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Chlorophyll meter – a decision-making tool for nitrogen application in wheat under light soils

M.M. Akhter[a], Akbar Hossain[a,*], Jagadish Timsina[b], Jaime A. Teixeira da Silva[c], M.S. Islam[d]

[a]*Wheat Research Centre, Bangladesh Agricultural Research Institute, Dinajpur-5200, Bangladesh.*
[b]*Faculty of Veterinary and Agricultural Sciences, University of Melbourne, Melbourne, Australia.*
[c]*P.O. Box 7, Miki-cho post office, Ikenobe 3011-2, Kagawa-ken, 761-0799, Japan.*
[d]*Hajee Mohammad Danesh Science and Technology University, Dinajpur-5200, Bangladesh.*
*Corresponding author. E-mail: akbar.wrc@bari.gov.bd

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Abstract

Nitrogen (N) in plants is generally diagnosed by a soil test and plant tissue analysis. However, such analyses are costly in terms of time and money and are not easily accessible by researchers and extension workers, let alone farmers. Alternative cost-effective methods are required for rapid analysis of the N status of crops and to guide N management in wheat. The objective of this study was to assess whether the SPAD values using a leaf chlorophyll meter could be used to apply N at the maximum tillering (MT) stage of irrigated spring wheat grown under light soils of South Asia. Experiments were conducted over two years under light soils at the Wheat Research Centre (WRC), Dinajpur, Bangladesh. Treatments were 80, 100 and 120 kg N ha^{-1}, applied two-thirds as basal and one-third at the crown root initiation (CRI) stage along with additional 10, 20 and 30 kg N ha^{-1} (first year) and 0, 10, 20 and 30 kg N ha^{-1} (second year) at MT. Rates at MT were determined on the basis of SPAD values, which fell below the critical value of 37.5, recorded at 45 days after sowing (DAS). SPAD values recorded at 55 and 65 DAS were positively correlated with grain yield (GY), indicating that the application of extra N at MT influences wheat GY. Our results have two major implications: (i) SPAD values based on a leaf chlorophyll meter can be used for N application and (ii) extra 30 kg N ha^{-1} at MT is recommended for achieving maximum GY of irrigated spring wheat under the light soils of South Asia.

Keywords: Grain yield; Maximum tillering; Nitrogen; Leaf chlorophyll meter; SPAD values; Wheat.

Introduction

Nitrogen (N) is the most important nutrient for plant growth, yield and grain quality (Sarker et al., 2015). In developing countries, farmers likely apply excess N fertilizer to achieve higher yield. However, excessive N fertilizers can lead to elevated NO_3-N concentrations in groundwater which often exceeds the maximum level of drinking water standards, damaging human health (Liang et al., 2011). In plants, excessive N application prolongs crop duration, the period between leaf appearance and leaf yellowing (Wang et al., 2011; Hall et al., 2014), decreases grain yield (GY) and increased N loss (Wang et al., 2011). Nitrogen toxicity in cereal crops leads to low N recovery efficiency and the risk of groundwater pollution while over-fertilizing increases maintenance and labour costs (Wang et al., 2011).

However, the determination of an optimal N dose is a difficult task. Many studies have revealed a positive correlation between N uptake, leaf N concentration and leaf chlorophyll content and the GY of many crops (Timsina et al., 2006; El-Habbal et al., 2010; Guendouz et al., 2014). Plant N is traditionally diagnosed by a soil test and plant tissue analyses (Timsina et al., 2006) but these are costly, time consuming and require sophisticated laboratory equipment that is often unavailable to users. Delays as a result of sample collection and laboratory work may also prevent a timely remedial response. The SPAD-502 chlorophyll meter (Minolta Camera Co., Osaka, Japan) can serve as a diagnostic tool for this purpose. It is a simple, portable and nondestructive light-weight device used to estimate leaf chlorophyll content (Minolta, 1989) that also serves as a diagnostic tool for identifying crop N status (Ling et al., 2011). This technique saves time and resources (Netto et al., 2005) and offers a new strategy for synchronizing N application with actual crop demand (Babu et al*.,* 2000). Chlorophyll meter readings (CMRs) have been positively correlated with destructive chlorophyll measurements in many crops (Zhu et al., 2012) and are considered to be a useful indicator of N top-dressing during crop growth (Naderi et al., 2012).

