

Non-destructive analysis of photosynthetic pigments in *Avena strigosa* and *Avena sativa*

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Abstract

Chlorophylls and carotenoids are the main photosynthetic pigments in plants. The photosynthetic potential of crop plants is used to determine the correct rate of nitrogen fertilization. To date, no studies have been conducted to understand the relationship between different methods of measurement of photosynthetic pigments in oats. The objective of this study was to quantify and compare the levels of photosynthetic pigments in *Avena strigosa* and *A. sativa* using two different methods, the extraction method and portable chlorophyll meter, and to determine whether the results of these two methods showed a significant correlation. Photosynthetic pigments were measured using both methods in a greenhouse and the laboratory at four developmental stages: tillering [28 days after sowing (DAS)], vegetative stage I (55 DAS), vegetative stage II (75 DAS), and reproductive stage (120 DAS). The same leaves were used to measure the relative chlorophyll content using a portable chlorophyll meter and extractable chlorophyll using the laboratory extraction method. The readings of the chlorophyll index differed for each developmental stage of both *A. sativa* and *A. strigosa*. The contents of chlorophyll a, chlorophyll b, and carotenoids determined using the extraction method showed high coefficients of correlation with the total chlorophyll index determined using the portable chlorophyll meter. Thus, the measurement of chlorophyll using the portable chlorophyll meter can be used for the accurate evaluation of the photosynthetic potential of oats, thus saving time and reagents.

Keywords: *Avena sativa*, *Avena strigosa* Schreb, Carotenoids, Chlorophyll, Portable chlorophyll.

Abbreviations: DAS_days after sowing, DMS_difference minimum significant, DMSO_dimethyl sulfoxide.

Introduction

Avena strigosa Schreb and *Avena sativa* are multipurpose cereals that are considered important cultivation alternatives for the winter period in southern Brazil. Both of these species are the main forage crops used in the formation of winter pastures, either in isolation or intercropping with other temperate climate species, owing to their high dry biomass and forage quality, trampling resistance, and low production costs (Macari et al., 2006). Both crop species, especially *A. sativa*, are also used as soil cover crops in the no-till system for the production of grains, which are high in proteins, vitamins, minerals, and fiber and are also used as human nutrition (Silva and Ciocca, 2005).

The production potential, mass accumulation, and grain yield of *A. strigosa* and *A. sativa* are related to their competitive capacity, speed of establishment, and use of environmental resources. Thus, the photosynthetic potential of these crop species and higher levels of leaf pigments are linked to high rates of accumulation of photoassimilates as well as the rapid establishment and development of plants

in different environments (Engel & Poggiani, 1991; Fleet et al., 2003). The photosynthetic efficiency of crop plants is used to determine the rate of nitrogen fertilization because the total chlorophyll content is associated with the organic nitrogen content of leaves and consequently crop yield in many cultures (Smeal & Zhang, 1994; Rigon et al., 2012; De Castro et al., 2014).

The levels of photosynthetic pigments are measured mostly using traditional methodology, which comprises the destruction of leaf samples. Moreover, it is a costly procedure as it involves the use of laboratory reagents, thus making it impractical because it does not produce instant results. In contrast, portable chlorophyll meters use non-destructive, simple and instantaneous principles (Salla et al., 2007). In recent years, portable chlorophyll meters have been successfully used to measure the levels of photosynthetic pigments and leaf nitrogen content in different species (Ciganda et al., 2009; Rigon et al., 2012;

Rigon et al., 2013; Kaspary et al., 2014a; Kaspary et al., 2014b; Cavalcante et al., 2016).

Chlorophyll meters indirectly determine the relative chlorophyll content, which is calculated by the amount of light transmitted by the leaf measured at wavelengths with variable absorbance, providing a unique reading proportional to the contents of chlorophyll a, chlorophyll b, and carotenoids (Falker Automação Agrícola, 2009; Minolta Camera Company, 1989). Models of chlorophyll meters describe the relationship between portable chlorophyll meter readings and extractable chlorophyll according to the species, their intrinsic characteristics, demanding independent calibration (Lee, 1988; Markwell et al., 1995; Uddling et al., 2007). To date, no studies have been conducted to understand the relationship between different methods of measurement of photosynthetic pigments in *A. strigosa* and *A. sativa*.

The objective of the present study was to quantify and compare the levels of photosynthetic pigments in *A. strigosa* and *A. sativa* using two different methods, the extraction method and portable chlorophyll meter, and to determine whether the results of these methods were significantly correlated.

