SPAD is the Proxy for Estimation of Chlorophyll content in wheat leaves

ABSTRACT

A chlorophyll meter measures the greenness of leaves and can indicate the chlorophyll content and N status. Therefore, by using a chlorophyll meter, proxy information related to the leaf N content could be obtained and this would avoid the need of laborious laboratory analysis. Keeping in this view a field experiment was conducted during winter *(Rabi)* 2019 growing season at the experimental field of JNKVV, Jabalpur to estimate crop nitrogen (N) and chlorophyll content in wheat using SPAD. The treatment comprised of 3 sources of nutrient M1- Inorganic sources (NPK fertilizers), M2- organic sources (FYM, vermicompost, biofertilizers *Azotobacter* and PSB) and M3- Integrated sources (50% Inorganic + 50% organic) as main treatments and 5 NPK levels S1- control, S2- 100% RDF, S3- 150% RDF, S4- 200% RDF, S5- Based on soil test value for target yield of 6t ha^{-1} as sub treatments were replicated thrice in a split plot design. The results showed that the correlation (r = 0.94, 0.90, 0.92 and 0.94, respectively) and coefficient of determination (R^2 = 0.96, 0.94, 0.83 and 0.94, respectively) was significantly and positively correlated with SPAD and nitrogen concentration in leaf. Similarly significant and positive correlation (r = 0.84, 0.76, 0.78 and 0.71, respectively) found with SPAD and total chlorophyll values. Coefficient of determination (R^2) SPAD with chlorophyll and leaf N concentration proved to be significant (R^2 = 0.75, 0.76, 0.54 and 0.85, respectively) at different growth stages. It was concluded that SPAD meter are useful for the determination of proxy chlorophyll and total nitrogen content of wheat plant.

Keywords: SPAD, Chlorophyll, total N, Inorganic, INM, organic

1. INTRODUCTION

"Nitrogen (N) is a key constituent of all living cells and is essential for the growth and development of plants" [1]. "The most important role of N in the plant is its presences in the structure of protein, the most important building substances from which the living material or protoplasm of every cell is made. In addition, nitrogen is also found in chlorophyll, the green colouring matter of leaves. Chlorophyll enables the plant to transfer energy from sunlight by photosynthesis. Therefore, the nitrogen supply to the plant will influence the amount of protein, protoplasm and chlorophyll formed" [2]. Nitrogen is one of the major nutritional elements that limits crop yields. Insufficient N supply results in smaller leaf area [3,4] and lower leaf photosynthesis, chlorophyll content and biomass production [4] leading to the loss of yield and quality.

"On the other hand, excessive use of N fertilizer increases not only production cost but also environmental pollution" [5]. "Increased N fertilizer application does not always imply an increase of grain yield; on the contrary, even a decrease in crop yields is seen in poorly managed production systems" [6]. "In the quest of achieving high yield of wheat, farmers tend to apply nitrogen in excess of the requirements. It is well known that excess N availability for crops like wheat and barley can adversely affect plant health, yield, values of the final product and environmental pollution" [7]. As [8] showed, "about 67% of total applied