To increase N use efficiency in the irrigated spring wheat of South Asia, N fertilizer should be applied at growth stages when crop N needs are high so that applied N is least lost from the soil-plant system. Sufficient N is needed for high yield and quality of wheat. In much of South Asia, N fertilizer is applied to irrigated wheat in two split doses: two-thirds as basal and the rest at the crown root initiation (CRI) stage just after first irrigation, i.e., 17-21 days after sowing (DAS), but some farmers also apply at the maximum tillering stage (MT) after a second irrigation at 50-55 DAS (Sarker et al., 2015). However, there is a lack of suitable criteria to determine whether N application at MT is in fact needed. Therefore, it is necessary to test N diagnostic tools in wheat under varying soil and weather conditions. The SPAD-502 chlorophyll meter can help to establish the need to apply N at MT, which will largely depend on soil N supply. Therefore, a two-year field experiment was conducted to observe the effect of extra N application at MT, to establish the role of CMRs in guiding N application at MT and to determine the critical values of CMRs for N application of irrigated spring wheat for light soils of South Asia.

Materials and Methods

Site description

Location

The experiment was conducted in the *rabi* (winter) season of 2011-2012 (Y_1) and 2012-2013 (Y_2) under light soils at the research field of the Wheat Research Centre (WRC), Dinajpur, Bangladesh. The geographical position of the area is between 25° 38′ N, 88° 41′ E and 40 m above sea level.

Soil characteristics

Soil of the experimental field was analysed before sowing wheat in Y_1. Soil pH was measured in soil/water (1:2, w/v) using a glass electrode pH meter. Organic carbon was determined by the Walkley and Black oxidation method (Walkley and Black, 1934), total

N by the micro Kjeldhal method (Jackson, 1958), Ca and Mg by the extractable method (Hunter, 1972), P, K, S and Zn by a modified Hunter's method (BARC, 1984) and B determined colorimetrically by the Azomethine-H method (Sippola and Ervio, 1977).

The pH of the experimental site was 5.4 and organic matter was 1.34%. Soil total N was 0.07%, which was much lower than the critical level, indicating N deficiency. Soil K was 0.18 meq 100 g^{-1} soil, while P, S, Zn and B were 18.55, 5.55, 0.79 and 0.45 µg g^{-1} soil. Based on the critical levels of these plant nutrients, soil K, S, Zn and B were low, but soil P was high.

Weather information in 2011-12 and 2012-13

Weather data, specifically temperature, humidity and rainfall in both years, were recorded regularly by the HOBO U12 Family of Data Loggers (MicroDAQ.com) at the meteorological observatory of the WRC and are presented in Figure 1.

