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## Evaluation of SPAD meter values for estimating rice nitrogen status

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### Abstract

A field experiment with six rice genotypes were conducted to relate SPAD chlorophyll meter readings to variables reflecting nitrogen (N) status at different developmental stages. These indices can be used to predict foliar N concentration in rice without significant interferences of rice genotypes and growth stages. Dependence of nitrogen content from mineral element in the soil was established at the same time. Investigations were guided on six fertilization variants (N1: 0; N2: 40; N3: 60; N4: 80; N5: 100; N6: 150 kg N ha<sup>-1</sup>). The results indicated that nitrogen content influenced from presence and ratio mineral elements in the soil. SPAD value increased with increase in N level and growth stages upto FL stage. However no clear trend is observed among cultivars, since ratio of SPAD readings to predict rice N status is not influenced by rice genotypes and is based on the principle of N translocation from old to young leaves when the plant is nitrogen deficient.

**Keywords:** Soil-plant analyses development (SPAD), nitrogen (N), maximum tillering (MT), panicle initiation (PI), flowering (FL), grain filling (GF), chlorophyll meter

### Introduction

From all metabolic elements which plants use from soil, nitrogen is an absolutely essential for crop growth and plant need nitrogen in the largest amounts (TUCKER, 2004) [29]. Under sufficient amounts of available nitrogen plants develop, grow rapidly with healthy green colour. Nitrogen exists in organic and inorganic form and the greatest nitrogen content is in seeds, leaves, shoots and roots. Leaves exhibit a structural and functional accumulation of the photosynthetic apparatus to the light intensity experienced during their growth (PRILOUL *et al.*, 1980) [27]. Nitrogen supply has large effect on leaf growth because it increases the leaf area of plants and, on that way, it influences on photosynthesis. Photosynthetic proteins represent a large proportion to total leaf N (EVANS, 1989; FIELD and MOONEY, 1986) [6, 9]. Chlorophyll content is approximately proportional to leaf nitrogen content, too (EVANS, 1983) [7].

Nitrogen is the most deficient nutrient element in Indian soils and insufficient N supply results in smaller leaf area (Fernandez *et al.*, 1996; Zhao and Oosterhuis, 2000) [8], loss of green colour of leaves i.e., chlorophyll content, lower intensity of photosynthesis and lower biomass production (Zhao and Oosterhuis, 2000) [33], leading to the loss of yield and quality otherwise an adequate supply of nitrogen can increase as much as 60% rice yield (Mikkelsen *et al.*, 1995) [19] because of its key role in cell division. If cell division is stopped, the leaf area decreases and thereby loses its potential to produce an adequate yield.

Adding more nitrogen than needed for optimum yield will generally result in an increase in grain protein until an upper threshold is reached. Excessive application of N fertilizers in rice production is quite common in agriculture (Ju *et al.*, 2009) [16], which increases not only production cost but also environmental pollution (Jaynes *et al.*, 2001) [15], which is partly because of the lack of routine diagnostic analysis. Since nitrogen is one of the key limiting nutrients in crop growth; So, finding efficiency levels of nitrogen is important for growth, development, protein, and yield therefore, Crop-demand based N application is one of the important options to reduce N loss and to increase N use efficiency of a crop.

There have been no rapid diagnostic methods of N status for fertilizer application recommendation in the field, which can be operated easily and reproducibly by farmers (Wang

*et al.*, 2001; Peng *et al.*, 2006) [32, 24, 25]. Therefore, it is important to develop effective diagnosis of rice N status for sustainable management of rice production. Conventional wet-chemistry techniques for foliar N analysis are time-consuming, expensive and not easily to be affordable by farmers and reduce its utility for within-season management of N supply. Therefore, Several improved methods have been proposed for non-destructive estimation of plant N nutrition, including leaf color charts, chlorophyll meter, reflectance spectra, and chlorophyll fluorescence (Shukla *et al.*, 2004; Huang *et al.*, 2008; Nguyen and Lee, 2006) [28, 20].

