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Evaluation of potential of portable chlorophyll meter to quantify chlorophyll and nitrogen contents in leaves of wheat under different field conditions

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The chlorophyll meter is a simple, non destructive and portable tool that could be used to measure the greenness or relative chlorophyll and nitrogen contents in leaves during different developmental stages for efficient nutrient management. In this study, we tried to correlate SPAD index with chlorophyll (chl)/nitrogen(N) content in leaves of bread wheat (*Triticumaestivum* L., PBW 509) and durum wheat (*Triticum durum* L. PDW 233), and to find the variable (chl concentration *i.e.* µg Chl g^{-1} tissue, content *i.e.* µg Chl cm^{-2} tissue or N content) that could be best estimated with chlorophyll meter.The leaf samples collected from four different fields varying in N and manganese fertility levels, exhibited a wide range of SPAD index (26-47).The linear model was best fitted to describe the relationship of these variables with SPAD index and it was found to be the best measure of chl concentration (R^2= 0.59) as compared to chl content (R^2= 0.49) and nitrogen content (R^2= 0.37). The correlations improved when separate calibration curves were plotted for cultivar and field. SPAD index explained 70, 87 and 71% of variation in chl concentration, chl content and N content, respectively, at field 3 from pooled data of the two cultivars and the corresponding values of variables were 94, 93 and 91% for PBW 509 and 87, 83 and 91% for PDW 233 from pooled data of four fields. The chlorophyll meter can be used with caution to develop separate calibration curves for particular cultivar, field, hybrid and treatments to develop standards for interpretations and efficient fertilizer management.

Keywords: Chlorophyll content, Nitrogen content, SPAD index, SPAD meter, *Triticum durum*, Wheat

Photosynthesis determines growth and yield of crops. Chlorophyll (chl) is the photosynthetic pigment essential for the conversion of light energy into chemical energy. Thus, the leaf chl content is a parameter of significant interest from a physiological perspective. The amount of solar radiation absorbed by a leaf is largely a function of the foliar concentrations of photosynthetic pigments and low concentrations of chl can, therefore, limit photosynthetic potential and hence primary production[1]. There is a close link between leaf chlorophyll content and leaf N content, which makes sense because the majority of leaf N is contained in chlorophyll molecules. So quantification of chl content gives an indirect measure of N nutrient status[2].

Traditional methods used for chl extraction from plant material use organic solvents, such as acetone[3], dimethyl sulfoxide[4], methanol, N, N-dimethyl formamide, or petroleum ether[5] followed by spectrophotometric determination of absorbance by chl extract and conversion from absorbance to concentration using standard equations[6]. Absolute ethanol/acetone/ methanol and DMSO are commonly used to extract chlorophyll from a wide range of plants[7]. Potential biases in chl extraction may be attributable to solvent type, solvent impurity, tissue type polarity, temperature, incubation time, degree of maceration or the equation used to calculate chl concentration[8,9]. Further, these methods require destructive sampling and are time-consuming[10]. The evaluation of nutrient status for efficient management of cultural practices demands the streamlining of cost and time spent on tissue sampling or laboratory analyses to interpret data and use field diagnostic tools across numerous sites and species. The Minolta SPAD-502 meter (Spectrum Technologies, Plainfield, Ill.) is used for quick determination of relative greenness of leaves by measuring the transmittance of the leaf in two wave bands (600 to 700 and 400 to 500 nm). The SPAD is an acronym for soil plant analysis development[11]. The Minolta chlorophyll meter (model SPAD 502) enables users to quickly and easily measure potential photosynthetic activity, which is closely linked to leaf chlorophyll content, crop N status, and leaf greenness[12]. Leaf thickness has

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also been reported to interfere with chl distribution, so it is better to correlate SPAD index with chl per unit area rather than per unit weight[13,14]. The SPAD value indicates an excellent correlation with chl content that in turn is positively correlated with N status of crops. Thus, SPAD reading can be used as a rapid diagnostic tool for fertilizer N scheduling[15,16]. The device has been tested in coffee and papaya[17,18], rice[19], wheat[20], maize[21], cotton[22] and many trees[23].