N fertilizer in wheat production could be lost because of natural processes like denitrification, leaching, volatilization (NH_3), and greenhouse gas (N_2O, NO or NO_2) emissions". "Nitrogen application at the right amount contributes to higher yields" [9,10]. Leaf chloroplasts contain 70% of leaf N concentration [11] thus, the amount of chlorophyll present in plant leaves is often well correlated with leaf N [12,13]. "Assessment of crop N requirement is critical for optimum N fertilizer application [14]. The optimum rate and application timing of Nitrogen (N) fertilizer are crucial in achieving a high yield however, conventional laboratory testing of plant nutrients is time-consuming and expensive" [15]. "Plant tests performed with optical instruments can be a significant simplification of the methods applied to attain precise determination of the nutritional status of plants during a growing season" [16]. "As leaf nitrogen content is strongly correlated with chlorophyll content the use of Soil Plant Analysis Development (SPAD) meter has been introduced as a popular, fast, and cheap technique to estimate N levels from the measurement of leaf transmittance" [15]. Many studied suggests that "a positive correlation between N uptake, leaf N concentration, leaf chlorophyll content, and grain yield" [17, 18, 19, 20]. In this regard, [21] suggested that "the SPAD chlorophyll meter can be used as an alternative to nitrogen nutrition index (NNI) to measure N status in wheat". Therefore, SPAD values has been successfully used for N fertilizer management in rice [22, 23] wheat [22,24] and maize [25]. "The device can be used very simply and effectively for decision making in supplementary application of N fertilizer in wheat field. Wheat is very sensitive to insufficient nitrogen and very responsive to N fertilization" [26]. "The chlorophyll meter is faster than tissue testing for N and allows "fine tuning" of N management to field conditions, and consequently reduces risk of under- or over fertilizing the wheat crop" [27]. "There is no doubt that the nitrogen management practices can be adopted by crop growers if the practices are affordable, accurate in fertilization, easy to use, and environmentally friendly. The SPAD meter has been demonstrated and proved to be a viable and effective tool in developing and evaluating management recipes across a field" [28]. Keeping in this view, the objective of this work was to evaluate the potential for Minolta SPAD 502 chlorophyll meter readings in monitoring leaf chlorophyll content and its relation to N concentration in wheat leaves.

2. MATERIAL AND METHODS

2.1 Experiment details

A field experiment was conducted during winter (Rabi) 2019 growing season at the experimental field of JNKVV, Jabalpur. (23º 13' North latitude, 79º 57' East longitudes; 393 m elevation). The soil of the experimental site was Typic Haplustert, clay in texture neutral in reaction, non-calcareous, medium in organic carbon content, medium in available nitrogen, phosphorus, and potassium and low in DTPA extractable Zn. The treatment comprised of 3 sources of nutrient M1- Inorganic sources (NPK fertilizers), M2- organic sources (FYM, vermicompost, biofertilizers Azotobacter and PSB) and M3- Integrated sources (50% Inorganic + 50% organic) as main treatments and 5 NPK levels S1- control, S2- 100% RDF, S3- 150% RDF, S4- 200% RDF, S5- Based on soil test value for target yield of 6t ha^{-1} as sub treatments were replicated thrice in a split plot design. The wheat crop (GW-366) sown on with spacing of 22.5 cm row to row.

The experimental treatments were arranged in split plot design with three replications. Each experimental plot was 4 m long and 3 m wide consisting of three rows with one m inter-row spacing. The plots were kept weed-free and maintained in an optimum soil moisture regime throughout the experimental period. The specific quantity of each fertilizer was calculated based on gross plot size as per treatment. Treatment wise 1/3 quantity of nitrogen and entire dose of phosphorus and potassium were applied as basal. While, the 1/3 dose of nitrogen was top dressed in two equal splits, after CRI and third irrigation (65 DAS). Urea (46% N),

Single super phosphate (16% P2O5) and muriate of potash (60% K2O) were used as sources of nitrogen, phosphorus and potassium, respectively. FYM, VC and biofertilzers were applied as per treatment 15 days before sowing. The observations were recorded from each plot at crown root initiation (CRI) (21 DAS), tillering (45 DAS), flowering (65 DAS) and milking stages (90 DAS) of wheat.

2.3 Estimation of Chlorophyll using SPAD-502

A chlorophyll meter (SPAD-502, Minolta, Japan) was used to obtain readings estimating leaf chlorophyll concentration (SPAD value). Five plants per treatment were selected randomly and SPAD values were recorded from the fully matured leaves counted from the top of the plants, the youngest fully expanded leaf. The observations were recorded from each plot at crown root initiation (CRI) (21 DAS), tillering (45 DAS), flowering (65 DAS) and milking stages (90 DAS).

