Journal of Root Crops, 2018, Vol. 44 No. 1, pp. 37-40
Indian Society for Root Crops
ISSN 0378-2409, ISSN 2454-9053 (online)

Rapid Method for Estimation of Total Chlorophyll, Chlorophyll a and b and Carotene Content in Leaves of Cassava and Sweet potato Using SPAD Meter

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Abstract

A rapid, non-destructive method for determining total chlorophyll, chlorophyll a and b and carotene content in leaves of cassava and sweet potato using SPAD (Soil Plant Analysis Development) meter readings was developed at Central Tuber Crops Research Institute, Thiruvananthapuram, India. In cassava leaves, the SPAD readouts varied between 31.4 and 55.9 whereas in sweet potato leaves it varied between 22.1 and 47.8. In cassava leaves, the total chlorophyll, chlorophyll a, chlorophyll b, carotenoid and nitrogen content varied between 2.12 and 4.72 mg g^{-1}, 1.53 and 3.71 mg g^{-1}, 0.54 and 2.95 mg g^{-1}, 0.34 and 0.8 mg g^{-1} and between 1.26 and 5.54% respectively. In sweet potato leaves, the total chlorophyll, chlorophyll a, chlorophyll b, carotenoid and nitrogen content varied between 0.58 and 1.69 mg g^{-1}, 0.38 and 1.12 mg g^{-1}, 0.2 and 0.5 mg g^{-1}, 0.15 and 0.34 mg g^{-1} and between 1.4-4.77% respectively. The correlation between SPAD readings and total chlorophyll, chlorophyll a, chlorophyll b, carotenoid and nitrogen content, the coefficients of determination (R^2) and the linear regression equations (y = a + bx) derived between SPAD readings and total chlorophyll, chlorophyll a and b, carotene and nitrogen content are given for cassava and sweet potato separately and together. The factors (F values) derived between SPAD readings and total chlorophyll, chlorophyll a and b and carotene and nitrogen content are given for cassava and sweet potato separately and together. The total chlorophyll, chlorophyll a and b and carotene content in leaves of cassava and sweet potato can be directly estimated by multiplying the SPAD readings with F values.

Key words: Chlorophyll, carotene, SPAD, cassava, sweet potato

Introduction

SPAD is acronym for Soil Plant Analysis Development. This meter was designed originally in 1963 for N management in rice in Japan. This instrument determines the "greenness" of a leaf by measuring the transmittance / absorbance of the leaf between 400 and 500 nm (blue region) and between 600 and 700 nm (red region), which are the absorption maxima of chlorophyll a and b and carotenoid pigments. Sunlight conditions in the field do not affect its readings and so measurements can be taken in different weather conditions. However, on each measurement it measures only one spot in a leaf. Therefore, many measurements must be taken to get a reliable average. The SPAD meter gives readings in arbitrary unit which is proportional to the amount of these pigments in leaves. The device is pressed onto the leaf surface and a relative non-destructive greenness reading is recorded in few seconds. The SPAD readout has been widely used to measure leaf chlorophyll content in leaves of many crops *viz*., corn, papaya, soybean, rice, coffee, wheat, peanut, sugarcane, dry bean and sorghum and well correlated with N status of crops (as most of leaf N is present within chlorophyll) and photosynthetic activity of plant leaves (Hussain, et al. 2000; Loh et al., 2002; Netto et al., 2005; Kapotis et al., 2003; Guler and Ozcelik, 2007; Uddling et al., 2007;

Varvel et al. 2007; Steel et al., 2008; Hawkins et al., 2009; Jangpromma et al., 2010; Ruiz-Espinoza et al., 2010). A non-destructive method for determining total chlorophyll, chlorophyll a, chlorophyll b and carotenoid in elephant foot yam leaves was reported (Ravi et al. 2011). The present paper reports the rapid method for determining total chlorophyll, chlorophyll a and b and carotene content in leaves of cassava and sweet potato using SPAD meter.