Wheat is the staple food crop of the world's population and India is among the leading producers of wheat in world. There is need to estimate time course changes in the absolute chl content of photosynthetic tissue to have an insight into various physiological processes determining crop growth and yield. In view of the above and the need for non destructive estimation of chl content, interference of leaf thickness in chl content and N fertilizer scheduling using chlorophyll meter, the present study was planned to (i) correlate SPAD index to chl concentration (*i.e.* µg Chl g^{-1} tissue) or content (*i.e.* µg Chl cm^{-2} tissue) and nitrogen content of two wheat cultivars growing in fields with different fertility level; (ii) evaluate the need of separate calibration curves under different conditions; and (iii) find the variable that can be best estimated/correlated with SPAD index.

Materials and Methods

Experimental design

To span a wide range of chl content, leaves of two wheat cultivars (PBW 509 and PDW 233) were collected from four ongoing experiments at four fields having different fertilizer N/Mn levels. Field 1 comprised four fertilizer N levels (0, 40, 80, or 150 kg ha^{-1} N as urea); field 2 four fertilizer N levels (0, 50, 100, or 150 kg ha^{-1} N as urea,); field 3 three fertilizer N levels (50, 100, or 150 kg ha^{-1} N as urea) supplemented with manganese (20 kg ha^{-1}Mn as manganese sulfate) whereas field 4 had three fertilizer N levels (50, 100, or 150 Kg ha^{-1} N as urea) and Mn-deficient soil. All the treatments at the four fields had three replications.

Sampling and analysis

The SPAD index was recorded from the middle of 10 leaf blades (about halfway between the tip and the base), excluding the midrib, in each plot using chlorophyll meter (SPAD-502, Minolta Corp; Spectrum Technologies, Plainfield, Ill) at the flag leaf stage. The same leaf samples, as used for recording the SPAD readings, were collected to determine the chl and N content. Leaf area of a sample weighing 100 mg was recorded (CI-203 CA, Leaf Area Meter, Conveyer Attachment, CID, Inc., USA). Leaf slices weighing 100 mg were placed in a stoppered vial containing 10 mL dimethyl sulfoxide (DMSO). The vials were incubated at 40°C with constant shaking. The extract was filtered after 24 h when the leaf samples had become colorless, indicating complete extraction. The absorbance of filtrate was recorded at 649 nm and 665 nm. The chlorophyll concentrations were calculated using the equations given by Wellburn[24]:

$$Chl_a = 12.19A_{665} - 3.45A_{649} \times V/W$$

$$Chl_b = 21.99A_{649} - 5.32\ A_{665} \times V/W$$

where V is the volume of DMSO in litres and W is the weight of tissue in grams.

Leaf samples were dried and their total N content was determined by Micro kjeldahl's method. Dried plant material weighing 0.5 g was taken in digestion flask. To this, 2 g of digestion mixture (K_2SO_4:$CuSO_4$:10:1 W/W) and 10 mL of concentrated sulphuric acid was added. The flasks were heated till clear solution was obtained. After cooling, the volume was made to 50 mL with distilled water. 5 mL of aliquot was distilled in micro kjeldahl distillation apparatus with 5 mL of 40% sodium hydroxide. The liberated ammonia was trapped in 5 mL of 1 % boric acid containing 2-3 drops of mixed indicator (0.5 g bromocresol green and 0.1 g methyl red dissolved in 100 mL of 95% alcohol and pH adjusted to 4.5 with diluted HCl). About 10 mL of distillate was collected in 250 mL conical flask and titrated with N/100 hydrochloric acid till end point (pink to yellow). A blank without sample was also run simultaneously.

N content was calculated as:

$$\% \text{ N in sample} = \frac{(T-B) \times 0.00028 \times V_1}{V_2 \times S} \times 100$$

where T = volume of N/100 HCl used for sample titration; B = volume of N/100 HCl used for blank titration; S = sample weight (g); V_1 = total volume made; V_2 =volume used for distillation

Statistical analysis

Regression analysis with SPAD index as the independent variable (X) and chl concentration/chl content/N content as the dependent variables (Y), was done to determine regression equations, test the

Table 1—Range of SPAD index and total chlorophyll concentration (mg/g fresh weight), chlorophyll content (mg/dm^2 leaf area) and nitrogen content (%) for leaves of two wheat cultivars growing at different fields

