NON-DESTRUCTIVE ESTIMATION OF CHLOROPHYLL CONTENT IN YELLOW PASSION FRUIT LEAVES

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ABSTRACT – The chlorophyll content in leaf of yellow passion fruit is usually determined by destructive methodologies. Portable chlorophyll meters make indirect measuring of leaf chlorophyll concentration possible in rapid and non-destructive way. The chlorophyll content of yellow passion fruit leaves were determined fifty times by dimethylsulfoxide extraction and estimated by the chlorophyll meter SPAD-502. Linear relations among the SPAD readings and the chlorophyll concentrations were established. The estimated chlorophyll contents were, $Y_a = 2.7990 + 0.3208X$; $Y_b = -11.7934 + 1.2815X$, and $Y_t = 9.0497 + 1.3967X$, with determination coefficients (r^2) of 0.9942, 0.9893, and 0.9948, respectively for chlorophyll a, b and total. It is concluded that SPAD readings had good accuracy in estimating the leaf chlorophyll concentration of the passion fruit plant, in a non-desctructive way, by linear regression models.

Key Words: chlorophyll meter, dimethylsulfoxide, leaf chlorophyll, Passiflora edulis.

ESTIMAÇÃO NÃO DESTRUTIVA DO CONTEÚDO DE CLOROFILA FOLIAR EM MARACUJÁ AMARELO

RESUMO – A concentração de clorofila nas folhas é, usualmente, determinada por metodologias que são destrutivas. No entanto, a utilização do clorofilômetro portátil SPAD-502 possibilita mensurações indiretas rápidas e não destrutivas da concentração de clorofila foliar. Estudou-se a existência de relação linear entre leituras SPAD-502 e conteúdo de clorofila em folhas de maracujazeiro amarelo com extrator dimetilsulfóxido (DMSO). Cinquenta leituras SPAD foram, significativamente, relacionadas com a concentração de clorofila nas folhas de maracujá e o modelo linear de primeiro grau descreveu melhor esta relação. Quais sejam: $Y_1 = 2,7990X + 0,3208$; $Y_2 = -11,7934X + 1,2815$ e $Y_3 = 9,0497X + 1,3967$, com coeficientes de determinação (r^2) de 0,9942, 0,9893 e 0,9948, respectivamente para clorofila a, clorofila b e clorofila total. Leituras SPAD podem ser usadas com boa acurácia na estimativa da concentração de clorofila foliar de maracujazeiro, de forma não destrutiva, através dos modelos de regressão adotados.

Palavras-chave: clorofilômetro, dimetilsulfóxido, clorofila foliar, Passiflora edulis.

1. INTRODUCTION

Chlorophyll is a green pigment that absorbs light and is directly related with the photosynthetic efficiency of the plants, making possible its growth and adaptability to different environments.

The chlorophyll content makes an indirect estimation of the nutritional status possible, since it has great amounts of nitrogen (Fillela et al., 1995). In addition, the leaf chlorophyll content is closely related with stress and senescence in plants (Hendry et al., 1987; Merzlyak and Gitelson, 1995; Peñuelas and Fillela, 1998; Merzlyak et al. 1999).

The methodologies traditionally used for quantification of chlorophyll are based on methods that destruct the leaf tissue to extract chlorophyll molecules, using organic solvents such as, acetone (Bruisna, 1961), dimethylsulfoxide (DMSO) (Hiscox &

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Israelstam, 1979), N,N-dimethylformamide (DMF) and petroleum ether (Moran & Porath, 1980; Lichtentahler & Wellburn, 1983; Inskeep and Bloom, 1985). Shoaf & Lium (1986), using DMSO, have modified the chlorophyll extraction methodology eliminating the maceration and centrifugation procedures, which made the analysis more simple and less expensive.

Although widely used, these methods are not only destructive and time consuming, they also imply the use of spectrophotometer. Alternative solutions have been developed to estimate photosynthetic pigment concentration using optical methods that are not tissue destructive, making the analysis cheaper and faster to be used in field conditions (Buschmann & Nagel, 1993; Gitelson & Merzlyak, 1994; Gitelson et al., 2003).

The portable equipment SPAD (Soil Plant Analysis Development)-502 measures the intensity of green color in the leaf surface by inserting the leaf blade in the end of the equipment, which flashes light at 650 nm (red) and at 940 nm (infrared) diodes. The 940 nm transmittance compensates leaf variations, such as thickness and water content, while 650 nm detects the intensity of green color. The excess of light from leaf blade is received by a silicone photodiode, which converts it into analog electrical signals. These signals are amplified and converted into digital data by an A/D converter, (Minolta, 1989), and a microprocessor calculates the SPAD values, which are shown in a display. The values can be proportional to the chlorophyll content in the leaf.

Several researches have been using SPAD readings to estimate leaf chlorophyll concentration in different species, such as:apple (Campbell et al., 1990), coffe (Torres Neto et al., 2005), eucalyptus (Datt, 1999) soy and corn (Gitelson et al., 2005) and vine (Mark et al., 2008). However, data is scarce to passion fruit plant.

The objective of this study was to adjust a mathematical model that expresses the linear ratio existing between SPAD-502 readings and the chlorophyll content in yellow passion leaves (*Passiflora edulis* f. *flavicarpa*).

2. MATERIAL AND METHODS

Leaf samples were taken from yellow passion fruit plants (*Passiflora edulis* f. *flavicarp*a) in the breeding program of Universidade Federal de Viçosa, Viçosa city, Minas Gerais state, Brazil. The plants were two years old and have received conventional cultural practices.

