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Short Communication

Development of SPAD value-based linear models for non-destructive estimation of photosynthetic pigments in wheat (*Triticum aestivum* L.)

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Abstract

Chlorophyll content in leaf which can be measured and expressed as SPAD values, is a good indicator of plant health. The present study aimed to develop linear models for studying relationship between SPAD values and spectrophotometrically determined chlorophylls and carotenoid contents in leaves of 468 wheat genotypes. The SPAD values were taken in the flag leaves at heading stage. Chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*), total chlorophyll (total Chl) and total carotenoids (total Car) contents were assessed using dimethyl sulfoxide (DMSO) extraction method. To estimate photosynthetic pigment content in leaves, SPAD value-based linear models were developed and Chl_a (Chl_b = 0.0690 x SPAD Value - 1.082), Chl_a (Chl_b = 0.021 x SPAD Value 0.396), total Chl (Total chlorophyll = 0.090 x SPAD Value - 1.477) and total carotenoids (Total Carotenoids = 0.013 x SPAD Value 0.074) were estimated. The present study reports development of linear model for quantification of the photosynthetic pigment content in wheat leaves using chlorophyll meter.

Keywords: Carotenoids, chlorophyll, photosynthetic pigments, SPAD measurement, wheat

Chlorophylls and carotenoids are the most abundant pigment molecules found in green plants which trap the solar energy for photosynthetic process and control optical properties of leaf. The amount of chlorophyll present in the chloroplasts of mesophyll cells of leaf is a key indicator of the photosynthetic capacity (Cannella et al. 2016). Photosynthetic capacity and leaf area index have been found to be critical proxy for vegetation productivity (Gitelson et al. 2006; Sanglard et al. 2016). Carotenoids, another key group of photosynthetic pigments, being essential structural components of the photosynthetic antenna, take part in harvesting of solar energy for photosynthesis and also participate in the defense mechanism against oxidative stress (Zakar et al. 2016). They also play an essential role in the dissipation of excess light energy and provide protection to reaction centers (Santa Barbara et al. 2013).

Estimation of the photosynthetic pigment content in plants essentially required two steps i.e., extraction of pigment and determination of its content using spectrophotometer. This method is time-consuming, destructive in nature and difficult to carry out continuously under field conditions. To overcome these limitations, a variety of optical techniques including SPAD meter have been developed to evaluate leaf chlorophyll content rapidly and non-destructively (Richardson et al. 2002). The Soil-Plant Analyses Development (SPAD) unit of Minolta Camera Co. Osaka, Japan has manufactured a portable, handy, lightweight, self-calibrating device SPAD-502 Plus chlorophyll meter to estimate quantity of chlorophyll accurately, rapidly, in non-destructive manner in plant leaves (Minolta 1989). The present study was aimed to develop the linear models of the relationship between the SPAD values and spectrophotometrically determined chlorophylls and carotenoids content in leaves of wheat.

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A field experiment was conducted using 468 wheat genotypes in augmented design at experimental farm, ICAR-Indian Agricultural Research Institute New Delhi. Field was watered at regular intervals depending upon the rainfall. Recommended package of agronomic practices were followed to raise the healthy crop. SPAD values were estimated on flag leaves using the chlorophyll meter SPAD-502Plus during the day between 9 to 12AM (Nauš et al. 2010). The meter head was put at upper leaf surface avoiding main mid-vein. Three readings were taken and averaged per leaf in each genotype. Thereafter, photosynthetic pigments were extracted from the same flag leaves which were used for the SPAD determination. Leaf was cut, weighed and put immediately into test tube consisting 4 ml dimethyl sulphoxide (DMSO). Chlorophyll and carotenoid contents were extracted as per method of Hiscox and Israelstam (1979). DMSO renders plasmalemma permeable resulting in the leaching of the pigments. The absorbance of the known volume of solution containing known quantity of leaf tissue at two respective wavelengths (663 and 645 nm) was determined for chlorophyll content and at 480 nm for total carotenoids contents. Chlorophyll a, chlorophyll b and total chlorophyll contents were estimated using the formula given by Arnon (1949) while carotenoids content was determined the method given by Lichtenthaler and Welburn (1983). Thirty mg fresh leaf samples were added to the test tubes containing 4 ml DMSO and were kept in dark for 4 h at 65^{o}C. Then the samples were taken out cooled at room temperature and the absorbance was recorded at 663, 645 and 480 nm using DMSO as blank and was expressed as mg g^{-1} dry wt.

