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Quick Determination of Leaf Photosynthetic Pigments Using SPAD Readings

Pramod Kumar[1*] and RK Sharma[2]

*[*1]Division of Plant Physiology, ICAR-Indian Agricultural Research Institute, New Delhi, India*

[2]Division of Genetics, ICAR-Indian Agricultural Research Institute, New Delhi, India.

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ABSTRACT

SPAD meter is a simple, portable diagnostic device that measures the greenness or relative chlorophyll content of leaves. Compared with the traditional destructive methods of chlorophyll extraction, the use of this tool device saves time, space, and resources. The objective of this article is to review the associations between the photosynthetic pigments content (chlorophylls, carotenoids) extracted in dimethylsulfoxide or acetone, with the SPAD readings in leaves for estimating their content non-destructively. To estimate photosynthetic pigment content in flag leaves of wheat using the chlorophyll meter, linear models were developed by Kumar and Sharma (2019) from the relationship between the Chlorophyll meter SPAD readings and photosynthetic pigments, i.e., Chl_a (Chl_a=0.0690 × SPAD Value - 1.082), Chl_b (Chl_b=0.021 × SPAD Value - 0.396), total Chl (Total chlorophyll=0.090 × SPAD Value - 1.477) and total carotenoids (Total Carotenoids=0.013 × SPAD Value - 0.074). Therefore, it is obvious that the portable chlorophyll meters, SPAD-502 produced readings are associated with leaf photosynthetic pigments and thus allow for a quick determination of the concentration of photosynthetic pigments in the leaves of crop plants with high accuracy and avoiding the use of chemical reagents and extensive laboratory protocols.

Keywords: Carotenoids, Chlorophyll, Photosynthetic pigments, SPAD readings

INTRODUCTION

Chlorophylls and carotenoids are the most abundant pigment molecules occurred in the chloroplast of leaf mesophyll cells of green plants which trap the solar energy for photosynthetic process. The amount of leaf photosynthetic pigments is key variables in characterizing photosynthetic capacity and gross primary production in the biosphere [1-4]. Besides light harvesting, photosynthetic pigments play a central role in photosystem protection and other growth functions [5,6]. Carotenoids are composed of carotenes and xanthophylls and represents another key photosynthetic pigment group. Being essential structural components of the photosynthetic antenna, carotenoids participate in harvesting light energy for photosynthesis [7,8]. In addition to the direct contribution in the photosynthetic process, carotenoids are also involved in the defense mechanism against oxidative stress [9,10] and play an essential role in the dissipation of excess light energy and provide protection to reaction centers [11].

In eco-physiological studies amount of chlorophylls indicates responses of plants to different stresses such as nitrogen deficit [12,13], water deficit [14,15] and high irradiance [16-19]. Carotenoids play a role in protection of the photosynthetic machinery against excess energy and their high content in leaf may indicate a photo inhibitory stress [20-22]. In addition, the amount of chlorophyll content present in plants, gives an indirect estimation of the nutrient status of the plants because much of the leaf nitrogen is incorporated in chlorophylls [23]. Furthermore, leaf chlorophyll concentration is strongly associated to plant stress and senescence [16].

Conventionally, the methods used to estimate the photosynthetic pigment in laboratory as suggested by Porra et al. [24] and Wellburn [25], are based on extraction of pigments from the plants followed by estimation using spectrophotometry. Essentially, these methods are destructive in nature, time consuming, requiring specific equipment's and solvents which are toxic to human health and environments. Moreover, the methods are not applicable in the conditions, under which researchers want to retain the whole plant as such.

Therefore, non-invasive, inexpensive and rapid methods of

Corresponding author: Pramod Kumar, Division of Plant Physiology, ICAR-Indian Agricultural Research Institute, New Delhi, India, E-mail: pramodk63@yahoo.com

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measuring chlorophyll have been developed based on optical leaf properties. For this purpose a portable chlorophyll meter (SPAD-502Plus, Minolta Camera Co., Osaka, Japan) has been often used in plant physiology, agriculture and less frequently in forestry research and practice [26-31]. This handy device allows a convenient estimation of total chlorophyll content in leaves, especially with repeated number of measurements on the same leaf [32-34].