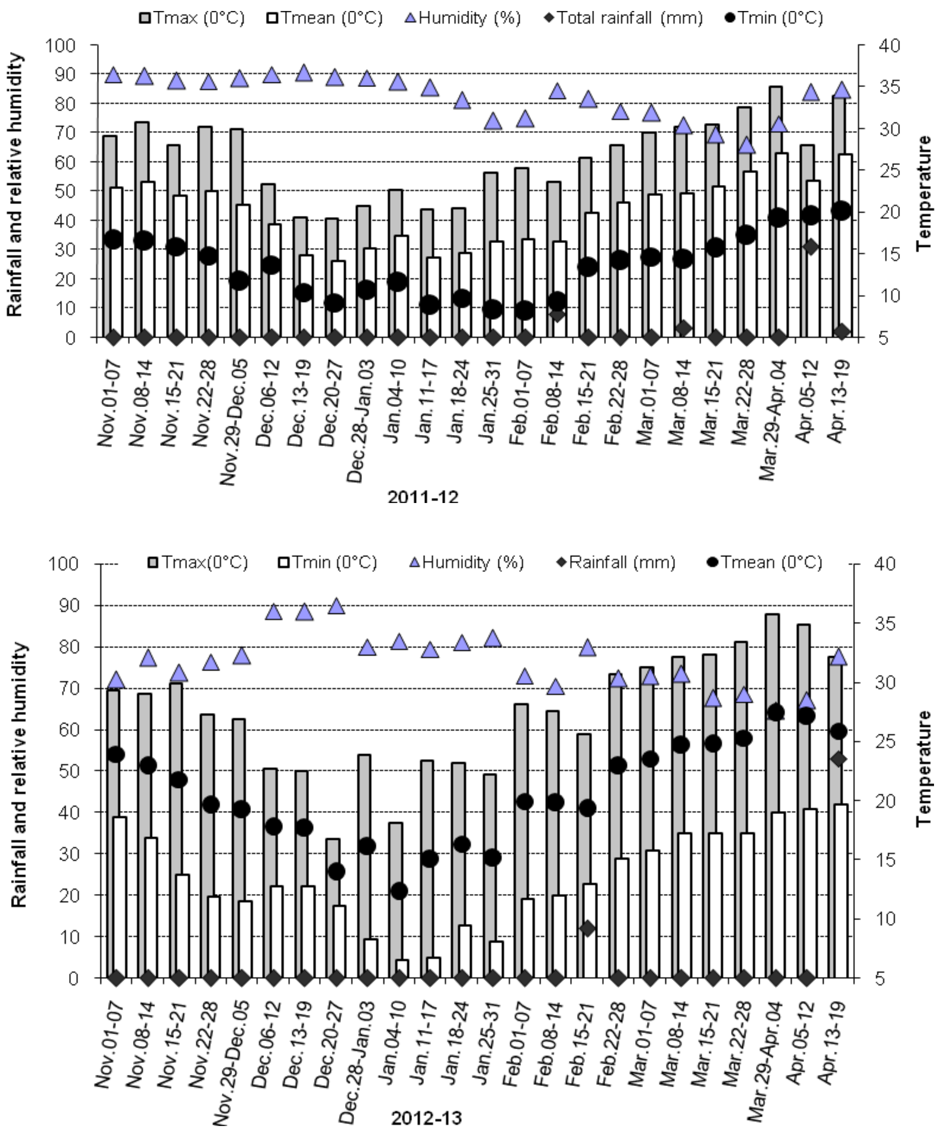

Figure 1. Weather information in both years of the experiment.

Experimental design and treatments

The experiment was laid out in a randomized complete block design with three replications. Three treatments (80, 100 and 120 kg N ha^{-1}), applied two-thirds as basal and one-third at the CRI stage along with extra 10, 20 and 30 kg N ha^{-1} in 2011-12 and 0, 10, 20 and 30 kg N ha^{-1} in 2012-13 at MT. The rates at MT were based on SPAD values if they fell below the critical value of 37.5, which was recorded at 45 DAS. The unit plot size was 4×5 m in Y_1 and 4×4 m in Y_2.

Experimental procedure and crop management

Variety, seed rate, sowing time and seed treatment

Wheat variety BARI Gom 26, a popular variety among Bangladeshi farmers, was sown at 120 kg ha^{-1} in lines 20 cm apart on 5 December 2011 (Y_1) and 28 November 2012 (Y_2). To achieve excellent germination and protecting against fungal attack during the seedling stage, seeds were pre-treated with a fungicide (Provax-200 WP containing Carboxin and Thiram). To control soil-borne insects, Furadan 5G containing Carbofuran (systemic pesticide; FMC Corp., Philadelphia, PA, USA) was broadcast at 10 kg ha^{-1} during final land preparation.

Fertilizer and irrigation

A blanket dose of P-K-S-B-Zn at 27-50-20-1-1.25 kg ha^{-1}, recommended by the WRC, was applied as basal during final land preparation (Sarker et al., 2015). Basal N application was carried out as indicated for each treatment while top dressing was applied after the first irrigation at CRI and after the second irrigation at MT. Soil was irrigated four times at 21, 45, 75 and 85 DAS (Y_1) and at 21, 45, 76 and 88 DAS (Y_2).

Data collection

The following data were recorded from an area of 0.5×0.4 m at 60 DAS in both years: initial plant population m^{-2} (PP) at 12 DAS, number of tillers m^{-2} (NT) at 40, 50 and 60 DAS, leaf area index (LAI) and above-ground dry matter (g m^{-2}) (DM). Leaf area was calculated with an Automatic Leaf Area Meter (Model AAM-9, Hayashi Denko Co. Ltd., Tokyo, Japan) and the DM of above-ground parts was determined after drying in an oven at 70 °C for 72 h. CMRs were taken three times at 45, 55 and 65 DAS in both years with a Minolta SPAD-502. Readings were taken from 10 leaves from 10 independent plants $plot^{-1}$, with three readings per leaf and then averaged. Yield and yield-contributing characters were recorded after harvesting the crop on 5 April 2012 and 1 April 2013. A yield sample was taken from a 2×4 m area (i.e., 10 middle rows) in 2011-12 and from a 3×2.8 m area (i.e., 14 middle rows) in 2012-13. Data on number of spikes m^{-2} (NS), spike length (SL), number of spikelets $spike^{-1}$ (NSS), number of grains $spike^{-1}$ (NGS), 1000-grain weight (TGW), GY and straw yield (SY) were recorded, the latter on a sun-dry basis for a large volume of straw. Data on GY and TGW were determined and adjusted to 12% moisture using the following equation (Hellevang, 1995):

$$Y(M_2) = \frac{100 - M_1}{100 - M_2} \times Y(M_1)$$

where $Y(M_2)$ = weight of grain at 12% moisture; $Y(M_1)$ = weight of grain at actual moisture %; M_1 = actual moisture %; M_2 = expected moisture %.

Data analysis

Data on PP at 12 DAS, NT at 40, 50 and 60 DAS, LAI, DM, CMRs at 45, 55 and 65 DAS, NS, SL, NSS, NGS, TGW, GY and SY were analysed by partitioning the total variance and treatment means were compared with the help of R package 'stats' (version 2.15.3) "R" at the 5% level of significance (R Core Team, 2013). Correlation analysis between GY and SPAD values at different DAS in both years was conducted by STAR (Statistical Tool for Agricultural Research; IRRI, 2014).

