PHOTOSYNTHETIC PIGMENTS DISTRIBUTIONS ALONG THE LENGTH OF THE WHEAT LEAF; CASE STUDY IN DACIC CULTIVAR

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Received 15 May 2023; accepted 21 July 2023

ABSTRACT
The study evaluated the spatial variation of the photosynthetic pigments along the length of the wheat leaf. The biological material was represented by the 'Dacic' wheat cultivar, cultivated under the conditions of SCDA Lovrin, Romania. The fully developed leaf, third leaf, stage BBCH 30, was considered for analysis. 50 leaves were taken at random, from which 10 leaves with a length of 30 cm were selected. The photosynthetic pigments (chlorophyll - Chl, carotenoids - Car) were determined in 29 positions along the length of the leaf, at intervals of 1 cm (p1 to p29). Based on the determined values, the Chl/Car and Car/Chl ratios were calculated. The chlorophyll content (Chl) varied between 36.70 – 67.80±0.51 units, the carotenoid content (Car) varied between 4.90 – 17.10±0.17 units. In the case of the calculated ratios, the Chl/Car ratio values were between 3.95 – 7.65±0.04, and the Car/Chl ratio values were between 0.13 – 0.25±0.001. The analysis of the data was done on the basis of a proposed logical scheme. The distribution of Chl and Car along the length of the leaf was described by polynomial equations of degree 4, under statistical safety conditions (R^2=0.963 for Chl; R^2=0.928 for Car, $p<0.001$). The cluster analysis facilitated the grouping of the determination positions (p1 to p29) based on similarity in relation to Chl values (Coph.corr.=0.738). Starting from the equations that described Chl and Car variation in relation to the position on the leaf (p1 to p29) were found the areas on the leaf where pigment content values were determined at the level of the calculated average values (Chl_Avg, Car_Avg; p8-p9, p24-p25), and the area with the maximum level of pigment content (p16 – p18). These areas were also identified in the dendrogram resulted from the cluster analysis.
KEY WORDS: *Chl/Car ratio, distribution model, leaf, photosynthetic pigments, wheat*

INTRODUCTION

The content of photosynthetic pigments in the leaves varies in relation to the plant species, the vegetation conditions, the age of the leaves, the position of the leaves on the plant, or various other influencing factors (Esteban *et al*., 2015; Li *et al*., 2018). In the case of economic interest plants, the content of photosynthetic pigments is especially important in relation to the productivity of the cultivated genotypes, productions and production quality indices (Hosseini *et al*., 2021; Abd-Elkader *et al*., 2022; Simkin *et al*., 2022).

Different methods have been developed to determine the content of photosynthetic pigments, and non-destructive methods are increasingly promoted due to the facilities they offer (Liang *et al*., 2017; Wood *et al*., 2020; Zeng *et al*., 2021; Petibon and Wiesenberg, 2022). In crop plants, the variation of photosynthetic pigments was studied in relation to different technological input elements, such as fertilizers, irrigation water, protective substances (Fornari *et al*., 2020; Peng *et al*., 2021; Sala, 2021; Abd-Elkader *et al*., 2022). The variation in the content of photosynthetic pigments in the leaves was studied from the interspecific and intraspecific perspective, in relation to the anatomical-morphological elements of the leaves (Feng *et al*., 2017; Borsuk and Brodersen, 2019).

In the case of grasses, variations in the content of photosynthetic pigments were communicated in relation to the plant species, the territorial location, the anatomical structure of the leaves, the method of determination (Borsuk and Brodersen, 2019; Zielewicz *et al*., 2020; Zhang *et al*., 2022). As a result of the importance of photosynthetic pigments in relation to the capture of solar energy and the conversion into biochemical energy, some ecological predictions, productivity in agricultural crops, but also other important aspects, the development of different distribution models of photosynthetic pigments in leaves has shown interest (Feng *et al*., 2017; Borsuk and Brodersen, 2019; Zhang *et al*., 2020; Zhang *et al*., 2022).

The purpose of this study was to evaluate the distribution of photosynthetic pigments, chlorophyll and carotenoids, along the length of the wheat leaf, to describe the variation through mathematical models and to find out the optimal areas for determination in relation to the calculated average value.

MATERIAL AND METHODS

The study analyzed the distribution of the content of photosynthetic pigments along the length of the wheat leaf, *Triticum aestivum* L. The biological material was represented by the 'Dacic' wheat cultivar, cultivated in the SCDA Lovrin conditions, Romania.

In relation to the purpose of the study, leaf samples were taken, the third leaf, fully developed, in the BBCH 30 stage. Ten leaves with a length of 30 cm were selected. The photosynthetic pigments (chlorophyll - Chl, carotenoids - Car) were determined on different positions along the length of the leaf (position on the leaf - p), at intervals of 1 cm (29 positions, p1 to p29). The SPAD-502Plus chlorophyll meter (KONICA MINOLTA) and the ACM-200 Plus device (OPTI-SCIENCES) were used for the determinations. 29 determinations were made for Chl and Car for each leaf and for the ten leaves selected in the study. Data series (290 values) were recorded for chlorophyll (Chl) and carotenoids (Car). Based on the respective values, the ratios Chl/Car and Car/Chl were calculated, for each position on the leaf (p1 to p29) for the ten leaf samples. In relation to the purpose and objectives of the study, the flow diagram was generated, figure 1, based on which the stages of sampling, determination of the content of photosynthetic pigments, data analysis and interpretation, comparative analysis and validation of the results were carried out.

