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CHLOROPHYLL AND CAROTENOID CONTENT VARIATION IN CELERY LEAVES

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ABSTRACT
The study analyzed the distribution of the photosynthetic pigments content in celery leaves, in relation to the position of the leaves on the plant. The biological material was represented by the 'Rex' celery variety. Plant nutrition was provided by organic products, namely Naturamin Plus (V2), Naturvital Plus (V3), and Naturcomplet G (V4). To compare the results, a control variant (V1), unfertilized, was considered. The experiment was established by planting seedlings on March 22, 2023, in open field conditions. On May 25, 2023, 34 days after planting, plants from each variant were randomly sampled to determine the content of photosynthetic pigments. A variable number of leaves were recorded in relation to the experimental variants (11 leaves at V1, 13 leaves at V2, and 12 leaves at V3 and V4). The variation of the content of chlorophyll (Chl) and carotenoids (Car) in the leaves, in relation to leaf position on the plant, was described by polynomial equations of the 3rd degree ($p<0.001$). Young leaves (L1 – L4) showed low content of pigments, and high values were recorded in leaves L8 – L13. According to PCA, PC1 explained 94.833% of variance, and PC2 explained 4.1989% of variance. The Cluster Analysis led to the grouping of the leaves in relation to the pigment content in two distinct clusters (Coph.corr. = 0.844). Positions L12 and L13 of the leaves presented a high level of similarity, with SDI = 1.345. For consumption, in different "green diets" it is recommended to harvest the mature leaves, with an external position (L8 - L13, in the conditions of the present study), which have a high content of pigments.
KEY WORDS: *celery, distribution model, PCA, photosynthetic pigments*

INTRODUCTION

Through the intake of dietary fiber, mineral elements, vitamins, as well as other phytochemical compounds, vegetables are of high importance in human nutrition (Dias, 2012). Different food programs have analyzed the improvement of the nutrition index through the consumption of vegetables (Kim et al., 2021). Vegetable production was analyzed in relation to elements of productivity, quality, the impact on agro-food chains, processing and consumption (Spiker et al., 2023).

Biodiversity studies (agrobiodiversity, food biodiversity) have taken into account different species of vegetable plants (Harris et al., 2022). Within the diversity of vegetable plants, root crops are known and appreciated, both from a technological point of view as culture plants and important in horticultural crops, as well as from a food and phytopharmaceutical point of view due to the high content of nutritional principles (Knez et al., 2022). Different species of root plants have been studied in terms of nutritional composition, through the content of dietary fibers, vitamins, minerals, and various other nutrients (Que et al., 2019).

Celery (*Apium graveolens* L.), Apiaceae family, is a vegetable plant of high importance, for the production of roots, petioles, seeds, with a multitude of active, food and aromatic principles (e.g. phenolic compounds, volatile oils), with multipurpose use (Malhotra, 2006; Li et al., 2018).

Celery has been studied in relation to bioactive compounds and antioxidant capacity (Kooti et al., 2017). Bioactive compounds from four varieties of celery were studied in relation to antioxidant activity (Liu et al., 2020). Quality indices and yield were compared between different genotypes of celery for leaves, stems and roots (Golubkina et al., 2020). The authors of the study reported different values for the indices considered, in relation to the varieties studied and their classification categories.

Some studies and researches based on classic and modern techniques (e.g. molecular biology, sequencing technology) have led to the improvement of celery in terms of crop and chemical and nutritional composition (Li et al. 2018). Important elements in celery breeding programs were analyzed in accordance with genetic aspects of celery, with importance for the active principle, petiole and hypocotyl production, nutraceutical compounds, phytoremediation, etc. (Bruznican et al., 2020).

The growth and development of celery plants was analyzed in relation to vegetation factors (Chu et al., 2023). The authors of the study communicated the variation of some morphological parameters and physiological indices (e.g. root length, plant height, shoot weight, chlorophyll content, electron transfer rate, etc.) and the active principle in relation to the photoperiod. Similar studies were conducted by Qin et al. (2024), who evaluated the synthesis of bioactive compounds in relation to light intensity.

