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Oil and protein content in the grain of soybean cultivars at different sowing seasons

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ABSTRACT: The objective of this work was to evaluate the oil and protein contents of soybean cultivars at different sowing times in Tocantins. Four trials of sowing season were carried out, being two (first sowing season on 03/11 and second sowing season on 17/12) at Fazenda Serra Azul, in Porto Nacional - TO, Brazil, and two (first sowing season at 15/11 and second sowing season on 05/12) at the Fazenda Mariana in Santa Rosa - TO, Brazil, in two agricultural years 2014/15 and 2015/16. The experimental design was a randomized block design with three replications and seven treatments, with seven commercial cultivars. The sowing time, year and location were also evaluated, and in a factorial way. The chemical analyzes were carried out: oil and protein content and finally their respective yields. The concentrations of oil and protein varied according to the different sowing times. The cultivar 8579RSF IPRO presented the best averages for the oil content at all times of sowing. The cultivar 8579RSF IPRO presented the best means for the oil yield in the first sowing season in Porto Nacional and Santa Rosa. Only the cultivar M9144 RR presented the best averages for protein content in all sowing seasons. The cultivars 8579RSF IPRO, M8644 IPRO and M9144 RR presented the best means for protein yield for second sowing season in Porto Nacional and Santa Rosa.

Key words: cultivar evaluation; *Glycine max*; Tocantins

Teor de óleo e proteína no grão de cultivares de soja em diferentes épocas de semeadura

RESUMO: Este trabalho teve como objetivo avaliar teores de óleo e proteína no grão de cultivares de soja em diferentes épocas de semeadura no Tocantins. Foram realizados quatro ensaios de época de semeadura, sendo dois (primeira época de semeadura em 03/11 e segunda época de semeadura em 17/12) na Fazenda Serra Azul, em Porto Nacional – TO, e dois (primeira época de semeadura em 15/11 e segunda época de semeadura em 05/12) na Fazenda Mariana em Santa Rosa – TO, em dois anos agrícolas 2014/15 e 2015/16. O delineamento experimental utilizado foi de blocos ao acaso com três repetições e sete tratamentos, sendo sete cultivares comerciais. A época de semeadura, ano e local também foram avaliados, e de maneira fatorial. Foram realizadas as análises químicas: teor de óleo e proteína e por fim seus respectivos rendimentos. As concentrações de óleo e proteína apresentaram variações em função das diferentes épocas de semeadura. A cultivar 8579RSF IPRO apresentou as melhores médias para o teor de óleo em todas as épocas de semeadura. A cultivar 8579RSF IPRO apresentou as melhores médias para o rendimento de óleo na primeira época de semeadura em Porto Nacional e Santa Rosa. Apenas a cultivar M9144 RR apresentou as melhores médias para teor de proteína em todas as épocas de semeadura. As cultivares 8579RSF IPRO, M8644 IPRO e M9144 RR apresentou as melhores médias para rendimento de proteína para segunda época de semeadura em Porto Nacional e Santa Rosa.

Palavras-chave: avaliação de cultivar; *Glycine max*; Tocantins

Introduction

Brazil is the second largest producer and exporter of soybean and its products (soybean oil and soybean meal), with a production of 96.5 million tons (t) and a productivity of 2,988 kg ha^{-1}. However, the expansion of soybean cultivation has spread significantly throughout the country, but with great emphasis on the Cerrado biome, where new agricultural frontiers have been established, such as MATOPIBA (Maranhão, Tocantins, Piauí and Bahia), with emphasis on the State of Tocantins with the greatest advance in planted area and production, with a total of 877.2 thousand hectares (Conab, 2016).

Soybean and its by-products have been studied with greater emphasis due to nutritional and functional values since they are rich in proteins of high biological value and low cost, possessing polyunsaturated fatty acids, photochemical compounds and being an excellent source of minerals and B vitamins. Its use in healthy diets aimed at reducing the risks of chronic diseases, as well as its technological properties for the food industry and the chemical industry is quite efficient (Gonçalves et al., 2014).

The bran is an important protein source and it has been used in the human food chain, still in small quantity, and in the animal chain where it has been established as one of the main protein sources in the elaboration of rations with high nutritional value. The vegetable oil extracted from the grain has excellent quality, used for human food, in the preparation of foods representing 92% of the edible oils (Sediyama et al., 2015).