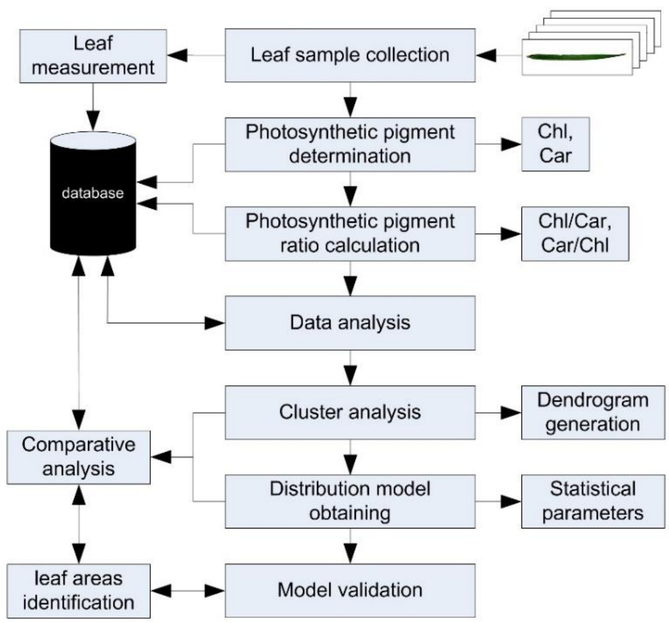


FIGURE 1. Flow diagram in the evaluation of photosynthetic pigments in wheat leaf, 'Dacic' cultivar

Appropriate mathematical and statistical methods were used for data analysis, in relation to the purpose of the study (Hammer *et al*., 2021; Wolfram Alpha, 2020; JASP, 2022).

RESULTS AND DISCUSSION

The complete series of data (290 values for each parameter) was statistically analyzed (Descriptive statistics) in order to find statistical parameters for the general characterization of the data. The obtained results are presented in table 1. The chlorophyll content (Chl) varied between 36.70 – 67.80±0.51 units, and the carotenoid content (Car) varied between 4.90 – 17.10±0.17 units. In the case of the calculated ratios, the Chl/Car ratio values were between 3.95 – 7.65±0.04, and the Car/Chl ratio values were between 0.13 – 0.25±0.001. The ANOVA test certified the presence of variance and the statistical reliability of the complete series of data, F>F crit, under conditions of Alpha=0.001, table 2.

TABLE 1. Descriptive statistics, general data analysis, wheat leaves, 'Dacic' cultivar

Statistical parameters	Chl	Car	Chl/Car	Car/Chl
Valid	290	290	290	290
Missing	0	0	0	0
Median	57.05	12.7	4.59	0.22
Mean	56.202	11.998	4.841	0.21
Std. Error of Mean	0.51	0.174	0.041	0.001
Std. Deviation	8.689	2.962	0.702	0.025
Coefficient of variation	0.155	0.247	0.145	0.121
Variance	75.506	8.775	0.493	6.415×10^{-4}
Range	31.1	12.2	3.7	0.12
Minimum	36.7	4.9	3.95	0.13
Maximum	67.8	17.1	7.65	0.25
25th percentile	50.1	9.9	4.402	0.2
50th percentile	57.05	12.7	4.59	0.22
75th percentile	64.3	14.5	5.027	0.23

TABLE 2. ANOVA test

Source of Variation	SS	df	MS	F	P-value	F crit
Rows	18730.51	28	668.9467	64.04647	1.1E-150	2.09132
Columns	283396.5	19	14915.61	1428.054	0	2.365002
Error	5556.585	532	10.44471			
Total	307683.6	579				

Alpha=0.001

In relation to the purpose of the study, the distribution of the values of chlorophyll pigments along the length of the wheat leaf, at the determined positions (p1 to p29) at a distance of 1 cm, was analyzed, table 3.

TABLE 3. Distribution of photosynthetic pigments, average values, along the length of the leaf wheat, 'Dacic' cultivar

Position on the leaf	Chl	Car	Chl/Car	Car/Chl
p1	38.50±0.34	5.55±0.13	6.94±0.121	0.14±0.002
p2	39.17±0.33	6.20±0.18	6.32±0.137	0.16±0.003
p3	42.63±0.73	7.53±0.37	5.66±0.199	0.18±0.006
p4	44.29±0.45	8.54±0.43	5.19±0.245	0.19±0.008
p5	46.84±0.64	9.34±0.41	5.01±0.210	0.20±0.007
p6	50.08±0.47	9.99±0.43	5.01±0.213	0.20±0.007
p7	52.69±0.46	10.89±0.33	4.84±0.128	0.21±0.005
p8	54.81±0.23	11.44±0.34	4.79±0.139	0.21±0.005
p9	56.38±0.12	12.27±0.21	4.59±0.080	0.22±0.003
p10	59.12±0.26	12.93±0.24	4.57±0.079	0.22±0.004
p11	60.69±0.28	13.42±0.19	4.52±0.056	0.22±0.003
p12	62.86±0.19	13.87±0.19	4.53±0.056	0.22±0.003
p13	64.28±0.17	14.29±0.14	4.50±0.041	0.22±0.002
p14	65.20±0.12	14.54±0.15	4.48±0.045	0.22±0.002
p15	65.85±0.13	14.97±0.17	4.40±0.047	0.23±0.002
p16	66.28±0.18	15.21±0.17	4.36±0.048	0.23±0.003
p17	66.34±0.32	15.53±0.19	4.27±0.039	0.23±0.002
p18	66.09±0.50	15.49±0.23	4.27±0.044	0.23±0.002
p19	65.55±0.69	15.38±0.29	4.26±0.048	0.23±0.003
p20	64.49±0.81	14.97±0.29	4.31±0.059	0.23±0.003
p21	63.16±0.84	14.42±0.30	4.38±0.049	0.23±0.003
p22	61.96±0.94	13.90±0.29	4.46±0.045	0.22±0.002
p23	59.56±0.99	13.45±0.28	4.43±0.045	0.23±0.002
p24	57.40±0.93	12.74±0.26	4.51±0.046	0.22±0.002
p25	54.99±0.78	11.79±0.27	4.66±0.069	0.21±0.003
p26	53.19±0.56	11.06±0.16	4.81±0.059	0.21±0.002
p27	51.61±0.48	10.24±0.10	5.04±0.055	0.20±0.002
p28	49.12±0.44	9.30±0.06	5.28±0.038	0.19±0.001
p29	46.73±0.35	8.70±0.11	5.37±0.091	0.19±0.003