Variety	Field	SPAD index range	Chlorophyll concentration range (mg/g)	Chlorophyll content range (mg/dm^2)	Nitrogen content range (%)
PBW 509	1	32.2 – 47.0 (39.0)	1.83 – 2.42 (2.08)	3.5 – 4.7 (4.10)	1.14 – 3.97 (2.82)
	2	28.0 – 45.0 (40.0)	1.33 – 2.52 (2.10)	2.5 – 4.9 (3.91)	2.25 – 3.95 (3.08)
	3	30.3 – 46.8 (38.3)	1.36 – 2.65 (2.00)	2.8 – 4.3 (3.75)	2.05 – 2.17 (2.33)
	4	32.2 – 41.2 (37.3)	1.68 – 2.24 (1.82)	2.8 – 4.8 (3.83)	2.23 – 3.07 (2.68)
PDW 233	1	34.6 – 42.9 (38.0)	1.38 – 2.20 (1.98)	3.5 – 4.4 (3.82)	2.50 – 3.99 (2.92)
	2	23.0 – 44.0 (37.0)	1.75 – 2.74 (2.26)	2.8 – 4.4 (4.01)	2.05 – 3.76 (2.96)
	3	34.2 – 42.4 (39.4)	1.73 – 2.93 (2.26)	2.6 – 4.5 (3.79)	1.59 – 2.21 (2.27)
	4	26.2 – 41.5 (35.6)	1.61 – 2.46 (1.97))	2.9 – 4.8 (3.94)	1.65 –3.29 (2.19)

[Values in parentheses indicate the mean]

Table 2—Relationship between total chlorophyll concentration (mg/g fresh weight), chlorophyll content (mg/dm^2 leaf area) and nitrogen content(%) (Y) and SPAD index (X) in leaves of wheat irrespective of cultivars and fields/growing location

Variable	Regression Equation	r^2	r
Chl concentration	Y = 0.0519*X+0.0931	0.592**	0.77*
Chl content	Y = 0.0760*X+0.9451	0.489**	0.70*
N content	Y = 0.0781*X–0.324	0.369**	0.61*

[Y = aX + b where Y is dependent variable and X is SPAD index. a*X means slope is significant; **Significant at P <0.01, r^2 = coefficient of determination, r = correlation coefficient]

significance of slopes, coefficient of correlation (r), determination (r^2) and their significance using software Graph Pad Prism (5.01).

Results

Data recorded on leaf samples from all the field experiments gave a wide range of SPAD index, chl concentration (mg/g fresh leaf tissue), chl content (mg/cm^2 leaf area) and N content (%). SPAD index ranged from 26.2 to 47, chl concentration from 1.33 to 2.93 mg/g, chl content from 2.5 to 4.9 mg/dm^2, and N content from 1.14 to 3.99% (Table 1). Data were subjected to regression analysis and among different mathematical models, the linear model best fitted the data to describe the relationship between the independent variable X (SPAD index) and dependent variable Y (chl concentration/chl content/N content, (Table 2).

Table 3—Relationship between total chlorophyll concentration (mg/g fresh weight), chlorophyll content (mg/dm^2 leaf area) and nitrogen content (%) (Y) and SPAD index (X) in leaves of wheat cultivars growing at different fields irrespective of type of cultivar

Variable	Field	Regression Equation	r^2	R
Chlconc	1	Y=0.257*+ 0.046*X	0.622*	0.79*
	2	Y=−0.261*+ 0.061*X	0.631*	0.79*
	3	Y=−0.773*+ 0.075*X	0.699*	0.84*
	4	Y= 0.628* + 0.035*X	0.582*	0.76*
Chl content	1	Y = 1.024*+ 0.075*X	0.439*	0.66*
	2	Y= 1.241* + 0.066*X	0.413*	0.64*
	3	Y = −0.003*+ 0.001*X	0.866*	0.93*
	4	Y= −0.002*+ 0.001*X	0.686*	0.83*
N content	1	Y= −3.801* + 0.172*X	0.695*	0.83*
	2	Y= 1.001*+ 0.034*X	0.456*	0.68*
	3	Y= 0.791* + 0.039*X	0.705*	0.84*
	4	Y= 0.293* + 0.059*X	0.542*	0.74*

[Y = a + bX where Y is dependent variable and X is SPAD index. a* and b*X means intercept and slope, respectively, are significant. *Significant at P < 0.05, r^2 = Coefficient of determination, r = Correlation coefficient]

Chlorophyll concentration and SPAD index

The regression analysis of chlorophyll concentration (Y) and SPAD index (X) with the data sets of different field experiments was done. The results revealed that SPAD index could explain 59% of variation in chl concentration.