2.4 Chlorophyll content measurement

The material was processed in the fresh state immediately after collection. After fine chopping, portions weighing 0.5 g were measured off on an analytical balance. The measured-off material was then homogenized in a homogenizer with the addition of 10 ml of 80 % acetone. A primary acetone extract containing all chloroplast pigments was obtained in this way. The extract was then centrifuged at 2500 rpm for 5 min. Since the concentration of pigments was in most cases too great for reading to be performed on a spectrophotometer, the obtained extract was diluted by adding 9 ml of 80% acetone per ml of extract. The extract produced in this way was subjected to reading on a spectrophotometer at 645, and 663 and 470 nm using acetone (80%) blank. The amount of chlorophyll ‘a’ and ‘b’ are determined using the formula given by Arnon (1949)

Chl ‘a’= ((12.7 A 663) - (2.69 A 645))

Chl ‘b’= ((22.9 A 645) - (4.68 A 643))

Total chlorophyll (a+b) = ((20.2 (A 645) +8.02(A 663)))

Where, A = Absorbance, V = Final volume of 80% acetone (in ml), W= Weight of plant tissue (in grams) µg g^{-1} fresh weight (µg/ml final volume)/leaf weight (g)

The observations were recorded from each plot at crown root initiation (CRI) (21 DAS), tillering (45 DAS), flowering (65 DAS) and milking stages (90 DAS).

2.4 Nitrogen analysis in plant

The nitrogen content in plant sample was estimated by micro Kjeldahl digestion and distillation method using KEL PLUS system. For digestion, a known weight of plant sample in the presence of sulfuric acid with catalyst mixture (5:1) under high temperature was digested and distilled in presence of sodium hydroxide, the released ammonia was condensed and absorbed in boric acid with mixed indicator (Bromocresol cresol green and methyl red indicator) to form ammonium borate, the excess of which was titrated with standard sulfuric acid (0.1N) (AOAC, 1995). The observations were recorded from each plot at crown root initiation (CRI) (21 DAS), tillering (45 DAS), flowering (65 DAS) and milking stages (90 DAS).

2.5 Statistical analysis:

Data were analyzed using SPSS for analysis of variance and Fisher's LSD multiple range test was employed for the means comparisons. The correlations between fertilizer application levels and grain yield, SPAD value and grain yield were tested at 5% level of significance.

3. RESULTS AND DISCUSSION

3.2 SPAD readings at different growth stages

It can be seen from the data (Table 2) that the SPAD reading, was significantly maximum (8.15, 12.38, 28.58 and 30.65, respectively) with the application of inorganic sources of nutrient (M1) over integrated (M3) and organic sources of nutrient (M2) at CRI, tillering, jointing and milking stages, respectively. The SPAD reading of 6.36, 9.71, 25.54 and 25.00 were registered in M3 and it was also found significantly superior over M2 (4.47, 6.74, 17.26 and 16.65, respectively) at each stage, respectively.

However, the application of S3 (150% RDF) and S4 (200% RDF) significantly increased SPAD reading over S2 (100% RDF) and S5 (STV based RDF) at CRI and jointing but at par at tillering. S4 Significant S2 (100% RDF) and S5 (STV based RDF) but at par with S3. At milking. The highest SPAD reading was registered in S4 (200% RDF) which was 7.97, 11.07, 27.45 and 28.02 and 7.29, 11.11, 26.60 and 27.24 with 150% RDF at each stage, respectively.