Results

Non-destructive method of chlorophyll measurement

The readings of chlorophyll content obtained using the portable chlorophyll meter differed significantly with the developmental stage of *A. strigosa* and *A. sativa* ($P \leq 0.05$; Table 1). For *A. strigosa*, the chlorophyll index measured at the tillering stage was the lowest (48.83), whereas that measured at the vegetative stage I was the highest (62.42). For *A. sativa*, the mean chlorophyll index measured at the vegetative stage II was the highest (69.56), whereas that measured at the tillering stage was the lowest (47.84). For both species, the chlorophyll index measured during the reproductive stage was intermediate between those measured during other stages.

Destructive method of chlorophyll measurement in the laboratory

The evaluation of photosynthetic pigments in *A. strigosa* and *A. sativa* using the extraction method revealed that the contents of chlorophyll a, chlorophyll b, total chlorophyll, and carotenoids varied with the developmental stage of the plants (Table 1). For *A. strigosa*, the contents of chlorophyll a, chlorophyll b, total chlorophyll, and carotenoids were the lowest at the peeling stage (1.5583, 0.3334, 2.7540, and 0.4293 mg.g⁻¹ FLW, respectively) and highest at the vegetative stage I (2.2922, 0.4618, and 0.6828 mg.g⁻¹ FLW chlorophyll a, chlorophyll b, and carotenoids, respectively) (Table 1). At the other stages, values of these pigments were intermediate, with the exception of chlorophyll a, which did not differ between the vegetative and reproductive stages. The total chlorophyll content behaved similarly to chlorophyll a and b contents measured individually. For *A. sativa*, the contents of chlorophyll a, chlorophyll b, total chlorophyll, and carotenoids at vegetative stage II were

2.602, 0.4237, 3.1979, and 0.6176 mg.g⁻¹ FLW, respectively, thus revealing superior photosynthetic performance at this stage (Table 1). The values of these pigments were the lowest during the cutting stage and intermediate during vegetative stage I and reproductive stage. These data indicate a greater accumulation of photosynthetic pigments in *A. sativa* than in *A. strigosa*.

Comparison between destructive and non-destructive methods of chlorophyll measurement

Regression analysis of the results obtained using two different methodologies (portable chlorophyll meter and laboratory extraction method) showed a high correlation, with all determination coefficients above 0.86 (Figures 1 and 2). The correlation between the chlorophyll index obtained using the chlorophyll meter and that obtained using the laboratory extraction method for chlorophyll a was high, with determination coefficients of 0.91 for both species of oats (Figure 1A and 1B). For chlorophyll b, data showed quadratic behavior and determination coefficients of 0.86 and 0.95 for *A. strigosa* and *A. sativa*, respectively (Figure 1C and 1D). The chlorophyll index obtained using the chlorophyll meter showed a high capacity to indirectly measure the total chlorophyll content in both oat species, with determination coefficients of 0.86 and 0.95 for *A. strigosa* and *A. sativa*, respectively (Figure 1E and 1F).

The measurement of carotenoids using the laboratory extraction method showed a strong correlation with the chlorophyll index obtained using the chlorophyll meter, with determination coefficients of 0.89 and 0.86 for *A. strigosa* and *A. sativa*, respectively (Figure 2A and 2B). Based on the high determination coefficients, chlorophyll a and b contents could be correlated with the index obtained using the chlorophyll meter, and a highly reliable mathematical model was obtained for these variables when analyzed in *A. strigosa* and *A. sativa* (Figure 2C and 2D).

Discussion

Our findings showed that the chlorophyll index increased in the vegetative stage I for *A. strigosa* and vegetative stage II for *A. sativa* with subsequent reduction in the reproductive stage (Table 1). Higher content of photosynthetic pigments facilitate the use of luminosity to the higher rate of liquid accumulation of photoassimilates, which is also linked to high growth rate (Fleck et al., 2003). This suggests that *A. strigosa* has a superior capacity to utilize luminosity in vegetative stage I, thus increasing the accumulation of dry matter and competitive potential, whereas *A. sativa* is more efficient and competitive in vegetative stage II. Thus, both oat species show different photosynthetic potential.

The results of measurement of photosynthetic pigments using the laboratory extraction method corroborated with the index obtained using the chlorophyll meter (Table 1). This trend has been reported in studies using the chloroLOG index and laboratory extraction in *Sesamum indicum*, *Ricinus communis*, *Lolium multiflorum*, and *Conyza bonariensis* (Rigon et al., 2012; Rigon et al., 2014; Kaspary et al., 2014a; Kaspary et al., 2014b).