The chlorophyll content in vegetables was analyzed in comparisons, between "in vivo" and "in vitro" data (Limantara et al., 2015). The content of pigments in celery leaves (chlorophyll, carotenoids) were studied from the perspective of food importance, in relation to the conditions of storage and use by freezing (Chen et al., 2024).

The present study analyzed the content of pigments (chlorophyll, carotenoids) in celery leaves, in relation to the leaves position on the plant, and with different variants of organic fertilization.

MATERIAL AND METHODS

The study evaluated the content of photosynthetic pigments in celery leaves, in relation to experimental variants and the leaf position on the plant. The biological material was represented by the 'Rex' celery variety, in open field conditions (Gottlob, Timis County, Romania).

Considering the increasing preference for ecological products, organic fertilizers were used, respectively: Naturamin Plus (V2), Naturvital Plus (V3), and Naturcomplet G (V4). Comparatively, a control variant was considered, without fertilizers (V1, control). The celery crop was achieved by seedlings, planted on March 22, 2023. Plant samples for study were taken on May 25, 34 days after planting. Examples of studied plants, by variants, with the distribution of the leaves, are presented in figure 1.

Fig. 1. Celery leaves on experimental variants, 'Rex' celery variety

The leaves were detached from the plants and were analyzed individually, on each experimental variant, in order from young to mature leaves, as the order of arrangement on the plant. Chlorophyll content (Chl, SPAD-502Plus chlorophyll meter) and carotenoid content (Car, ACM-200 Plus) were determined. The data series were recorded, and based on them the Chl/Car ratio was calculated for each position of the leaves and the experimental variant. The experimental data were properly analyzed, through the statistical calculation module in EXCEL and the PAST software (Hammer et al., 2001).

RESULTS AND DISCUSSIONS

The celery plant samples were analyzed in terms of the number of leaves (Ln), the chlorophyll content (Chl) and the carotenoid content (Car). The determination was made from young plants, at the beginning of root formation, on series of 11-13 leaves. A variable number of leaves on the plants were recorded. 11 leaves were recorded for the plants from V1, 13 leaves for the plants from the V2 variant, and 12 leaves each for the plants from the V3 and V4 variants. The content of photosynthetic pigments showed a variation in relation to the experimental variant, as well as in relation to leaves position on the plant. The data series for photosynthetic pigments, associated with the position of the leaves, are presented in table 1. The ANOVA Test confirmed the reliability of the experimental data (Alpha = 0.001), table 2.

Table 1. The values of the content of photosynthetic pigments in the leaves of celery, 'Rex' variety

Leaf position	V1		V2		V3		V4	
	Chl	Car	Chl	Car	Chl	Car	Chl	Car
L1	7.60	1.50	7.80	1.60	9.60	1.70	10.60	1.80
L2	14.80	2.30	12.90	1.90	20.10	2.60	21.44	2.70
L3	31.44	3.40	18.05	2.50	32.30	3.30	39.42	4.40
L4	41.32	5.75	29.43	3.20	43.90	4.96	44.25	5.56
L5	47.48	6.75	40.90	5.15	48.25	6.55	52.34	8.10
L6	52.95	8.83	46.73	6.63	54.16	8.32	55.63	9.20
L7	54.98	9.90	51.02	7.30	58.53	10.96	57.10	9.50
L8	56.81	10.37	54.33	8.40	59.56	11.61	58.37	11.02
L9	57.12	10.55	56.48	9.62	60.96	11.84	59.62	11.37
L10	57.73	10.44	57.74	10.90	61.20	12.38	60.47	11.78
L11	52.28	8.60	58.46	11.38	58.45	11.85	61.30	12.20
L12	-	-	60.62	11.45	57.20	11.31	58.54	11.53
L13	-	-	60.28	10.87	-	-	-	-
SE	±5.34	±1.03	±5.32	±1.05	±5.02	±1.18	±4.80	±1.08

The data series regarding the content of photosynthetic pigments in celery leaves showed normal distributions. In the case of chlorophyll, the values r = 0.902 for the V1 variant, r = 0.926 for the V2 variant, r = 0.899 for the V3 variant, and r = 0.878 for the V4 variant were recorded. In the case of the carotenoid content, the values r = 0.948 for variant V1, r = 0.960 for variant V2, r = 0.937 for variant V3 and r = 0.948 for variant V4 were recorded.