The recorded values for Chl and Car, for the ratios calculated for each photosynthetic pigment and determination position (29 positions), as well as the calculated standard errors (SE) are presented in table 3. The data are presented in the form of average values calculated for each position (p1 to p29) from the individual values for the 10 analyzed wheat leaves.

The variation of the chlorophyll content (Chl) along the length of the wheat leaf (interval of 1 cm) was described by equation (1), under conditions of R^2=0.963, p<0.001, with the graphic distribution of the data series and the expression of the equation (1) in figure 2. The distribution of Chl values in the leaf samples, on the 29 determined positions, is shown in the form of a matrix plot in figure 3. The variation of carotenoid content (Car) along the length of the wheat leaf (interval of 1 cm), was described by equation (2), under conditions of R^2=0.928, p<0.001, with the graphic distribution of the data series and the equation (2) expression in figure 4. The distribution of Chl values in the leaf samples, on the 29 determined positions, is shown in the form of a matrix plot in figure 5.

$$\text{Chl} = 0.0002806\,x^4 - 0.01805\,x^3 + 0.2591\,x^2 + 1.176\,x + 36.75 \qquad (1)$$

where: x – position on the leaf (p1 to p29), cm

$$\text{Car} = 3.894E-05x^4 - 0.002837\,x^3 + 0.02639\,x^2 + 0.7941\,x + 4.822 \qquad (2)$$

where: x – position on the leaf (p1 to p29), cm

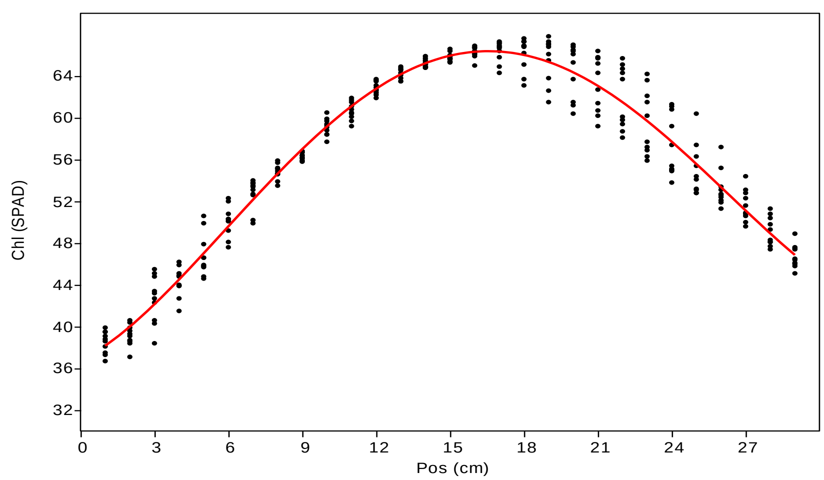


FIGURE 2. The variation of Chl in relation to the position along the length of the wheat leaf, 'Dacic' cultivar

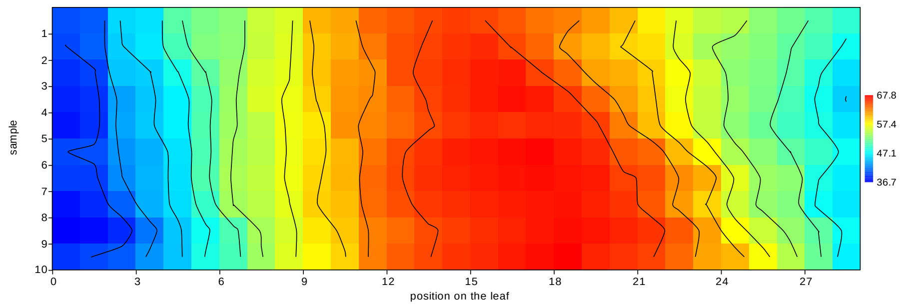


FIGURE. 3. Matrix plot regarding the distribution of Chl along the length of the wheat leaf, 'Dacic' cultivar

