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GENOTYPIC SPECIFICITY OF SOYBEAN [*Glycine max* (L.) MERR.] PLASTID PIGMENTS CONTENT UNDER SOWING DATE AND INTERROW SPACING

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Soybean [*Glycine max* (L.) Merr.] is a legume and the second most planted crop after maize in worldwide. The objective of this study was to evaluate the change in the total plastid pigments content in three soybean varieties depending on the date of sowing and the row spacing was studied during four consecutive years (2018-2021). The Avigea, Isidor and Richi varieties, sown on three dates (I - 28 March - 05 April; II - 19-24 April; III - 09-14 May) at row spacing of 25, 45 and 70 cm were studied in field conditions. The plastid pigments (chlorophyll a, chlorophyll b, carotenoids) was determined in fresh plant samples in the beginning of pod formation stage. The total content of plastid pigments and the chlorophyll a/chlorophyll b and chlorophyll a + chlorophyll b/carotenoids ratios were calculated. The total plastid pigments content varied by years and varieties depending on the date of sowing and row spacing. The date of sowing was found as a factor with stronger effect on the plastid pigments content compared to the row spacing. On average for the period it was found that the total plastid pigments content in the Avigea variety decreased compared to the first date of sowing at all three row spacing, most pronounced at 45 cm. The reduction reached 25.59% on the second date and up to 19.02% on the third sowing date. The strongest effect of the date of sowing on the plastid pigments content was found in the Richi variety on the third date of sowing, where the increase was up to 64.33% at a row spacing of 25 cm and up to 36.02% at a row spacing of 70 cm compared to the first date. The row spacing factor had a smaller effect - for Avigea variety a decrease of 12.31% was reported on the second sowing date and a row spacing of 45 cm compared to the 70 cm accepted row spacing and for Richi variety by

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15.79% on the first date and row spacing 45 cm. The chlorophyll a/chlorophyll b ratio decreased at a row spacing of 25 at the second (for Isidor and Richi) and third sowing dates (for Avigea and Isidor).

Key words: soybean, plastid pigments, sowing date, row spacing, genotypic specificity, crop quality

INTRODUCTION

Soybean [*Glycine max* (L.) Merr.] is an annual grain legume with many uses - for feed, food, industrial, medicinal and environmental purposes. It is a legume and the second most planted crop after maize in worldwide. Soybean is grown in more than 35 countries around the world and dominates international markets as a major protein and oilseed crop. Its ability to absorb nitrogen from the air based on nitrogen fixation makes it an indispensable predecessor to major crops and an important source of free nitrogen and energy (KING and PURCELL, 2001). About 60% of the required nitrogen is obtained biologically by nodule bacteria (THIÉBEAU *et al.*, 2003), and under optimal conditions it can fix up to 450 kgN ha^{-1} (GILLER, 2001).

Soybean is a self-pollinated species with less than one per cent out-crossing. Breeders attempt several types of crosses between varieties or germplasm lines to alter gene frequency in the breeding population via gene recombination. Breeders choose methods which help in simultaneous improvement of yield and component traits. In soybean, traditional breeding methods involving hybridization and phenotypic selection are responsible for all the genetic gain in yield (CORYELL *et al.*, 1999). Many breeding methods of generation advance viz. bulk, pedigree, single seed descent, early generation testing and their modifications have been proposed and used for soybean improvement. Efficiency of these methods has been compared based on generation of superior lines (KHOSLA *et al.*, 2019). Assessment of genetic variability through molecular markers retained in the populations advanced by different methods can be used to compare the efficiency of breeding methods (KERVELLA and FOUILOUX, 1992; KHOSLA *et al.,* 2022). Identification of stable sources for breeding for important agronomic traits is prerequisite for providing a continuous and long-term progress in breeding (PERIĆ *et al.*, 2021).

Soybeans are also one of the few plants that have a lot of important of amino acids in their protein compositions to be considered "complete" proteins, on par with meats, milk products, and eggs (POPOVIC *et al.*, 2016; 2019a; 2020). The interest in this crop is justified (POPOVIC *et al.*, 2019b).

The process of photosynthesis is one of the most important factors determining productivity. Through this process, green plants accumulate organic matter and energy (SMIRNOVA, 2013). The absorption and transformation of solar energy is carried out by photosynthetic pigments - chlorophyll a and b, and carotenoids. Chlorophyll a and chlorophyll b are essential components of chlorophyll and responsible capturing the solar energy required for photosynthesis. Thus they are essential components for plant growth. Both are the main components of chlorophyll and affect the capacity and speed of its photosynthetic activities. Although both are active components, chlorophyll a has significant potential for light binding, energy acquisition and sugar production, especially in photosystem I and photosystem II (SARIEVA *et al*., 2010). Carotenoids has a role in photoprotection (LI RUI *et al*., 2014). They perform a protective function against chlorophyll photooxidation and prevent destructive photooxidation of organic compounds of the protoplasm in the presence of free oxygen (GILMORE and GOVINDJEE, 1999).

The plastid pigments content in the leaf mass is an indicator of the reaction of plants to changes in the environment and their adaptation to environmental conditions (TITOVA, 2010; NURMAKOVA, 2013; SMIRNOVA, 2013). YOKOYA *et al.* (2007) and ZHAO *et al.* (2016) reported that these photosynthetic pigments are responsible for collecting and transmitting absorbed light to photosynthetic reaction centres, and their concentration is linked to the effectiveness of photosynthesis. In addition, according to ZHAO *et al.* (2016), increased content of these pigments may be one of the factors increasing photosynthetic activity. Synthesis of plastid pigments is of great importance for photosynthetic activity of plants (YAO *et al.,* 2017).

There are many factors that affect the activity of photosynthesis. In the present study, we aimed to determine the total plastid pigments content of three soybean varieties depending on the date of sowing and row spacing.