Table 2. ANOVA test

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	34299.24	7	4899.892	29.66708	1.25E-20	3.879876
Within Groups	14534.31	88	165.1626			
Total	48833.55	95				

The variation in the content of photosynthetic pigments in celery leaves, from young leaves to mature leaves, was analyzed. The variation of the chlorophyll content, in relation to the position of the leaf on the plant, was described by equation (1) for variant V1, R^2 = 0.989, p<0.001, figure 2, and the variation of the carotenoid content was described by equation (2), R^2 = 0.994, p<0.001, figure 3.

$$\mathrm{Chl}_{V1} = 0.01099\,x^3 - 1.125\,x^2 + 16.76\,x - 10.03 \tag{1}$$

$$\mathrm{Car}_{V1} = -0.02787\,x^3 + 0.3609\,x^2 + 0.1027\,x + 0.9491 \tag{2}$$

Chlorophyll content variation, in the case of variant V2, was described by equation (3), R^2 = 0.986, p<0.001, figure 2, and the variation of carotenoid content was described by equation (4), R^2 = 0.995, p<0.001, figure 3.

$$\mathrm{Chl}_{V2} = -0.00906\,x^3 - 0.2982\,x^2 + 10.34\,x - 5.168 \tag{3}$$

$$\mathrm{Car}_{V2} = -0.0149\,x^3 + 0.2804\,x^2 - 0.4066\,x + 1.692 \tag{4}$$

The variation of chlorophyll content, in the case of variant V3, was described by equation (5), R^2 = 0.997, p<0.001, figure 2, and the variation of carotenoid content was described by equation (6), R^2 = 0.991, p<0.001, figure 3.

$$\mathrm{Chl}_{V3} = 0.0229\,x^3 - 1.226\,x^2 + 16.73\,x - 6.912 \tag{5}$$

$$\mathrm{Car}_{V3} = -0.02434\,x^3 + 0.376\,x^2 - 0.2208\,x + 1.522 \qquad (6)$$

In the case of variant V4, the variation of chlorophyll content was described by equation (7), $R^2 = 0.990$, $p<0.001$, figure 2, and the variation of carotenoid content was described by equation (8), $R^2 = 0.991$, $p<0.001$, figure 3.

$$\mathrm{Chl}_{V4} = 0.06621\,x^3 - 2.028\,x^2 + 20.52\,x - 8.814 \qquad (7)$$

$$\mathrm{Car}_{V4} = -0.00844\,x^3 + 0.07105\,x^2 + 1.313\,x + 0.1597 \qquad (8)$$

In equations (1) - (8), "*x*" represents the position of the leaf, starting from young leaves (inner) to mature leaves (outer). The graphic distribution of the chlorophyll content (Chl) in relation to the position of the leaves, and the experimental variants, is shown in figure 2, and the distribution of the carotenoid content (Car) is shown in figure 3.

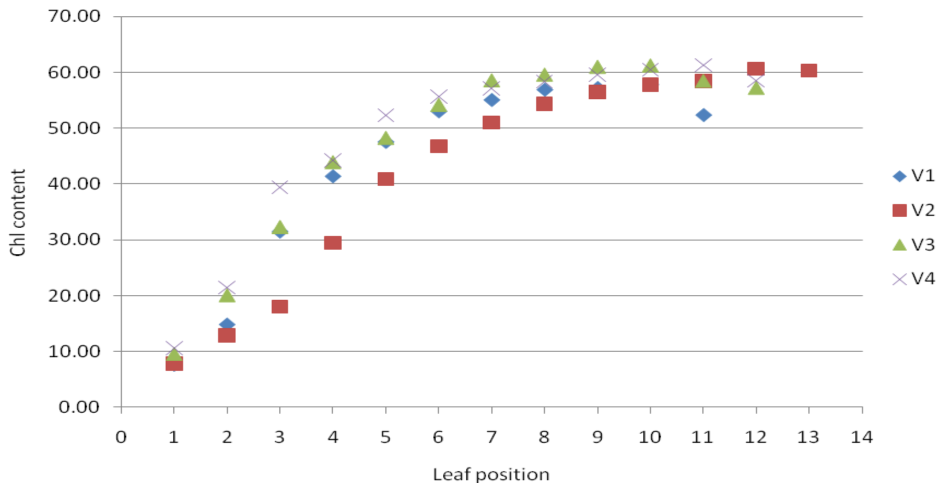

Fig 2. The graphic distribution of the chlorophyll content in relation to the position of the celery leaves, and the experimental variants, 'Rex' celery variety