The interaction effect was found to be significant at all growth stages. At CRI stage of wheat the combination of M1S4 (10.50) was recorded the highest SPAD reading, which was significantly superior over all the combination and followed by M1S3 (9.23), M1S2 (8.40), M1S5 (8.40), M3S4 (7.93) and M3S3 (7.52) all were at par among themselves. At tillering stage, the combination M1S5 (16.11), M1S4 (15.64), M1S3 (15.28) were found significant over all combination. On comparing the treatment combination at jointing stage, applied to wheat, performance of M1S3 (33.70), M1S4 (33.48), M1S5 (33.45) were found significant over all combinations. However, at milking stage, the interaction of M1S4 (38.92) causes significant difference and statistically similar with M1S3 (32.44) and M1S5 (32.86), M1S2 (31.86). Maximum SPAD reading were obtained about jointing stage (65 DAS) then the chlorophyll content gradually decreased till the end of the vegetation season similar results also reported by Juśko et al [29]. Maximum SPAD reading found in inorganic fertilizers because there was more nitrogen available to the NPK treated plants, there was more chlorophyll in the leaves, which speeds up photosynthesis [30]. Our results show that the application of nitrogen in inorganic form at the right time enhances chlorophyll content which resulted to higher SPAD values [15]. Nitrogen is part of the enzymes associated with chlorophyll synthesis [31] and the chlorophyll concentration reflects relative crop nitrogen status and yield level [32]. "Very close link between chlorophyll and nitrogen content was observed as investigated by many investigators" [33, 34]. "It is understandable, because nitrogen is a structural element of chlorophyll and protein molecules, and thereby affects formation of chloroplasts and accumulation of chlorophyll in them" [35, 36]. Our results supported by Islam et al. 2014 [37] reported that the SPAD values drastically reduced in treatments treated with only organic fertilizers. The values were higher in treatment receiving full dose of NPK through inorganic fertilizers as compared with reduced inorganic fertilizers and/or organically supplied fertilizers. These results agree with that obtained [37] in potato. "This might be because N supply in organic treatment is generally restricted for slow N mineralization as compared to crop N demand" [38].

3.2 Total chlorophyll in leaves (mg g^{-1} leaf tissue) at different growth stages

Table 3 showed that the total chlorophyll of 1.27, 1.92, 2.14 and 1.24 mg g^{-1} leaf tissue was significantly more where, inorganic sources of nutrient (M1) was applied followed by integrated sources of nutrients (M3) with value of 1.08, 1.75, 1.78 and 1.04 mg g^{-1} leaf tissue over organic sources of nutrient (M2) which recorded lowest total chlorophyll of 0.93, 1.33, 1.41 and 0.87 mg g^{-1} leaf tissue at CRI, tillering, jointing and milking stages,.

However, the application of S3 (150% RDF) and S4 (200% RDF) significantly increased total chlorophyll over S2 (100% RDF) and S5 (STV based RDF) at each growth stages. The highest total chlorophyll was recorded with the application of S4 at CRI and tillering stage which was statistically similar with S3 (150% RDF) with the values of 1.26 and 1.84 mg g^{-1} leaf tissue at CRI and tillering stage. However, S3 (150% RDF) was highest at jointing and milking stage with the values of 1.35 and 1.86 mg g^{-1} leaf tissue. Treatment S2 (100% RDF) was found significant over S5 (STV based RDF) at tillering, jointing and milking stage but at par at CRI stage.

The interaction effect was found to be significant at CRI stage of wheat. The combination of M1S3 (1.61 mg g^{-1} leaf tissue) was recorded as the highest CCI, which was significantly superior over all the combination and at par with M1S4 (1.54 mg g^{-1} leaf tissue). At tillering stage, the combination M1S4 (2.27) was found maximum and significant over all combination and at par with M1S3 (2.16 mg g^{-1} leaf tissue). On comparing the treatment combination at jointing stage, applied to wheat, performance of M1S4 (2.58 mg g^{-1} leaf tissue), was found significant over all combinations. However, at milking stage, the interaction of M1S4 (1.54 mg g^{-1} leaf tissue) causes significant different and statistically similar treatment M1S3 (1.38 mg g^{-1} leaf tissue).

The maximum chlorophyll in inorganic sources of nutrients is usually immediately and fast containing all necessary nutrients that are ready for plants [39]. Earlier study [40] indicated that abundant nutrient supplementation increases chlorophyll concentration in Sorghum bicolor and *Zea mays*. However, organic sources of nutrients perform poorly. This may be due to disappearance of mineral N in the soil system was caused by microbial immobilization under organic sources [41] which cause lower availability of N and chlorophyll. “Organic fertilizer improves physical, biological, and chemical properties of a soil but the nutrients may not be as readily available to the plants” [39].