Table 1. Relative chlorophyll index by chlorofiLOG and chlorophyll a, chlorophyll b, total chlorophyll and carotenoids (mg.g⁻¹flw) by the extraction method, in *A. strigosa* and *A. sativa*. 2017.

-----Chlorophyll index-----				
	Tillering	Vegetative - I	Vegetative - II	Reproductive
<i>Avena strigosa</i>	48.83 B ²	62.42 A	51.97 B	53.30 B
CV (%) ¹		5.78		
<i>Avena sativa</i>	47.84 C	64.00 B	69.56 A	62.00 BC
CV (%)		6.92		
-----Chlorophyll a-----				
	Tillering	Vegetative - I	Vegetative - II	Reproductive
<i>Avena strigosa</i>	1.5583 B	2.2922 A	1.,007 B	1.7553 B
CV (%)		4.08		
<i>Avena sativa</i>	1.4594 C	2.1837 AB	2.6002 A	1.9184 B
CV (%)		6.01		
-----Chlorophyll b-----				
	Tillering	Vegetative - I	Vegetative - II	Reproductive
<i>Avena strigosa</i>	0.3334 B	0.4618 A	0.4237 A	0.4312 A
CV (%)		8.43		
<i>Avena sativa</i>	0.4032 B	0.5018 B	0.5977 A	0.4689 B
CV (%)		7.65		
-----Chlorophyll total-----				
	Tillering	Vegetative - I	Vegetative - II	Reproductive
<i>Avena strigosa</i>	1.8917 C	2.7540 A	2.0244 B	2.1865 B
CV (%)		7.32		
<i>Avena sativa</i>	1.8626 C	2.6855 B	3.1979 A	2.3878 B
CV (%)		8.12		
-----carotenoids-----				
	Tillering	Vegetative - I	Vegetative - II	Reproductive
<i>Avena strigosa</i>	0.4293 C	0.6828 A	0.6176 B	0.6369 B
CV (%)		6.98		
<i>Avena sativa</i>	0.6249 B	0.6993 A	0.7283 A	0.6655 AB
CV (%)		7.25		

¹Coefficient of Variation. ²Similar upper case letters in the row do not differ from one another by the DMS test at 5% significance.

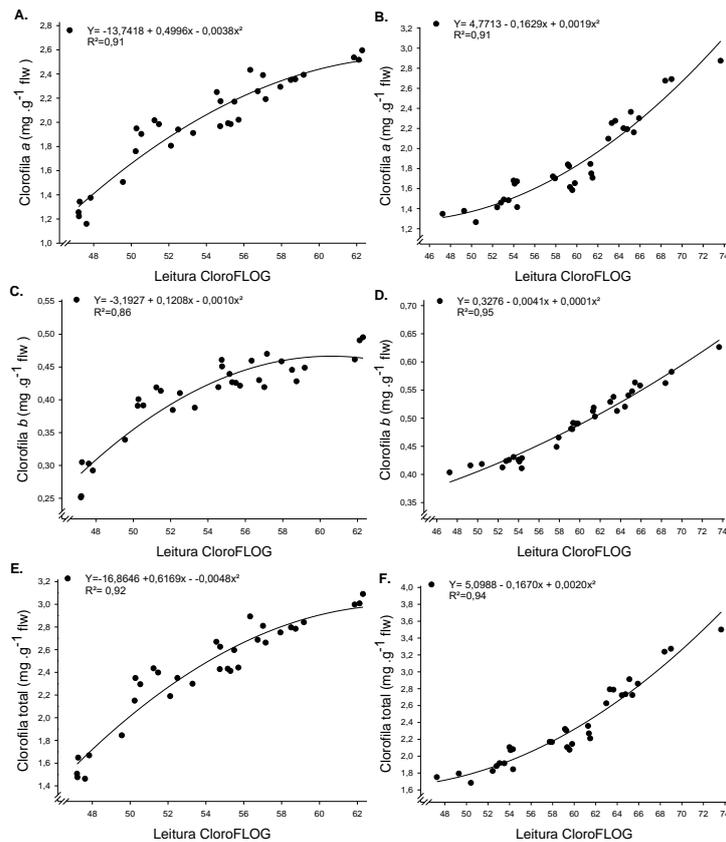


Fig 1. Relationship between the readings of the portable ClorofiLOG® for chlorophyll a, chlorophyll b, and total chlorophyll contents in *A. strigosa* (A, C, and E) and *A. sativa* (B, D, and F).

