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COMPARATIVE ANALYSIS OF CHICKPEA (*CICER ARIETINUM* L.): PHOTOSYNTHETIC ACTIVITY, ANTIOXIDANT CAPACITY, PHENOLIC AND PIGMENT CONTENT

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Abstract

Biochemical and physiological parameters, such as: the antioxidant capacity, the content of total polyphenols and photosynthetic pigments, and the activity of the photosynthesis (the light phase), as measured by means of chlorophyll fluorescence were studied in 22 varieties of chickpeas (*Cicer arietinum* L.). At the start of the blooming stage, a strong decrease of the air temperatures was recorded, suggesting an impact of chilling stress. It was found out that PI_{ABS} (out of the fluorescence parameters) was mostly affected by the decrease of temperatures. Conversely, individual varieties of chickpea were able to stand out from the whole on the basis of their increased photosynthetic activity during this cold period. The cultivar, showing the highest increase in photosynthesis (133%) under chilling was "Blanco Lechoso" with 7.73 a.u. of the performance index immediately after the chilling. Concerning the antioxidant activity and phenolic content, it was discovered that only the antioxidant activity, as measured by the FRAP method, correlated to the phenolic content. On the other hand, both antioxidant methods strongly correlated to the concentration of carotenoids. These parameters had a weak inverse relation to the seed yield.

Key words: *Cicer arietinum* L., photosynthesis, antioxidants, polyphenols, chilling stress

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Introduction. Chickpea (*Cicer arietinum* L.) is an important food legume crop which is grown in semi-arid regions. It is the second most important legume in the world [1], covering at least 37 countries and about 15% of legume growing areas. Usually, chickpeas are used for human consumption, however, they are also used in the livestock industry as an alternative protein source, whereas the straw residue can be a high-protein forage for ruminants [2]. In the context of the current global changes of climate, the increased productivity and tolerance of crops to certain types of abiotic stress (heat, drought) is essential for contemporary agriculture [3]. However, several constraints limit desired goals of chickpea productivity: low genetic variability, low and unstable yield, and low resistance to biotic and abiotic stresses [4].

Photosynthesis is one of the most important physiological and anabolic processes in plants, thus is the foundation of plant productivity and crop yield [3]. Prompt fluorescence of chlorophyll *a* is a fast, cheap and reliable means to analyze the activity of the light phase (and its components), based on the fluorescence emission of illuminated leaves [5]. Since fluorescence is a competitive process to photochemistry in the photosystem II (PSII), it can indicate changes occurring there.

The antioxidant (AO) capacity of plants relates to photosynthesis as it has a crucial role in oxidative stress responses, since photosynthesis is a primary source of reactive oxygen species (ROS), increasing after environmental stress and inducing the damage of the cell [6]. Total content of polyphenols, a very large group of secondary metabolites, with multiple and diverse functions and one of the largest groups of antioxidants [7] were also measured. The isoflavonoids, a large subgroup of polyphenols, is abundant in legumes [8]. Isoflavonoids control the nodulation of roots by means of recognition by different symbiotic microorganisms [9]. Like most legumes, chickpeas contain large amounts of isoflavones and flavonols, which were observed mainly in the fractions of the seed coat [10]. However, the phenolic content and AO capacity can vary greatly among chickpea varieties. Two main types of chickpeas exist, comprising most of the varieties: 1) with dark-coloured hulls, containing larger amounts of polyphenols and flavonoids, and 2) beige-coloured seeds, having lower levels of phenolics, flavonoids and antioxidants [1].

Materials and methods. ***C. arietinum cultivation.*** Table 1 shows the names of the 22 studied chickpea cultivars. Plants were sown on mollisol (chernozem) and grown by organic means: without the use of fertilizers, irrigation, or chemical treatment. The sowing rate was 50 cm between rows and 20 cm inside the row (15 crops per row in two repetitions). The crops were grown in field conditions in Krushovitsa village, Pleven region, which is located within a humid continental climate zone (Dfa) according to the Köppen classification.

Obtaining the samples and measurements. The samples were obtained in the spring of 2024, with the exception of the AO capacity, as measured by the phosphomolybdate method, which was measured in both 2023 and 2024. Samples were taken and measurements were performed at two stages of development of *C. arietinum* cultivars: buttoning and early blooming. On April 26 (buttoning stage) samples from every cultivar were taken but only the chlorophyll fluorescence was measured. The blooming stage began at different dates for each cultivar. Thus, five different measurements of the photosynthetic activity, the AO capacity, and the phenolic and pigment content were performed: 08.V; 14.V; 17.V; 21.V; 28.V (Table 2).