On the contrary, chlorophyll meters is simple, low-cost and affordable by farmers and portable diagnostic tool that measures the greenness or relative chlorophyll content of leaves (Inada, 1963 and 1985; Kariya *et al.*, 1982). Chlorophyll meter equipments such as Soil-Plant Analyses Development (SPAD, Minolta Camera Co., Osaka, Japan) are designed to determine chlorophyll concentration of leaves, and have become a popular method for estimating leaf N in rice (Turner and Jund, 1991, 1994). Since, there is a strong linear relationship between SPAD values and leaf nitrogen concentration, It is possible to monitor leaf N status using SPAD thresholds and guide fertilizer-N timing on irrigated rice (Peng *et al.*, 1996b, 2006; Huang *et al.*, 2008; Balasubramanian *et al.*, 1999) [24, 25, 11, 12, 2], but this relationship can be influenced by many factors such as rice variety, crop growth stage, leaf position and the sampling point on the leaf (Huang *et al.*, 2008; \*Peng *et al.*, 2006; Hoel and Solhaug, 1998; Esfahani *et al.*, 2007) [11, 12, 24, 25], mostly because of leaf thickness or specific leaf weight (Peng *et al.*, 1993) [23]. The linear relationship between nitrogen and SPAD values has led to the adaptation of the SPAD meter to assess crop nitrogen status and to determine the plant's need for additional nitrogen fertilizer (Peng *et al.*, 1995 and 1996; Balasubramanian *et al.*, 1999) [22, 26, 2]. SPAD readings indicate that plant nitrogen status and the amount of nitrogen to be applied are determined by the physiological nitrogen requirement of crops at different growth stages.

Chlorophyll meter (SPAD) can be used for adjustment of fertilizer N application based on actual plant N status (Balasubramanian *et al.*, 1999) [2]. Need based N application would result in greater agronomic efficiency of N fertilizer than the commonly practiced method (Hussain *et al.*, 2000) [13]. Ali *et al.*, 2005 [1] reported that the requirement of N fertilizer based on SPAD reading was found 15 and 40 kg N/ha lower compared to conventional N management during wet and dry seasons, respectively. The SPAD meter-based N management appeared to be more efficient and would save 20-30 kg N/ha than the conventional N management practices to produce similar grain yield (Miah and Ahmed, 2002) [18].

The overall objective of this research was to describe the temporal variability present in a typical rice paddy field and also to determine that chlorophyll meter readings of leaves at which stage of rice growth shows higher amount, to assess whether the chlorophyll meter analysis of rice leaves at different growth stages would be useful in making nitrogen fertilizer recommendations

### Material and Methods

One field experiment was conducted at research farm of ICAR- National Rice Research Institute, Cuttack, Odisha, India (20°25'N, 85°55'E; elevation 24 m above mean sea level) having sub-humid tropical climate during Kharif season. The soil had the following properties: pH 6.7, organic carbon 0.54%, available N 259 kg ha<sup>-1</sup> air-dry soil, available P

21 kg ha<sup>-1</sup> air-dry soil, available K 189 kg ha<sup>-1</sup> air-dry soil. Treatments were arranged in a factorial randomized block design with N rates as main plots and variety as subplot. Experimental plot is especially designed to carry out nitrogen experiments. Cemented bunds are having plot size – 6m\*4m (24m<sup>2</sup>) which is one meter deep from the surface to avoid seepage across the bunds and half meter high from the surface to allow submergence condition to the crop. There were three replications in each of the treatments.