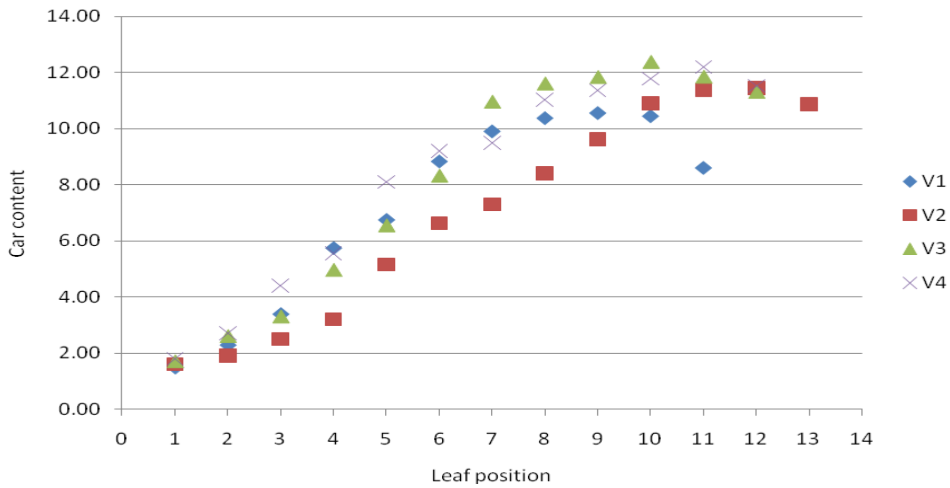

Fig 3. The graphic distribution of the carotenoid content in relation to the position of the celery leaves, and the experimental variants, 'Rex' celery variety

Table 3. Values of the Chl:Car ratio in celery leaves, 'Rex' variety

Leaf number (Ln)	Experimental variants			
	V1	V2	V3	V4
L1	5.07	4.88	5.65	5.89
L2	6.43	6.79	7.73	7.94
L3	9.25	7.22	9.79	8.96
L4	7.19	9.20	8.85	7.96
L5	7.03	7.94	7.37	6.46
L6	6.00	7.05	6.51	6.05
L7	5.55	6.99	5.34	6.01
L8	5.48	6.47	5.13	5.30
L9	5.41	5.87	5.15	5.24
L10	5.53	5.30	4.94	5.13
L11	6.08	5.14	4.93	5.02
L12	-	5.29	5.06	5.08
L13	-	5.55	-	-

High interdependence was recorded between the content of pigments (Car compared to Chl) at the leaf level, $R^2 = 0.994$ in the case of V1; $R^2 = 0.985$ in the case of V2; $R^2 = 0.979$ in the case of V3; $R^2 = 0.987$ in the case of V4.

The ratio between the content of chlorophyll and carotenoids (Chl:Car) in the celery leaves was calculated, and the values presented in table 3 resulted.

The variability of the content of photosynthetic pigments in the leaves was evaluated, as well as the calculated ratio, on experimental variants, figure 4. The high value of the coefficient of variation showed the content of carotenoids in variant V2 (CV = 54.0667) and the lowest value was recorded in the case of the Chl/Car ratio, for variant V1 (CV = 19.0640).

The PCA analysis facilitated obtaining the diagram in figure 5, in which the leaf samples were distributed in relation to the experimental variants, based on the content of photosynthetic pigments. PC1 explained 94.833% of variance, and PC2 explained 4.1989% of variance. The independent arrangement of the leaves L1 to L4 was observed, as a result of the low content of pigments.

The Cluster Analysis led to the dendrogram in figure 6, in which the positions of the leaf samples were associated based on similarity, in relation to the content of photosynthetic pigments (Coph.corr = 0.844). The formation of two distinct clusters was found.