MATERIAL AND METHODS

The study was conducted on the experimental field of the Experimental Station on Soybean and Cereals – Pavlikeni (43º23'N, 25º32'E, 144 m above sea level), Bulgaria during four consecutive years (2018-2021) under non-irrigated conditions and without the use of herbicides. The object of study were the soybean varieties Avigea (Bg) - early, Richi (Bg) – mid-early and Isidor (Euralis) - early. Three terms of sowing in 20 days were applied: first date - early (March 28-April 5); second date - optimal (April 19-24) and third date - late (May 9-14). Size of the experimental plot was 40 m2, the harvest plot 5 m2 in 3 replications. In the variants with 70 cm row spacing 4 rows of 30 seeds per 1 m^2 were sown, in the variants with 45 cm row spacing 6 rows of 45 seeds per 1 m^2 were sown and in the variants with 25 cm row spacing 8 rows of 55 seeds were sown.

The plastid pigments content (chlorophyll a, chlorophyll b, carotenoids) (mg/100 g FW), the total plastid pigments content ([chlorophyll a + chlorophyll b) + carotenoids)] was determined in fresh plant samples by the method of ZELENSKY and MOGILEVA (1980) and chlorophyll a/chlorophyll b, (chlorophyll a + chlorophyll b)/carotenoid ratios were calculated. The samples were taken in the beginning pod formation stage as follows: in 2018 on 13.06. of the variants with the first date of sowing, on 26.06. of those with a second sowing date and on 13.07. of the variants with a third sowing date; in 2019 on 02.07. of the variants with first and second sowing dates, and on 17.07. of those with a third sowing date; in 2020 on 25.06. of the variants with first and second sowing dates and on 06.07. of those with a third sowing date; in 2021 on 30.06. of the variants with first and second sowing dates, and on 20.07. of those with a third sowing date. The experimental data are presented by years and averaged for the four experimental years. The analysis of variance (average, standard deviation, minimum, maximum) were applied with the SPSS 20.0 statistical program for Windows. Peason's correlation coefficient was calculated for the most important traits.

RESULTS AND DISCUSSION

The study period is characterized by two consecutive moderately wet years (2018 and 2019) and two consecutive dry years (2020 and 2021) (Table 1). The first two experimental years (2018 and 2019) according to the precipitation for the period April-September are characterized as moderately wet with precipitation above the norm and with a favorable distribution by months. In April, May and August of 2018 they are much less than the norm, but in June and July, when the beginning of flowering - early ripening (R1-R7) period - critical in terms of water consumption, they are well above the norm.

Table 1. Agro meteorological conditions for the soybean vegetation period of study (2018-2021)

Rainfall, mm	April	May	June	July	August	September	Sum	Difference
2018	10.3	44.3	134.6	126.6	12.6	49.2	377.6	+23.5
2019	76.0	45.8	130.4	76.0	8.0	2.4	338.6	-15.2
2020	22.0	42.4	103.8	6.6	33.6	21.0	229.4	-123.4
2021	51.8	104.2	65.7	10.1	26.4	23.6	281.8	-70.4
Av. for 126 yrs.	43.8	70.2	80.8	67.5	48.7	41.4	352.4	0.0
Air temp. °C								
2018	16.6	19.4	21.5	22.6	23.5	19.3	3756.1	+268.0
2019	12.4	17.1	22.0	22.5	24.3	19.9	3608.5	+118.3
2020	12.0	17.0	20.3	23.6	24.5	21.3	3626.1	+136.0
2021	10.3	17.9	20.8	24.4	24.8	18.6	3573.2	+81.6
Av. for 56 yrs.	12.0	16.5	22.4	22.6	22.5	18.1	3491.6	0.0

In the second experimental year in April, June and July the precipitation was above the norm, and in May - significantly below the norm. The beginning flowering - early ripening period took place with very good moisture supply and normal temperatures in June and July.

The years 2020 and 2021 are characterized by precipitation well below the norm and with an unfavorable distribution by months. Only in June 2020 they are above the norm, and in the other five months they are well below the norm, especially in July. In 2021, the period of beginning flowering - beginning of ripening (R1-R7) took place with reduced moisture supply. The average daily temperatures in the four years of study were higher in July, September and especially in May and August, only for June they are slightly lower.

Leaf chlorophyll pigment contents were different between the years and cultivars.

First experimental year, 2018

The data for the first experimental year are presented in Table 2. The total plastid pigments content at the first sowing date was highest at a row spacing of 45 cm for the Avigea (353.07 mg 100^{-1} g FW) and Isidor (308.67 mg 100^{-1} g FW) varieties.

On average for the varieties studied the ranking was as follows: Avigea> Isidor> Richi, with almost no difference between the first two varieties.

On the second sowing date, there was the same tendency. The highest total plastid pigments content at a row spacing of 45 cm was found for the Avigea (322.92 mg 100^{-1} g FW) and Isidor (372.81 mg 100^{-1} g FW) varieties. On average, the differences between the varieties studied were found small. On the third sowing date, the highest total plastid pigments content was reported. The highest values at a row spacing of 25 cm for all varieties were recorded. We found a higher content of chlorophyll and carotenoids at the smaller line spacing, at which the shading of plants was greater.

Probably there is a compensatory mechanism of plants in the later sowing with a higher content of plastid pigments, due to the larger photosynthetic area. In wide-row crops, the temperature in the area occupied by plants is higher, which doesn't favor the synthesis of plastid pigments. Our results are in agreement with the results obtained by FEN *et al*. (2019). They reveal that soybean leaves under shading treatment exhibited increased chlorophyll and carotenoid contents per unit mass. In the first experimental year as a whole the total content of plastid pigments for all varieties was lowest on the first sowing date, but the values for the second and third dates were close.