3.3 Total N content in leaf at different growth stages

Data presented in table 4 showed that the total N content in leaves of 2.39, 2.29, 1.80 and 1.38%, respectively was significantly more where, inorganic sources of nutrient (M1) was applied followed by integrated sources of nutrients (M3) with value of 2.26, 2.15, 1.76 and 1.34%, respectively over organic sources of nutrient (M2) which recorded lowest total chlorophyll of 2.22, 2.13, 1.60 and 1.19%, respectively at CRI, tillering, jointing and milking stages. However, M1 is significant over M3 except milking stage.

Among the different levels of NPK, the application of S3 (150% RDF) and S4 (200% RDF) significantly increased N content over S2 (100% RDF) and S5 (STV based RDF) at CRI, jointing and milking stage. S3 (150% RDF) and S4 (200% RDF) were found at par among themselves. All levels were found at par among themselves.

The interaction effect was found to be significant at jointing and milking stage of wheat. The combination of M1S4 (2.13%) was recorded as the highest N content, which was significantly superior over all the combination and at par with M1S3 (2.04%) at jointing stage. However, at milking stage, the interaction of M1S4 (1.56%) causes significant different and statistically similar treatment M1S3 (1.45%) and M1S5 (1.48%)

"The percentage of total N concentration in dried leaf increased as larger N doses in mineral fertilization were applied. The pattern of nitrogen accumulation during the vegetation season deviated from the pattern of chlorophyll synthesis. The highest N accumulation was observed during the first measurement. Subsequent analyses showed lesser N concentration in the leaves. The lowest N concentration was noted later stages, and then it remained roughly at this level till the end of the vegetation season. The fact that the N concentration stayed roughly at the same level throughout the whole period till the end of the vegetation season, while chlorophyll content in the leaves fell, points to the accumulation of nitrogen compounds other than pigments" [28]. "Maximum N concentration in inorganic fertilizers may be because inorganic fertilizers releases nutrients for the plants instantly and in readily available forms for the plants during its growth, development and reproductive phases where the nutrient demand is at its peak. Higher levels of inorganic fertilizers have been increases the activity of photosynthesis and enzymes which responsible for transformation of energy, carbohydrates, fat metabolism and respiration of plant" [42]. "The nitrogen containing organic compounds in organic manures especially in FYM are more resistant to decomposition and only about one third of the nitrogen is easily released. The remaining amount of nitrogen persisted in the soil for a long period" [43].

3.4 Correlation and Coefficient of determination of SPAD readings with total chlorophyll and total N content in leaves at different growth stages

Pearson correlation coefficients between total N concentration in leaf, total chlorophyll content, SPAD values at CRI, tillering, jointing and milking stages of wheat crop are presented in Table 1 and fig 1. Good correlations were found for both meters for determination of chlorophyll content and N content The SPAD values were positively correlated with grain yield of wheat at differ good stages of plants. The SPAD values were also significantly correlated (r = 0.94, 0.90, 0.92 and 0.94, respectively) with leaf N concentration at different growth stages in this study. Maximum correlation was found in CRI and milking stage followed by jointing and tillering respectively. Coefficient of determination R2 between chlorophyll content in SPAD units and nitrogen concentration and leaf N concentration proved to be significant (R2 = 0.96, 0.94, 0.83 and 0.94, respectively respectively). Highest value of Coefficient of determination was found in CRI and followed by milking, jointing and tillering, respectively. A close correlation (r = 0.93) was established between the measurements of chlorophyll content using the N-tester and those obtained by laboratory tissue testing, for soybean, rice and spring wheat leaves [44], potato (r = 0.97) [45], rice [46]. Also, Piekielek and Fox [47], Smeal and Zhang [48], Waskom et al. [49] confirm that "the measurement results obtained using the SPAD optical method reflect the actual chlorophyll content in various crop plants".

Table showed that the relationship between leaf chlorophyll and SPAD value at different growth stages. The SPAD values were also significantly correlated (r =0.84, 0.76, 0.78 and 0.71, respectively) with leaf chlorophyll at different growth stages in this study. Maximum correlation was found in CRI and jointing followed by tillering and milking stage, respectively. Coefficient of determination R2 between chlorophyll content in SPAD units and nitrogen concentration and leaf N concentration proved to be significant (R2 =0.75, 0.76, 0.54 and 0.85, respectively). Highest value of Coefficient of determination was found in milking and followed by tillering, CRI and jointing, respectively.