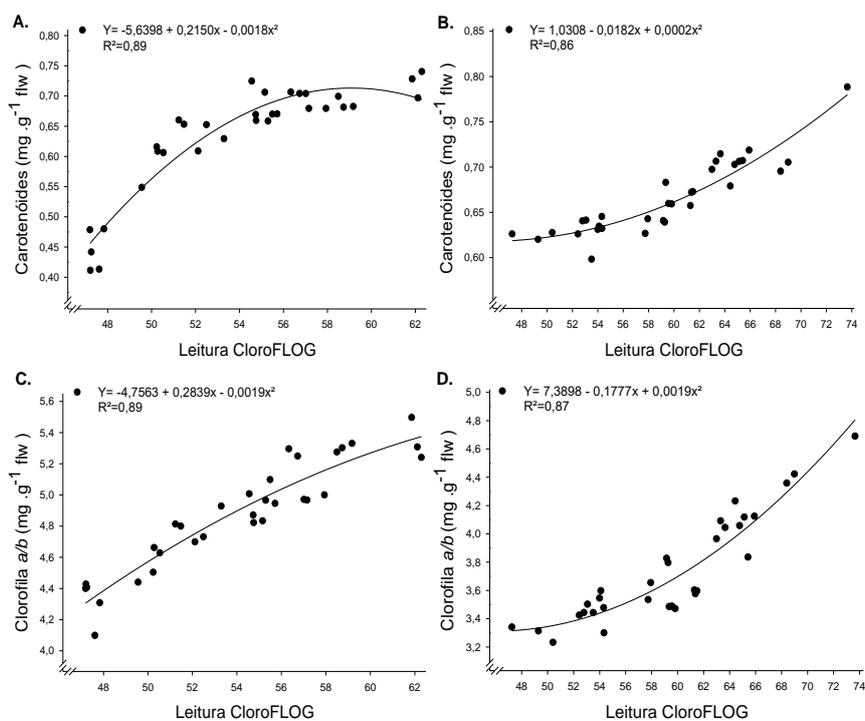


Fig 2. Relationship between the readings of the portable ClorofiLOG® for carotenoid contents and chlorophyll a/b ratio in *A. strigosa* (A and C) and *A. sativa* (B and D).

High correlations were observed between the two measurement methods for all analyzed photosynthetic pigments (Figures 1 and 2). Chlorophyll a, which showed a determination coefficient of 0.91 in both species of oats, also showed a high correlation in *S. indicum* and *L. multiflorum*, with determination coefficients of 0.98 and 0.96, respectively (Rigon et al., 2012, Kaspary et al., 2014). Thus, it is possible to efficiently estimate chlorophyll a content using portable chlorophyll meters and mathematical models generated in comparison to the laboratory extraction method.

High determination coefficients for chlorophyll b (>0.86 for both species of oats) between the chlorophyll index measured using the chlorophyll meter and the laboratory extraction method corroborates with those observed in *C. bonariensis* (>0.90 in the two evaluated biotypes) (Kaspary et al., 2014b). However, measuring chlorophyll b content using chlorophyll meters is more difficult than measuring chlorophyll a content (Neves et al., 2005) because the wavelength emitted by the apparatus is closer to the absorption peak of chlorophyll a (660 nm), whereas the absorption of chlorophyll b has two peaks (500 and 650 nm) (Divittorio, 2009). However, in the present study, high efficiency was observed in the chlorofiLOG readings in relation to the laboratory analyses for *A. strigosa* and *A. sativa*. Similarly, as observed in the present study, high reliability coefficients were obtained for chlorophyll b and chlorofiLOG readings in *L. multiflorum* and *C. bonariensis* (Kaspary et al., 2014a; Kaspary et al., 2014b).

The total chlorophyll content also showed a high correlation with the chlorophyll index, with determination coefficients of 0.92 and 0.94 for *A. strigosa* and *A. sativa*, respectively (Figure 1E and 1F). These values corroborate with those obtained using the correlation method in *Gossypium hirsutum* and *S. indicum*, with coefficients of 0.91 and 0.98,

respectively (Brito et al., 2011, Rigonet al., 2012). This confirms that it is possible to adjust a mathematical model of high reliability for this variable, when comparing the measurements of pigments using different methods in *A. strigosa* and *A. sativa* (Figure 1E and 1F).