Second experimental year, 2019

In the second experimental year, the total plastid pigments content on the first sowing date was highest at 45 cm row spacing for Avigea (444.54 mg 100^{-1} g FW) and Richi (347.32 mg 100^{-1} g FW), Table 3. In general, the data varied greatly. The varieties according to the values (average data) of this indicator were arranged as follows: Avigea> Richi> Isidor.

On the second sowing date, the total plastin pigments content was highest at a row spacing of 70 cm for the varieties Avigea (419.76 mg 100^{-1} g FW) and Richi (420.80 mg 100^{-1} g FW). On the third date of sowing, the total content of plastid pigments was higher as a whole, and the variation of the data was stronger. The Avigea (517.64 mg 100^{-1} g FW) and Richi (658.45 mg 100^{-1} g FW) varieties showed the highest total content of plastid pigments at a row spacing of 25 cm. On average for the varieties they were arranged in the next order: Richi> Isidor> Avigea.

The total content of plastid pigments for all varieties was lowest on the first sowing date and increased with the advancing sowing dates.

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The total content of plastid pigments for all varieties was lowest on the first sowing date and increased with the advancing sowing dates.

Table 2. Plastid pigments content, (mg 100^{-1} g FW), 2018

Variants	Cl a	Cl b	Cl a+b	Carotin	(Cl a+b)+Car	Cla/Clb	(Cl a+b)/Car
First date (April 05)							
Avigea, 25 cm	132.45	150.30	282.75	31.41	314.16	0.8812	9.0019
Avigea, 45 cm	143.52	186.24	329.76	23.31	353.07	0.7706	14.1467
Avigea, 70 cm	93.27	130.68	223.95	18.42	242.37	0.7137	12.1580
Average	123.08	155.74	278.82	24.38	303.20	0.7885	11.7689
Izidor, 25 cm	124.80	141.00	265.80	22.86	288.66	0.8851	11.6273
Izidor, 45 cm	134.40	143.13	277.53	31.14	308.67	0.9390	8.9123
Izidor, 70 cm	111.57	129.36	240.93	21.09	262.02	0.8625	11.4239
Average	123.59	137.83	261.42	25.03	286.45	0.8955	10.6545
Richi, 25 cm	112.74	114.48	227.22	26.28	253.50	0.9848	8.6461
Richi, 45 cm	106.23	124.17	230.40	26.70	257.10	0.8555	8.6292
Richi, 70 cm	115.41	168.57	283.98	27.57	311.55	0.6846	10.3003
Average	111.46	135.74	247.20	26.85	274.05	0.8417	9.1919
STDEV	14.18	20.66	31.79	3.94	33.20	0.0926	1.7681
Max	143.52	186.24	329.76	31.41	353.07	0.9848	14.1467
Min	93.27	114.48	223.95	18.42	242.37	0.6846	8.6292
Second date (April 24)							
Avigea, 25 cm	130.92	153.87	284.79	31.98	316.77	0.8508	8.9053
Avigea, 45 cm	127.41	163.29	290.70	32.22	322.92	0.7803	8.0109
Avigea, 70 cm	129.87	132.75	262.62	35.67	298.29	0.9783	6.9112
Average	129.40	149.97	279.37	33.29	312.66	0.8698	7.9424
Izidor, 25 cm	125.16	139.44	264.60	33.03	297.63	0.8976	9.0223
Izidor, 45 cm	159.69	171.81	331.50	41.31	372.81	0.9295	8.0247
Izidor, 70 cm	138.69	140.07	278.76	35.43	314.19	0.9901	5.5442
Average	141.18	150.44	291.62	36.59	328.21	0.9391	7.5304
Richi, 25 cm	141.54	159.51	301.05	43.56	344.61	0.8873	7.3625
Richi, 45 cm	146.91	135.51	282.42	50.94	333.36	1.0841	7.8679
Richi, 70 cm	131.25	121.83	253.08	50.10	303.18	1.0773	5.0515
Average	139.90	138.95	278.85	48.20	327.05	1.0163	6.7606
STDEV	10.31	14.76	21.19	6.95	22.24	0.0930	1.2384
Max	159.69	171.81	331.5	50.94	372.81	1.0841	9.0223
Min	125.16	121.83	253.08	31.98	297.63	0.7803	5.0515
Third date (May 14)							
Avigea, 25 cm	137.13	189.09	326.22	24.27	350.49	0.7252	13.4413
Avigea, 45 cm	99.18	118.59	217.77	26.85	244.62	0.8363	8.1106
Avigea, 70 cm	113.64	132.93	246.57	28.98	275.55	0.8549	8.5083
Average	116.65	146.87	263.52	26.70	290.22	0.8055	10.0201
Izidor, 25 cm	178.20	238.40	416.60	45.00	461.60	0.7475	9.2578
Izidor, 45 cm	138.81	184.77	323.58	36.66	360.24	0.7513	8.8265
Izidor, 70 cm	105.96	131.76	237.72	29.52	267.24	0.8042	8.0528
Average	140.99	184.977	325.967	37.06	363.03	0.7676	8.7124
Richi, 25 cm	150.84	180.36	331.20	35.92	367.12	0.8363	9.2205
Richi, 45 cm	99.87	115.50	215.37	27.36	242.73	0.8647	7.8717
Richi, 70 cm	116.55	136.08	252.63	34.83	287.46	0.8565	7.2532
Average	122.42	143.98	266.40	32.70	299.10	0.8525	8.1151
STDEV	24.44	38.23	62.34	6.25	67.10	0.0496	1.6505
Max	178.20	238.40	416.60	45.00	461.60	0.8647	13.4413
Min	99.18	115.50	215.37	24.27	242.73	0.7252	7.2532