Within the government's plan to expand production and use of biofuels in an economically viable way, soy is responsible for 90% of the supply of these biofuels. This is possible due to a number of advantages; with a well-structured production chain, well-defined production technologies, a broad research network; a quick cycle, and the crop is adapted to produce uniformly throughout the country (Petter et al., 2012).

The grain consists of oil, protein, carbohydrates, minerals and ash, and moisture content, which range around 20%, 40%, 25%, 10% and 5% respectively. The expression of these contents is very dependent on this interaction with the environment, especially during the grain maturation stage. This fact hinders to obtain cultivars with high oil, protein, carbohydrate, mineral and ash contents (Pípolo, 2002).

Therefore, nutritional and climatic factors should be considered to obtain higher levels of oil or protein in a soybean crop (Albrecht et al., 2008; Barbosa et al., 2011). According to Pípolo (2002), after studies related to the planting season and chemical composition of the grain, the environmental factors can contribute strongly to the concentration of oil and protein in the grains.

The sowing time is a determinant factor to obtain positive results in the soybean crop, mainly in the search for greater expression of oil or protein contents for soybean cultivars (Albrecht et al., 2008).

Environmental factors vary greatly from year to year, and there are few studies related to environmental interactions in soybean that aim to quantify or estimate this effect in soybean breeding programs in the state of Tocantins (Peluzio et al. 2012).

Therefore, the objective of this study is to evaluate the effect of sowing time on the chemical composition of the grain in soybean cultivars in the closed biome.

Material and Methods

Four trials of sowing times were carried out. Two of them were carried out at the Serra Azul farm, in the municipality of Porto Nacional - TO (234 m altitude, 10°42'27"S and 48°24'51"W) - L1 and two of them (288m altitude, 11°26'31''S and 48°7'2''W) - L2, were repeated in two agricultural years, 2014/2015 and 2015/2016.

The sowing was between November and December, according to the climatic zoning and planting window at each site, with the opening of the harvest in Porto Nacional occurring first in Santa Rosa, due to the anticipation of the rainy season in this region.

Thus, the first sowing season was carried out at the opening of the planting in each place, that is, November 3 in Porto Nacional and November 15 in Santa Rosa. The second season was held between 15 and 20 days after the first planting, November 17th in Porto Nacional and December 5th in Santa Rosa.

The experimental design was a randomized block design with seven treatments and three replicates. The treatments consisted of seven cultivars: 8576 RSF (RR Race), IPRO 8579RSF (IPRO Bonus), ST 820 RR, TMG 132 RR, IPRO 9086RSF, IPRO M8644 and M9144 RR), with groups of maturation of 7.9; 7.9; 8.2; 8.5; 8.6; 8.6; 9.3, respectively. As for the growth habit, the Opus IPRO cultivar has a determined growth habit and the other habit is indeterminate.

The climatological data, rainfall, registered in the agricultural year of 2014/2015 and 2015/2016 were obtained through the daily data collection at the farm (Place of the test), and are presented in Figure 1.

The experimental plot used for each cultivar was composed of a total area of 300 m^2, consisting of ten rows of 60 m, spaced 0.5 m apart. At harvest, the two lateral lines and 23.75 m of the ends of each central row were discarded.

At the time of sowing, the seeds were treated with fungicides, followed by seed inoculation with *Bradyrhizobium japonicum* strains. The sowing was done mechanically with the purpose of obtaining a final stand of 350, 320, 280, 300, 320, 200 and 200 thousand plants per hectare^{-1}, respectively, for the seven cultivars, following the recommendation of each material.

Fertilization and liming were performed according to recommendations for soybean cultivation (Sediyama et al., 2015) to correct the deficits presented in soil chemical analysis. In general, 90-120 kg ha^{-1} of phosphorus (P) was used in each plot, according to the need presented in the