Different regressions for four fields irrespective of cultivars showed that linear regression equations were best fitted to explain the relationship between chl concentration and SPAD index at different fields with graded doses of N (Table 3). The values of

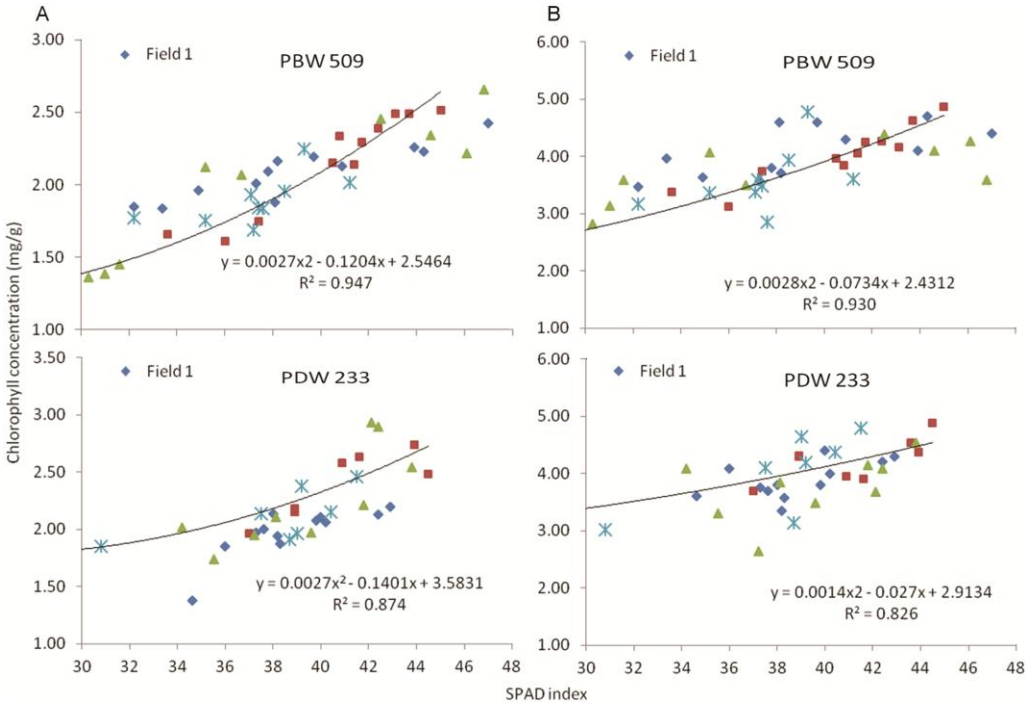

Fig. 1—Relationship between (A) total chlorophyll concentration (mg/g fresh weight, Y) and SPAD index (X); and (B) total chlorophyll content (mg/dm^2 leaf area, Y) and SPAD index (X) in leaves of two wheat cultivars irrespective of growing in different fields

coefficients for all regressions were significant at 0.1% level and r^2 value was highest for field 3 (0.70). For each change of one unit in SPAD index at field 3, the average change in mean of chl concentration is 0.075 mg/g fresh weight.

The regression equations developed separately for two cultivars irrespective of different fields revealed significant polynomial relationship between chl concentration and SPAD index (Fig. 1). The relationship between chl concentration and SPAD index was stronger for PBW 509 than PDW 233 (r^2 = 0.95 and 0.87, respectively) indicating SPAD index as a better estimator of chl concentration of PBW 509 than PDW 233.

The relationship between chl concentration and SPAD index from the complete range of data sets of two cultivars at four fields was quadratic and positive (Table 4). The coefficient of determination, r^2 ranged between 0.751 and 0.947 (P <0.001). The relationship was strongest for PBW 509 at field 1 and weakest for PDW 233 at field 3.

Different equations for two cultivars developed under four fields revealed that both PBW 509 and PDW 233 had comparatively higher mean chl concentration and chl per unit SPAD index (Table 4) at field 2. The values of coefficients of the regression quadratic model were significant at 1% level and varied considerably among cultivars at different fields. For estimating chlorophyll concentration, the value of intercept 'a' ranged from −8.757 to 14.254 and of coefficient 'b' from −0.722 to 1.169. The same cultivar growing at different fields revealed different regression lines. The regression analysis explained 84, 95, 92 and 84 % variation in chl concentration with SPAD index at field 1, 2, 3 and 4, respectively for cv PBW 509 whereas the corresponding values for cultivar PDW 233 were 82, 77, 65 and 75% (Table 4).