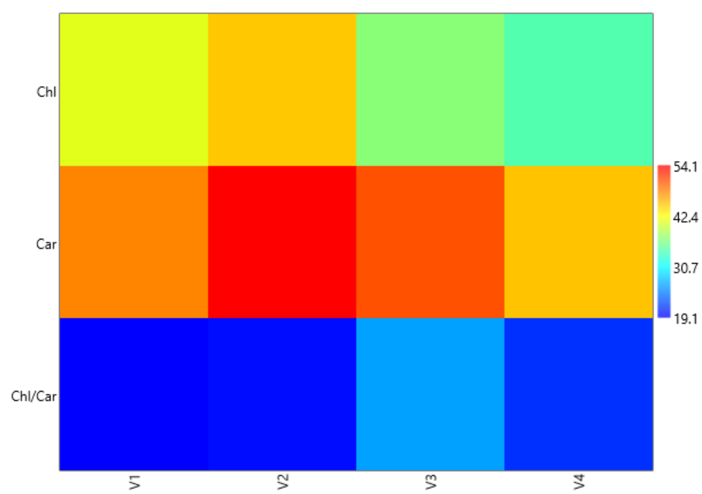

Fig. 4. Graphic representation of the coefficient of variation (CV) values for photosynthetic pigments and the calculated ratio, celery leaves, 'Rex' variety

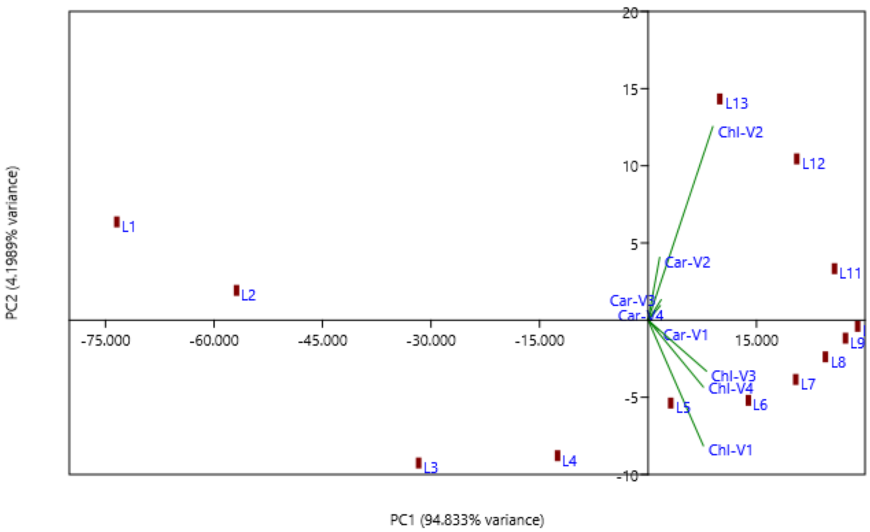

Fig. 5. PCA diagram regarding the distribution of leaf samples in relation to the experimental variants and the content of photosynthetic pigments, celery 'Rex' variety

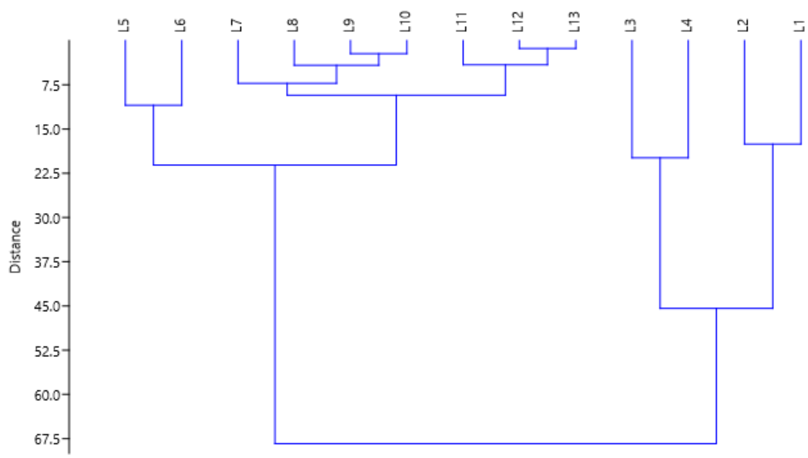

Fig. 6. Dendrogram of leaf position based on Euclidean distances, in relation to the content of photosynthetic pigments, celery 'Rex' variety

Positions L12 and L13 presented a high level of similarity, with SDI = 1.345. The recorded SDI values are presented in table 3.