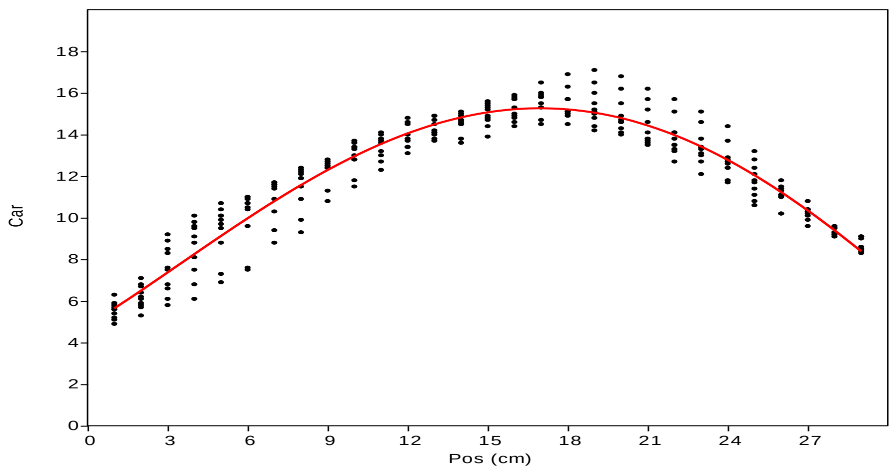


FIGURE 4. The Car variation in relation to the position along the length of the wheat leaf, 'Dacic' cultivar

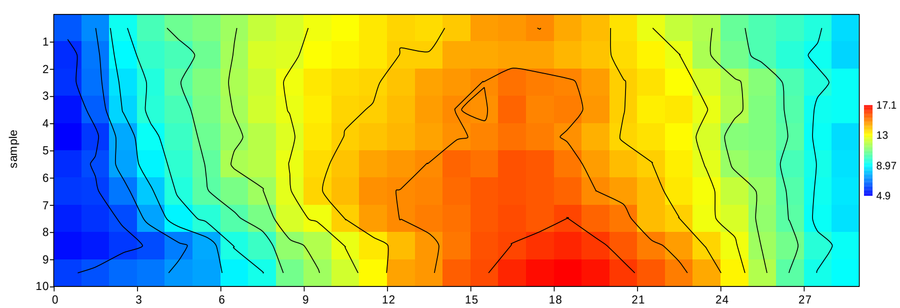


FIGURE 5. Matrix plot regarding the distribution of Car along the length of the wheat leaf, 'Dacic' cultivar

The variation of carotenoid pigments (Car) in relation to chlorophyll (Chl) was described by the linear equation (3), under conditions of $R^2=0.956$, $p<0.001$, represented in figure 6.

$$Car = 0.3334 \cdot Chl - 6.739 \qquad (3)$$

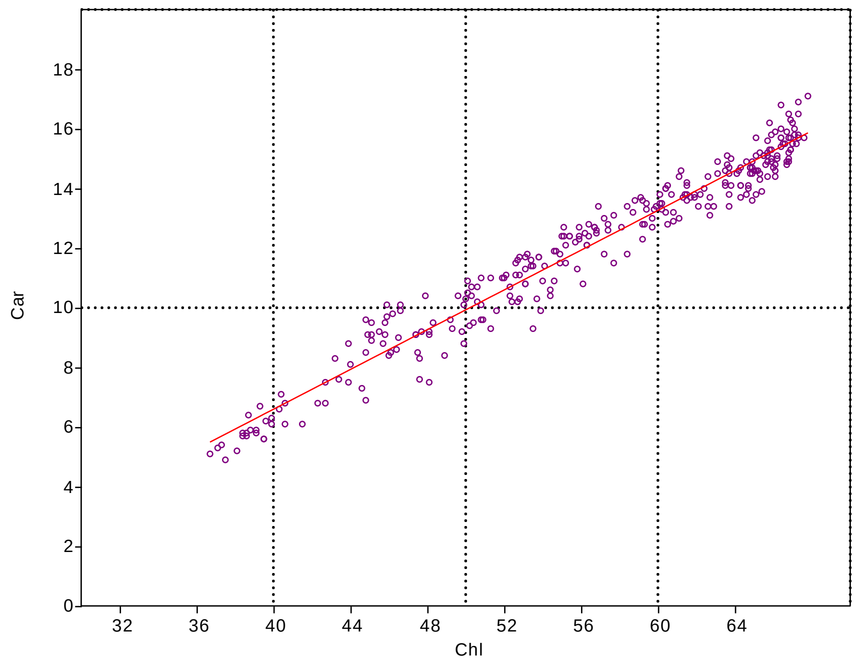


FIGURE 6. Variation of Car in relation to Chl in wheat leaves, 'Dacic' cultivar

The cluster analysis led to the dendrogram in figure 7 (Coph.corr.=0.738), in which the positions on the length of the leaves (p1 to p29) were associated based on similarity in relation to the chlorophyll content determined at each point. Two distinct clusters resulted, with several sub-clusters each.

A C1 cluster comprised points on the leaf blade with a higher level of chlorophyll. The highest chlorophyll content was recorded in the area of points p16, p17, p18 (marked in red on the dendrogram). The area of points p8, p9, p24 and p25 corresponded to the average value of the chlorophyll content (Chl_Avg, 56.20 units), calculated at the level of the analyzed leaves (marked in green on the dendrogram).

Based on the calculated SDI values, it turned out that the highest level of similarity was recorded between positions (p16,p17) SDI=0.06, followed by positions (p16,p18) SDI=0.19.