4. 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 to increase N use efficiency [50,51]. 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 wheat.

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Table 1. Correlation and Coefficient of variance of SPAD with total chlorophyll and total N content

Stages	Correlation of SPAD with total chlorophyll and total N content		Coefficient of variance of SPAD with total chlorophyll and total N content	
	Total chlorophyll	Total N content	Total chlorophyll	Total N content
CRI	0.94	0.96	0.84	0.75
Tillering	0.90	0.94	0.76	0.76
Jointing	0.92	0.83	0.78	0.54
Milking	0.94	0.94	0.71	0.85

Table 2. Effect of different sources and NPK levels on SPAD readings at different growth stages

	SPAD															
	CRI				Tillering				Jointing				Milking			
M/S	M1	M2	M3	Mean	M1	M2	M3	Mean	M1	M2	M3	Mean	M1	M2	M3	Mean
S1	4.20	3.83	4.17	**4.06**	2.70	6.60	4.10	**4.47**	13.95	17.38	15.82	**15.72**	17.17	13.47	17.86	**16.17**
S2	8.40	5.03	5.76	**6.40**	12.15	7.53	10.58	**10.09**	28.31	18.14	26.48	**24.31**	31.86	16.13	25.42	**24.47**
S3	9.23	5.10	7.52	**7.29**	15.28	7.15	10.90	**11.11**	33.70	18.49	27.63	**26.60**	32.44	20.27	29.01	**27.24**
S4	10.50	5.47	7.93	**7.97**	15.64	6.11	11.44	**11.07**	33.48	19.85	29.01	**27.45**	38.92	16.48	28.65	**28.02**
S5	8.40	2.90	6.40	**5.90**	16.11	6.34	11.53	**11.33**	33.45	12.43	28.29	**24.72**	32.86	16.90	24.04	**24.60**
Mean	**8.15**	**4.47**	**6.36**	**6.32**	**12.38**	**6.74**	**9.71**	**9.61**	**28.58**	**17.26**	**25.45**	**23.76**	**30.65**	**16.65**	**25.00**	**24.10**
SEm±	0.19				0.48				0.90				1.18			
CD(p=0.05)	**0.76**				**1.90**				**3.56**				**4.64**			
SEm±	0.20				0.45				0.93				1.14			
CD(p=0.05)	**0.60**				**1.31**				**2.71**				**3.34**			
Int I	0.35				0.77				1.61				1.98			
CD(p=0.05)	**1.03**				**2.27**				**4.70**				**5.79**			
Int II	0.50				1.19				2.31				2.95			
CD(p=0.05)	**1.46**				**3.48**				**6.76**				**8.62**			

M1- *Inorganic sources (NPK fertilizers),* ***M2****- organic sources (FYM, vermicompost, biofertilizers Azotobacter and PSB) and* ***M3-*** *Integrated sources (50% Inorganic + 50% organic)* ***S1-*** *control,* ***S2****- 100% RDF,* ***S3****- 150% RDF****, S4****- 200% RDF,* ***S5-*** *Based on soil test value for target yield of 6t ha*$^{-1}$

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Table 3. Effect of different sources and NPK levels on total chlorophyll at different growth stages