Results

Effect of N on wheat growth

In both years, PP was not influenced by different N levels (Table 1). NT at 40 DAS in both years and at 50 DAS in Y_1 were not influenced by N level. However, at 60 DAS in both years, NT was influenced by N level. At 40 DAS, highest NT was observed with 100 kg N ha^{-1} when applied as basal and at CRI in both years. NT at 50 DAS in Y_2 was influenced by different N doses (0 to 30 kg ha^{-1}), when applied at MT with all N levels applied as basal and at CRI. The highest NT was observed both in 20 and 30 kg N ha^{-1} when applied at MT with 100 and 120 kg N ha^{-1} applied as basal and CRI stages. In both years, NT at 60 DAS was influenced by N level and increased as the dose increased from 0 to 30 kg ha^{-1} at MT with different N levels (80, 100, 120 kg ha^{-1}) applied as basal and at CRI. At 60 DAS, highest NT was observed with 30 kg N ha^{-1} when applied at MT with 120 kg N ha^{-1} applied as basal and at CRI, which was statistically similar to 20 kg N ha^{-1} at MT with 80 kg N ha^{-1} applied as basal and at CRI.

In Y_1, DM recorded at 60 DAS was not influenced by different N doses, when applied at MT, as basal and at CRI, but increased as the N dose increased. In Y_2, DM was highly influenced by the N level applied at MT, as basal and at CRI. The highest DM was recorded when 30 kg N ha^{-1} was applied at MT with 120 kg N ha^{-1} applied as basal and at CRI. As for PP, PH was not influenced by different N doses, but increased numerically as the N dose increased from 0 to 30 kg ha^{-1} applied at MT and with different N levels applied as basal and at CRI. Tallest plants formed in response to 30 kg N ha^{-1} at MT with 120 kg N ha^{-1} applied as basal and at CRI. LAI recorded at 60 DAS was influenced by N levels (from 10 to 30 kg ha^{-1}) when applied at MT in both years. Highest LAI was achieved with 30 kg N ha^{-1}, applied at MT in Y_1 (Figure 2).

Table 1. Growth of wheat as influenced by N, when applied at 80, 100, 120 kg N ha^{-1} as basal and CRI stages along with extra 10, 20 and 30 kg N ha^{-1} applied at MT.

Amount of N and time of application			Plants m^{-2}		Tillers m^{-2}						*Dry matter ($g\ m^{-2}$)		Plant height (cm)	
As basal and CRI stage	MT	Total			40 DAS		50 DAS		60 DAS					
(kg ha^{-1})			Year of experiment											
			11-12	12-13	11-12	12-13	11-12	12-13	11-12	12-13	11-12	12-13	11-12	12-13
80	0	80	-	224	-	651	-	670^{c}	-	650^{d}	-	213^{j}	-	93.8
80	10	90	263	239	786	689	729	700^{bc}	370^{b}	658^{cd}	203	218^{g}	106.9	95.3
80	20	100	269	234	726	661	697	732^{abc}	374^{b}	720^{a-d}	215	224^{gh}	107.5	95.5
80	30	110	239	246	682	679	641	742^{abc}	375^{b}	733^{ab}	217	226^{fg}	107.5	95.4
100	0	100	-	244	-	690	-	715^{abc}	-	710^{bcd}	-	222^{h}	-	94.2
100	10	110	263	239	774	709	741	723^{abc}	420^{a}	726^{abc}	220	226^{f}	108.1	95.1
100	20	120	278	241	822	714	692	780^{ab}	433^{a}	766^{abc}	222	236^{d}	107.1	96.2
100	30	130	239	262	718	740	691	782^{ab}	436^{a}	770^{abc}	225	239^{c}	108.1	96.9
120	0	120	-	227	-	687	-	768^{ab}	-	762^{abc}	-	232^{e}	-	93.8
120	10	130	235	246	674	720	656	778^{ab}	429^{a}	763^{ab}	224	238^{c}	107.9	96.1
120	20	140	251	235	703	704	664	785^{a}	437^{a}	780^{ab}	228	249^{b}	107.3	96.9
120	30	150	262	240	726	705	683	793^{a}	450^{a}	793^{a}	236	262^{a}	108.1	97.7
CV (%)			7.21	7.32	9.91	4.79	10.19	4.22	4.74	3.98	6.47	0.42	1.00	1.57

* Dry matter was taken at 60 DAS.

In a column, means followed by the same letter(s) or without letter are not significantly different at 5% level by DMRT.