In order to establish the relationship between the SPAD- 502Plus meter reading and the leaf chlorophyll content, it was desirable to have larger number of diverse genotypes which possesses wider range of chlorophyll content; therefore, we took 468 contrasting wheat genotypes. Wide range of variability was observed in these genotypes for SPAD values and for amount of different photosynthetic pigments. The SPAD values ranged from 21.0 to 54.7 with mean value of 44.09. The photosynthetic pigments content (mg g^{-1}FW) [Chl*a* varied from 0.0893 to 4.0279 with mean value of 2.119), Chl*b* (ranged from 0.0144 to 1.3825 with mean value of 0.573), total chlorophylls (varied from 0.1037 to 4.458 with mean 2.693) and total carotenoids (varied from 0.0204 to 0.9889 with mean value of 0.499)]. Similarly, sufficient level of genetic variability was noted earlier (Kumar et al. 2013) among the wheat genotypes for photosynthetic pigments. The chlorophylls and carotenoids contents in wheat leaf were found to be significantly correlated with the SPAD value measured using the Chlorophyll meter SPAD-502Plus (Figs. 1-4). The linear regression was found to be significant in sorghum (Yamamoto et al. 2002) *Malus domestica* Borkh. (Campbell et al. 1990) and some other tree species (Samsone et al. 2007).

Recorded SPAD 502Plus readings were significantly correlated with spectrophotometrically estimated Chl*a* content in diverse wheat genotypes. It is because in both the techniques chlorophyll absorption property is used for its measurement. Our results are consistent with the finding of other workers (John Mark Well et al. 1995; Briton et al. 2011; Shah et al. 2017). They reported that SPAD -502 meter gives differing prediction responses for different plant species, the calibration lines found species specific. Therefore, calibration models demand individual regression for particular species. Fig. 1 shows the relationships between the readings obtained in wheat leaf by chlorophyll meter and the estimated concentrations of Chl*a*. The relationship between the chlorophyll readings from SPAD and the Chl*a* contents was fit in linear model (Chl*a* = 0.0690 x SPAD Value - 1.082), and an R^2 value of 0.302** (n = 468) was obtained for SPAD chlorophyll meter. Similarly, measured SPAD values were found to be significantly associated with Chl_b content in diverse wheat

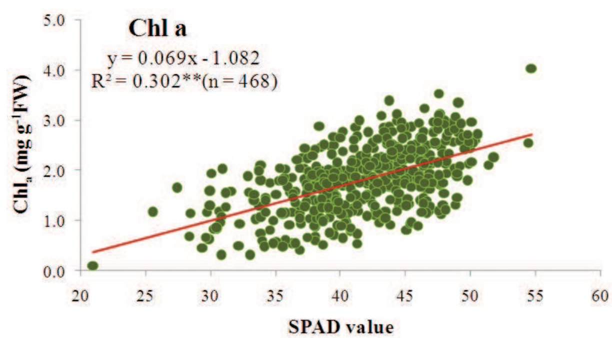

Fig. 1. Linear association between chlorophyll a content (Chl*a*) and SPAD values

genotypes. Our results are in agreement with findings of earlier workers (Markwell et al. 1995; Brito et al. 2011; Shah et al. 2017). Fig. 2 shows the association between the SPAD values noted and the Chl*b* content obtained in wheat leaves. The correlation between the SPAD values and the estimated contents of Chl*b* was fit in linear model (Chl*b* = 0.021 x SPAD Value – 0.396), and an R^2 value of 0.240** (n = 468) was obtained for

SPAD chlorophyll meter (Fig. 2).