The relationship between chlorophyll and carotenoid contents of leaves in *A. strigosa* and *A. sativa*, shown in Figures 2A and 2B, present equations of high representativeness, making it possible to estimate the actual content of these plant pigments from the readings of chlorofiLOG. Similar results have been reported for corn and cotton (Ciganda et al., 2009; Brito et al., 2011). The measurement of carotenoids is important because these pigments act as photoprotectors in photosystem II and dissipate excess light energy, thus avoiding chlorophyll damage via their high antioxidant potential (Li et al. 2010). The ratio of chlorophyll content to carotenoid content is altered under stress conditions affecting a plant, especially under water stress (Young and Britton, 1990). Thus, the rapid and accurate measurement of carotenoid content helps in making inferences about the state of the plant. Using reliable models, the carotenoid content can be determined using readings obtained from portable chlorophyll meters.

The correlation analysis between the chlorophyll index measured using chlorofiLOG and extracted chlorophyll a and b content easured using the laboratory extraction method revealed high determination coefficients of 0.89 and 0.87 in *A. strigosa* and *A. sativa*, respectively. The relationship between chlorophyll a and chlorophyll b is important in assessing the plant's ability to capture light under shade (Nakazono et al., 2001). The results obtained for the two species of oats in the present study corroborate with those observed for *R. communis*, *L. multiflorum*, and *C. bonariensis*, which showed determination coefficients of

0.70, 0.88, and 0.93, respectively (Rigon et al., 2012; Kaspary, et al., 2014a, Kaspary et al., 2014b).

The measurement of photosynthetic pigments in *A. strigosa* and *A. sativa* using a portable chlorophyll meter and adjusted mathematical models is accurate, efficient and economical in comparison to the laboratory extraction method. Thus, the chlorophyll meter is an important tool to determine the photosynthetic potential of oats.

Materials and Methods

Plant material

This study was conducted in a greenhouse and in the Laboratory of Chemical Analysis of the Department of Agronomic and Environmental Sciences of the Federal University of Santa Maria (UFSM), Campus of Frederico Westphalen-RS from May 2013 to October 2013. Seeds of *A. strigosa* (cultivar EMBRAPA 29) and *A. sativa* (cultivar URS Guapa) were sown in 6 L plastic pots filled with agricultural substrate. After seed germination, only one seedling was retained per pot. Photosynthetic pigments were measured using both the methods at four developmental stages, including tillering [28 days after sowing (DAS)], vegetative stage I (55 DAS), vegetative stage II (75 DAS), and reproductive stage (120 DAS). To measure photosynthetic pigments at each developmental stage, two leaves of the middle third of four plants of each oat species were analyzed and collected, totaling eight replicates per treatment.

Estimation of pigment contents

The relative chlorophyll content was measured individually using ClorofiLOG1030[®] portable meter, and the index reading was obtained directly from the leaf without removing it from the plant. The relative chlorophyll content determined using portable chlorophyll meters was calculated from the amount of light transmitted by the leaf based on the wavelength with variable absorbance, thus instantaneously providing a single reading proportional to the content of chlorophyll a, chlorophyll b, and carotenoids (Rigon et al., 2012). Portable ClorofiLOG1030 operates at the following three wavelengths: 635, 660, and 850 nm (Rigon et al., 2012).

Measurement of chlorophyll using the destructive method

Leaves of *A. strigosa* and *A. sativa* were collected and taken to the laboratory for analysis using the extraction methodology, as described previously (Hiscox and Israelstam, 1979). To dissolve the samples, 0.05 g of fresh leaf weight (FLW) was placed in test tubes containing 3 ml of dimethyl sulfoxide (DMSO) and incubated in at 65°C in a water bath for 45 min. Subsequently, 2 ml of DMSO was added, and samples were manually shaken for 30 s. Then, the absorbance of the final solution (2.5 ml) containing the pigments was measured using a Biomate[®] tm3 spectrophotometer at 470, 645, and 663 nm for carotenoids, chlorophyll b, and chlorophyll a, respectively. Readings obtained at each wavelength were used in equations reported by Lichtenthaler (1987), based on the FLW, to determine the content of total chlorophyll, chlorophyll a, chlorophyll b, and carotenoids in mg g^{-1} .

Statistical analysis

Data obtained for *A. strigosa* and *A. sativa* using the two methods were subjected to analysis of variance using the F test, and the means of the treatments were compared using the DMS test, with a 5% probability level. The correlation between the readings obtained using the extraction method and portable chlorophyll meter was verified via regression analysis. Curve adjustments were performed by the quadratic function, according to the determination coefficient, using Sigmaplot[®] version 11.2.

Conclusion

Our study suggests that the measurement of photosynthetic pigments in *A. strigosa* and *A. sativa* using a portable chlorophyll meter and adjusted mathematical models is accurate and saves time and reagents in comparison to the laboratory extraction method.

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