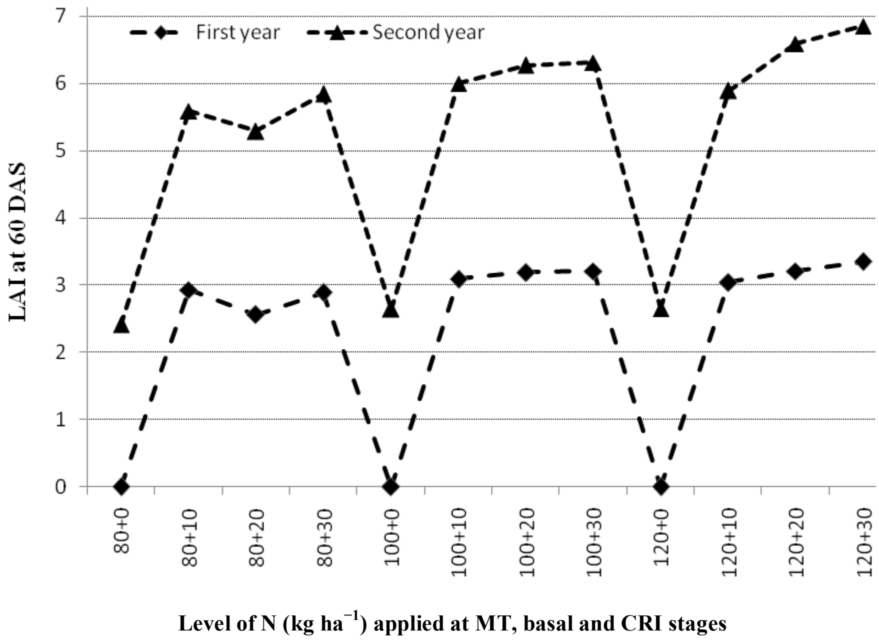

Figure 2. LAI of wheat recorded at 60 DAS influenced by different N levels, applied at MT, basal and CRI stages.

Effect of N on yield and yield-contributing characters of wheat

Yield and yield-contributing characters of wheat in different treatments were influenced by different N levels applied at MT plus different levels as basal and at CRI (Tables 2 and 3) in both years, except for SL, NSS and TGW, which were not influenced by N level in Y_1. In both years, NS, NGS, GY and SY increased as N dose increased from 10 to 30 kg ha^{-1} at MT. The highest NS and NGS were obtained in response to 30 kg N ha^{-1} applied at MT with 120 kg N ha^{-1} applied as basal and at CRI, which was statistically similar to 20 kg N ha^{-1} at MT with 120 kg N ha^{-1} applied as basal and at CRI. GY and SY were influenced by different N levels at MT from 10 to 30 kg ha^{-1} with different N levels applied as basal and at CRI. The highest GY was obtained from 30 kg N ha^{-1} applied at MT with 120 kg N ha^{-1} as basal and at CRI, which was statistically similar to 30 kg N ha^{-1} at MT with both 80 and 100 kg N ha^{-1} applied as basal and at CRI. Higher GY at 30 kg N ha^{-1} at MT with 120 kg N ha^{-1} as basal and at CRI was attributed to higher NS, NGS and SY (Tables 2 and 3).

In Y_1, SL, NSS and TGW were not influenced by different N levels applied at MT, as basal and at CRI, but were significantly influenced in Y_2. TGW, SL and NSS increased as N dose increased in MT from 0 to 30 kg ha^{-1} with different N levels applied as basal and at CRI. The highest SL and NSS were observed in response to 30 kg N ha^{-1} at MT with 120 kg N ha^{-1} as basal and at CRI, similar with 80 kg N ha^{-1} applied as basal and at CRI. Similar to SL and NSS, highest TGW was obtained from 30 kg N ha^{-1} at MT with 120 kg N ha^{-1} as basal and at CRI and was similar to 20 kg N ha^{-1} at MT and to 80 kg N ha^{-1} applied as basal and at CRI (Tables 2 and 3).

Table 2. Yield and yield contributing characters of wheat as influenced by N, when 80, 100, 120 kg ha^{-1} was applied as basal and CRI stages along with extra 10, 20 and 30 kg ha^{-1} applied at MT.