The variation of the carotenoid content (Car) along the length of the wheat leaf, in relation to the determination position (p1 to p29) and the chlorophyll content (Chl) related to each position, as a direct and interaction effect, was described by equation (4) under conditions of R^2=0.999, p<0.001. The graphic distribution of Car in relation to the position on the leaf (p) and the chlorophyll content (Chl) is presented in figure 8, a and b.

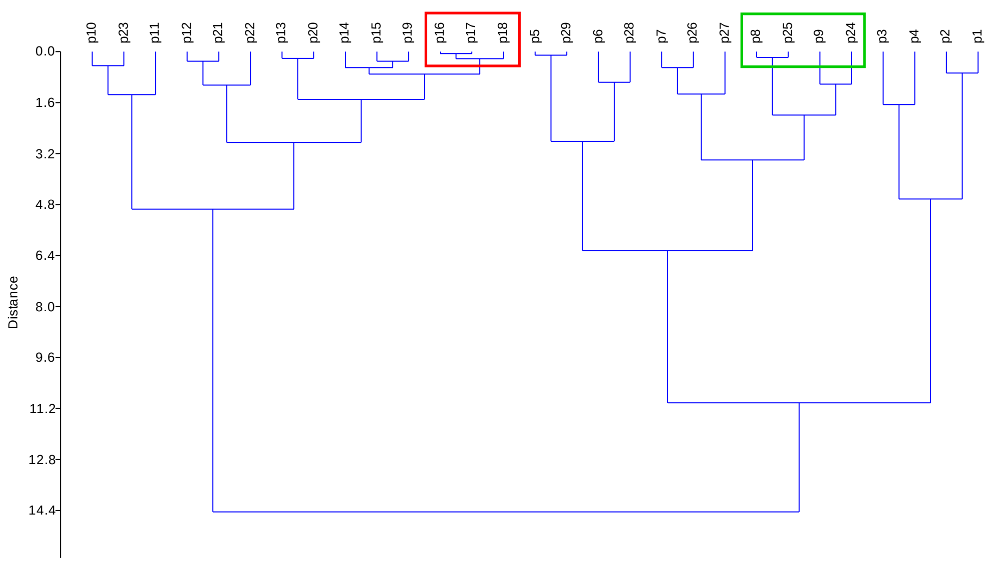


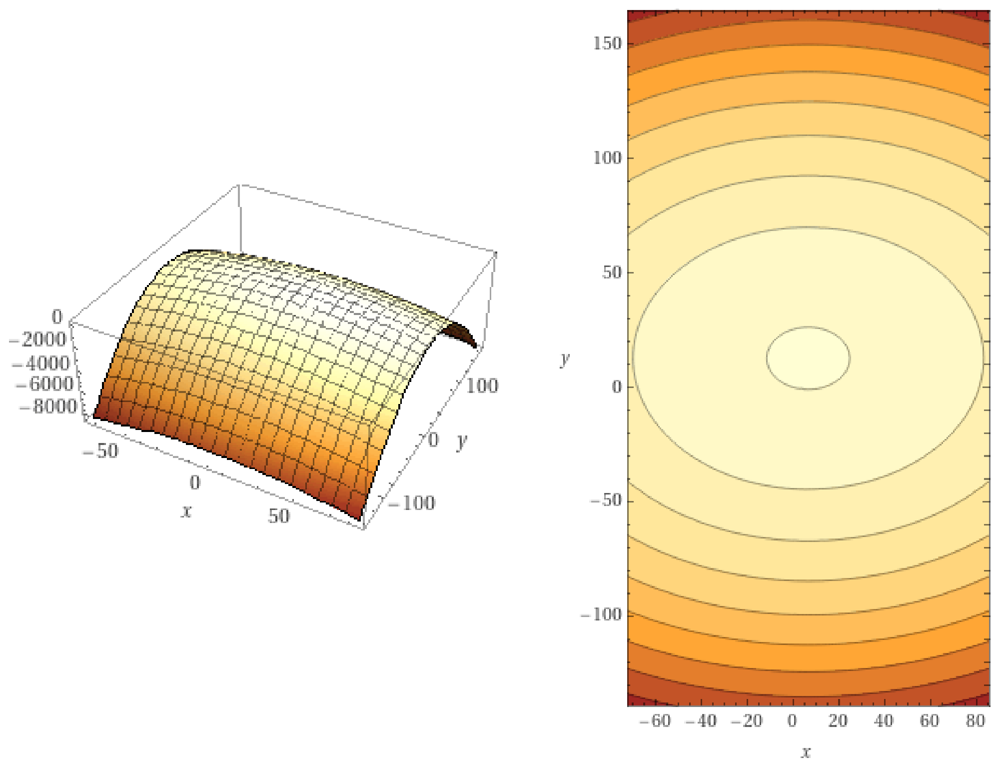
FIGURE 7. Dendrogram of the p1 to p29 points distribution on the length of the leaves, 'Dacic' wheat cultivar, in relation to the chlorophyll content; red marking - the area with maximum pigment content; green marking – area with average pigment values

$$\text{Car} = ax^2 + by^2 + cx + dy + exy + f \qquad (4)$$

where: Car – carotenoid content;
x – p – the position on the leaf;
y – Chl – chlorophyll content;

a, b, c, d, e, f – coefficients of the equation (4);
a= -0.02282336;
b= -0.00022622;
c= 0.59778020;
d= 0.13771975;
e= 0.00316391;
f= 0

From the graphic analysis of the Car data distribution (figure 8 a), it was found that the chlorophyll content has a much stronger influence than the p position (p1 to p29) on the leaf in the variation of the carotenoid content.