Table 4—Relationship between total chlorophyll concentration (chl c, mg/g fresh weight, Y) and SPAD index (X) in leaves of two wheat cultivars growing at different fields

Cultivar	Field	a	b	c	r^2
PBW-509	1	0.909	0.023	0.0002	0.840*
	2	2.546	−0.120	0.0027	0.947*
	3	−8.757	0.505	−0.0057	0.921*
	4	0.565	0.016	0.0005	0.844*
PDW-233	1	−2.2102	1.169	−0.0141	0.823*
	2	2.8975	−0.0937	0.0020	0.769*
	3	14.254	−0.722	0.0105	0.651*
	4	2.683	−0.093	0.002	0.751*

[a, b and c are the coefficients of polynomial equation: $Y = a + bX + cX^2$; r^2= coefficient of determination; *Significant at P <0.05]

Chlorophyll content and SPAD index

The regression analysis with all the data sets of different experiments indicated that 49% of variation in chl content can be estimated with SPAD index. The regression equations developed separately for four fields irrespective of cultivars revealed a linear relationship between chl content and SPAD index (Table 3). The regression coefficients were significant at 0.1% level and the correlation coefficient was greater at field 3 (0.87) than 1 (0.44), 2 (0.41) and 4 (0.69). The slopes for different fields varied from 0.001 to 0.075 whereas intercepts varied from 0.002 to 1.241.

Regression equations for two cultivars irrespective of fields revealed higher, significant and positive correlation between chl content and SPAD index for PBW 509 (R^2 = 0.930) than PDW 233 (R^2 = 0.826). The regressions were significant at 0.1% level and the values of coefficients varied among cultivars with slope - 0.498 for PBW 509 and −0.163 for PDW 233 (Fig. 1).

The quadratic regression equations were best fitted to explain the relationship between chl content and SPAD index for two cultivars separately at different fields (P <0.001). The values of coefficient of determination were high and significant indicating that SPAD index is a good estimator of chl content for both cultivars grown at four fields (Table 5). The relationship was strongest for PBW 509 at field 2 (R^2 = 0.947) and weakest for PDW 233 at field 1 (R^2 = 0.627). The chl content ranged from 2.5 to 4.9 mg/dm^2 for two cultivars grown at different fields. The mean chl content was highest for PBW 509 at field 1 and for PDW 233 at field 2 (Table 5). The highest chl content per unit SPAD index was 0.108 for PDW 233 at field 2 and 0.105 for PBW 509 at field 1 (Table 5). For estimating chlorophyll content, the value of intercept 'a' ranged from −0.034 to 0.255 and of coefficient 'b' from −0.120 to 0.008. The regression analysis explained 79, 94, 70 and 71 % of the variation in chl content with SPAD index at fields1, 2, 3 and 4, respectively for PBW 509 and 63, 83, 59 and 60% for PDW 233 (Table 5).

Table 5—Relationship between chlorophyll content (mg/dm^2 leaf area, Y) and SPAD index (X) in leaves of two wheat cultivars growing at different fields

Cultivar	Field	a	b	c	r^2
PBW-509	1	−0.034	0.003	-3×10^{-5}	0.788*
	2	2.550	−0.120	3×10^{-3}	0.947*
	3	−0.129	0.008	-1×10^{-4}	0.709*
	4	0.109	−0.005	8×10^{-5}	0.711*
PDW-233	1	0.131	−0.006	9×10^{-5}	0.627*
	2	0.029	−0.003	1×10^{-5}	0.826*
	3	0.590	−0.029	4×10^{-4}	0.588*
	4	0.114	−0.006	1×10^{-4}	0.595*

[a, b and c are the coefficients of polynomial equation: $Y = a+bX+cX^2$. r^2= coefficient of determination. *Significant at P <0.05]

Nitrogen content and SPAD index

The regression analysis with pooled data of two cultivars from four fields revealed a positive linear relationship between N content and SPAD index and 37% of variation in N content can be estimated by SPAD index (Table 2).

The SPAD index could explain 70% of the variation in N content at field 1 and 3, 54 and 46%, respectively at field 4 and 2 (Table 3). The values of coefficients for all regressions were significant at 0.1% level. The slope of the regression equation was positive except at field 1. The values of intercept ranged from 0.034 to 0.172. The highest rate of change in mean of N content for one unit change in SPAD index was 0.172 at field 1.