Table 3. SDI values calculated based on Chl and Car in relation to the position of the leaves

	L1	L2	L3	L4	L5	L6	L7	L8	L9	L10	L11	L12	L13
L1	0.000	17.549	45.089	62.921	77.624	88.108	94.432	98.350	101.012	102.648	99.382	100.916	106.585
L2	17.549	0.000	27.937	45.723	60.616	71.196	77.583	81.609	84.295	85.974	82.737	83.865	96.443
L3	45.089	27.937	0.000	19.878	35.259	46.050	52.837	57.082	59.909	61.659	58.904	63.171	86.103
L4	62.921	45.723	19.878	0.000	16.360	26.742	33.428	37.775	40.669	42.522	40.396	44.691	63.578
L5	77.624	60.616	35.259	16.360	0.000	10.978	17.971	22.124	24.966	26.770	24.553	27.846	40.413
L6	88.108	71.196	46.050	26.742	10.978	0.000	7.241	11.356	14.269	16.156	15.254	18.193	28.396
L7	94.432	77.583	52.837	33.428	17.971	7.241	0.000	4.598	7.531	9.575	10.330	12.515	19.849
L8	98.350	81.609	57.082	37.775	22.124	11.356	4.598	0.000	3.152	5.196	7.801	8.549	12.885
L9	101.012	84.295	59.909	40.669	24.966	14.269	7.531	3.152	0.000	2.202	6.638	6.938	8.001
L10	102.648	85.974	61.659	42.522	26.770	16.156	9.575	5.196	2.202	0.000	6.523	6.275	5.080
L11	99.382	82.737	58.904	40.396	24.553	15.254	10.330	7.801	6.638	6.523	0.000	4.411	3.780
L12	100.916	83.865	63.171	44.691	27.846	18.193	12.515	8.549	6.938	6.275	4.411	0.000	1.345
L13	106.585	96.443	86.103	63.578	40.413	28.396	19.849	12.885	8.001	5.080	3.780	1.345	0.000

Chlorophyll is an important physiological index in the description and characterization of plants in relation to cultivated species and genotypes, growing conditions, vegetation status, biomass production and yield (Pîrvulescu et al., 2015; Constantinescu et al., 2018; Zulkarnaini et al., 2019; Zhang et al., 2022).

Physiological indices, represented by dimensional foliar parameters, content of photosynthetic pigments, represent the level of plant growth and development in relation to vegetation conditions (Zhang et al., 2022; Li et al., 2023). Also, the respective indices, correlated with the development of leaves for consumption (green diets) and marketing, can indicate which leaves should be harvested, ensuring a balance in the growth and development of plants (periodic harvesting of leaves for consumption).

Based on the results of the present study, the leaves from the range L8 - L13 presented higher values of pigment content, with a high nutritional value for harvesting and consumption.

CONCLUSIONS

In the study conditions on celery plants, the 'Rex' variety, the number of leaves (L11 to L13) and the content of photosynthetic pigments in the leaves expressed the differentiated response of the plants to organic fertilization.

The distribution of the content of chlorophyll (Chl) and carotenoids (Car) in the leaves was described by models in the form of polynomial equations of the 3rd degree, under statistical safety conditions ($p<0.001$). Very strong interdependence was recorded between the content of chlorophyll and that of carotenoids in the leaves ($R^2 = 0.979$ to $R^2 = 0.994$, $p<0.001$).

According to PCA, PC1 explained 94.833% of variance, and PC2 explained 4.1989% of variance. According to CA, two distinct clusters resulted, based on similarity (SDI = 1.345 in the case of L12 and L13).

For consumption in different "green diets", and valorization on the market, it turned out that the mature leaves (L8 - L13, in the conditions of the present study) presented highest content of pigments and are suitable to be harvested.

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