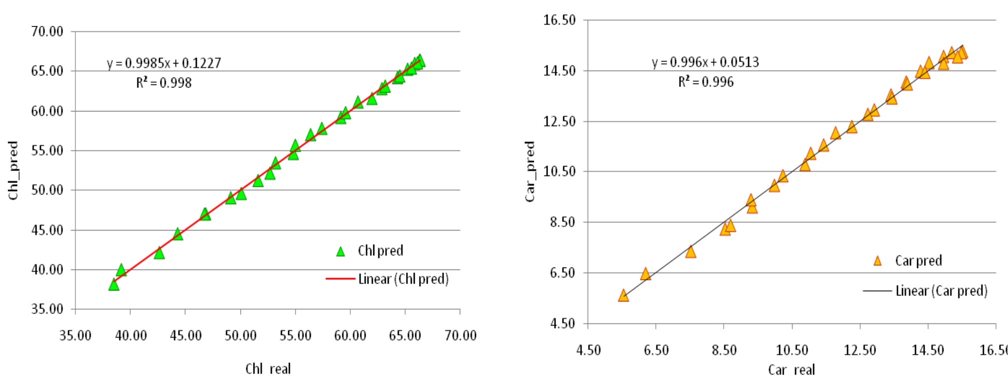
a b

FIGURE 8. Graphic distribution of the carotenoid content (Car) in relation to the position on the leaf p (x – axis) and Chl (y – axis); a – 3D model; b – model in the form of isoquants

The graphic analysis of the distribution of chlorophyll (Chl) and carotenoids (Car) content along the length of the wheat leaf, 'Dacic' cultivar showed a variation of the photosynthetic pigments in relation to the position on the leaf, equations (1) and (2), figures 2 and 3. Starting from equation (1), an analysis was considered to find the position on the leaf (x value in the equation) where the chlorophyll content should be the calculated average value (56.2 SPAD units).

Starting from equation (1) and giving to x the concrete values of p (position on the leaf in cm, 1 to 29), a series of Chl values resulted from which two areas on the leaf limb were identified, where average values was recorded (56.20 SPAD units). Thus, position p8-p9 and position p24-p25 resulted, where, by calculation, average Chl values at the leaf level (Chl_Avg = 56.20 units) was obtained. The fitting analysis between real Chl and predicted Chl, based on equation (1), was described by linear equation (5), under conditions of R^2=0.998, p<0.001, figure 9 a.

In a similar way, the analysis was made for the carotenoid content, starting from equation (2). Concrete values were given to *x*, a series of Car values resulted, and two zones were identified the length of the wheat leaf, where the average value for the carotenoid content (12.00 units) was recorded. Thus, the p8-p9 area and the p24-p25 area resulted, with which was associated the average Car value at the level of the entire leaf (Car_Avg = 12.00 units). The fitting analysis between real Car and predicted Car based on equation (2) was described by linear equation (6), under conditions of R^2=0.996, p<0.001, figure 9 b.



(a) (b)

FIGURE 9. The fitting lines between the real and predicted values of the photosynthetic pigments in the wheat leaves, 'Dacic' cultivar; (a) Chl; (b) Car

The graphic representation of the areas along the length of the wheat leaves, where the content of chlorophyll and carotenoids recorded the average value, is presented in figure 10.

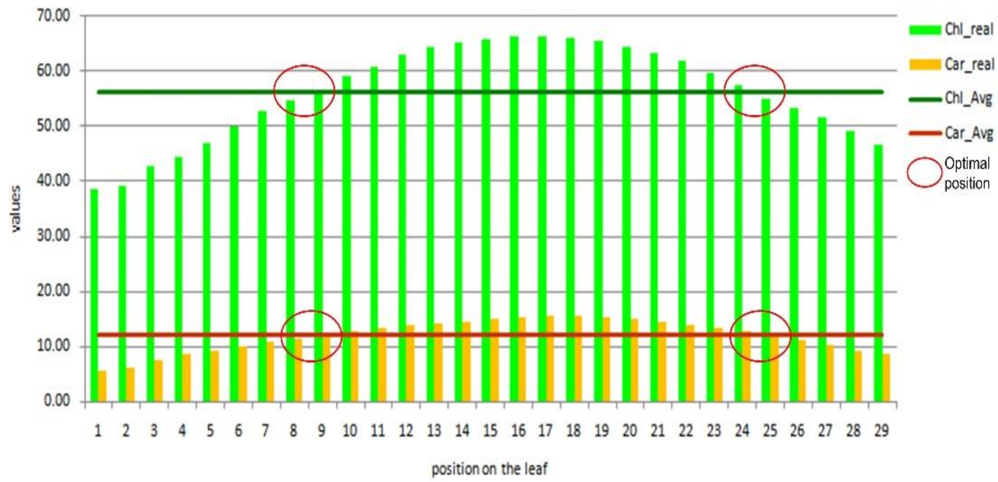


FIGURE 10. The variation of Chl and Car content (bar format) in relation to the position on the wheat leaf, 'Dacic' cultivar, the average Chl and Car values (line format), and the optimal areas identified

An important aspect for determining the content of photosynthetic pigments by non-destructive methods, such as with a portable chlorophyll meter, is in which area on the leaf to take the readings to capture the average chlorophyll content as faithfully as possible.