Table 3. Plastid pigments content, (mg 100^{-1} g FW), 2019

Variants	Cl a	Cl b	Cl a+b	Carotin	(Cl a+b)+Car	Cla/Clb	(Cl a+b)/Car
			First date (March 28)				
Avigea, 25 cm	163.68	179.76	343.44	39.36	382.80	0.911	8.726
Avigea, 45 cm	196.35	241.45	437.80	72.35	510.15	0.813	6.051
Avigea, 70 cm	185.72	202.56	388.28	52.40	440.68	0.917	7.410
Average	181.92	207.92	389.84	54.70	444.54	0.880	7.396
Izidor, 25 cm	169.04	201.88	370.92	48.76	419.68	0.837	7.607
Izidor, 45 cm	105.75	131.22	236.97	42.12	279.09	0.806	5.626
Izidor, 70 cm	110.49	176.04	286.53	21.09	307.62	0.628	13.586
Average	128.43	169.71	298.14	37.32	335.46	0.757	8.940
Richi, 25 cm	111.93	132.54	244.47	38.28	282.75	0.844	6.386
Richi, 45 cm	130.76	173.40	304.16	43.16	347.32	0.754	7.047
Richi, 70 cm	114.63	139.86	254.49	34.92	289.41	0.820	7.288
Average	119.11	148.60	267.71	38.79	306.49	0.806	6.907
STDEV	34.36	34.50	67.22	13.12	77.92	0.082	2.159
Max	196.35	241.45	437.80	72.35	510.15	0.917	13.586
Min	105.75	131.22	236.97	21.09	279.09	0.628	5.626
			Second date (April 19)				
Avigea, 25 cm	140.28	154.60	294.88	30.93	325.81	0.907	9.534
Avigea, 45 cm	112.08	186.72	298.80	43.76	342.56	0.600	6.828
Avigea, 70 cm	157.52	219.12	376.64	43.12	419.76	0.719	8.735
Average	136.63	186.81	323.44	39.27	362.71	0.742	8.366
Izidor, 25 cm	201.88	239.72	441.60	50.48	492.08	0.842	8.748
Izidor, 45 cm	143.60	165.36	308.96	43.76	352.72	0.868	7.060
Izidor, 70 cm	200.96	209.48	410.44	57.88	468.32	0.959	7.091
Average	182.15	204.85	387.00	50.71	437.71	0.890	7.633
Richi, 25 cm	152.10	171.96	324.06	44.85	368.91	0.885	7.225
Richi, 45 cm	143.60	165.36	308.96	45.52	354.48	0.868	6.787
Richi, 70 cm	169.00	200.12	369.12	51.68	420.80	0.844	7.142
Average	154.90	179.15	334.05	47.35	381.40	0.866	7.052
STDEV	27.94	26.11	49.89	7.12	55.31	0.103	0.942
Max	201.88	239.72	441.60	57.88	492.08	0.959	9.534
Min	112.08	154.60	294.88	30.93	325.81	0.600	6.787
			Third date (May 09)				
Avigea, 25 cm	206.24	259.00	465.24	52.40	517.64	0.796	8.879
Avigea, 45 cm	215.85	215.95	431.80	43.25	475.05	1.000	9.984
Avigea, 70 cm	212.65	257.30	469.95	26.45	496.40	0.826	17.767
Average	211.58	244.08	455.66	40.70	496.36	0.874	12.210
Izidor, 25 cm	181.95	206.50	388.45	46.60	435.05	0.881	8.336
Izidor, 45 cm	220.10	237.30	457.40	67.55	524.95	0.928	6.771
Izidor, 70 cm	239.10	308.15	547.25	56.65	603.90	0.776	9.660
Average	213.72	250.65	464.37	56.93	521.30	0.862	8.256
Richi, 25 cm	282.00	301.30	583.30	75.15	658.45	0.936	7.762
Richi, 45 cm	199.05	210.55	409.60	55.30	464.90	0.945	7.407
Richi, 70 cm	287.45	268.90	556.35	77.90	634.25	1.069	7.142
Average	256.17	260.25	516.42	69.45	585.87	0.983	7.437
STDEV	32.85	33.62	61.74	15.25	71.84	0.088	3.157
Max	287.45	308.15	583.30	77.90	658.45	1.069	17.767
Min	181.95	206.50	388.45	26.45	435.05	0.776	6.771

Third experimental year, 2020

In the third experimental year, the total plastin pigments content on the first sowing date was highest at 45 cm row spacing for Avigea (487.56 mg 100^{-1} g FW) and Richi (457.24 mg 100^{-1} g FW) (Table 4). It decreases with advancing sowing dates. The varieties according to the values of this indicator (average data) were arranged as follows: Avigea> Isidor> Richi.

On the second sowing date, the total plastid pigments content was highest at a row spacing of 45 cm for the Isidor (465.48 mg 100^{-1} g FW) and Richi (399.45 mg100^{-1} g FW) varieties.

On the third sowing date, the total plastin pigment content was highest at a row spacing of 70 cm for the varieties Avigea (417.72 mg100^{-1} g FW) and Richi (668.25 mg100^{-1} g FW). It increased with advancing sowing dates. On average for the varieties they were arranged: Richi> Isidor> Avigea. The total content of plastid pigments for all varieties was highest on the third sowing date.

Fourth experimental year, 2021

The total content of plastid pigments at the first sowing date was highest at a row spacing of 45 cm for Isidor (473.00 mg100^{-1} g FW) and Richi (433.89 mg100^{-1} g FW) varieties, Tab. 5.

The strongest variation was observed and the varieties (average data) were arranged as follows: Avigea> Isidor> Richi. At the second sowing date, the values for total plastid pigments content were highest at a row spacing of 25 cm for Avigea (384.03 mg100^{-1} g FW) and Isidor (396.92 mg100^{-1} g FW).

On the third sowing date, the total content of plastid pigments was found the lowest, and the variation of the data was greater. The highest content of plastid pigments was highest in the Richi variety - respectively when sowing at 70 cm (448.96 mg100^{-1} g FW). On average for the varieties they were arranged: Richi> Avigea> Isidor.