The six tested rice cultivars with varying N response viz- Naveen, Indira, Ratna, , Surendra, Birupa and Daya were grown under different six levels of nitrogen (N1: 0; N2: 40; N3: 60; N4: 80; N5: 100; N6:150 kg N ha<sup>-1</sup>), and the urea were split-applied in three times with one third of recommended dose. Before two to three day of transplanting plots were flooded followed by puddling, 25 days seedling were transplanted with plant to plant distance 15 cm and row to row spacing 20 cm. Nitrogen was applied in three splits one third on basal and remaining two third in equal splits at maximum tillering and panicles initiation stage. Phosphorous and potassium were applied once (40 kg ha<sup>-1</sup>) at the time of transplanting through single super phosphate and murate of potash respectively. Flood irrigation was applied to keep 3-5 cm standing water during the cropping season. Standard crop management practices were followed to control weed, pest and diseases. Data related to soil and plant parameters were collected at important growth stages.

**SPAD value:** The SPAD-502, a hand held chlorophyll meter (Minolta corporation, Ramsey, N.J.) was used for rapid non-destructive estimation of extractable chlorophyll in green leaves. This instrument uses a silicon photo-iodide to detect transmittance of light emitted by two light emitting diodes-through a leaf sample, one with peak emittance at 650 nm, where absorbance by chlorophyll is high and relatively unaffected by carotene and another with peak emittance at 940 nm, where absorbance by chlorophyll is negligible. The SPAD (soil plant analysis development) reading that is correlated with leaf chlorophyll concentration is calculated from the transmittance through leaf tissue at these two wavelengths. Chlorophyll content of randomly selected leaves from various rice plants in net plot area was measured at MT, PI, FL, stage of the crop. Finally, the average value on chlorophyll content were computed and expressed in SPAD value.

### Result and Discussion

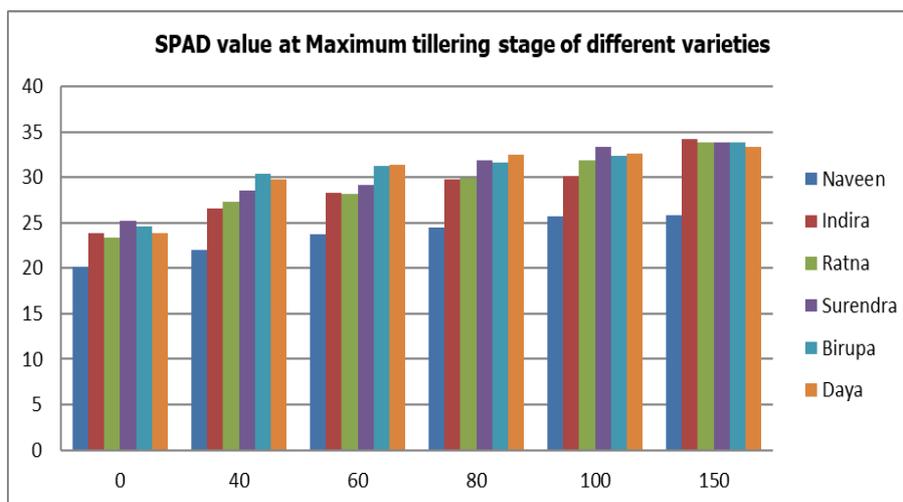
Nitrogen is a structural element of chlorophyll and protein molecules, and thereby affects formation of chloroplasts and accumulation of chlorophyll in plant (Tucker, 2004; Daughtry, 2000) [28, 4]. Leaf N content and greenness of leaf are often positively related (Cabrera, 2004) [3]. The SPAD value provides immediate, on-site, quantitative measurements of leaf greenness that have been correlated ( $r^2 = 0.62$ ) with rice plant N needs during different growth stages not measure N or chlorophyll concentration. The SPAD meter measurements taken on the most recently matured leaf can range from 25 to 44 depending on N uptake and growth stage. Though studies reported positive correlation of SPAD value with leaf N concentration in rice, (Shukla *et al.*, 2004) [28] it does not measure N or chlorophyll concentration; rather SPAD values were significantly affected by rice plant growth stage, cultivar, leaf thickness, plant population, and other soil or climate factor causing leaf chlorosis. SPAD value increased with increase in N level and growth stages upto FL

stage shown in table.1. However no clear trend is observed among cultivars (figure.1 to figure.3), since ratio of SPAD readings to predict rice N status is not influenced by rice