In terms of determining the content of photosynthetic pigments by non-destructive methods, with portable devices, they have a small active reading surface of a few mm^2, and choosing the right area on the leaf blade leads to finding out the average value of the content of photosynthetic pigments.

A large number of determinations requires a greater consumption of time, which is possible in the case of a small number of samples.

However, if there is a large number of samples, such as in a field of genetics and breeding of grass cereal plants (wheat, barley, rye, oats, etc.), or in the case of a large number of fertilization variants, knowing some areas along the length of the leaves that express average values of the content of photosynthetic pigments in the leaf has high practical importance.

The variation in the content of photosynthetic pigments from leaves to grasses has been communicated in different studies, and different models of spatial distribution of chlorophyll have been communicated in conditions of statistical safety. Different models have been communicated regarding the distribution of photosynthetic pigments in the leaves of wheat or other grassy cereals, in relation to influencing factors and de-ermination methods.

Based on imaging analysis (Visible, and Near-infrared), Zhang *et al*. (2016) estimated with high precision (confirmed by RMSE values, correlation coefficient) the content of chlorophyll in wheat using different image processing methods and analysis models the data.

Jhanji and Sekhon (2018) communicated results, under statistical safety conditions, regarding the content of chlorophyll and nitrogen in wheat in different field conditions, based on SPAD values resulting from the analysis with a portable chlorophyll meter.

Constantinescu *et al*. (2018) communicated models for estimating the chlorophyll content and the biomass production in cereal grasses, based on UAV images and some calculated indices, under statistical safety conditions ($p<0.01$). Mathematical models were used to describe the wheat crop through imaging analysis, in relation to the conditions for taking the images given at the time of day (Sala *et al*., 2020).

Song *et al*. (2021) communicated models based on imaging analysis and multiple linear regression for the estimation of chlorophyll content in wheat, correlated with SPAD values, under statistical safety conditions, based on the recorded R^2 and RMSE values.

SPAD values were analyzed compared to spectral data from the remote sensing technique and estimation models were obtained under statistical safety conditions given by R^2 and RMSE (Wu *et al*., 2023).

Considering the portable chlorophyll meter (SPAD meter) as a reliable tool for determining the chlorophyll content and associated nitrogen content in plants, Yuan *et al.* (2016) used a comparative analysis (SPAD, flat color scanner, calculation algorithms) to optimize rice leaf measurements.

From the study, based on the results obtained, the authors communicated that 2/3 from the base of the leaf was the area where the chlorophyll content was representative of the entire leaf. It was the area where the SPAD values were consistent with the content of chlorophyll a and b extracted, and also representative of the N content in the leaf.

In the present study on wheat, 'Dacic' cultivar, the third fully developed (expanded) leaf, the average value of chlorophyll (SPAD units) was found at positions p8-p9 (8-9 cm) from the base of the leaf, which represents approx. 30% of the length of the leaf (1/3), and at positions p24-p25 (24-25 cm) from the base of the leaf, which represents approx. 80% of the length of the leaf (4/5).

The results obtained in the present study can be useful in other research studies as well as for agricultural practice, in order to consider the areas identified on the third leaf of wheat as landmarks for finding out the average chlorophyll content (SPAD) through measurements with chlorophyll meters portable.

CONCLUSIONS

The variable distribution of the content of photosynthetic pigments (Chl, Car) was identified along the length of the wheat leaves (third fully developed leaf), and was described by mathematical models in the form of polynomial equations under statistical safety conditions (R^2=0.963, R^2=0.928, $p<0.001$).

Starting from equations (1) and (2) that described the variation in the content of chlorophyll and carotenoids (Chl and Car) in relation to the position on the leaf (p1 to p29, cm) the representative areas on the wheat leaf were found, where were obtained by measurement (SPAD reader) values of the pigment content at the level of average values (Chl_Avg, Car_Avg; p8-p9, p24-p25), as well as the area with the maximum level of pigment content (p16 – p18).

These areas were also identified in the resulted dendrogram, and confirmed by cluster analysis.

The results can be useful for research and practice in the study of photosynthetic pigments in wheat, 'Dacic' cultivar, and the model can be adapted to other cereal grasses, starting from the flow diagram proposed by the recent study.

ACKNOWLEDGMENTS

The authors thank SCDA Lovrin for facilitating this study.

REFERENCES

- Abd-Elkader D.Y., Mohamed A.A., Feleafel M.N., Al-Huqail A.A., Salem M.Z.M., Ali H.M., Hassan H.S. 2022. Photosynthetic pigments and biochemical response of zucchini (*Cucurbita pepo* L.) to plant-derived extracts, microbial, and potassium silicate as biostimulants under greenhouse conditions. *Front. Plant Sci.* 13: 879545.
- Borsuk A.M., Brodersen C.R. 2019. The spatial distribution of chlorophyll in leaves. *Plant Physiol.* 180(3): 1406-1417.
- Constantinescu C., Herbei M., Rujescu C., Sala F. 2018. Model prediction of chlorophyll and fresh biomass in

cereal grasses based on aerial images. *AIP Conference Proceedings*, 1978: 390003.