In our study, we found a higher total content of plastid pigments in years with less rainfall. The total content of plastid pigments decreased from the first to the third date. Our findings are in agreement with the findings of KELEŞ and ÖNCEL (2002).

They also indicated increased carotenoid contents under different stress conditions. In our study there is the same tendency found. An opposite of our findings, several researchers found that the stress factors as warm and dry weather conditions adversely affect the chlorophyll content, and a lower chlorophyll content was reported (FERRI *et al.,* 2004; FRITSCHI and RAY, 2007; MAKBUL *et al.,* 2011). DEMIR (2021) consider the chlorophyll a, b and total chlorophyll contents differed between the years, and were found lower when the precipitation was lower, and the temperature was higher.

Averaged for the period of study 2018-2021

Data on plastid pigments content on average for the period are presented in Table 6. The total plastin pigments content on the first sowing date on average of the cultivars studied was highest in the Avigea variety (411.858 mg100^{-1} g FW), with a row spacing of 45 cm, a value of 435.038 mg100^{-1} g FW was reported. The varieties according to the values of this indicator were arranged as follows: Avigea> Isidor> Richi. It is noticed that in all three varieties the highest total plastid pigments content was reported when sowing at a row spacing of 45 cm.

The Pearson's coefficient between chlorophyll a and chlorophyll b was found r=0.703 and between chlorophyll a+ chlorophyll b and carotenoids r=0.9096, respectively. Similar to our findings, many researchers reported that photosynthetic pigments have significant correlations with each other and the increase in pigment content had a positive effect on photosynthesis rate (NOURIYANI *et al.*, 2012; JAN *et al.*, 2013).

At the second sowing date, the values for total plastid pigments content were similar for the varieties Richi (348.304 mg100^{-1} g FW) and Avigea (346.458 mg100^{-1} g FW). Higher values on average for the varieties were marked by the total plastid pigments content in the variety Isidor (386.704 mg100^{-1} g FW), Table 6.

The highest total content of plastid pigments was found in the Isidor, sown at a row spacing of 45 cm (394.743 mg100^{-1} g FW) - a trend found on the first sowing date in terms of row spacing. Two of the varieties viz. Avigea and Richi had the highest total plastid pigments content at a row spacing of 70 cm.

On the third date of sowing, as we noted, the total content of plastid pigments was higher in general, and the variation of the data was greater. The highest content of plastid pigments was highest in the Richi variety - respectively when sowing at 25 cm (456.298 mg100^{-1} g FW). On average for the varieties they were arranged: Richi> Isidor> Avigea. In all three varieties, the highest total content of plastid pigments was reported at a row spacing of 25 cm. It is probably a result of the compensatory ability of soybeans, as well as the more active photosynthetic activity at this row spacing, due to the formed denser leaf area and more efficient use of light. The photosynthetic potential is highly variable by variety and age.

The photosynthetic activity of plants in the crop is closely dependent on the size and duration of activity work on the leaf area. With optimal leaf area, plants use solar radiation effectively, and the vitality of the leaves is preserved for a longer time. The total content of plastid pigments for all varieties was highest on the third sowing date.

We also determined some plastid pigments ratios. It is believed that the chlorophyll a/chlorophyll b ratio and the chlorophyll a + b/carotenoids ration indicate the physiological status of plants (PETKOVA and PORYAZOV, 2007). The chlorophyll a/chlorophyll b ratio is a relatively constant value and is considered to be genetically determined (TITOVA, 2010). This ratio is related to the activity of the basic chlorophyll a. In our study, we found a decrease in the chlorophyll a/chlorophyll b ratio at a row spacing of 25 on the second and third sowing dates. On the second date for the Isidor and Richi varieties and on the third date of sowing for the Avigea and Isidor varieties. Similar results were obtained by YAO *et al.* (2017) and JOVANOVIĆ-TODOROVIĆ *et al.* (2020). According to the authors, the reduction of chlorophyll a/chlorophyll b ratio in shade were most likely due to changes in the organization of both light-intercepting and electron transport components.