Amount and time of N application			Spikes m^{-2}		Spike length (cm)		Spikelets $spike^{-1}$		Grains $spike^{-1}$		1000- grain weight (g)		Straw yield (kg ha^{-1})	
As basal and CRI stage	MT	Total	Year of experiment											
(kg ha^{-1})			11-12	12-13	11-12	12-13	11-12	12-13	11-12	12-13	11-12	12-13	11-12	12-13
80	0	80	-	236^{c}	-	9.85^{c}	-	16.1^{b}	-	46.1^{c}	-	40.07^{c}	-	8997^{c}
80	10	90	275^{c}	253^{bc}	9.77	9.99^{bc}	17.9	17.0^{ab}	51.3^{c}	47.4^{abc}	45.35	41.55^{bc}	9820^{c}	9484^{bc}
80	20	100	282^{bc}	263^{abc}	9.72	10.11^{bc}	17.8	17.4^{a}	54.7^{bc}	48.6^{abc}	45.35	42.88^{ab}	10302^{bc}	9493^{bc}
80	30	110	285^{bc}	271^{ab}	9.70	10.53^{abc}	17.7	17.2^{a}	58.1^{ab}	51.1^{abc}	46.10	43.33^{ab}	10716^{ab}	9469^{bc}
100	0	100	-	259^{abc}	-	10.02^{bc}	-	17.2^{a}	-	47.2^{bc}	-	42.68^{ab}	-	9141^{c}
100	10	110	287^{bc}	264^{abc}	9.86	10.49^{abc}	17.8	17.3^{a}	54.9^{bc}	49.3^{abc}	46.28	42.72^{ab}	10769^{ab}	9611^{bc}
100	20	120	293^{abc}	272^{ab}	9.82	10.62^{abc}	17.7	17.7^{a}	58.1^{ab}	51.9^{ab}	46.27	43.03^{ab}	11005^{ab}	9998^{abc}
100	30	130	299^{abc}	274^{ab}	9.40	10.99^{ab}	17.8	17.8^{a}	58.9^{a}	51.7^{ab}	45.25	43.82^{a}	10877^{ab}	10593^{ab}
120	0	120	-	270^{ab}	-	10.23^{bc}	-	17.5^{a}	-	51.8^{ab}	-	42.88^{ab}	-	9693^{abc}
120	10	130	296^{abc}	273^{ab}	9.72	10.71^{abc}	17.8	17.6^{a}	56.3^{ab}	51.4^{ab}	45.77	43.68^{ab}	11087^{ab}	10457^{ab}
120	20	140	302^{ab}	280^{ab}	9.95	11.01^{ab}	17.8	17.7^{a}	59.8^{a}	51.9^{ab}	45.67	43.78^{ab}	11272^{a}	10617^{ab}
120	30	150	314^{a}	284^{a}	9.65	11.35^{a}	17.9	17.9^{a}	59.0^{a}	52.7^{a}	44.95	43.96^{a}	11456^{a}	10809^{a}
CV (%)			4.33	4.30	4.37	3.89	4.13	2.40	3.50	4.08	2.15	1.99	4.30	4.48

In a column, means followed by the same letter(s) or without letter are not significantly different at 5% level by DMRT.

Table 3. Increase in grain yield and CMR (SPAD value) over the control when 80, 100, 120 kg N ha^{-1} was applied as basal and CRI stages along with extra 10, 20 and 30 kg N ha^{-1} applied at MT.

Amount and time of N application			Grain yield (kg ha^{-1})		Increase at MT stage (%)		Chlorophyll meter reading (CMR)									
As basal and CRI stage	MT	Total					45 DAS		55 DAS		Increase at MT stage (%)		65 DAS		Increase at MT stage (%)	
(kg ha^{-1})			Year of experiment													
			11-12	12-13	11-12	12-13	11-12	12-13	11-12	12-13	11-12	12-13	11-12	12-13	11-12	12-13
80	0	80	-	3751^{d}	-	-	-	34.96	-	37.83^{b}	-	-	-	36.97^{b}	-	-
80	10	90	4158^{c}	3950^{cd}	-	5.29	36.13	34.20	38.06^{d}	37.95^{bc}	-	0.32	38.80^{b}	38.9^{ab}	-	5.32
80	20	100	4707^{b}	4061^{bcd}	13.20	8.26	35.66	36.00	38.6^{cd}	38.37^{ab}	1.31	1.43	38.86^{b}	39.89^{a}	0.17	7.91
80	30	110	5145^{ab}	4422^{abc}	23.74	17.87	36.76	34.30	39.1^{bcd}	40.27^{ab}	2.81	6.45	40.53^{a}	40.43^{a}	4.46	9.38
100	0	100	-	3878^{cd}	-	-	-	35.13	-	38.17^{ab}	-	-	-	39.67^{a}	-	-
100	10	110	4823^{ab}	4067^{bcd}	-	4.87	37.80	34.06	39.2^{bcd}	39.17^{ab}	-	2.62	40.76^{a}	40.40^{a}	-	1.85
100	20	120	4904^{ab}	4359^{a-d}	1.68	12.40	36.73	36.53	39.7^{a-d}	40.37^{ab}	1.46	5.76	41.23^{a}	40.23^{a}	1.15	1.42
100	30	130	5033^{ab}	4656^{ab}	4.35	20.07	36.33	34.93	40.1^{abc}	40.88^{ab}	2.40	7.10	41.43^{a}	40.30^{a}	1.64	1.60
120	0	120	-	4236^{a-d}	-	-	-	34.20	-	39.70^{ab}	-	-	-	40.08^{a}	-	-
120	10	130	4993^{ab}	4402^{abc}	-	3.92	37.10	34.93	40.40^{ab}	40.40^{ab}	-	1.76	41.10^{a}	40.15^{a}	-	0.17
120	20	140	5231^{a}	4689^{ab}	3.93	10.69	36.53	35.77	40.76^{ab}	40.97^{ab}	0.89	3.21	41.46^{a}	41.30^{a}	0.88	3.04
120	30	150	5283^{a}	4712^{a}	5.81	11.42	36.73	35.37	41.20^{a}	41.77^{a}	1.98	5.21	41.73^{a}	41.43^{a}	1.53	3.37
CV (%)			5.25	5.81				4.34	2.21	3.53			2.01	3.26		