Materials and Methods

In the present study a rapid, non-destructive method for determining total chlorophyll, chlorophyll a and b and carotene content in leaves of cassava and sweet potato using handheld (portable) SPAD meter readings has been developed. SPAD -502 Minolta meter was used for recording greenness of randomly selected 31 leaves showing greenness of different density in 14 varieties of cassava *viz*., Sree Vijaya, Sree Athulya, Ambakkadan, Thailand, Quintal kappa, H-226, Sree Swarna, Burma, Sree Jaya, M-4, PDP-CMR-1, Sree Reksha, Sree pavithra, Aromal and 43 leaves in 6 varieties of sweet potato *viz*., (*Ipomoea batatas* (L.) Lam.) *viz*., Bhu krishna, Sree Arun, Sree kanaka, Bhu Sona, Sree Varsha, Sree Badhra, cultivated under field conditions at Central Tuber Crops Research Institute, Thiruvananthapuram, India during January – October 2018. ICAR-CTCRI recommended package of practices was followed for cultivation of cassava and sweet potato. In each leaf, five SPAD read outs were recorded and the mean of these values were used for calculation. Chlorophyll and carotenoid was extracted from leaves of known weight and estimated according to Leichtenthaler (1987). The nitrogen content was estimated in dried leaf samples using Kjeldahl method.

Results and Discussion

In cassava leaves, the SPAD read outs among 31 leaf samples varied between 31.4 and 55.9 with an average of 43.84. The estimated total chlorophyll content varied between 2.12 and 4.72 mg g^{-1} fresh leaf with an average of 3.39 mg g^{-1} fresh leaf. The chlorophyll a content varied between 1.53 and 3.71 mg g^{-1} fresh leaf with an average of 2.49 mg g^{-1} fresh leaf. The chlorophyll b content varied between 0.54 and 2.95 mg g^{-1} fresh leaf with an average of 0.9 mg g^{-1} fresh leaf. The carotene content varied between 0.34 and 0.8 mg g^{-1} fresh leaf with an average of 0.6 mg g^{-1} fresh leaf. The nitrogen content of dry leaf samples varied between 1.26% and 5.54% with an average of 3.68%. The coefficients of determination (R^2) values derived between SPAD readings and total chlorophyll, chlorophyll a and b, carotene and nitrogen content, the linear regression equations derived between SPAD readings and total chlorophyll, chlorophyll a and b, carotene and nitrogen content are given in Table 1. There was significant positive correlation between SPAD readings and total chlorophyll (r = 0.43), chlorophyll a (r = 0.63) and nitrogen (r = 0.372) content in cassava leaves. Nevertheless, the correlation as well as coefficients of determination (R^2) values between SPAD readouts and nitrogen content were poor. The factors (F values) derived between SPAD readings and total chlorophyll, chlorophyll a and b, carotene and content were 0.078, 0.057, 0.021, 0.014 and nitrogen 0.087, respectively.

In sweet potato leaves, the SPAD read outs among 43 leaf samples varied between 22.1 and 47.8 with an average of 34.76. In sweet potato leaves, the total chlorophyll content varied between 0.58 and 1.69 mg g^{-1} fresh leaf with an average of 1.095 mg g^{-1} fresh leaf. The chlorophyll a content varied between 0.38 and 1.12 mg g^{-1} fresh leaf with an average of 0.738 mg g^{-1} fresh leaf. The chlorophyll b content varied between 0.2 and 0.5 mg g^{-1} fresh leaf with an average of 0.357 mg g^{-1} fresh leaf. The carotene content varied between 0.58 and 1.69 mg g^{-1} fresh leaf with an average of 0.224 mg g^{-1} fresh leaf. The nitrogen content of dry leaf samples varied between 1.4% and 4.77% with an average of 3.1%. The coefficients of determination (R^2) values derived between SPAD readings and total chlorophyll, chlorophyll a and b, carotene and nitrogen content, the linear regression equations derived between SPAD readings and total chlorophyll, chlorophyll a and b, carotene and nitrogen content are given in Table 1. There was significant positive correlation between SPAD readings and total chlorophyll (r = 0.302), chlorophyll a (r = 0.316 and nitrogen (r = 0.242) content in sweet potato leaves. Nevertheless, the correlation as well as coefficients of determination (R^2) values between SPAD readouts and nitrogen content were poor. The factors (F values) derived between SPAD readings and total chlorophyll, chlorophyll a and b, carotene and content were 0.032, 0.021, 0.011, 0.007 and nitrogen 0.089, respectively.