Table 4. Plastid pigments content, (mg 100^{-1} g FW), 2020

Variants	Cl a	Cl b	Cl a+b	Carotin	(Cl a+b)+Car	Cla/Clb	(Cl a+b)/Car
First date (March 30)							
Avigea, 25 cm	179.70	179.04	358.74	57.39	416.13	1.004	6.251
Avigea, 45 cm	164.52	263.20	427.72	59.84	487.56	0.625	7.148
Avigea, 70 cm	156.00	245.32	401.32	46.24	447.56	0.636	8.679
Average	166.74	229.19	395.93	54.49	450.42	0.755	7.359
Izidor, 25 cm	214.52	176.68	391.20	72.24	463.44	1.214	5.415
Izidor, 45 cm	174.44	225.12	399.56	56.88	456.44	0.775	7.025
Izidor, 70 cm	111.44	186.84	298.28	46.28	344.56	0.596	6.445
Average	166.80	196.21	363.01	58.47	421.48	0.862	6.295
Richi, 25 cm	114.21	140.10	254.31	40.02	294.33	0.815	6.355
Richi, 45 cm	158.68	242.00	400.68	56.56	457.24	0.656	7.084
Richi, 70 cm	144.56	184.72	329.28	52.44	381.72	0.783	6.279
Average	139.15	188.94	328.09	49.67	377.76	0.751	6.573
STDEV	28.96	37.23	52.33	8.53	58.15	0.184	0.845
Max	214.52	263.20	427.72	72.24	487.56	1.214	8.679
Min	111.44	140.10	254.31	40.02	294.33	0.596	5.415
Second date (April 19)							
Avigea, 25 cm	133.98	177.27	311.25	47.97	359.22	0.756	6.488
Avigea, 45 cm	124.86	163.32	288.18	43.77	331.95	0.765	6.584
Avigea, 70 cm	135.48	156.09	291.57	59.61	351.18	0.868	4.891
Average	131.44	165.56	297.00	50.45	347.45	0.796	5.988
Izidor, 25 cm	131.76	174.45	306.21	46.68	352.89	0.755	6.560
Izidor, 45 cm	172.88	227.76	400.64	64.84	465.48	0.759	6.179
Izidor, 70 cm	133.17	185.85	319.02	53.25	372.27	0.717	5.991
Average	145.94	196.02	341.96	54.92	396.88	0.744	6.243
Richi, 25 cm	129.60	169.53	299.13	62.79	361.92	0.764	4.764
Richi, 45 cm	154.10	183.50	337.60	61.85	399.45	0.840	5.458
Richi, 70 cm	112.53	170.16	282.69	53.70	336.39	0.661	5.264
Average	132.08	174.40	306.47	59.45	365.92	0.755	5.162
STDEV	15.95	19.69	34.00	7.02	38.24	0.056	0.659
Max	172.88	227.76	400.64	64.84	465.48	0.868	6.584
Min	112.53	156.09	282.69	43.77	331.95	0.661	4.764
Third date (May 08)							
Avigea, 25 cm	135.57	187.89	323.46	37.68	361.14	0.722	8.584
Avigea, 45 cm	106.26	175.68	281.94	33.87	315.81	0.605	8.324
Avigea, 70 cm	139.80	225.32	365.12	52.60	417.72	0.620	6.941
Average	127.21	196.30	323.51	41.38	364.89	0.649	7.950
Izidor, 25 cm	171.16	244.12	415.28	53.60	468.88	0.701	7.748
Izidor, 45 cm	116.82	158.97	275.79	28.47	304.26	0.735	9.687
Izidor, 70 cm	204.44	228.36	432.80	57.16	489.96	0.895	7.572
Average	164.14	210.48	374.62	46.41	421.03	0.777	8.336
Richi, 25 cm	181.96	234.52	416.48	55.72	472.20	0.776	7.475
Richi, 45 cm	244.60	334.50	579.10	89.15	668.25	0.731	6.496
Richi, 70 cm	143.12	240.68	383.80	39.52	423.32	0.595	9.712
Average	189.89	269.90	459.79	61.46	521.26	0.701	7.894
STDEV	41.01	46.66	84.93	16.40	100.85	0.090	1.009
Max	244.60	334.50	579.10	89.15	668.25	0.895	9.712
Min	106.26	158.97	275.79	28.47	304.26	0.595	6.496

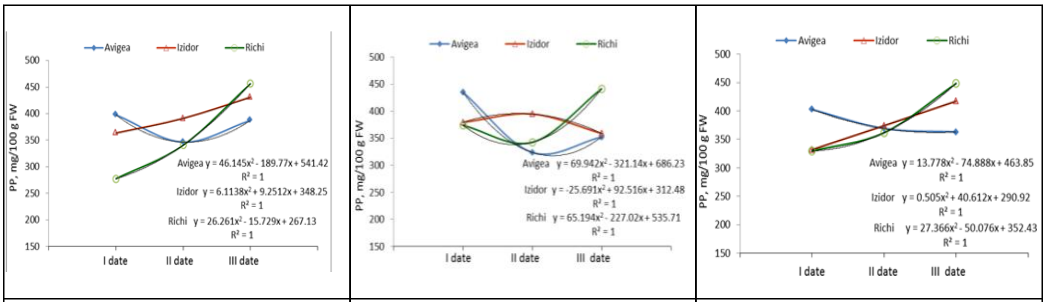
Table 5. Plastid pigments content, (mg/100 g FW), 2021

Variants	Cl a	Cl b	Cl a+b	Carotin	(Cl a+b)+Car	Cla/Clb	(Cl a+b)/Car
First date (April 01)							
Avigea, 25 cm	162.92	259.56	422.48	55.60	478.08	0.628	7.599
Avigea, 45 cm	125.55	219.93	345.48	43.89	389.37	0.571	7.871
Avigea, 70 cm	140.60	258.36	398.96	81.40	480.36	0.544	4.901
Average	143.02	245.95	388.97	60.30	449.27	0.581	6.790
Izidor, 25 cm	104.40	146.88	251.28	31.38	282.66	0.711	8.008
Izidor, 45 cm	165.00	250.24	415.24	57.76	473.00	0.659	7.189
Izidor, 70 cm	147.96	219.00	366.96	47.00	413.96	0.676	7.808
Average	139.12	205.37	344.49	45.38	389.87	0.682	7.668
Richi, 25 cm	88.32	163.48	251.80	28.28	280.08	0.540	8.904
Richi, 45 cm	148.08	239.97	388.05	45.84	433.89	0.617	8.465
Richi, 70 cm	121.24	185.92	307.16	29.04	336.20	0.652	10.577
Average	119.21	196.46	315.67	34.39	350.06	0.603	9.315
STDEV	23.51	38.58	60.29	15.73	73.54	0.058	1.388
Max	165.00	259.56	422.48	81.40	480.36	0.711	10.577
Min	88.32	146.88	251.28		280.08	0.540	4.901
Second date (April 23)							
Avigea, 25 cm	127.72	219.40	347.12	36.92	384.04	0.582	9.402
Avigea, 45 cm	113.24	176.80	290.04	32.72	322.76	0.640	8.864
Avigea, 70 cm	135.92	180.48	316.40	44.80	361.20	0.753	7.063
Average	125.63	192.23	317.85	38.15	356.00	0.659	8.443
Izidor, 25 cm	126.80	223.52	350.32	46.60	396.92	0.567	7.518
Izidor, 45 cm	159.96	182.52	342.48	45.48	387.96	0.876	7.530
Izidor, 70 cm	121.28	168.72	290.00	32.72	322.72	0.719	8.863
Average	136.01	191.59	327.60	41.60	369.20	0.721	7.970
Richi, 25 cm	103.04	215.92	318.96	14.80	333.76	0.477	21.551
Richi, 45 cm	109.72	157.96	267.68	34.00	301.68	0.695	7.873
Richi, 70 cm	138.08	201.00	339.08	47.52	386.60	0.687	7.136
Average	116.95	191.63	308.57	32.11	340.68	0.620	12.187
STDEV	15.71	21.33	26.69	9.44	32.05	0.106	4.136
Max	159.96	223.52	350.32	47.52	396.92	0.876	21.551
Min	103.04	157.96	267.68	14.80	301.68	0.477	7.063
Third date (May 12)							
Avigea, 25 cm	125.40	157.59	282.99	37.41	320.40	0.796	7.565
Avigea, 45 cm	136.83	196.71	333.54	40.17	373.71	0.696	8.303
Avigea, 70 cm	112.24	112.20	224.44	38.64	263.08	1.000	5.808
Average	124.82	155.50	280.32	38.74	319.06	0.831	7.225
Izidor, 25 cm	134.79	184.95	319.74	38.82	358.56	0.729	8.236
Izidor, 45 cm	81.63	134.43	216.06	29.70	245.76	0.607	7.275
Izidor, 70 cm	119.56	146.16	265.72	42.40	308.12	0.818	6.267
Average	111.99	155.18	267.17	36.97	304.15	0.718	7.259
Richi, 25 cm	126.18	155.82	282.00	45.42	327.42	0.810	6.209
Richi, 45 cm	143.68	206.04	349.72	40.00	389.72	0.697	8.743
Richi, 70 cm	150.44	240.24	390.68	58.28	448.96	0.626	6.704
Average	140.10	200.70	340.80	47.90	388.70	0.711	7.218
STDEV	18.63	36.15	52.46	7.02	57.71	0.110	0.940
Max	150.44	240.24	390.68	58.28	448.96	1.000	8.743
Min	81.63	112.20	216.06	29.70	245.76	0.607	5.808