In a column, means followed by the same letter (s) or without letter are not significantly different at 5% level by DMRT.
In 2011-12 % increased of grain yield and chlorophyll meter reading were calculated on the basis of control i.e., 10 kg N ha^{-1}.

Chlorophyll meter reading is influenced by the level of N when applied at MT

In both years, GY and CMR were higher when 30 kg N ha^{-1} was applied at MT with 80, 100 and 120 kg N ha^{-1} applied as basal and at CRI (Table 3). This increment was higher with a lower N level (80 kg N ha^{-1}, applied as basal and at CRI). CMR at 55 and 65 DAS increased as N dose increased from 10 to 30 kg ha^{-1} at MT with different N levels applied as basal and at CRI. At 55 DAS, the highest CMR was found in response to 30 kg N at MT with 120 kg N ha^{-1} as basal and at CRI, which was similar to 20 kg N ha^{-1} at MT with 80 kg N ha^{-1} as basal and at CRI. At 65 DAS, highest CMR was also found in response to 30 kg N ha^{-1} at MT with 120 kg N ha^{-1} as basal and at CRI, statistically similar to 30 kg N ha^{-1} at MT with 80 kg N ha^{-1} as basal and at CRI. However, at 45 DAS, CMR was not influenced by different N levels applied as basal and at CRI (Table 3).

In both years, although 30 kg N ha^{-1} was applied at MT with 120 kg N ha^{-1} applied as basal and at CRI, giving highest GY, this was statistically similar with 30 kg N ha^{-1} applied at MT with 80 kg N ha^{-1} applied as basal and at CRI. For higher GY, at least 30 kg N ha^{-1} should be applied at MT with 80 kg N ha^{-1} and for maximum GY the amount should be 30 kg N ha^{-1} at MT with 120 kg N ha^{-1} applied as basal and at CRI.

Correlation between GY and SPAD values

The correlation matrix between GY and SPAD values of wheat at different DAS is presented in Table 4. The SPAD values recorded at 55 and 65 DAS were positively correlated (Y_1; r=0.839**, 0.837** and Y_2; r=0.971**; 0.813**) with GY in both years. However, at 45 DAS, SPAD values were not positively correlated with GY in both years due to lower SPAD values than at 55 and 65 DAS (Table 4). This indicates that application of extra N at MT (after recording the SPAD values at 45 DAS) influenced the growth and development of wheat final GY in both years (Table 4).

Table 4. Correlation matrix between grain yield and SPAD values at different DAS in both years.

Parameters	Yield (kg ha^{-1})		SPAD value at 45 DAS		SPAD value at 55 DAS		SPAD value at 65 DAS		Standard deviation	
	11-12	12-13	11-12	12-13	11-12	12-13	11-12	12-13	11-12	12-13
Yield (kg ha^{-1})	1.00	1.00	0.308^{ns}	0.254^{ns}	0.839**	0.971**	0.837**	0.813**	341.48	327.47
SPAD value at 45 DAS			1.00	1.00	0.297^{ns}	0.236^{ns}	0.535^{ns}	0.197^{ns}	0.60	0.78
SPAD value at 55 DAS					1.00	1.00	0.897**	0.796**	1.03	1.33
SPAD value at 65 DAS							1.00	1.00	1.10	1.16

** Significant at 1% level; ^{ns} Non significant.

Discussion

Weather parameters such as maximum and minimum air and soil temperature, rainfall and relative humidity are the most important climatic factors for the growth and development of plants and hence their fluctuations affect crop growth and development and ultimately yield. The ideal timing for sowing wheat is linked to favorable climatic conditions for wheat that prevail in November until mid-December. In this study, the wheat crop was sown on 5 December 2011 (first year; Y_1) and 28 November in 2012 (second year; Y_2). Hossain and Teixeira da Silva (2012) and Hossain et al. (2013) reported optimum sowing times for existing wheat varieties of Bangladesh as being mid-November to the first week of December in the agro-ecological zone 1 of Bangladesh. According to their findings, the weather conditions in our experimental period were suitable for good wheat yield (Figure 1) because in the vegetative stage, average temperature was below 25 °C and at the grain-filling stage average temperature was below 20 °C.

