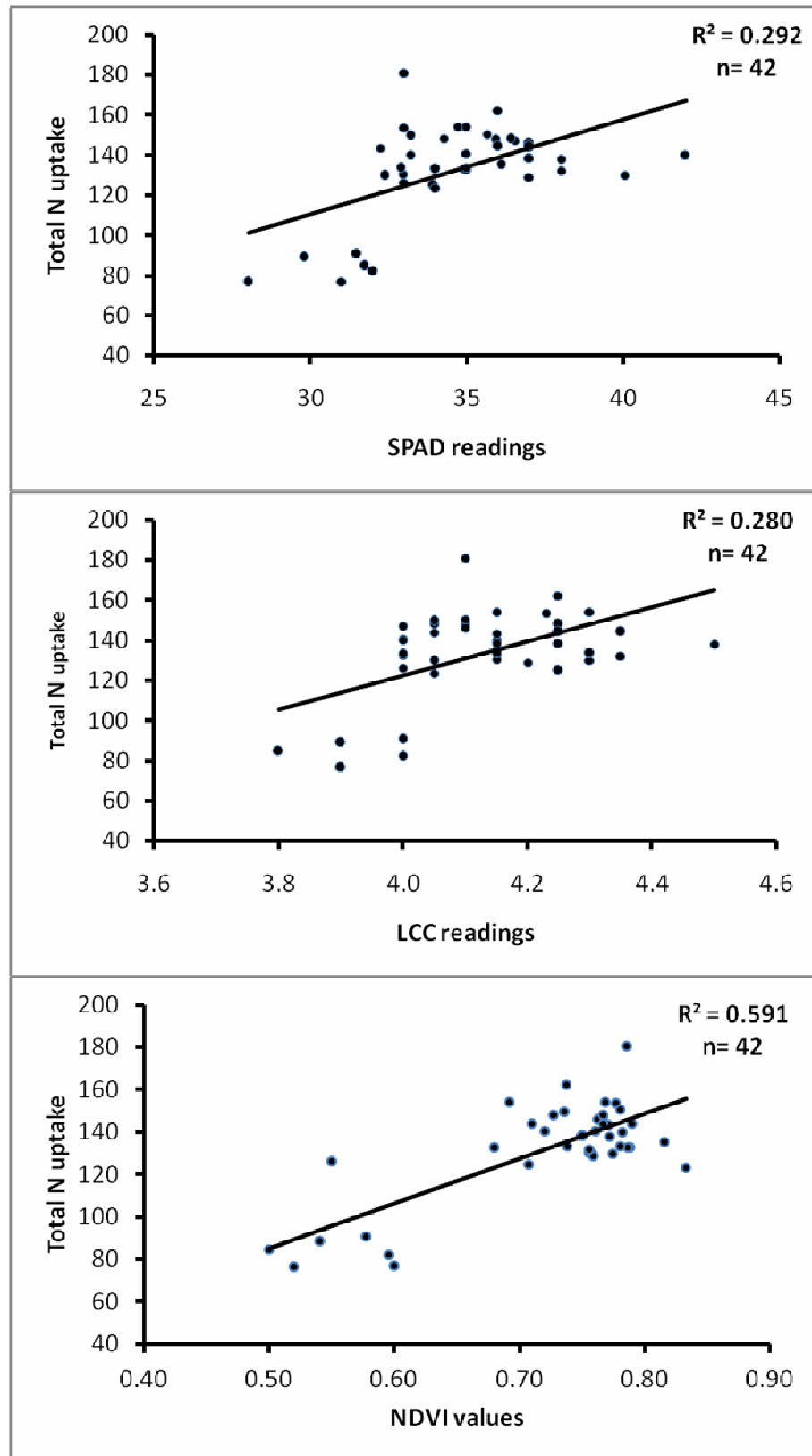


Fig. 4.6.7. Relationship of LCC, SPAD meter readings and NDVI values with total N uptake at boll formation stage