Table 6. Plastid pigments content, average for the period (mg100^{-1} g FW)

Variants	Cl a	Cl b	Cl a+b	Carotin	(Cl a+b)+Car	Cla/Clb	(Cl a+b)/Car
First date (28 March - 05 April)							
Avigea, 25 cm	159.688	192.165	351.853	45.940	397.793	0.831	7.659
Avigea, 45 cm	157.485	227.705	385.190	49.848	435.038	0.692	7.727
Avigea, 70 cm	143.898	209.230	353.128	49.615	402.743	0.688	7.117
Average	153.690	209.700	363.390	48.468	411.858	0.737	7.501
Izidor, 25 cm	153.190	166.610	319.800	43.810	363.610	0.919	7.300
Izidor, 45 cm	144.898	187.428	332.325	46.975	379.300	0.773	7.075
Izidor, 70 cm	120.365	177.810	298.175	33.865	332.040	0.677	8.805
Average	139.484	177.283	316.767	41.550	358.317	0.790	7.726
Richi, 25 cm	106.800	137.650	244.450	33.215	277.665	0.776	7.360
Richi, 45 cm	135.938	194.885	330.823	43.065	373.888	0.698	7.682
Richi, 70 cm	123.960	169.768	293.728	35.993	329.720	0.730	8.161
Average	122.233	167.434	289.667	37.424	327.091	0.735	7.734
STDEV	16.899	24.654	38.675	6.132	44.372	0.073	0.495
Max	159.688	227.705	385.190	49.848	435.038	0.919	8.805
Min	106.800	137.650	244.450	33.215	277.665	0.677	7.075
Second date (19-24 April)							
Avigea, 25 cm	133.225	176.285	309.510	36.950	346.460	0.756	8.376
Avigea, 45 cm	118.835	166.570	285.405	38.320	323.725	0.713	7.448
Avigea, 70 cm	142.615	178.800	321.415	47.773	369.188	0.798	6.728
Average	131.558	173.885	305.443	41.014	346.458	0.756	7.517
Izidor, 25 cm	146.963	200.245	347.208	43.995	391.203	0.734	7.892
Izidor, 45 cm	159.033	186.863	345.895	48.848	394.743	0.851	7.081
Izidor, 70 cm	150.580	174.890	325.470	48.698	374.168	0.861	6.684
Average	152.192	187.333	339.524	47.180	386.704	0.815	7.219
Richi, 25 cm	128.653	172.540	301.193	39.528	340.720	0.746	7.620
Richi, 45 cm	136.528	161.723	298.250	44.200	342.450	0.844	6.748
Richi, 70 cm	137.715	173.278	310.993	50.750	361.743	0.795	6.128
Average	134.298	169.180	303.478	44.826	348.304	0.795	6.832
STDEV	11.785	10.656	20.422	4.748	23.487	0.051	0.637
Max	159.033	200.245	347.208	50.750	394.743	0.861	8.376
Min	118.835	161.723	285.405	36.950	323.725	0.713	6.128
Third date (09-14 May)							
Avigea, 25 cm	151.085	198.393	349.478	37.940	387.418	0.762	9.211
Avigea, 45 cm	139.530	176.733	316.263	36.035	352.298	0.789	8.777
Avigea, 70 cm	144.583	181.938	326.520	36.668	363.188	0.795	8.905
Average	145.066	185.688	330.753	36.881	367.634	0.782	8.964
Izidor, 25 cm	166.525	218.493	385.018	46.005	431.023	0.762	8.369
Izidor, 45 cm	139.340	178.868	318.208	40.595	358.803	0.779	7.839
Izidor, 70 cm	167.265	203.608	370.873	46.433	417.305	0.822	7.987
Average	157.710	200.323	358.033	44.344	402.377	0.788	8.065
Richi, 25 cm	185.245	218.000	403.245	53.053	456.298	0.850	7.601
Richi, 45 cm	171.800	216.648	388.448	52.953	441.400	0.793	7.336
Richi, 70 cm	174.390	221.475	395.865	52.633	448.498	0.787	7.521
Average	177.145	218.708	395.853	52.879	448.732	0.810	7.486
STDEV	15.629	17.144	32.300	6.803	38.696	0.025	0.649
Max	185.245	221.475	403.245	53.053	456.298	0.850	9.211
Min	139.340	176.733	316.263	36.035	352.298	0.762	7.336

Comparing the total content of plastid pigments compared to the first date of sowing, the data showed that in the Avigea variety there is a decrease in the values for all three row spacing, with the strongest expression at 45 cm (Figure 1).