The coefficients of determination (R^2) values derived between SPAD readings and total chlorophyll, chlorophyll a and b, carotene and nitrogen content were too low and not near to 1 in both cassava and sweet potato leaves. Therefore, the total chlorophyll, chlorophyll a and b, carotene and nitrogen content data of cassava and sweet potato were pooled together and r, R^2 y = a + bx were derived (Figs. 1, 2, 3, 4, 5, Table 1). The pooled data indicated significant positive correlation between SPAD readings and total chlorophyll (r = 0.64), chlorophyll a (r = 0.67), chlorophyll b (r = 0.43), carotenoid (r = 0.61). The coefficients of determination (R^2) values derived between SPAD readings and total chlorophyll, chlorophyll a and b, carotene were also greater viz., 0.412, 0.454, 0.181, and 0.326, respectively. Nevertheless, the correlation as well as coefficient of determination (R^2) values between SPAD readouts and nitrogen content were poor. Although the correlation as well as coefficients of determination (R^2) values between nitrogen content and total chlorophyll content were poor in the case of cassava and sweet potato, the pooled data of nitrogen and total chlorophyll content of cassava and sweet potato indicated positive correlation (r = 0.43) and coefficients of determination (R^2 = 0.592). The factors (F values) derived between SPAD readings and total chlorophyll, chlorophyll a and b, and carotene content

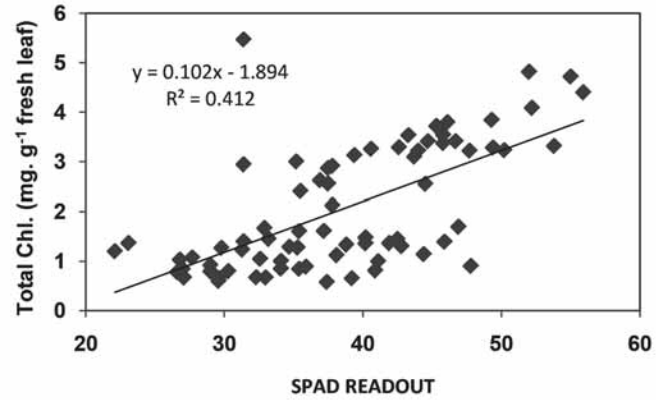

Fig.1. Relation between SPAD readout and total Chl. content in leaves of cassava and sweet potato

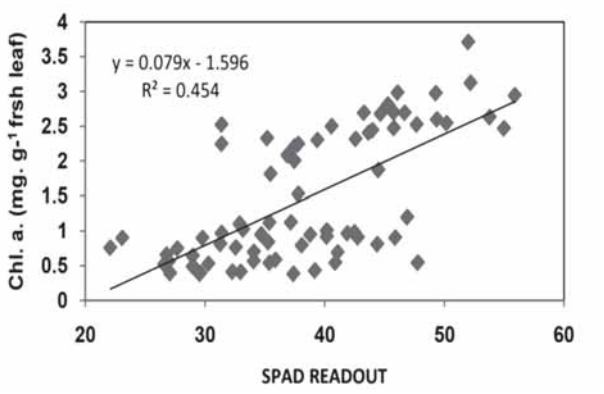

Fig. 2. Relation between SPAD readout and Chl.a. content in leaves of cassava and sweet potato

Table 1. Statistical relation between SPAD readouts and total chlorophyll, chlorophyll a, chlorophyll b, carotenoid and nitrogen content in leaves of cassava and sweet potato