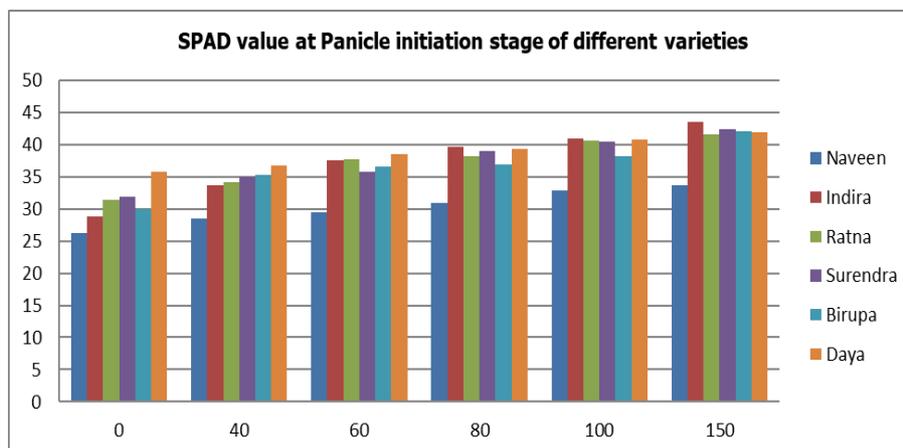
genotypes and is based on the principle of N translocation from old to young leaves when the plant is nitrogen deficient (figure.4).

**Table 1:** SPAD value from maximum tillering to flowering stage of different varieties

SN	Treatment	N doses (kg ha <sup>-1</sup> )						mean
	Varieties(V)	0	40	60	80	100	150	
<b>A</b>	<b>SPAD value at Maximum tillering stage of different varieties</b>							
	Naveen	20.2	22	23.75	24.45	25.65	25.8	23.64
	Indira	23.8	26.6	28.3	29.8	30.15	34.25	28.82
	Ratna	23.35	27.35	28.15	29.9	31.85	33.9	29.08
	Surendra	25.2	28.6	29.15	31.85	33.4	33.9	30.35
	Birupa	24.55	30.35	31.25	31.65	32.3	33.8	30.65
	Daya	23.85	29.75	31.35	32.45	32.65	33.35	30.57
	Mean	23.64	27.36	28.54	30.26	31.26	32.62	
<b>B</b>	<b>SPAD value at Panicle initiation stage of different varieties</b>							
	Naveen	26.2	28.55	29.45	30.95	32.85	33.7	30.28
	Indira	28.8	33.7	37.6	39.65	41	43.6	37.39
	Ratna	31.45	34.1	37.65	38.25	40.65	41.65	37.29
	Surendra	31.85	35	35.75	39.05	40.5	42.4	37.43
	Birupa	29.95	35.25	36.65	36.95	38.2	42.05	36.51
	Daya	35.7	36.75	38.5	39.3	40.85	41.9	38.83
	Mean	30.14	33.94	36.04	37.71	39.35	41.07	
<b>C</b>	<b>SPAD value at flowering stage of different varieties</b>							
	Naveen	27.5	35.15	39.2	41.95	45.5	47.5	39.47
	Indira	32.3	38.95	39.8	40.8	41.9	44.85	39.77
	Ratna	35.45	36.05	37.85	39	42.1	47.05	39.58
	Surendra	36.55	37.25	38.25	40.1	41.05	45.85	39.84
	Birupa	36.75	38.65	39.35	39.55	41.35	42.85	39.75
	Daya	30	37.75	39.8	42.85	43	44.25	39.61
	Mean	33.64	37.44	39.16	40.73	42.43	45.21	