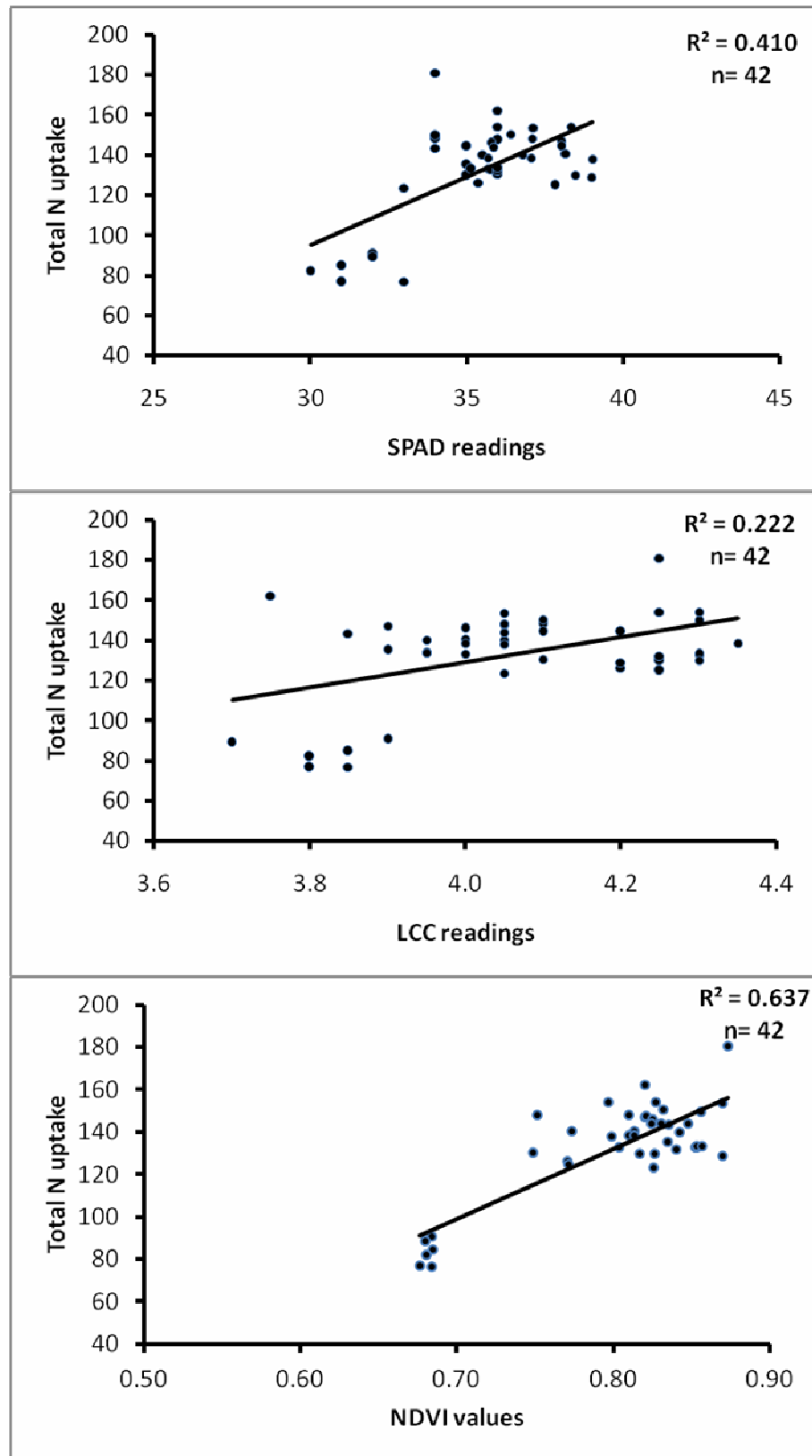


Fig. 4.6.8. Relationship of LCC, SPAD meter readings and NDVI values with total N uptake at boll filling stage