Crop and parameters	Statistical relation with SPAD readout			
Cassava	*Y = a + bx*	*r*	R^2	F
Chlorophyll a	y = 0.040x + 0.736	0.63	0.400	0.057
Chlorophyll b	y = 0.006x + 0.604	0.09	0.008	0.021
Total Chlorophyll	y = 0.017x + 2.646	0.43	0.062	0.078
Carotenoid	y = 0.001x + 0.539	0.167	0.026	0.014
Nitrogen	y = -0.008x + 4.028	0.372	0.011	0.087
Sweet potato				
Chlorophyll a	y = 0.011x + 0.328	0.316	0.099	0.021
Chlorophyll b	y = 0.003x + 0.237	0.24	0.057	0.011
Total Chlorophyll	y = 0.015x + 0.566	0.302	0.091	0.032
Carotenoid	y = 0.000x + 0.201	0.188	0.014	0.007
Nitrogen	y = 0.026x + 2.123	0.242	0.058	0.089
Cassava and sweet potato together				
Chlorophyll a	y = 0.079x - 1.596	0.67	0.454	0.036
Chlorophyll b	y = 0.022x - 0.298	0.43	0.181	0.015
Total Chlorophyll	y = 0.102x - 1.894	0.64	0.412	0.052
Carotenoid	y = 0.012x - 0.099	0.61	0.326	0.01
Nitrogen	y = 0.020x + 2.513	0.2	0.041	0.09

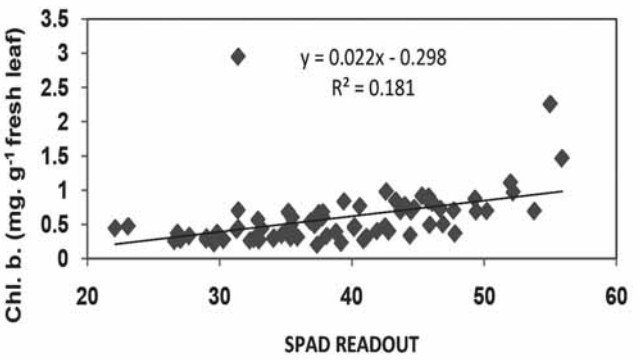

Fig. 3. Relation between SPAD readout and Chl.b. content in leaves of cassava and sweet potato

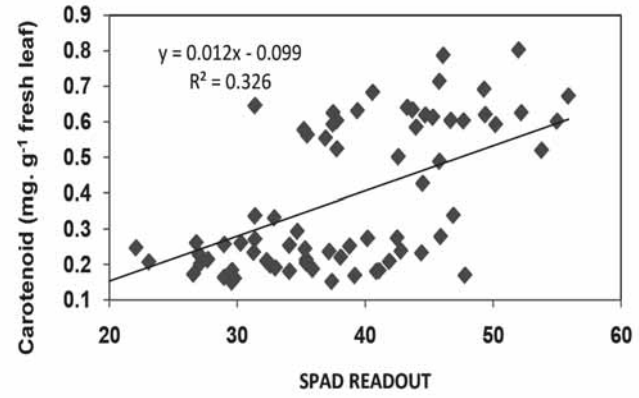

Fig. 4. Relation between SPAD readout and carotenoid content in leaves of cassava and sweet potato

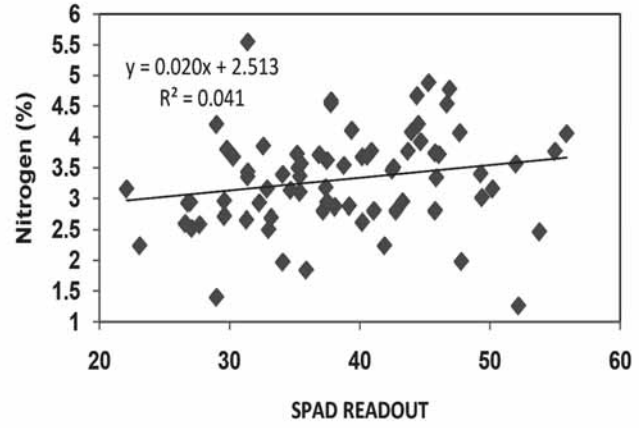

Fig. 5. Relation between SPAD readout and nitrogen content in leaves of cassava and sweet potato

were 0.052, 0.036, 0.015 and 0.01, respectively. Therefore, using the F value, the total chlorophyll, chlorophyll a and b and carotene content in leaves of cassava sweet potato can be directly calculated by multiplying the SPAD readings with F values 0.052, 0.036, 0.015 and 0.01, respectively.

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