All samples were taken in the morning (around 8:30 h). It should be noted that around the middle of May (9–19) a significant decrease of temperatures, preceded by intense rainfall was recorded (Table 1). Almost all rain occurred on 8–9 of May (39 mm). Afterwards, the average temperature decreased by 5 degrees and remained lower for around a week until the May 19 (the lowest was recorded on May 14, 6.6 °C).

Photosynthetic parameters. The light phase of photosynthesis was analyzed by measuring the parameters of prompt fluorescence of chlorophyll *a*, as described in [5]. Measurements were conducted using the FluorPen FP 110 device from PSI (Brno, Czech Republic), equipped with an LED that emits a 455 nm measuring pulse. Measuring flash at 3000 µmol m^{-2} s^{-1} (100%) was utilized. Leaves, freshly collected and dark-adapted for 30 min, served as samples for the chlorophyll fluorescence analyses after the method of [11].

Antioxidant (AO) capacity and total polyphenolic content (TPC). To assess the capacity of the plant to respond to oxidative stress, AO capacity was measured using two methods: the ferric reducing antioxidant power (FRAP) assay, based on Benzie and Strain [12], and the phosphomolybdate (PM) reduction method as per Prieto et al. [13]. TPC was determined following the method of Zhang et al. [14].

Pigment content. The content of photosynthetic pigments, including the chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*), and carotenoids (Car), was quantified according to the method of Lichtenthaler [15].

Statistical analysis. For statistical analysis, the Tukey test at $p \leq 0.05$ was utilized. Three independent measurements were taken from each crop variant to assess photosynthetic activity, and two replicates were taken to analyze the AO capacity, as well as the photosynthetic pigment and the TPC. Python 3.8 libraries, *numpy* and *scikit-learn*, were used for statistical analysis, whereas graphs were drawn by means of the library *matplotlib*. The online service of Google 'Colab' was used for compiling purpose: `https://colab.research.google.com/`.

Results and discussion. The results from the determination of the AO capacity, as well as the TPC are shown in Table 1, together with information about the type and husk colour of every cultivar. Some authors have found a prominent difference between the two main types of chickpeas, in terms of their seed husk colour, corresponding to the TPC of the husks [1]. However, the relation between both indicators in the measurements was insignificant, most probably since the leaves were analyzed and not the husks (Table 1, TPC). On the other hand, the seed colour was found to be related to some degree to the AO capacity of the leaves (Table 1, FRAP and PM). The other important and large group of compounds with AO nature are the carotenoids (Fig. 1A). They are involved mainly in the mechanisms of photoprotection (the xanthophyll cycle), by dissipating the excess light and they can also neutralize the singlet oxygen formed in PSII.

Table 1

AO capacity, as measured by the method of the ferric reducing antioxidant power (FRAP) and the method of the phosphomolybdate (PM) reduction (measured in 2023 and 2024). The measurements of the total polyphenolic content (TPC) were performed after flowering (early bloom stage) of each cultivar in May. The values are shown in mM/mg ascorbic acid equivalent, AAE (for the PM, FRAP) and mM/mg gallic acid equivalent, GAE (for the TCP). Lowercase letters denote the statistical significance at $p \leq 0.05$ (Tukey)