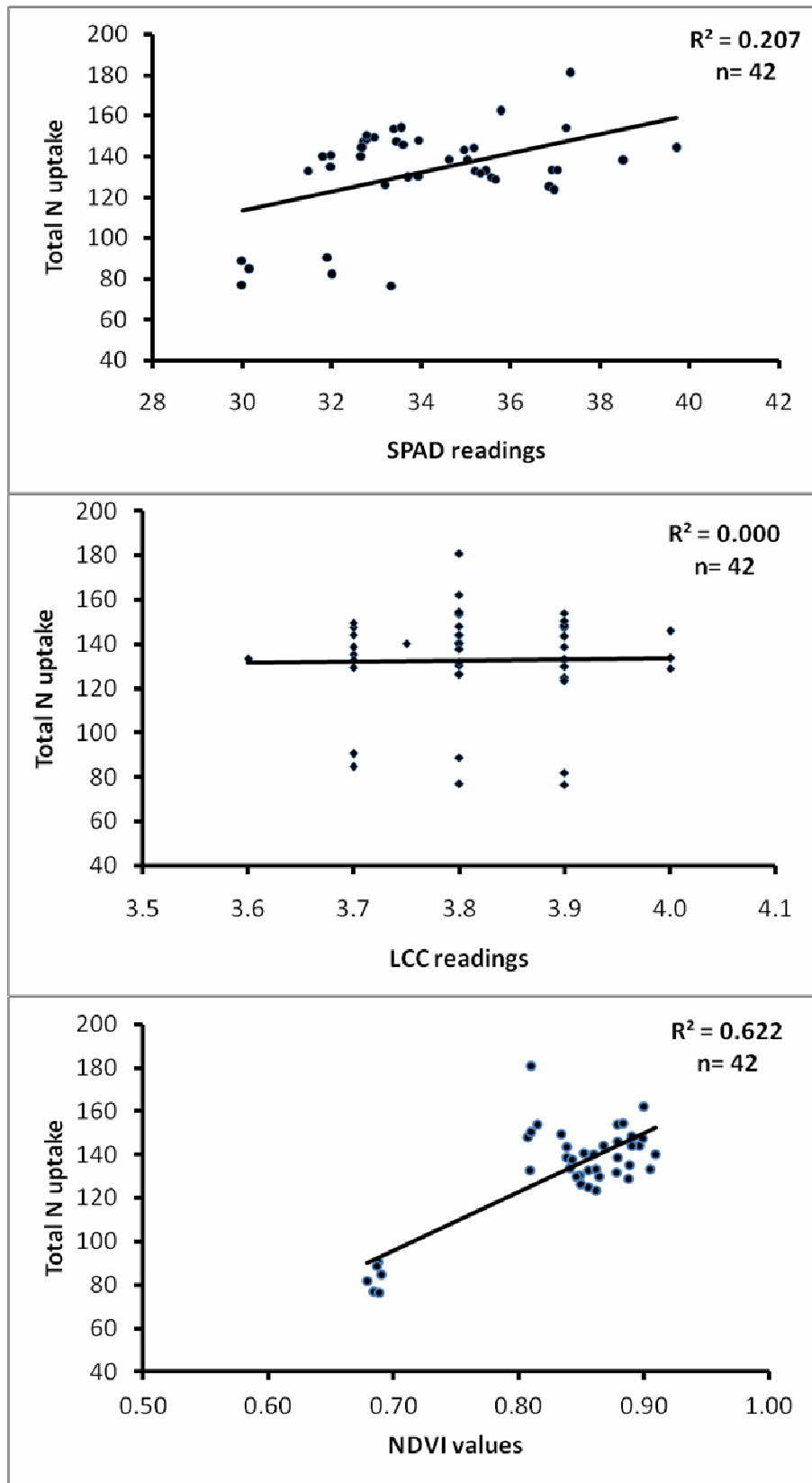


Fig. 4.6.9. Relationship of LCC, SPAD meter readings and NDVI values with total N uptake at boll opening stage

4.6.1 to Fig. 4.6.9. The LCC readings were correlated to total uptake at $p=0.01$ level of significance at squaring, early flowering and mid flowering growth stages and maximum R^2 value (0.582) was observed at early flowering stage (Fig.4.6.4). The coefficient of determination for total N uptake at maturity when measured using SPAD meter readings was more than LCC reading at different growth stages of cotton. The SPAD meter readings were correlated to total N uptake at $p=0.01$ level of significance at first squaring, squaring, early flowering, mid flowering, peak flowering and boll filling stages. The maximum R^2 value (0.576) was observed at early flowering stage (Fig.4.6.4). The SPAD meter proved to be more accurate in predicting total N uptake as in case of leaf N content and seed cotton yield. The NDVI values were correlated to total N uptake at $p=0.01$ level of significance at mid flowering, peak flowering, boll formation, boll filling and boll opening stages and maximum R^2 value (0.655) was observed at peak flowering stage (Fig. 4.6.6).

The coefficient of determination obtained with NDVI values gave better understanding of total N uptake than SPAD meter and LCC readings at growth stages after mid flowering; however NDVI values at early growth stages are not better indicator of total N uptake. The results support the correlations of leaf N content (section 4.4) and seed cotton yield (section 4.5) with NDVI values. The uncovered soil surface at early crop growth stages interfere with the NDVI values recorded using GreenSeeker optical sensor and thus lead to poor correlation with the growth and N uptake parameters. Li *et al* (2001) observed positive correlation with N accumulation and NDVI values calculated from reflectance at 2 m above the cotton canopy. Bronson *et al* (2011) reported that total N uptake, N recovery efficiency and cotton lint yield was significantly at par in soil test based and reflectance based N management treatments with the use of 50 per cent less N in reflectance based N management.