The N fertilizer application in two equal splits, half at sowing and half at CRI (along with first irrigation), has been shown to increase GY and N uptake of wheat and is a general recommendation for wheat in Bangladesh as well as over a vast area of the Indo-Gangetic Plain of South Asia, encompassing India, Bangladesh, Nepal and Pakistan (Timsina and Connor, 2001; Timsina et al., 2001; Hossain and Teixeira da Silva, 2013). The uptake of N of irrigated wheat proceeds very slowly until tillering begins and N flux (kg N ha^{-1} day^{-1}) increases to a maximum around Feeke's stage 6 (Singh et al., 2011). Also, N management in irrigated wheat should not only consider crop demand but also the specific irrigation schedule. Timsina et al. (2006) and Sarker et al. (2015) suggested that N be applied at the wheat growth stage when crop needs are high and such timing would also reduce the chances of N losses from the soil-plant system. Our study assessed whether there was a relationship between leaf SPAD readings and GY under different fertilizer management options in two wheat seasons in light soils of Bangladesh. CMRs, obtained from a SPAD meter, served as a guide for N application at MT of wheat. This study thus provided a simple way of using CMRs to assess the N requirements of wheat.

After two years' observation, the growth and yield components, namely GY and CMR, were higher when 30 kg N ha^{-1} was applied at MT with 80, 100 and 120 kg N ha^{-1} applied as basal and at CRI (Tables 1, 2 and 3). This increment was higher when lower N levels were used (80 kg N ha^{-1}, applied as basal and at CRI). CMR at 55 and 65 DAS increased as N dose increased from 10 to 30 kg ha^{-1} at MT with different N levels applied as basal and at CRI. However, at 45 DAS, CMR was not influenced by the N level applied as basal and at CRI. The higher GY at 30 kg N ha^{-1} at MT, with 120 kg N ha^{-1} as basal and at CRI, was attributed to higher NS, NGS and SY. El-Habbal et al. (2010) also observed that a higher N rate maintained higher SPAD values showing a mean of 48.8 between the tillering and grain-filling stage (46-130 DAS) and, irrespective of the fertilizer treatment, SPAD values increased from 67 DAS onwards, peaking at 74 DAS. Singh et al. (2011) observed high N-use efficiency and higher GY in irrigated wheat by applying a moderate amount of fertilizer N at planting and at MT and CMR-guided fertilizer N dose at Feeke's stages 5-6 or 7-8. According to Vidal et al. (1999), SPAD meter readings of leaves at Zadok stages 45 and 69 (Zadoks et al., 1974) accounted for 85 and 72% of variation in GY, respectively. The SPAD values at 40 and 45 were the lower and upper limits, respectively. Fertilizer N management is important not only to achieve and sustain high GY but also to achieve high protein content in wheat grains.

Islam et al. (2014) conducted an experiment with an old wheat variety (Sonalika; released in 1974) to establish a relationship between leaf SPAD readings and GY under different fertilizer management options. They found strong significant effects of SPAD values at 50 DAS that continued to 75 DAS corresponding to the vegetative and reproductive stages of the crop. Similar to the present study (Table 4), SPAD values recorded at 55 and 65 DAS (r=0.983**, 0.973**) were also positively correlated with the GY of wheat. These relationships also suggest that extra N at MT could be made effectively by measuring leaf relative chlorophyll content. Some studies (Singh et al., 2011) on irrigated wheat grown in the IGP (as for our experimental site) in India showed that an N fertilizer needs to be applied at MT according to the crop's needs to achieve high N-use efficiency. Ramesh et al. (2002) also observed highly significant correlations of SPAD readings with the GY of direct wet seeded rice (*Oryza sativa* L.) at 79 DAS. Many studies using SPAD to assess N status have shown reliable indication of N stress and its relationship to relative yield (Fox et al., 2001).

Conclusion

Compared with earlier studies, results from this study provide strong evidence that the current N dose is not enough to maximize wheat yield in light soils of South Asia. An N dose needs to be applied at MT according to the crop's needs to achieve high fertilizer N-use efficiency. SPAD values can be used to guide N fertilizer applications for wheat in regions where winters are mild, soil is light and yields are relatively lower. The criteria used to decide the stage of N fertilizer application and its doses in the present investigation are based on data generated for two wheat seasons. This should be useful for achieving higher N fertilizer-use efficiency in light soils of irrigated spring wheat.

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