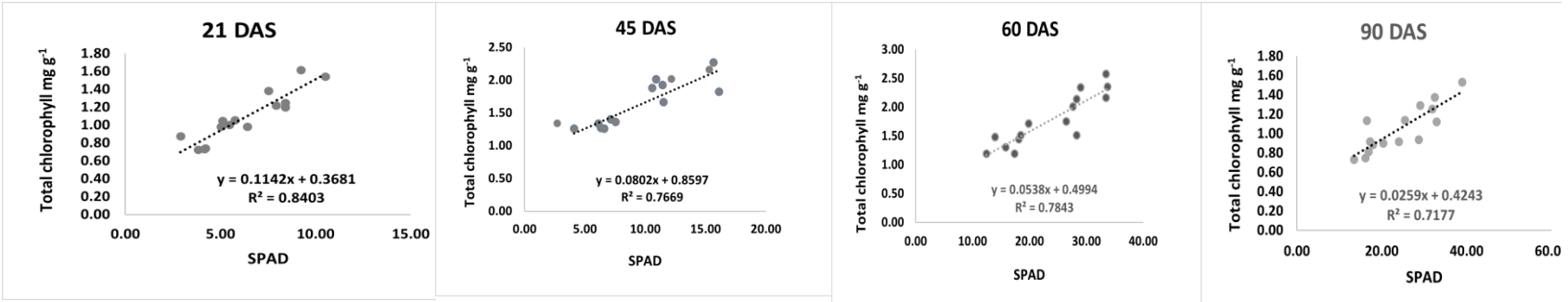
	Total chlorophyll															
	CRI				Tillering				Jointing				Milking			
M/S	**M1**	**M2**	**M3**	**Mean**	**M1**	**M2**	**M3**	**Mean**	**M1**	**M2**	**M3**	**Mean**	**M1**	**M2**	**M3**	**Mean**
S1	0.74	0.72	0.73	**0.73**	1.35	1.26	1.26	**1.29**	1.48	1.19	1.30	**1.32**	0.92	0.73	0.89	**0.85**
S2	1.20	0.98	1.06	**1.08**	2.02	1.36	1.88	**1.75**	2.14	1.45	1.75	**1.78**	1.26	0.75	1.14	**1.05**
S3	1.61	1.05	1.38	**1.35**	2.16	1.40	2.01	**1.86**	2.36	1.51	2.01	**1.96**	1.38	0.90	1.29	**1.19**
S4	1.54	1.00	1.22	**1.26**	2.27	1.34	1.92	**1.84**	2.58	1.72	2.34	**2.21**	1.54	1.13	0.94	**1.20**
S5	1.25	0.88	0.98	**1.04**	1.82	1.27	1.66	**1.59**	2.16	1.19	1.51	**1.62**	1.12	0.81	0.92	**0.95**
Mean	**1.27**	**0.93**	**1.08**	**1.09**	**1.92**	**1.33**	**1.75**	**1.67**	**2.14**	**1.41**	**1.78**	**1.78**	**1.24**	**0.87**	**1.04**	**1.05**
SEm±		**0.029**				**0.043**				**0.082**				**0.028**		
CD(p=0.05)		**0.113**				**0.169**				**0.321**				**0.109**		
SEm±		0.031				0.044				0.044				0.033		
CD(p=0.05)		**0.090**				**0.130**				**0.129**				**0.097**		
Int I		0.053				0.077				0.076				0.057		
CD(p=0.05)		**0.156**				**0.224**				**0.223**				**0.167**		
Int II		0.075				0.110				0.177				0.076		
CD(p=0.05)		**0.218**				**0.321**				**0.517**				**0.221**		

M1- *Inorganic sources (NPK fertilizers),* ***M2****- organic sources (FYM, vermicompost, biofertilizers Azotobacter and PSB) and* ***M3-*** *Integrated sources (50% Inorganic + 50% organic)* ***S1-*** *control,* ***S2****- 100% RDF,* ***S3****- 150% RDF****, S4****- 200% RDF,* ***S5-*** *Based on soil test value for target yield of 6t ha*$^{-1}$

Table 4. Effect of different sources and NPK levels on total N content (%) in leaves at different growth stages