4.7 Relationship between LCC, SPAD and NDVI at different growth stages of cotton.

A plot of correlation coefficient of SPAD meter readings vs. LCC readings across the cultivars is plotted at different growth stages in Fig. 4.7.1. The data revealed that SPAD meter and LCC readings are closely related at $p=0.01$ level of significance at first squaring, squaring, early flowering, mid flowering and boll formation stage of cotton. Close correlation of LCC readings with SPAD meter readings, plant N content (section 4.4), cotton seed yield (section 4.5) and plant N uptake (section 4.6) revealed that LCC can be used as an economical substitute of SPAD meter for poor and marginal farmers in developing countries to schedule in season fertilizer N application at squaring and flowering stages of cotton. Win (2003) reported close relationship in LCC and SPAD meter readings in rice ($r=0.94$) and wheat ($r=0.97$). Similarly, Shukla *et al* (2004) correlated LCC readings and SPAD readings and reported a strong positive correlation in rice ($r=0.84$) and wheat ($r=0.91$). Yang *et al* (2003) also found the close relationship between LCC and SPAD readings (R^2 range of 0.62–0.98) in rice.

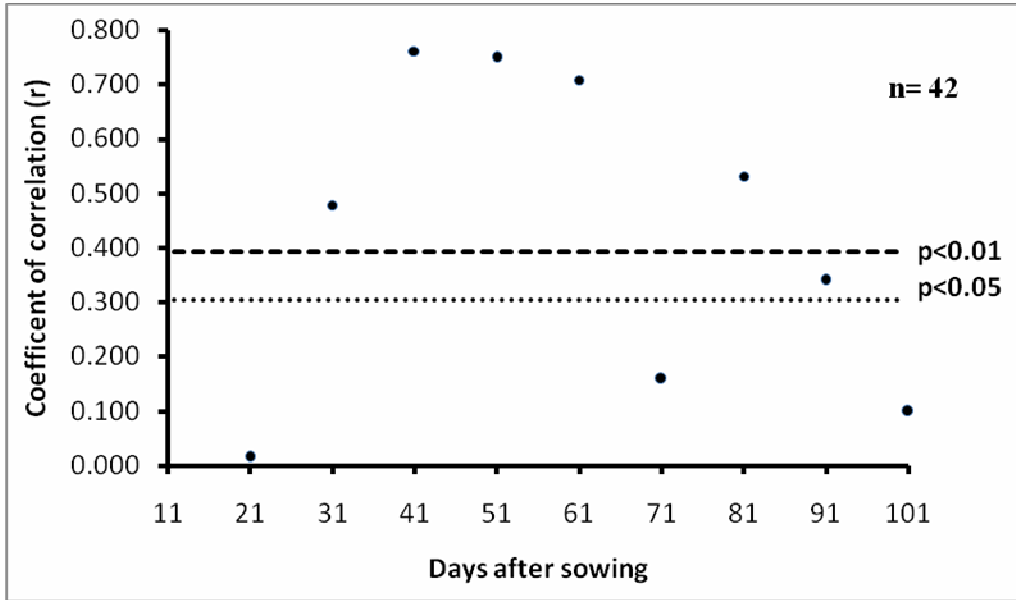


Fig. 4.7.1. A plot of correlation coefficient of SPAD meter readings vs. LCC readings across the cultivars at different growth stages of cotton