Cultivar (No. and name)	PM 2023	PM 2024	FRAP	TPC	Seed colour	Seed form
1) Alfa	10.53 ^{ab}	9.43 ^{abcde}	12.85 ^{abcde}	2.01 ^{abc}	beige	pealike
2) Beta	11.23 ^{abc}	9.61 ^{abce}	10.84 ^{b}	1.92 ^{abc}	beige	pealike
3) Businsky	13.13 ^{acde}	10.87 ^{afg}	13.74 ^{eg}	2.56 ^{c}	beige	pealike
4) Irenka	15.43 ^{dfg}	16.21 ^{g}	13.00 ^{acde}	1.73 ^{ab}	reddish brown	desi
5) Krajova z Kralovej	11.13 ^{abc}	11.65 ^{bcdeh}	12.29 ^{abcd}	1.56 ^{ab}	beige	pealike
6) Maskovsky Bagovec	12.96 ^{acde}	8.05 ^{cdh}	10.95 ^{b}	2.06 ^{abc}	beige	pealike
7) Slovak	12.04 ^{ace}	12.25 ^{abcde}	11.15 ^{ab}	1.57 ^{ab}	beige	pealike
8) FLIP 84-149 C	13.53 ^{cdef}	8.69 ^{bcdeh}	11.31 ^{abc}	2.09 ^{bc}	beige	pealike
9) VSP 11	11.78 ^{abce}	7.67 ^{dh}	11.55 ^{abc}	1.81 ^{ab}	brown	pealike
10) Progres	12.65 ^{acde}	12.52 ^{abcde}	11.96 ^{abcd}	1.48 ^{ab}	beige	pealike
11) Balkan	12.61 ^{acde}	14.57 ^{afg}	12.22 ^{abcd}	1.77 ^{ab}	white	pealike
12) Garbanzo 1	8.92 ^{b}	8.34 ^{bcdh}	8.48 ^{f}	1.73 ^{ab}	white	kabuli
13) Blanco Lechoso	13.03 ^{acde}	6.99 ^{h}	11.06 ^{ab}	1.83 ^{ab}	brown	kabuli
14) Caqui	14.68 ^{defg}	11.53 ^{bcdeh}	12.45 ^{abcde}	1.45 ^{ab}	black	desi
15) Garbanzo negro	20.79 ^{h}	12.16 ^{abcde}	12.99 ^{acde}	1.69 ^{ab}	black	desi
16) Tadzikskij 10	14.62 ^{defg}	12.85 ^{abce}	13.33 ^{cdeg}	1.96 ^{abc}	brown	desi
17) Sovhoznyj 14	17.11 ^{g}	15.51 ^{fg}	11.63 ^{abc}	1.38 ^{a}	beige	desi
18) Dneprovskij 1	17.44 ^{g}	12.15 ^{abcde}	11.15 ^{ab}	1.58 ^{ab}	beige	pealike
19) Garbanzo 2	11.86 ^{ace}	13.78 ^{aefg}	13.86 ^{deg}	1.67 ^{ab}	beige	pealike
20) Garbanzo 3	16.86 ^{g}	15.53 ^{fg}	15.07 ^{g}	2.03 ^{abc}	beige	pealike
21) Garbanzo 4	16.60 ^{g}	13.31 ^{abcf}	12.67 ^{abcde}	1.65 ^{ab}	beige	pealike
22) Plovdiv 8	16.09 ^{fg}	14.49 ^{afg}	13.97 ^{deg}	1.78 ^{ab}	beige	pealike

The values from the analysis of the photosynthetic pigments and activity are represented in Fig. 1A and B, respectively. The highest pigment concentration was determined in cultivars Nos. 21, 20, 1, 3, 22, whereas the lowest concentrations