WHAT IS SPAD?

The Soil-Plant Analyses Development (SPAD) unit of Minolta Camera Co. built up the SPAD-502 chlorophyll meter (Minolta Camera Co., Osaka, Japan). It is a portable, hand-held, light weight, self-calibrating device used to calculate the amount of chlorophyll non-destructively in plant leaves [35]. It is widely used for the rapid, accurate and non-destructive measurement of leaf photosynthetic pigments. It is being employed extensively in research as well as for agricultural applications with a range of different plant species.

PRINCIPLE INVOLVES IN SPAD CHLOROPHYLL METER

Markwell et al. [27] described the physical principles and equations on which the functioning of the SPAD Chl meter was based. In brief, this device measures the transmittance of light by leaves at two different wavelengths: red (650-660 nm) and near-infrared (930-940 nm). Red light is absorbed by chlorophylls and its absorption is associated with the chlorophyll content. The peak absorbance areas of chlorophyll are in blue and red regions. The wavelength ranges chosen to be used for measurements are the red area where chlorophylls *a* and *b* absorbance is high and unaffected by carotenoids (Chlorophyll Meter SPAD-502Plus; Instruction Manual 2009). Near-infrared absorption is used as a "reference value" for adjusting the differences in leaf structure. However, the values given by the chlorophyll meter are in SPAD units which have to be converted into physiological units (pigment concentration: mg g^{-1} fresh weight or dry weight and pigment content: mg m^{-2}). Thus, the calibration curve between the chlorophyll meter readings and chlorophyll content determined with an extraction method should be generated before an attempt to assess physiological responses of plants to environmental factors.

ASSOCIATION BETWEEN THE SPAD READINGS AND THE LEAF PHOTOSYNTHETIC PIGMENTS

Kumar and Sharma [36] using 468 contrasting wheat genotypes established the relationship between the SPAD-502Plus meter readings and the leaf photosynthetic pigments content estimated using spectrophotometer. Wide range of variability was recorded 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 value of 2.693) and total carotenoids (varied from 0.0204 to 0.9889 with mean value of 0.499)). 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, as exhibited in **Figures 1-4**. The linear regression was found to be significant in sorghum [37] and *Malus domestica* Borkh [38] and some other tree species [39].

SPAD 502Plus readings have been found significantly associated with laboratory estimated Chl_a content in diverse wheat genotypes because in both the techniques chlorophyll absorption property is used for chlorophyll measurement [27,36,40,41]. These research workers also 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.

Kumar and Sharma [36] depicted the relationship between the chlorophyll readings from SPAD and the Chl_a contents in linear model (Chl_a=0.0690 × SPAD Value - 1.082) and an R^2 value of 0.302** (n=468) was obtained for SPAD chlorophyll meter, as depicted in **Figure 1**. Similarly, measured SPAD values were found to be significantly associated with Chl_b content in diverse wheat genotypes. Other workers [27,40,41] also reported similar findings. **Figure 2** shows the association between the SPAD values noted and the Chl_b content obtained in wheat leaves. The association between the SPAD values and the estimated contents of Chl_b was fit in linear model (Chl_b=0.021 × SPAD Value - 0.396), and an R^2 value of 0.240** (n=468) was obtained for SPAD chlorophyll meter by Kumar and Sharma [36] as shown in **Figure 2**.