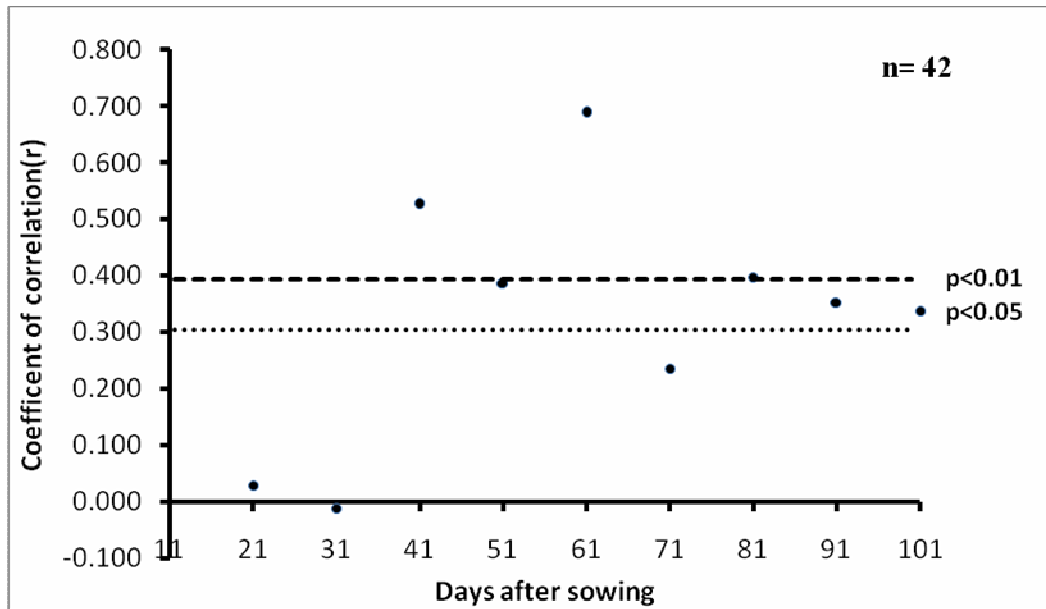


Fig. 4.7.2. A plot of correlation coefficient of NDVI values vs. LCC readings across the cultivars at different growth stages of cotton.

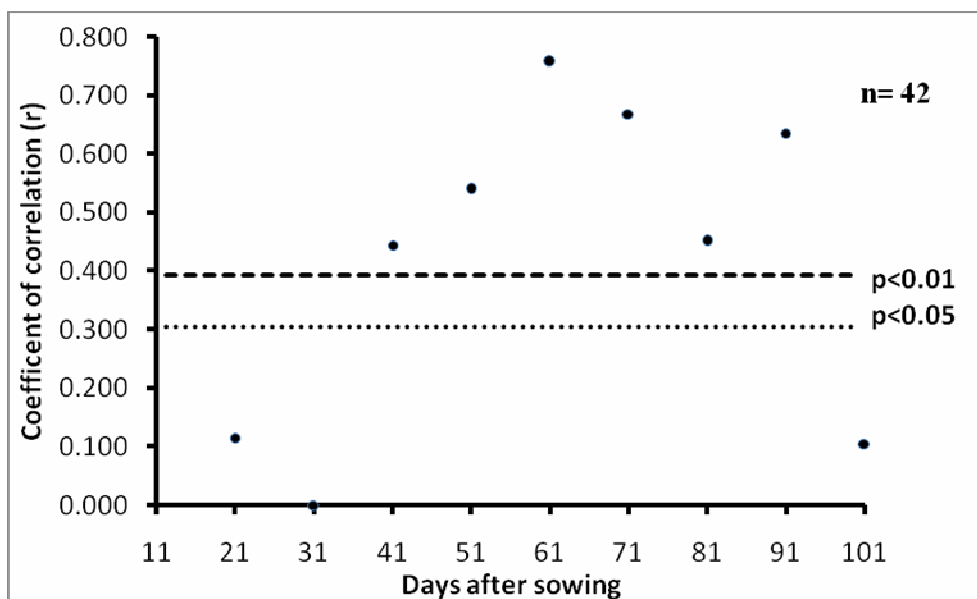


Fig. 4.7.3. A plot of correlation coefficient of NDVI values vs. SPAD readings across the cultivars at different growth stages of cotton

A plot of correlation coefficient of NDVI values vs. LCC readings across the cultivars at different growth stages is given in Fig. 4.7.2. The figure revealed a close relationship between NDVI values and LCC readings at $p=0.01$ level of significance at squaring and mid flowering stage of cotton. The LCC and NDVI were not closely related at other growth stages as LCC is a measure of only leaf greenness whereas NDVI values provide a measure for greenness and biomass production. The coefficient of correlation between SPAD readings and NDVI values (Fig. 4.7.3) was better than LCC readings vs. NDVI values. The SPAD and NDVI values were closely related at $p=0.01$ level of significance at squaring, early flowering, mid flowering, peak flowering, boll formation and boll filling stages. The data suggest that SPAD can be used as a reliable substitute of NDVI to guide in season fertilizer N topdressings in cotton.

4.8 Effect of level of fertilizer N application on cotton seed yield, stover yield, total N uptake, agronomic efficiency and recovery efficiency of applied fertilizer N, soil pH, EC and availability indices of soil nitrogen

The effect of fertilizer N application on cotton seed yield, stover yield, total N uptake, agronomic efficiency and recovery efficiency of applied N in two different cotton cultivars are provided in Table 4.8.1. In cotton cultivar MRC 7017, the highest cotton yield was recorded with the application of 75 kg N ha^{-1} which was at par with the application of 60 kg N ha^{-1} but higher than the no N control. The application of fertilizer N at levels higher than 75 kg N ha^{-1} did not lead to any improvement in seed cotton yield. However higher N application lead to increased biomass production and stover yield produced with the application of 240 kg