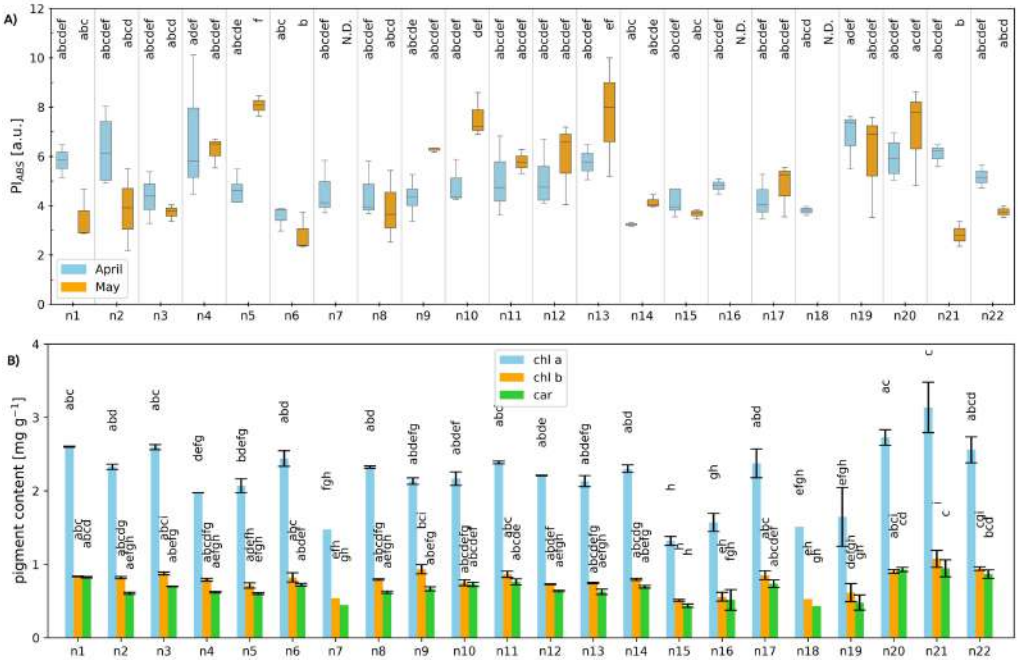

Fig. 1. A) Photosynthetic pigments (chlorophyll a – blue, b – yellow, carotenoids – green), measured after flowering (early bloom stage) of each cultivar in May. The cultivars were marked with lowercase 'n' and their corresponding number (see Table 1). B) Comparison between the first measurement of the photosynthetic activity by means of the performance index (PI_{ABS}) in the stage of buttoning (April, blue) and the next during the early blooming stage (May, red). N.D. denotes no data for the current cultivar. Lowercase letters denote the statistical significance at $p \leq 0.05$ (Tukey)

were found in Nos. 15, 7, 18, 16. The content of chlorophylls highly correlated to the carotenoid content, however, some cultivars had higher carotenoid content, as compared to chlorophylls. The highest ratio of the carotenoids to the chlorophylls was found in cultivars Nos. 10, 20, 22, whereas the lowest was in Nos. 2, 3, 18.

The photosynthetic activity of the light phase, as measured by the performance index (PI_{ABS}) is shown in Fig. 1B, whereas the change in PI_{ABS} after the cold period in May is shown in Table 2. The diversity in photosynthesis between the varieties and the drastic change in some of them can be clearly seen. Since in the middle of May a significant decrease of temperatures was recorded, the change of temperatures was one of the most important factors, affecting the photosynthesis and thus PI_{ABS} can be an indicator for the cold tolerance of these cultivars.

In addition, Table 2 shows the percentage change in the values of the parameter PI_{ABS}, between the measurements in April and May. It can be clearly seen that after May 8 (rainfall and decreased temperatures) most of the cultivars showed a lower photosynthetic activity, as compared with the measurements from

Table 2

Heat map of the change in the photosynthetic activity in May, presented as percentage of the initial value (measured on April 26)/original PI_{ABS} data in [a.u.]. The photosynthetic activity was represented by PI_{ABS} (performance index), as a measure for the photosynthetic activity. Source: Meteorological data (Pleven, April-May 2024) from the National institute of meteorology and hydrology `https://bulletins.cfd.meteo.bg/`

Date	26.IV	08.V	14.V	17.V	21.V	28.V
t°C 7:00h	9.9	16.9	9.7	12.7	16.7	14.2
Avg t°C	13.1	19.2	14.1	16	19.2	17.7
Chickpea cultivars Change of PI_{ABS} from April to May [% of the 1st measurement/original data]	First measurement (100 %)	9) VSP 11 144% / 6.28	13) Blanco Lechoso 133% / 7.73	5) Krajova z Kralovej 183% / 8.06	No data	10) Progres 156% / 7.56
			12) Garbanzo 1 117% / 5.94	14) Caqui 127% / 4.14		20) Garbanzo 3 118% / 7.08
			8) FLIP 84-149 C 86% / 3.87	19) Garbanzo 2 87% / 6.00		11) Balkan 114% / 5.79
			3) Businsky 85% / 3.72	15) Garbanzo negro 80% / 3.67		17) Sovhoznij 112% / 4.79
			6) Maskovsky Blagovec 79% / 2.82	21) Garbanzo 4 46% / 2.83		4) Irenka 91% / 6.25
			2) Beta 61% / 3.86			22) Plovdiv 72% / 3.75
			1) Alfa 59% / 3.48			

April (Table 2, red). Afterwards, with the progressively increasing temperatures, the photosynthetic activities began rising again, surpassing the measurements from April (Table 2, green). In addition, cultivars with higher, as well as lower than the control (measured in April) photosynthetic activity could be seen on the same date. This could be attributed to the individual characteristics of the cultivars and could be used as a tool to distinguish between cultivars, resistant to stress. Such a cultivar with outstanding performance after the chilling was "Blanco Lechoso" (No. 13), with 133% increase and PI_{ABS} of 7.73 a.u. (which is above the average). "Krajova z Kralovej" (No. 5) showed an even higher increase (183%, and PI_{ABS} of 8.06 a.u.), probably since it was measured further after the stress, when temperatures started to increase.