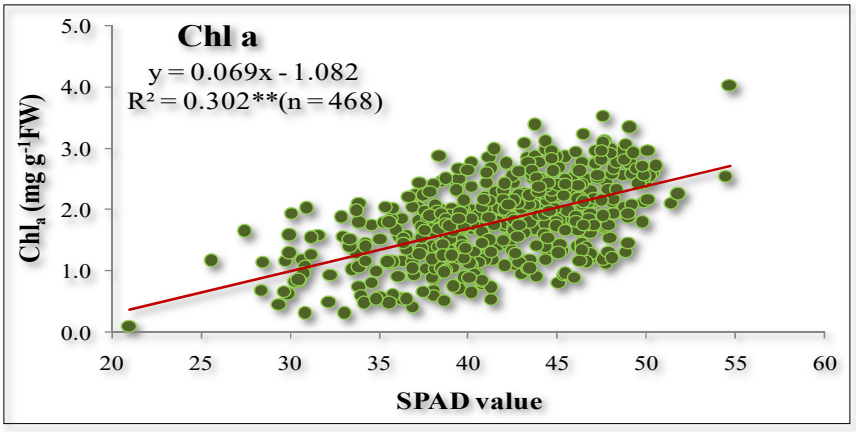

Figure 1. Linear association between chlorophyll a content (Chl$_a$) and SPAD values [36].

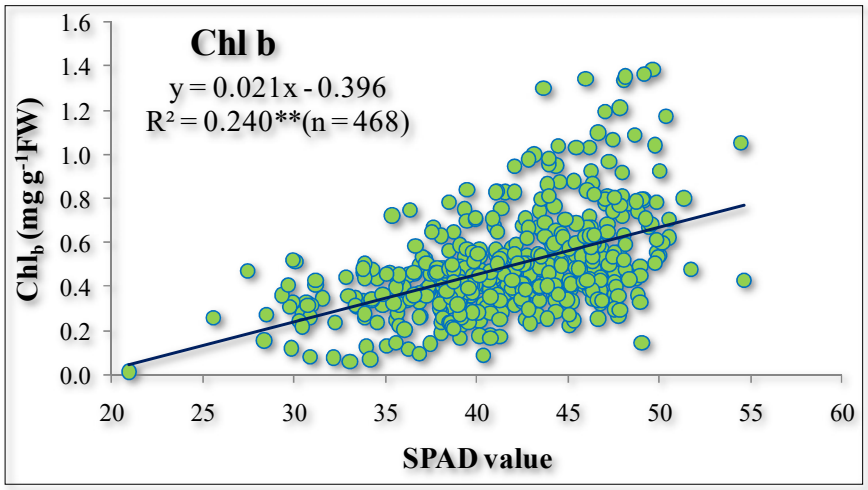

Figure 2. Linear association between chlorophyll b content (Chl$_b$) and SPAD values [36].

Earlier research workers like Brito et al. [40] and Shah et al. [41] also reported similar findings. The correlation between the SPAD values and the content of total chlorophyll was fit in linear model (Total chlorophyll=0.090 × SPAD Value - 1.477) and an R^2 value of 0.332** (n=468) was obtained by Kumar and Sharma [36] **(Figure 3)**.

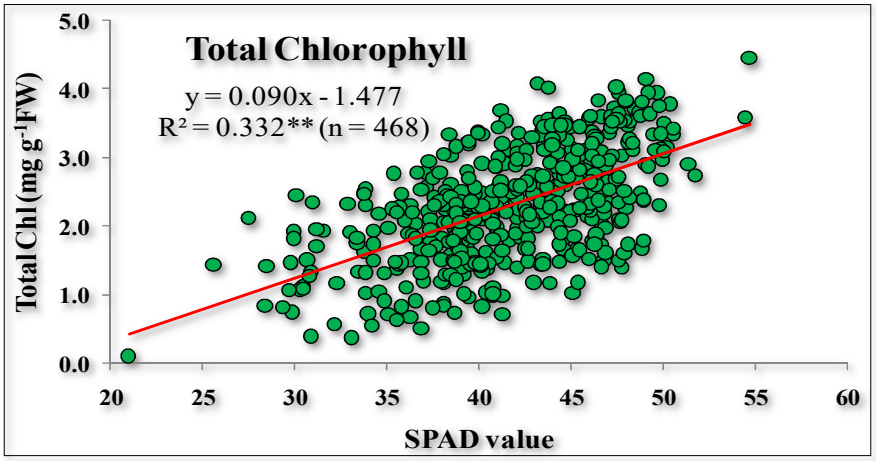

Figure 3. Linear association between total chlorophyll content and SPAD values [36].