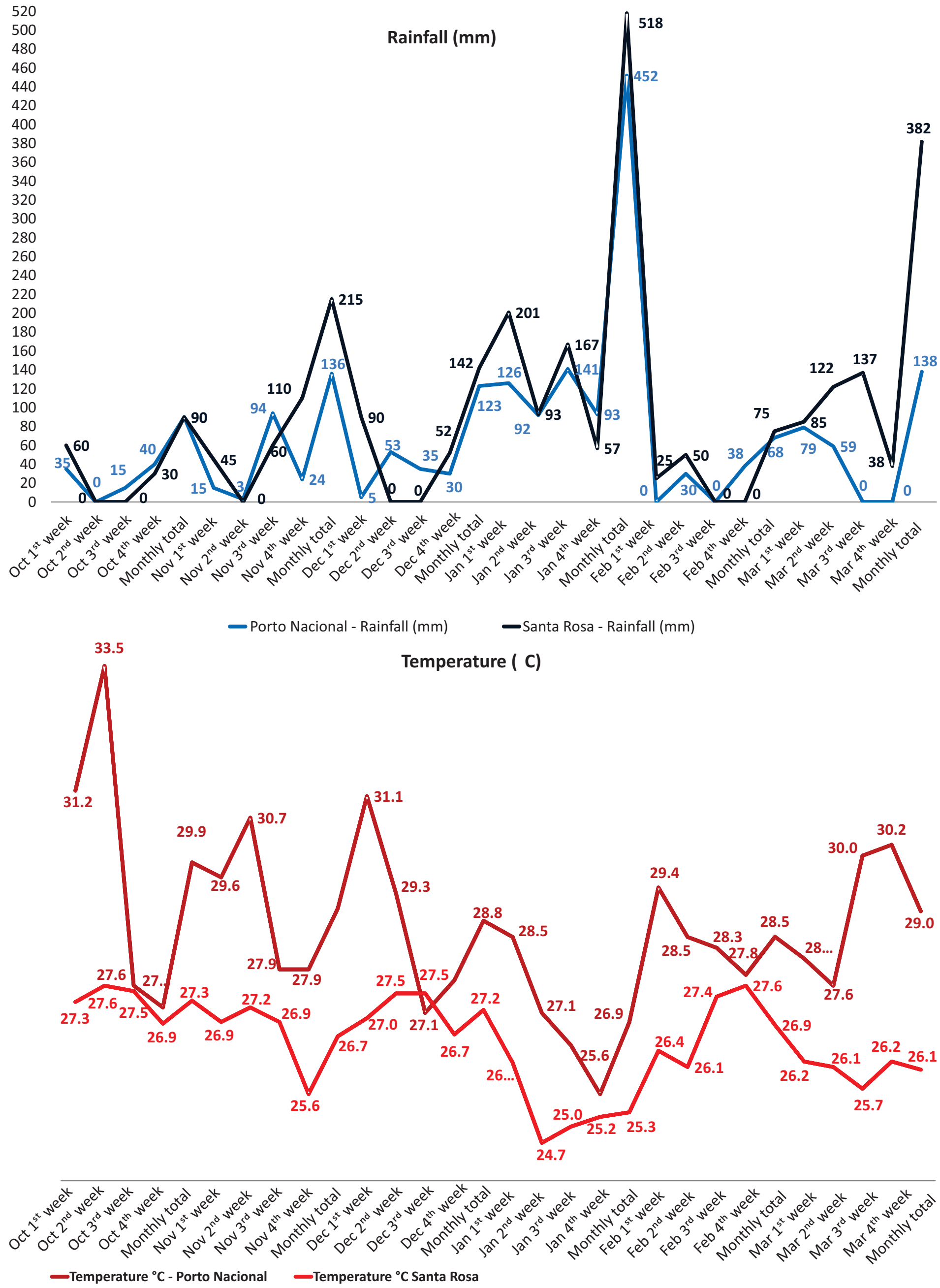

Figure 1. Graph with averages of temperature and precipitation weekly of two years agricultural 2014/15 and 2015/16, in the municipalities of Porto Nacional and Santa Rosa.

soil analysis, followed by 70 kg ha^{-1} coverage in the R2 stage (flowering) using as the source potassium chloride (KCl, 60% K_2O).

The control of pests, diseases, and weeds was carried out as needed to minimize the interference of these factors on the development of soybean cultivars (Sediyama et al., 2015).

The plants were harvested after 95% of the mature pods, that is, at the R8 stage. After the manual harvest, the plants were trod. After being harvested, the grain was dried and milled and the chemical analyses were carried out: oil and protein content and finally their respective yields.

Oil extraction was performed by the Bligh-Dyer method using three samples per cultivar in each of the tests, and each sample weighing 2.5 grams of the dried and milled material. The oil yield was obtained by the product between the oil content and the grain yield.