The correlation analysis (Table 3) showed the highest correlation among the three measured photosynthetic pigments. In addition, the prompt fluorescence parameters φ_{Do} and PI_{ABS} showed high inverse correlation (Table 3). Whereas φ_{Do} shows the amount of unused energy and thus stress exerted on plants, PI_{ABS}

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Correlation matrix of the main measured parameters. Statistical significance: $^{*}p \leq 0.05$; $^{**}p \leq 0.01$. Four of the measured fluorescence parameters are presented here: φ_{Do} – quantum yield of the energy dissipation; φ_{Ro} – quantum yield of the reduction of end PSI acceptors; PI_{ABS} – performance index (on absorptive basis); ABS/RC – absorption of light per reaction centre

	chl *a*	chl *b*	car	PM	FRAP	TPC	φ_{Do}	φ_{Ro}	PI_{ABS}	ABS/RC
chl *b*	**0.93									
car	**0.94	**0.86								
PM	**0.49	*0.44	**0.53							
FRAP	*0.43	0.33	**0.55	**0.72						
TPC	*0.48	* 0.39	* 0.39	0.3	* 0.47					
φ_{Do}	−0.27	−0.36	−0.23	−0.2	−0.07	−0.17				
φ_{Ro}	**0.53	**0.48	* 0.47	0.37	0.29	0.28	0.15			
PI_{ABS}	−0.01	0.12	−0.01	0.08	−0.08	−0.05	**−0.81	−0.31		
ABS/RC	0.13	−0.07	−0.09	−0.066	−0.01	0.09	* 0.48	* 0.4	**−0.85	
weight (seeds)	0.21	−0.1	0.18	− 0.15	−0.08	−0.13	−0.13	0.12	−0.02	−0.05

is a complex parameter, summarizing the overall activity of the light phase components. Higher levels of photosynthesis are a prerequisite for higher yields, thus PI_{ABS} has the potential to be used in the assessment of crop productivity. PI_{ABS} itself can sometimes be used as an indicator for the fixation of CO_2 under normal conditions, and even under stress [16]. Thus, it can indicate the transition of the light energy into biomass. However, higher biomass and higher seed yields can very often be inversely related. The next high correlation can be found between the AO capacity, as measured by both FRAP and PM (Table 3). It is also interesting to note that whereas both FRAP and PM correlate very well to the concentration of carotenoids (being important antioxidants), and to a lesser extent to the concentration of chlorophyll *a*, only FRAP correlated significantly to TPC (Table 3). An interesting tendency was that φ_{Ro}, a measure for the redox state of end acceptors of PSI [5] was highly correlated to the concentration of all photosynthetic pigments.

Conclusion. The information presented in this article shows some characteristics of chickpea varieties, which can be used in the selection of newer cultivars. There is a substantial difference in the photosynthetic activity not only between the varieties, but also between the two measurements, in April and May. In addition, measurements in May were most probably affected by the temperature decrease. Whereas most varieties showed a decrease in photosynthesis due to this weather change, a small amount of these cultivars actually increased their photosynthetic activity and could be candidates for a more detailed and advanced investigation. The following cultivars stand out as both early blooming and possessing higher photosynthesis in May: "Krajova z Kralovej" (5), "Blanco Lechoso" (13), "Garbanzo 1" (12), and "Caqui" (14). The analysis of the AO capacity and the content of phenols and pigments also shows diversity among the different cultivars of chickpea.

The method of prompt chlorophyll fluorescence can be used as a fast and reliable tool to study plant physiology earlier in the development, as compared with the traditional methods. It allows not only to detect cultivars with higher metabolic activity, but can be used to discern between resistant and sensitive to stress varieties. However, photosynthesis is often more closely related to the accumulation of raw biomass, rather than the seed yield, thus it should be used carefully in the selection process.

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