Table 4.8.1. Effect of level of fertilizer N application on cotton seed yield, stover yield, total N uptake, agronomic efficiency (AE_N) and recovery efficiency (RE_N) of applied fertilizer N

Cultivar	Treatment	Seed cotton Yield (t ha ⁻¹)	Stover Yield (t ha ⁻¹)	Total N uptake (kg ha ⁻¹)	AE _N [§]	RE _N [*]
MRC 7017	No-N control	1.1	5.9	81.2	-	-
	60 kg N ha ⁻¹	2.4	6.6	131.8	21.6	84.4
	75 kg N ha ⁻¹	2.6	6.2	136.6	19.7	73.9
	90 kg N ha ⁻¹	2.4	7.8	145.9	13.8	72.0
	120 kg N ha ⁻¹	2.4	7.9	144.4	11.0	52.7
	180 kg N ha ⁻¹	2.1	8.1	141.3	5.2	33.4
	240 kg N ha ⁻¹	2.1	9.1	150.7	3.9	28.9
Ankur 3028	No-N control	1.2	5.8	85.3	-	-
	60 kg N ha ⁻¹	2.4	6.7	134.5	19.6	82.0
	75 kg N ha ⁻¹	2.5	6.6	138.1	17.8	70.5
	90 kg N ha ⁻¹	2.3	7.1	134.4	12.6	54.6
	120 kg N ha ⁻¹	2.3	7.6	133.0	8.9	39.8
	180 kg N ha ⁻¹	2.2	8.1	147.6	5.8	34.6
	240 kg N ha ⁻¹	2.1	8.8	154.5	3.8	28.8
LSD (p=0.05):						
Cultivar		NS	NS	NS	NS	NS
Fertilizer treatment		0.30	0.69	12.3	3.57	12.0
Cultivar × Fertilizer treatment		NS	NS	NS	NS	NS

* Recovery efficiency of applied N (%)

§ Agronomic efficiency (Kg seed cotton yield kg⁻¹ N applied)

N ha⁻¹ was significantly higher than the application of 75 kg N ha⁻¹. Total N uptake does not differ significantly at fertilizer N application levels from 60-180 kg N ha⁻¹. The application of 240 kg N ha⁻¹ increased total N uptake attributed to more biomass and stover yield. The use of 60 kg N ha⁻¹ lead to highest agronomic efficiency (21.6 kg seed cotton yield per kg applied N) and recovery efficiency (84.4 per cent) of applied fertilizer N with the production of statistically higher seed cotton yield in cultivar MRC 7017. The application of higher N doses decreased the agronomic and recovery efficiency of applied N at every level of increased N

application.

The cotton cultivar Ankur 3028, also behaved similarly and highest cotton yield was recorded with the application of 75 kg N ha⁻¹ which was at par with the application of 60 kg N ha⁻¹ but higher than the no N control. There was no increase in seed cotton yield with further increase in fertilizer N application. The maximum stover yield was recorded with the application of 240 kg N ha⁻¹ without any improvement in seed cotton yield. The results indicate that cotton may not respond beyond 75 kg N ha⁻¹ to yield seed cotton. Increased plant bio mass at higher N application is not helpful to improve N use efficiency. The total N uptake with the application of 60 kg N ha⁻¹ was at par with the application of 180 kg N ha⁻¹, which however increase with the application of 240 kg N ha⁻¹. The maximum agronomic efficiency (19.6 kg seed cotton yield per kg applied N) and recovery efficiency (82.1 per cent) was obtained with the application of 60 kg N ha⁻¹. The results revealed that application of only 60 kg N ha⁻¹ in cotton may be sufficient to produce statistically maximum seed cotton yield with maximum agronomic and recovery efficiency of applied N in cotton. Johnson and Saunders (2003) reported insignificant differences in seed cotton yield at 60, 90, 120 and 150 lb ac⁻¹ fertilizer N application. Bronson *et al* (2011) reported that total N uptake, N recovery efficiency and seed cotton yield was significantly at par in soil test based (80 lb N ac⁻¹) and reflectance based (62 lb N ac⁻¹) fertilizer N management. Chua *et al* (2003) observed that chlorophyll meter based N management produced similar cotton lint yield as soil test based management by using 60 lb N ac⁻¹ less nitrogen.