For the protein content, the methodology proposed by Kjeldahl was used, finding the value of the total nitrogen (N) of the sample and later, converting to crude protein by the factor 6.25 - using three samples to cultivate in each of the tests, and each sample weighing 0.5 grams of dry and ground material. The protein yield obtained through the product between the protein content and grain yield.

After obtaining the data, the analysis of individual variance and later the joint analysis of the tests were performed, in which the lowest mean square residual did not differ by more than seven times the greater (Cruz et al., 2012).

The means were compared by the Scott-Knott test at 5% probability, after normality test of the data according to Kolmogorov-Smirnov. Statistical analyses were performed with the help of the Sisvar program (Ferreira, 2011).

Results and Discussion

The results of ANOVA showed significant differences for year, place and time in almost all the variables presented, except for the protein content (Table 1).

For the source of cultivar variation and the interaction between local × time × cultivar, all the variables had significant effects, showing the importance of the performance of regional works, related to different places, seasons and cultivars in the State of Tocantins (Peluzio et al. 2012) (Table 1).

The local interaction × season × cultivar indicated that the cultivars behave differently according to the environmental action from different periods, places and/or years, and the unfolding for all characteristics was performed.

The coefficients of variation (CV) obtained varied between 4.30 and 9.34, considered as low, demonstrating a good experimental precision (Table 1).

The mean values of oil contents varied between 20.7 and 26.7% (Table 2). These values were higher than those found in other studies that evaluated the oil content in soybean grains cultivated in the State of Tocantins (Barbosa et al., 2011, Sales et al., 2013, Santos et al., 2014), whose values ranged from (17.3 to 24.7%, 18.4 to 21.1%, 14.4 to 19.2%, 17.4 to 24.7%, 18.0 to 21.4%), respectively.

In Porto Nacional, the cultivars 8579RSF IPRO, 9086RSF IPRO and M9144 RR obtained the best oil content averages for sowing in 11/03 (season 1), and the cultivars 8579RSF IPRO and M8644 IPRO for sowing in 11/17, while in Santa Rosa for sowing in 11/15 the best means were of the cultivars 8576 RSF, 8579RSF IPRO and 9086RSF IPRO and in sowing in 12/05 did not present difference between the means (Table 2).

The cultivar 8579RSF IPRO had the highest averages for oil content at both sites and sowing times (Table 2).

For the tests carried out in Porto Nacional, sowing in 11/03 (season 1) presented an average higher than in the day 11/17 (season 2), but it was not statistically significant (Table 2). Similar results were found by Barbosa et al. (2011), where the first plantings obtained the best means but did not differ statistically among them.

For the Santa Rosa tests, the average value of the oil content in 11/15 (season 1) was higher than in the day 12/05 (season 2). It can be explained by the expression of higher averages of the cultivars, with the exception of M9144 RR in season 1, in relation to season 2. This reduction in season 2 occurred mainly due to the presence of milder temperatures

Table 1. Summary of the chemical variance analysis of the chemical components and their yields in seven soybean cultivars, in trials conducted in Porto Nacional and Santa Rosa - TO, with two sowing dates in the 2014/15 and 2015/16 seasons.

Source of variation	GL	QM			
		Oil contente	Oil yield	Protein contente	Protein yield
Year[1]	1	405.1 *	38905.3 *	0.4 ns	475298.3 *
Loca on	1	119.3 *	401738.9 *	1.4 ns	149980.2 *
Time	1	57.6 *	994261.7 *	5.7 ns	1006322.2 *
Cul var	6	10.7 *	130504 *	36 *	53735.1 *
Loca on × Time	1	12.3 **	24271.2 **	41.7 *	36011.7 *
Loca on × Cul var	6	10.5 *	69942.8 *	5.4 **	69149.6 *
Time × Cul var	6	9.4 *	45771.2 *	4.4 ns	54692.5 *
Loca on × Time × Cul var	6	16.5 *	52201.7 *	9.2 *	57157.5 *
Error	119	2	5303.9	2.1	8018.7
CV (%)		6.09	9.34	4.30	7.95
Average		23.5	779	34.2	1127

*, **Significant at 1% and 5% probability respectively; ns: Not significant; by the Scott Knott test.[1] The year factor has not been deployed.

Table 2. Average values of oil contents (%) of seven soybean cultivars at two sowing times in trials conducted in Porto Nacional and Santa Rosa - TO, in the 2014/15 and 2015/16 harvests.