**Fig 1:** SPAD value at Maximum tillering stage of different varieties



**Fig 2:** SPAD value at Panicle initiation stage of different varieties

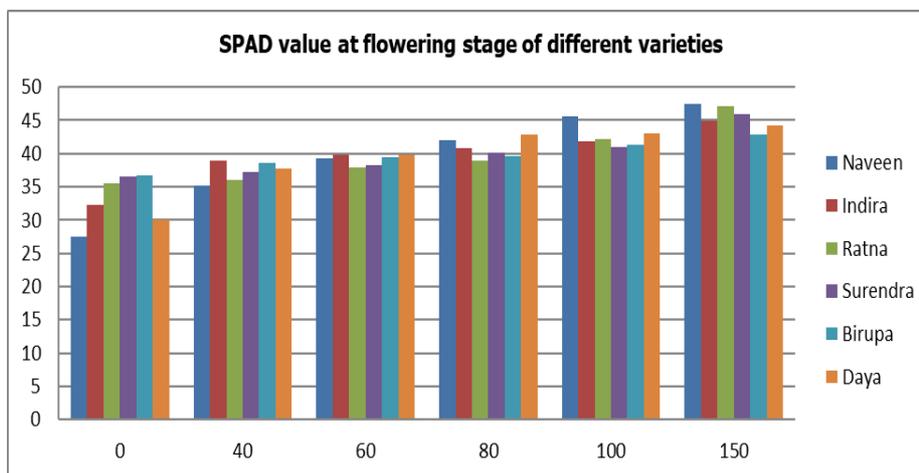


Fig 3: SPAD value at flowering stage of different varieties

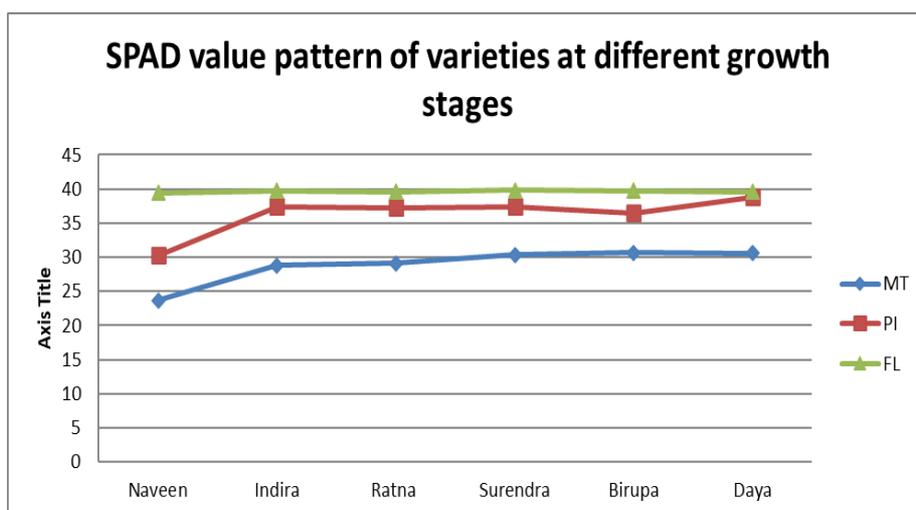


Fig 4: SPAD value pattern of varieties at different growth stages

## Conclusion

Since SPAD readings are closely related to leaf N content, the SPAD meter can be used to monitor the N status of rice and thereby to adjust the rate of N fertilization in order to increase N use efficiency (Hussain *et al.*, 2000 and Varvel *et al.*, 2007) [13, 31]. Analyses of data collected at different growth stages were used to determine when in the season SPAD data can be used to predict leaf N amount and future crop N need. The increasing of SPAD reading values with growth stage could be observed in this study. Although a chlorophyll meter (SPAD) has become a simple, quick and portable diagnostic tool for monitoring leaf N status and improving the timing of N topdressing in rice, the problem is that inconsistent SPAD critical indices exist in N fertilizer recommendation for different leaf positions, and different measurement points of the same leaf.

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