Since total chlorophyll includes both Chl*a* and

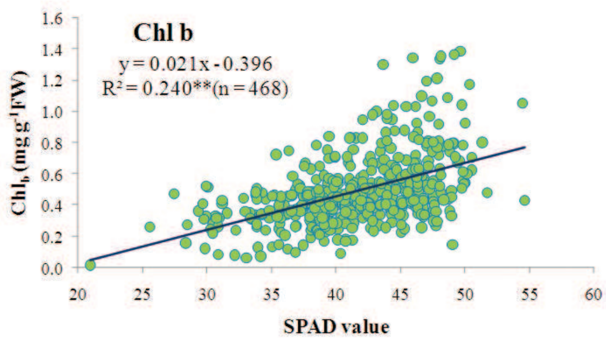

Fig. 2. Linear association between chlorophyll b content (Chl_b) and SPAD values

Chl*b*, therefore measured SPAD 502 readings were found significantly associated with estimated value of total Chl content in contrasting set of wheat genotypes. Results obtained in present study are in accordance with the findings of earlier workers (Markwell et al. 1995; Brito et al. 2011; Shah et al. 2017). Fig. 3 reveals the association between the SPAD values recorded and the content of total Chl obtained in wheat leaves. The correlation between the SPAD values and the contents of total Chl was fit in linear model (Total chlorophyll = 0.090 x SPAD Value –1.477) and an R^2 value of 0.332** (n = 468) was obtained for SPAD chlorophyll meter (Fig. 3).

Similarly, values recorded using SPAD 502 had

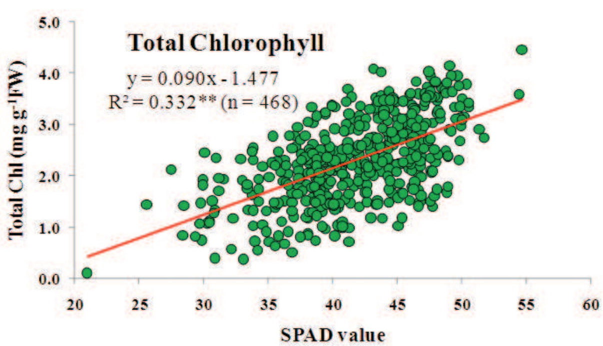

Fig. 3. Linear association between Total chlorophyll content (Total Chl) and SPAD values

significant association with total carotenoids content in a set of diverse wheat genotypes. The present findings are consistent with the findings of earlier workers (Markwell et al. 1995; Brito et al. 2011; Shah et al. 2017). Fig. 4 shows the association between the SPAD values obtained in wheat leaves and the estimated content of total carotenoids. The correlation between the SPAD values and the contents of total carotenoids was fit in linear model (Total Carotenoids = 0.013 x SPAD Value – 0.074), and an R^2 value of 0.147** (n = 468) was obtained for SPAD chlorophyll meter (Fig. 4). Comparatively lesser R^2 value with

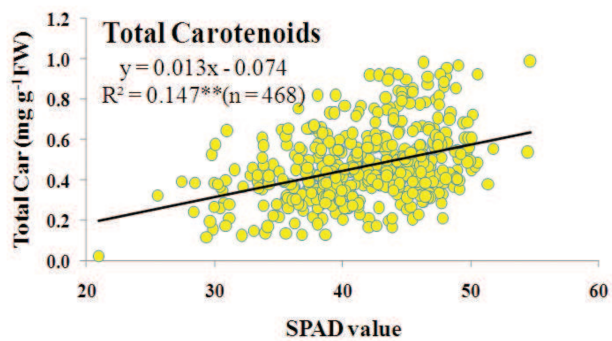

Fig. 4. Linear association between total carotenoids (Total Car) content and SPAD values

carotenoids indicated indirect association of carotenoids with SPAD values. It gives the impressions that indirect carotenoids quantification could be obtained with the chlorophyll meter due to the significant linear relationship between total chlorophyll and carotenoids content determined spectrophotometrically.

In general, it was found that the use of the portable chlorophyll meter SPAD-502Plus produced results associated with photosynthetic pigments in the form of linear models and allowed for a quick estimation of photosynthetic pigments in the leaves of wheat, reasonably accurate, rapidly and without the use of chemicals. Henceforth, it is concluded that SPAD-502Plus chlorophyll meter is able to provide a rapid and reasonably accurate estimate of leaf photosynthetic pigments in wheat and that may be used for faster screening of large number of wheat genotypes for environmental stresses under field conditions.

Authors' contribution

Conceptualization of research (PK); Designing of the experiments (PK, RKS); Contribution of experimental materials (PK, RKS); Execution of field experiment and data collection (PK, RKS); Analysis of data and interpretation (PK, RKS); Preparation of manuscript (PK, RKS).

Declaration

The authors declare no conflict of interest.

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