Five plants that had visual difference green color intensity were selected, which varied from yellow-green to dark-green, collecting from each one a branch segment with ten leaves with more than six centimeters of what in the main nervure.

SPAD readings and chlorophyll extraction

The yellow passion fruit plant had threelobed leaves; from each lobe were taken three discs of leaf blade of 0.785 cm², using a circular leaker. The 50 SPAD readingswere obtained from the mean of three lobules with three leaf discs in each one (Figure 01). After the SPAD reading, the discs of each lobule were immersed into 5 ml of DMSO under dark conditions. The bottles were sealed and kept at 25 ^{o}C ± 2 for 30 hours. After this period in the dark, a solution aliquot of 3 ml per bottle was taken and readied in the spectrophotometer at 663 and 645 nm. Concentrations of chlorophyll **a** (Clor a), chlorophyll **b** (Clor b) and **total** chlorophyll (Clor Total) were determined according to the Arnon (1949) and Lichtentahler (1987) equation with modifications to express the results based in the leaf area, as shown below:

Clor *a* = (12,7.A663 - 2,69 . A645/1000A).V

Clor b = (22,9 . A645 - 4,68 . A663/1000A).V

Clor. Total = (20,2 . A663 - 2,69 . A645/1000A).V

Where, A645 = absorbance at 645 nm; A663 = absorbance at 663 nm; V = sample volume (mL); A = area of the three discs, in cm². The chlorophyll concentration was expressed in mg/cm².

SPAD readings and chlorophyll concentration were submitted to regression analysis at SAS computer program, version 9.0 (SAS Institute, Cary, NC).

3. RESULTS AND DISCUSSION

The ratio between chlorophyll levels and SPAD readings had good linear adjustment in the mathematical model (Figure 01). To select the mathematical model the determination coefficient (r^2), significance of â's and biological description of the phenomenon were used. Equations $Y_a = 2.7990 + 0.3208X$; $Y_b = -11.7934$

+ 1.2815X; and Y_t = 9.0497 + 1.3967X with r^2 of 0.9942, 0.9893 and 0.9948, respectively, estimate accurately the concentration of chlorophyll **a**, **b** and **total**, of passion fruit leaves.

In the first grade linear model, all equation parameters were significant at 1% of significance, which was not observed in the quadratic model where some parameters were significant at 5% (Table 1).

The chlorophylometer allows readings in few seconds, without sending samples to a laboratory, saving time and financial resources, and also with non leaf destruction (Malavolta et al., 1997; Argenta et al., 2001).

The ratio between total chlorophyll concentration and SPAD readings has been demonstrated for different species (Yadava, 1986; Marquard &Tipton, 1987; Schaper & Chacko, 1991), in which the first grade linear models had better adjustments. However, in papaya leaves, the ratio between SPAD reading and photosynthetic pigments concentration was exponential for total chlorophyll and chlorophyll a and cubic for chlorophyll b (Torres Netto et al., 2002). These evidences show that it is needed research on mathematical models per specie or even per grown varieties.

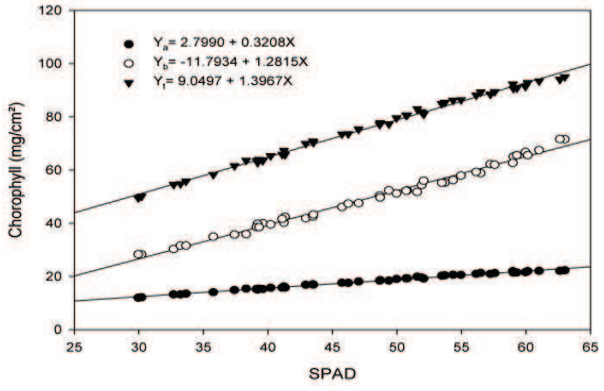

Figure 1 - Relationships between the SPAD-502 readings and chlorophyll a (Y_a), chlorophyll b (Y_b) and total chlorophyll (Y_t) in *P. edulis* f. *flavicarpa*.

The ratio between extractable chlorophyll and SPAD readings shows that chlorophylometer can be successfully used to accurately estimate the chlorophyll concentration of passion fruit leavesin replacement of traditional methods.

4. CONCLUSION

SPAD readings have an estimable linear relation for chlorophyll content estimation yellow passion fruit leaves.

The linear relation between SPAD-502 reading and chlorophyll concentration was better adjusted by the first grade linear model, as follows: Y_a = 2.7990x + 0.3208; Y_b = -11.7934x + 1.2815; and Y_t = 9.0497x + 1.3967 , with r^2 of 0.9942, 0.9893 and 0.9948, respectively, and accurately estimate concentration of chlorophyll **a**, **b** and **total** for passion fruit leaves.

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Table 1. Parameters of linear (y= a + bx) and quadratic equations (y= a + bx +c x^2) of chlorophyll a, b and total estimation

Model	Parameters of equation			R^2
	a	b	C	
		Chlorophyll a		
Linear	2.79896**	0.32083**	-	0.9942
Quadratic	-2.03985*	0.53519**	0.00228**	0.9901
		Chlorophyll b		
Linear	-11.79336**	1.28151**	-	0.9893
Quadratic	8.55635*	0.38001*	0.00959**	0.9935
		Total chlorophyll		
Linear	9.04968**	1.39668**	-	0.9948
Quadratic	6.58681**	2.08938**	0.00133*	0.9969

* P < 0.05 and ** P < 0,01.

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