Table 4.8.2. Effect of fertilizer N application on pH, EC and availability indices of soil nitrogen

Treatment	EC	pH	Organic carbon (%)	Ammonical-N (mg kg ⁻¹)	Nitrate- N (mg kg ⁻¹)	Available N (Kg N ha ⁻¹)
0 N	0.35	7.13	0.36	12.82	13.98	94.08
60 N	0.37	7.08	0.37	12.23	13.4	91.73
75 N	0.36	7.16	0.36	12.52	13.11	84.67
90 N	0.38	7.13	0.38	12.52	11.94	81.54
120 N	0.38	7.12	0.35	12.52	12.52	83.89
180 N	0.35	7.14	0.35	10.78	11.94	85.46
240 N	0.36	7.18	0.36	11.65	13.11	83.1
LSD(p=0.05)	NS	NS	NS	NS	NS	NS

The effect of different levels of fertilizer N application on properties of the surface soil sample (0-0.15 m) of experimental site is presented in Table 4.8.2. The data revealed that

soil pH and EC do not differ with the level of fertilizer N application in different cotton cultivars. Soil organic carbon, ammonical-N, nitrate-N and available N were also statistically at par irrespective of the level of fertilizer N application. Insignificant differences in N availability indices of no N control and 240 kg N ha⁻¹ treatment suggest that application of higher fertilizer N is neither helpful in improving inherent soil N nor N use efficiencies in cotton .

CHAPTER V

SUMMARY

Nitrogen (N) is the most used fertilizer nutrient in cotton and its consumption increased substantially in the last decades. One of the major causes of low N use efficiency in cotton is the application of fertilizer N in excess of the crop requirement and at the times when it is not required in large amounts. In northwestern India, the blanket fertilizer N recommendation consists of applying 150 kg N ha⁻¹ for Bt and non-Bt hybrids and 75 kg N ha⁻¹ for Desi cotton at fixed growth stages of the crop. These recommendations do not consider spatial and temporal variability in soil N supply and are established for large tracts. The synchronization between demand of N by cotton and supply from all available sources including fertilizers can help achieve higher N use efficiency. The gadgets like LCC, SPAD meter and GreenSeeker optical sensor have become available to measure different spectral properties of leaves and guide need based fertilizer N management in cotton. A field experiment was conducted during *khariif* 2012 to study the spectral properties of leaves using LCC, SPAD meter and GreenSeeker optical sensor in relation to yield, nitrogen content, uptake and use efficiencies in cotton at research farm, Department of Soil science at Punjab Agricultural University, Ludhiana, Punjab, India.

The experiment consisted of seven fertilizer N treatments (No-N control, 60 kg N ha⁻¹, 75 kg N ha⁻¹, 90 kg N ha⁻¹, 120 kg N ha⁻¹, 180 kg N ha⁻¹ and 240 kg N ha⁻¹) applied in two equal splits (at thinning and initiation of flowering) in two cotton cultivars (MRC 7017 and Ankur 3028). The layout of the experiment was arranged in split plot design with cultivars in main plot and N level in subplot. The objectives of the research programme include

- (i) The study of relationship of leaf greenness and biomass as measured by LCC, SPAD meter and GreenSeeker optical sensor with leaf N content, plant N uptake and seed cotton yield
- (ii) The study of relationship between estimates of LCC, SPAD and NDVI for guiding need-based fertilizer N applications in cotton
- (iii) The study of varietal differences in spectral properties of cotton leaves.

The crop was sown in first fortnight of May 2012. The LCC, SPAD, NDVI values, dry weight of leaves were recorded at different growth stages of cotton. The plant samples were analysed for N content at different growth stages. The SPAD meter readings of cotton leaves at node formation stage (21 DAS) were statistically at par irrespective of the level of fertilizer N application. The maximum SPAD reading was observed at squaring stage followed by steep decline during the flowering stages and then remains almost constant during boll formation, filling and opening stages of the cotton. The maximum LCC reading in fertilizer N application treatments was observed at early flowering stage which was almost at

par with the LCC readings at squaring and mid flowering followed by decline at later growth stages. The leaf color greenness in no N control treatment remain almost constant till peak flowering stage followed by slight decline during boll formation, boll filling and boll opening stages of cotton. The NDVI value showed an increasing trend with the growth of the crop at different levels of fertilizer N application and maximum value was observed at ball opening stage of cotton.