The relationship between the SPAD values and the contents of total carotenoids was also found fit in linear model (Total Carotenoids=0.013 × SPAD Value - 0.074), and an R^2 value of 0.147** (n=468) was obtained for SPAD chlorophyll meter by Kumar and Sharma [36] as depicted in **Figure 4**. Comparatively lesser R^2 value with carotenoids indicated indirect association of carotenoids with SPAD values. Such association findings were also reported earlier in cotton [41] and wheat [42]. 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.

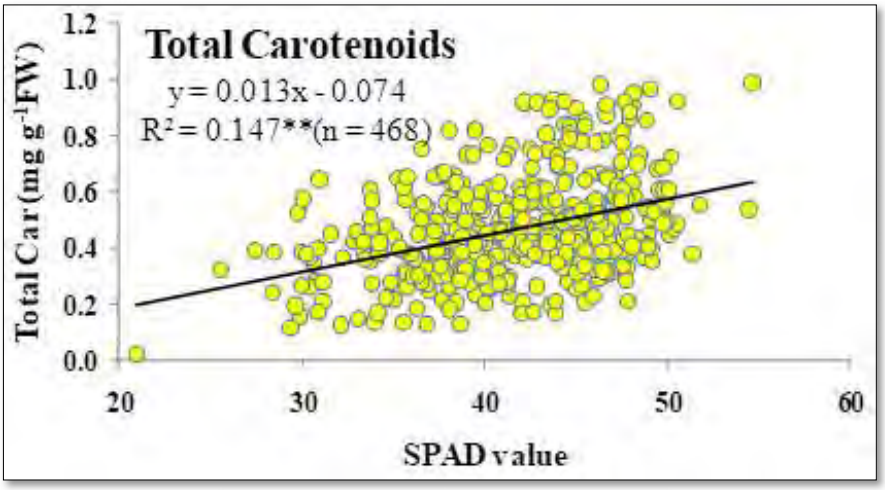

Figure 4. Linear association between total carotenoids (total car) content and SPAD values [36].

Relationships between SPAD-502 readings, the total Chl:Car ratio and the Chl *a*/*b* ratio have been developed in *Gossypim hirsutum* leaves by Brito et al. [41] and in Sycamore (*Acer pseudoplatanus*), English Oak (*Quercus robur*) and European Beech (*Fagus sylvatica*) by Percival et al. [13].

Association of SPAD readings with other leaf traits

Usually, non-invasive techniques for estimating chlorophyll content of foliage have significant importance to agricultural management operations, predominantly in the area of precision farming. The scientific interest was verified by Kaufman et al. [42] showing that chlorophyll content is key parameter with the highest frequency within investigations of hyper spectral studies carried out in agriculture.

The SPAD meter was initially developed in Japan to diagnose leaf N status and determine N fertilizer requirements in crops. Since then, this device was broadly tested in rice [43,44], wheat [45,46], maize [47-49], cotton [50], tomato [51], sorghum [37], groundnut [52], tall fescue [53] and others grown in variable seasons. There have been over 100 publications dealing with the application of the SPAD meter in predicting foliar chlorophyll or N status in the last few years alone. With a few exceptions most of the publications report good utility of the SPAD meter for predicting foliar N concentrations.

Few studies indicated that specific leaf area (SLA) and SPAD readings, which are easy to measure, are also associated with transpiration efficiency [54]. Moreover, both traits have considerable genetic variation in groundnut [55-58].

CONCLUSION

In general, portable chlorophyll meters, SPAD-502 produced results showed the association with photosynthetic pigments in their empirical models with ease. Thus, it may be concluded that SPAD readings allow for a quick determination of the concentration of photosynthetic pigments in the leaves of crop plants, with high accuracy and without disintegrated the plant material and avoiding the use of chemical reagents and extensive laboratory protocols.

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