25 cm	45 cm	70 cm

Figure 1. Curves, prognostic equations and relationship between plastid pigments content and sowing dates and row spacing, average for the period

The reduction reached 25.59% on the second date and up to 19.02% on the third sowing date and a row spacing of 45 cm. In the other two varieties tested this trend was not observed. In the Richi variety, the total content of plastid pigments on the second date and a row spacing of 25 cm increased to 22.71%. The largest increase was reported on the third sowing date, where the increase compared to the first date was up to 64.33% at a row spacing of 25 cm and up to 36.02% at a row spacing of 70 cm. Although smaller differences compared to the first date were also reported for the Isidor variety. The total content of plastid pigments at a row spacing of 70 cm on the second date and third date was 12.69% and 25.68%, respectively higher than the same on the first date of sowing.

Regarding the effect of the row spacing on the indicators tested, the data showed that it was not large. In the case of Avigea variety a decrease of 12.31% was reported on the second sowing date and a row spacing of 45 cm compared to the accepted row spacing of 70 cm, and in the Richi variety by 15.79% on the first date and a row spacing of 45 cm.

CONCLUSIONS

The total plastid pigments content on the first sowing date (28 March - 05 April) on average of the varieties studied was highest in the Avigea variety (435.038 mg 100^{-1} g FW) at a row spacing of 45 cm. In all three varieties, the highest total content of plastid pigments was reported when sowing at a row spacing of 45 cm. On the second sowing date (19-24 April) the highest total content of plastid pigments in the varieties Avigea and Richi was reported at a row spacing of 70 cm. On the third sowing date (09-14 May) the total content of plastid pigments was higher in general, and the variation of the data was greater. In all three varieties, the highest total content of plastid pigments was reported at a row spacing of 25 cm, reaching 456.298 mg 100^{-1} g FW for the Richi variety.

The date of sowing had a stronger effect on the plastid pigments content compared to the row spacing. It was found that the total content of plastid pigments in the Avigea variety decreased at all three row spacing compared to the first date of sowing, most pronounced at 45 cm. The reduction reached 25.59% on the second sowing date and up to 19.02% on the third sowing date. In the Richi variety, the total content of plastid pigments on the second date and a row spacing of 25 cm increased to 22.71%. The strongest effect of the date of sowing on the content of plastid pigments was found on the third date of sowing, where the increase compared to the first date was up to 64.33% at a row spacing of 25 cm and up to 36.02% at a row spacing of 70 cm.

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GENOTIPSKA SPECIFIČNOST SOJE [*Glycine max* (L.) MERR.] ZA SADRŽAJ PLASTIDNIH PIGMENTA PRI RAZLIČITIM DATUMIMA SETVE I MEĐUREDNOG RAZMAKA

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Izvod

Soja [*Glycine max* (L.) Merr.] je mahunarka i druga najzastupljenija gajena biljka posle kukuruza u svetu. Cilj ovog istraživanja bio je da se proceni promena sadržaja ukupnih plastidnih pigmenata u tri sorte soje u zavisnosti od datuma setve i međurednog razmaka tokom četiri uzastopne godine (2018-2021). U poljskim uslovima proučavane su sorte Avigea, Isidor i Richi, posejane u tri roka (I - 28. mart - 05. april; II - 19-24. april; III - 09-14. maj) na razmaku redova 25, 45 i 70 cm. Plastidni pigmenti (hlorofil a, hlorofil b, karotenoidi) određivani su u svežim biljnim uzorcima u početku faze formiranja mahuna. Izračunati su ukupan sadržaj plastidnih pigmenata i odnos hlorofil a/hlorofil b i hlorofil a + hlorofil b/karotenoidi. Ukupan sadržaj plastidnih pigmenata varirao je po godinama i sortama u zavisnosti od datuma setve i međurednog razmaka. Datum setve je utvrđen kao faktor sa snažnijim uticajem na sadržaj plastidnih pigmenata u odnosu na međuredni razmak. U proseku za navedeni period utvrđeno je da se ukupni sadržaj plastidnih pigmenata kod sorte Avigea smanjio u odnosu na prvi datum setve na sva tri reda, što je bilo najizraženije na 45 cm. Smanjenje je dostiglo 25,59% u drugom roku i 19,02% u trećem roku setve. Najjači uticaj roka setve na sadržaj plastidnih pigmenata uočen je kod sorte Riči trećeg roka setve, gde je povećanje iznosilo do 64,33% pri razmaku između 25 cm i do 36,02% pri razmaku između redova od 70 cm u odnosu na prvi rok setve. Faktor razmaka u redovima je imao manji efekat - za sortu Avigea zabeleženo je smanjenje od 12,31% na drugi datum setve i razmak između redova od 45 cm u poređenju sa razmakom između redova od 70 cm za sortu Richi za 15,79% na prvi datum i razmak redova 45 cm. Odnos hlorofil a/hlorofil b se smanjio kod razmaka između redova od 25 u drugom (za Isidor i Richi) i trećem roku setve (za Avigea i Isidor).

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