The effect of fertilizer N level on leaf N content was visible at first squaring, squaring, early flowering, mid flowering, peak flowering, boll formation and boll filling stages of cotton. The leaf N content increased with growth stage and was found maximum at squaring stage of cotton which remains almost constant during flowering stages followed by decline towards the maturity of the crop. The leaf dry weight increased with growth stage and was found maximum at mid flowering stage of cotton followed by decline towards the maturity of the crop. LCC readings were correlated to leaf N content at $p=0.05$ level of significance at squaring, early flowering, mid flowering, peak flowering, boll formation and boll filling stage and maximum R^2 value (0.583) was observed at early flowering stage. The SPAD meter readings were correlated to leaf N content at $p=0.05$ level of significance at squaring, early flowering, mid flowering, peak flowering, boll formation and boll filling stages and maximum R^2 value (0.635) was observed at mid flowering stage. The NDVI values recorded with GreenSeeker optical sensor were correlated to leaf N content at $p=0.01$ level of significance at mid flowering, peak flowering, boll formation and boll filling stages and maximum R^2 value (0.722) was observed at mid flowering stage.

LCC readings were correlated to seed cotton yield at $p=0.01$ level of significance at squaring and early flowering stages and maximum R^2 value (0.531) was observed at squaring stage. The SPAD meter readings were correlated to seed cotton yield at $p=0.01$ level of significance at first squaring, squaring, early flowering, mid flowering and boll filling stages and maximum R^2 value (0.572) was observed at squaring stage. The NDVI values recorded with GreenSeeker optical sensor were correlated to seed cotton yield at $p=0.01$ level of significance at mid flowering, peak flowering, boll formation and boll filling, boll opening stages and maximum R^2 value (0.613) was observed at boll opening stage. The coefficient of determination obtained with NDVI value after mid flowering stage gave better understanding of seed cotton yield than SPAD meter and LCC readings; however NDVI values at early growth stages are not better indicator of seed cotton yield.

The LCC readings were correlated to total N uptake at $p=0.01$ level of significance at squaring, early flowering and mid flowering stages and maximum R^2 value (0.582) was observed at early flowering stage. The coefficient of determination for total N uptake at maturity when measured using SPAD meter readings was more than LCC reading at different growth stages of cotton. The SPAD meter readings were correlated to total N uptake at

p=0.01 level of significance at first squaring, squaring, early flowering, mid flowering, peak flowering and boll filling stages. The maximum R^2 value (0.576) was observed at early flowering stage. The SPAD meter readings proved to be more accurate in predicting total N uptake, leaf N content and seed cotton yield than LCC readings. The NDVI values were correlated to total N uptake at p=0.01 level of significance at mid flowering, peak flowering, boll formation, boll filling and boll opening stages and maximum R^2 value (0.655) was observed at peak flowering stage. The coefficient of determination obtained with NDVI values gave better understanding of total N uptake than SPAD meter and LCC readings at growth stages after mid flowering; however NDVI values at early growth stages are not better indicator of total N uptake.

SPAD meter and LCC readings are closely related at p=0.01 level of significance at first squaring, squaring, early flowering, mid flowering and boll formation stage of cotton. Close correlation of LCC readings with SPAD meter readings, plant N content, cotton seed yield and plant N uptake revealed that LCC can be used as an economical substitute of SPAD meter for poor and marginal farmers in developing countries to schedule in season fertilizer N application at squaring and flowering stages of cotton. The NDVI values and LCC readings were closely related at p=0.01 level of significance only at squaring and mid flowering stage of cotton, however the correlation was poor at other growth stages as LCC is a measure of only leaf greenness whereas NDVI values provide a measure for greenness and biomass production. The SPAD readings and NDVI values were closely related at p=0.01 level of significance at squaring, early flowering, mid flowering, peak flowering, boll formation and boll filling stages. The data suggest that SPAD readings can be used as a reliable substitute of NDVI values to guide in season fertilizer N topdressings in cotton.

In both Cotton cultivars (MRC 7017 and Ankur 3028) the highest cotton yield was recorded with the application of 75 kg N ha⁻¹ which was at par with the application of 60 kg N ha⁻¹ but higher than the no N control. The application of fertilizer N at levels higher than 75 kg N ha⁻¹ did not lead to any improvement in seed cotton yield. The maximum stover yield was recorded with the application of 240 kg N ha⁻¹. The total N uptake with the application of 60 kg N ha⁻¹ was at par with the application of 180 kg N ha⁻¹. The maximum agronomic efficiency and recovery efficiency was obtained with the application of 60 kg N ha⁻¹ in both the cultivars. It was observed that application of only 60 kg N ha⁻¹ in cotton may be sufficient to produce potential seed cotton yield with maximum agronomic and recovery efficiency of applied N in cotton. Indices of soil N supply do not differ with the level of fertilizer N application. Insignificant differences in N availability indices of no N control and fertilizer N treatments suggest that application of higher fertilizer N is neither helpful in improving inherent soil N nor seed cotton yield.

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