- Esteban R., Barrutia O., Artexte U., Fernández-Marín B., Hernández A., García-Plazaola J.I. 2015. Internal and external factors affecting photosynthetic pigment composition in plants: a meta-analytical approach. *New Phytol.* 206: 268-280.
- Feng H., Chen G., Xiong L., Liu Q., Yang W. 2017. Accurate digitization of the chlorophyll distribution of individual rice leaves using hyperspectral imaging and an integrated image analysis pipeline. *Front. Plant Sci.* 8: 1238.
- Fornari E.Z., Gaviraghi L., Basso C.J., Pinheiro M.V.M., Vian A.L., Santi A.L. 2020. Relationship between photosynthetic pigments and corn production under nitrogen sources. *Pesqui. Agropecu. Trop.* 50: e63661.
- Hammer Ø., Harper D.A.T., Ryan P.D. 2001. PAST: Paleontological Statistics software package for education and data analysis. *Palaeontol. Electron.* 4(1): 1-9.
- Hosseini S.J., Tahmasebi-Sarvestani Z., Pirdashti H., Modarres-Sanavy S.A.M., Mokhtassi-Bidgoli A., Hazrati S., Nicola S. 2021. Investigation of yield, phytochemical composition, and photosynthetic pigments in different mint ecotypes under salinity stress. *Food Sci. Nutr.* 9(5): 2620-2643.
- JASP Team (2022). JASP (Version 0.16.2)[Computer software].
- Jhanji S., Sekhon N.K. 2018. Evaluation of potential of portable chlorophyll meter to quantify chlorophyll and nitrogen contents in leaves of wheat under different field conditions. *Indian J. Exp. Biol.* 56: 750-758.
- Li Y., He N., Hou J., Xu L., Liu C., Zhang J., Wang Q., Zhang X., Wu X. 2018. Factors influencing leaf chlorophyll content in natural forests at the biome scale. *Front. Ecol. Evol.* 6: 64.
- Liang Y., Urano D., Liao K.L., Hedrick T.L., Gao Y., Jones A.M. 2017. A nondestructive method to estimate the chlorophyll content of Arabidopsis seedlings. *Plant Methods* 13: 26.
- Peng J., Feng Y., Wang X., Li J., Xu G., Phonenasay S., Luo Q., Han Z., Lu W. 2021. Effects of nitrogen application rate on the photosynthetic pigment, leaf fluorescence characteristics, and yield of indica hybrid rice and their interrelations. *Sci. Rep.* 11: 7485.
- Petibon F., Wiesenberg G.L.B. 2022. Characterization of complex photosynthetic pigment profiles in European deciduous tree leaves by sequential extraction and reversed-phase high-performance liquid chromatography. *Front Plant Sci.* 13: 957606.
- Sala F., Popescu C.A., Herbei M.V., Rujescu C. 2020. Model of color parameters variation and correction in relation to "Time-View" image acquisition effects in wheat crop. *Sustainability* 12(6): 2470.
- Sala F. 2021. Variation of photosynthetic pigments and Chl/Car ratio in wheat in relation to fertilization rate. *AgroLife Scientific Journal*, 10(2): 160-169.
- Simkin A.J., Kapoor L., Doss C.G.P., Hofmann T.A., Lawson T., Ramamoorthy S. 2022. The role of photosynthesis related pigments in light harvesting, photoprotection and enhancement of photosynthetic yield in planta. *Photosynth Res.* 152(1): 23-42.
- Song Y., Teng G., Yuan Y., Liu T., Sun Z. 2021. Assessment of wheat chlorophyll content by the multiple linear regression of leaf image features. *Inf. Process. Agric.* 8(2): 232-243.
- Wood N.J., Baker A., Quinnell R.J., Camargo-Valero M.A. 2020. A simple and non-destructive method for chlorophyll quantification of chlamydomonas cultures using digital image analysis. *Front. Bioeng. Biotechnol.* 8: 746.
- Wolfram, Research, Inc., Mathematica, Version 12.1, Champaign, IL (2020).
- Wu Q., Zhang Y., Zhao Z., Xie M., Hou D. 2023. Estimation of relative chlorophyll content in spring wheat based on multi-temporal UAV Remote Sensing. *Agronomy* 13(1): 211.
- Yuan Z., Cao Q., Zhang K., Ata-Ul-Karim S.T., Tian Y., Zhu Y., Cao W., Liu X. 2016 Optimal leaf positions for SPAD meter measurement in rice. *Front. Plant Sci.* 7: 719.
- Zeng J., Ping W., Sanaeifar A., Xu X., Luo W., Sha J., Huang Z., Huang Y., Liu X., Zhan B., Zhang H., Li X. 2021. Quantitative visualization of photosynthetic pigments in tea leaves based on Raman spectroscopy and calibration model transfer. *Plant Methods* 17: 4.
- Zhang J., Han W., Huang L., Zhang Z., Ma Y., Hu Y. 2016. Leaf chlorophyll content estimation of winter wheat

based on visible and near-infrared sensors. *Sensors* 16(4): 437.

- Zhang Y., Li Y., Wang R., Xu L., Li M., Liu Z., Wu Z., Zhang J., Yu G., He N. 2020. Spatial variation of leaf chlorophyll in Northern Hemisphere grasslands. *Front. Plant Sci.* 11: 1244.
- Zhang R., Yang P., Liu S., Wang C., Liu J. 2022. Evaluation of the methods for estimating leaf chlorophyll content with SPAD chlorophyll meters. *Remote Sens.* 14: 5144.
- Zielewicz W., Wróbel B., Niedbała G. 2020. Quantification of chlorophyll and carotene pigments content in mountain melick (*Melica nutans* L.) in relation to edaphic variables. *Forests* 11(11): 1197.