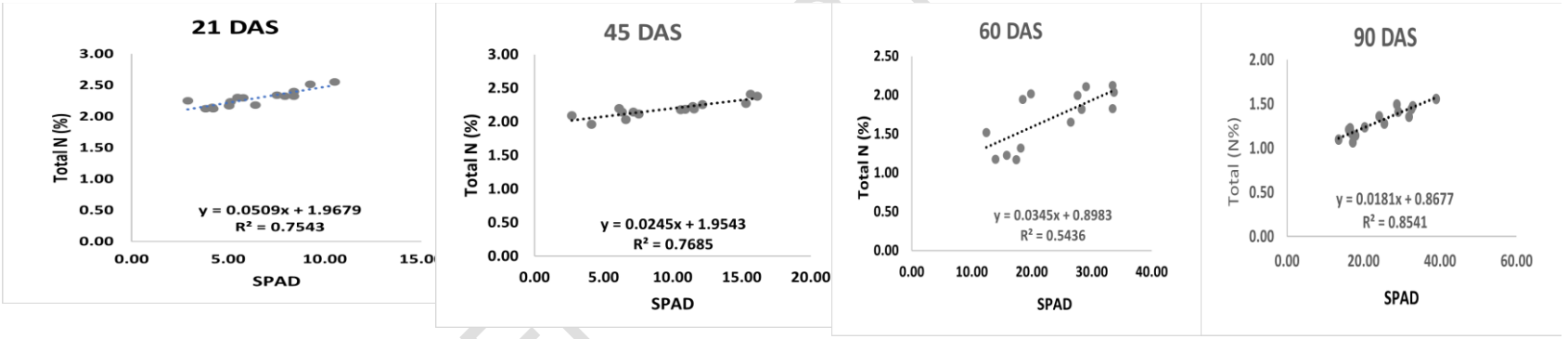
	Total N content (%)															
	CRI				Tillering				Jointing				Milking			
M/S	M1	M2	M3	Mean	M1	M2	M3	Mean	M1	M2	M3	Mean	M1	M2	M3	Mean
S1	2.13	2.13	2.15	**2.14**	2.09	2.04	1.97	**2.03**	1.17	1.17	1.23	**1.19**	1.07	1.10	1.14	**1.10**
S2	2.33	2.18	2.30	**2.27**	2.26	2.12	2.18	**2.19**	1.82	1.32	1.66	**1.60**	1.36	1.22	1.28	**1.29**
S3	2.52	2.24	2.35	**2.37**	2.28	2.15	2.19	**2.21**	2.04	1.95	2.00	**1.99**	1.45	1.24	1.41	**1.37**
S4	2.56	2.31	2.33	**2.40**	2.41	2.20	2.23	**2.28**	2.13	2.02	2.11	**2.09**	1.56	1.23	1.50	**1.43**
S5	2.40	2.25	2.19	**2.28**	2.38	2.15	2.20	**2.24**	1.83	1.52	1.83	**1.72**	1.48	1.17	1.36	**1.34**
Mean	**2.39**	**2.22**	**2.26**	**2.29**	**2.29**	**2.13**	**2.15**	**2.19**	**1.80**	**1.60**	**1.76**	**1.72**	**1.38**	**1.19**	**1.34**	**1.30**
SEm±	0.032				0.029				0.034				0.024			
CD(p=0.05)	**0.126**				**0.113**				**0.136**				**0.096**			
SEm±	0.027				0.032				0.038				0.025			
CD(p=0.05)	**0.080**				**0.093**				**0.111**				**0.072**			
Int I	0.047				0.055				0.066				0.043			
CD(p=0.05)	**NS**				**NS**				**0.192**				**0.126**			
Int II	0.077				0.076				0.091				0.062			
CD(p=0.05)	**2.13**				**2.13**				**2.15**				**2.14**			

M1- *Inorganic sources (NPK fertilizers),* ***M2****- organic sources (FYM, vermicompost, biofertilizers Azotobacter and PSB) and* ***M3-*** *Integrated sources (50% Inorganic + 50% organic)* ***S1-*** *control,* ***S2****- 100% RDF,* ***S3****- 150% RDF****, S4****- 200% RDF,* ***S5-*** *Based on soil test value for target yield of 6t ha*$^{-1}$



a **b** **c** **d**

Fig 1a. Relationship between SPAD and chlorophyll content at different growth stages



a **b** **c** **d**

Fig 1b. Relationship between SPAD and nitrogen content at different growth stages