Significant polynomial relationship between N content and SPAD index was recorded for two cultivars irrespective of different fields (Fig. 2). The

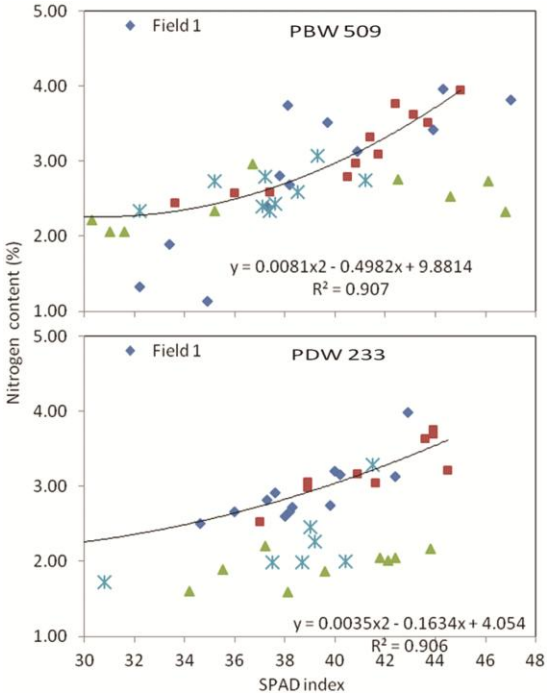

Fig. 2—Relationship between nitrogen content (%, Y) and SPAD index (X) in leaves of two wheat cultivars irrespective of growing in different fields

Table 6—Relationship between nitrogen content (%, Y) and SPAD index (X) in leaves of two wheat cultivars growing at different fields

Cultivar	Field	a	b	c	r^2
PBW-509	1	−23.021	1.142	−0.0121	0.463*
	2	9.881	−0.498	0.0081	0.907*
	3	−9.769	0.624	−0.0077	0.667*
	4	12.498	−0.634	0.0093	0.692*
PDW-233	1	23.406	−1.190	0.017	0.734*
	2	4.054	−0.1634	0.0035	0.906*
	3	6.131	−0.270	0.0040	0.687*
	4	4.223	−0.1786	0.0032	0.702*

[a, b and c are the coefficients of polynomial equation: $Y= a+bX+cX^2$. r^2= coefficient of determination. *Significant at P <0.05]

regression equations have significant coefficients at 0.1%. There was difference in the slope and intercept of two cultivars but the coefficient of determination, R^2 was 0.90. This revealed that SPAD index is a good estimator of N content irrespective of cultivar.

The regression equations developed separately for two cultivars at different fields revealed a strong positive relationship between N content and SPAD index. The regression equations were significant with 0.1% level and the coefficient of determination ranged from 0.463 to 0.907 (Table 6). The relationship was strongest for both the cultivars at field 2 and weakest for PBW 509 at field 1. The mean N content varied from 2.19 to 3.08% that was highest in both cultivars growing at field 2 (Table 1). The N content per unit SPAD index was 0.08 for PDW 233 at field 2 and 0.058 at field 3 (Table 6). The quadratic regressions for both cultivars in different fields were significant with 0.1% level indicating that the values of coefficients of quadratic regression model were affected by fields and cultivars. The value of intercept 'a' ranged from −23.021 to 23.406 and of coefficient 'b' from –0.270 to 1.142. The results revealed that SPAD index can explain 90% of variation in N content for both cultivars at field 2 in comparison to 46, 67 and 69% for PBW 509 and 73, 69 and 70% for PDW 233 at field 1, 3 and 4, respectively.

Comparison of different Variables as related to SPAD Index

Results in Table 2 revealed that SPAD index is best correlated to chl concentration (r = 0.77) in comparison to chl content (0.70) and N content (0.61). However, the correlation values altered when data of two cultivars was studied independent of field. The correlation coefficient was highest between SPAD index and N content at field 1 (r = 0.83), chl concentration at field 2 (r = 0.78) and chl content at field 3 (r = 0.93) and 4 (r = 0.83). The correlation coefficients of SPAD index with chl concentration, chl content and N content were high (r ≥0.90) for PBW 509 and PDW 233 (Table 4).

Discussion

SPAD index provided good estimates of chl concentration, chl content and nitrogen content in both cultivars tested in different fields. The linear relationship between chl concentration and SPAD index (Table 2) has been reported in many species[25,26]. However, the relationship improved when separate calibration curves were plotted for cultivars and fields. When regression equations for chl concentration/chl content/N content and SPAD index relation were developed separately for different fields, the regression coefficients became significant (P <0.001) and the value of coefficients of determination increased. This data imply that coefficient values that give good estimates under one field may not hold good for another field for the same cultivar under same climatic conditions. This implication was consistent with earlier reports for eucalyptus species[27] and for rice[28]. The variation in correlation between chl concentration and SPAD index of PBW 509 and PDW 233 growing under similar conditions could be explained by the difference in light-transmission pattern through the leaves of different cultivars of a crop. The relationship between SPAD index and chlorophyll concentration has been investigated in a variety of different species, and has been found to display considerable inter specific variation[29]. This variability is presumed to be attributable to structural differences between the leaves of different species, causing different light reflection or scattering effects. In rice leaves, Takebe and Yoneyama[30] reported a strong linear relationship between SPAD index and leaf chl concentration and this relationship varied with variety. Peng *et al.*[32] with rice and Thompson *et al.*[33] with soybean reported that the differences within the crop were mostly because of the differences in thickness of leaf or specific leaf weight. The confounding effect of leaf thickness can be eliminated if chl concentration is expressed on a leaf area basis[28]. The relationship between SPAD index and chlorophyll concentration in wheat and birch was similar when expressed as chlorophyll per unit leaf area but different when expressed as

chlorophyll per unit of leaf fresh weight[29]. A strong relationship between leaf area based chl content and SPAD values was recorded in 13 tree species[33]. A better correlation between SPAD index and total extractable chl per unit area (chl content), than per unit of weight (chl concentration) could be attributed to the sieve effect which resulted from non uniform distribution of chlorophyll. The leaves with high chlorophyll content have increased chlorophyll density in chloroplasts, rather than an increase in the number of chloroplast. In our studies, SPAD index explained 60% variation in chl concentration as compared to 49% in chl content. The variation in extent of relationship between SPAD index and chl concentration/content cultivar might be due to variation in leaf thickness and leaf dry weight per unit area under different environmental conditions/fields during leaf expansion[34].

The relationship between foliar N content and SPAD index based on pooled data of two cultivars and four fields was almost similar to chl content (R^2= 0.37; r = 0.61) although strong relationships have been observed in other species like wheat[35], rice[36], maize[11] and salvia[37]. In our studies, the coefficient of determination improved to 0.46 when separate calibration curves were plotted for PBW 509 whereas decreased to 0.30 for PDW 233. The N content also showed higher correlation with SPAD index at field 2 (r = 0.89) and 1 (r = 0.83) than 4 (r = 0.57) and 2 (r = 0.52). These results necessitate that separate calibration curves representing relationship between N content and SPAD index should be plotted for cultivars or fields[38]. The variation in relationship between SPAD index and N content might be due to the fact that leaf greenness does not always show a direct correlation between leaf N and photosynthetic activity. The pattern of allocation of leaf N between soluble proteins and the pigment –protein/reaction center complexes of thylakoids (on which light absorption by chlorophyll depends), vary with N supply and canopy light environment[39].

The correlation analysis revealed that from pooled data SPAD index can be considered as the best estimator of chl concentration in comparison to chl or N content, but the relationship varied when correlation was calculated separately for cultivar or field. Some studies reported SPAD index as the best estimator of chl content[25] whereas, other noted best correlation with N content[40,41]. Thus, the variable best estimated by SPAD index varies with the crop, cultivar, site of the experiment, and type of treatment[28].

The SPAD-502 is extensively used in several studies as a simple, non-destructive method for immediate assessment of physiological variables[42,43]. Our study indicated that a high degree of correlation existed for all the variables with SPAD index despite different regression equations for cultivars and fields, suggesting that SPAD meter could be a reliable tool for estimation of chl concentration, chl and N content in wheat but with a caution to develop calibration curves for each particular field, hybrid, and treatments to lend standards for interpretations[44].

Conclusion

The portable SPAD meter can be useful where relative rather than absolute values of foliar chl concentration/content and N content are required. It can serve as a reliable tool for a variety of research and management applications including rapid assessment of physiological changes across time, field diagnosis for assessment of relative health of crops and nutritional management practices (fertilizer recommendations).

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