Cultivars	Porto Nacional		Santa Rosa	
	03/11	17/11	15/11	05/12
8576 RSF	22.7 Ba*	22.7 Ca*	24.7 Aa**	22.3 Ab*
8579RSF IPRO	26.7 Aa**	26.7 Aa**	24.7 Aa*	21.1 Ab*
ST 820 RR	23.9 Ba*	23.9 Ba**	23.5 Ba*	20.7 Ab*
TMG 132 RR	23.1 Ba*	23.5 Ba*	22.7 Ba*	21.9 Aa*
9086RSF IPRO	25.9 Aa*	24.3 Ba**	25.1 Aa*	22.3 Ab*
M8644 IPRO	23.5 Bb*	26.7 Aa**	23.1 Ba*	21.9 Aa*
M9144 RR	26.7 Aa**	22.3 Cb*	20.7 Ca*	22.3 Aa*
Average	24.6 a*	24.3 a**	23.5 a*	21.8 b*
Average location	24.5 a*		22.6 a*	
F (C × L × T) = 0.001	CV = 6.09		Average = 23.5	

Capital letters in the same column and lowercase in the same row for each location do not differ statistically from one another by the Scott Knott test at 5% probability; *, ** comparison of the same times between sites, being **> *.

and higher precipitation in December (Figure 1), preceding the grain filling stage.

The higher temperatures and lower rainfall in February (Figure 1), as the final phase of filling the cultivars in 11/15, promoted a differential behavior between cultivars (season 1) with higher oil contents (Table 2) as a function of the occurrence of biochemical disturbances in oil biosynthesis (Albrecht et al., 2008).

The few studies have not yet clearly defined the real reason for variations in oil contents in soybeans, but the most of the studies point to climatic variations as the main factor, since high temperatures and the occurrence of water deficits promote reductions in the chemical and physiological quality of seeds (Greggio & Bonini, 2014).

The oil yield data (kg ha^{-1}) of the cultivars, as a function of location and time, are shown in Table 3. The average of the environments for the characteristic oil yield had significant differences between them and ranged from 656 to 893 kg ha^{-1}, values different from those found by Barbosa et al. (2011), ranging from 327 to 900 kg ha^{-1} and below those obtained by Moreira et al. (2017), which ranged from 948 to 962 kg ha^{-1}.

In the areas of Porto Nacional and Santa Rosa, sowing in the first season (11/03 and 11/15) presented the highest averages in oil yield than in the second season (11/17 and 12/5). This reduction in oil yield in all cultivars in season 2 was possibly due to the decline in precipitation from the end of January, coinciding with the beginning of the reproductive stage of grain filling (Figure 1).

A similar result was presented by Barbosa et al. (2011), where the late sowing times were less propitious for higher yields of oil. Thus, it is understood that the later sowing for the state of Tocantins is not recommended when it is sought to achieve high oil yields in the soybean crop.

In Porto Nacional for sowing in 11/03 (season 1) and in 11/17 (season 2), the cultivar 8579RSF IPRO had the best oil yield averages. In Santa Rosa, cultivars 8576 RSF and 8579RSF IPRO showed the highest average yield for sowing on November 15th (season 1) and for cultivar 8579RSF IPRO and M8644 RR for sowing day 12/05 (season 2). It is possible to highlight the cultivar 8579RSF IPRO with the highest oil yield in both seasons and sites (Table 3).

The results of the protein contents of the seven cultivars are presented in Table 4. In Porto Nacional and Santa Rosa, no significant effect was observed between sowing times. However, most the cultivars showed an increase in their protein content which extended the date of sowing.

Plant growth and productivity are direct competitors with the protein content by the nitrogen absorbed by the plant, and after the supply, for growth and productivity the accumulated N is translocated and used to increase the protein concentration in the grain.

Table 3. Average oil yields (kg ha^{-1}) of seven soybean cultivars at two sowing times in trials conducted in Porto Nacional and Santa Rosa - TO, in the 2014/15 and 2015/16 harvests.

Cultivars	Porto Nacional		Santa Rosa	
	03/11	17/11	15/11	05/12
8576 RSF	748 Da*	628 Cb*	1,014 Aa**	605 Bb*
8579RSF IPRO	1,072 Aa*	942 Ab**	1,034 Aa*	744 Ab*
ST 820 RR	916 Ba**	801 Bb**	724 Ca*	574 Bb*
TMG 132 RR	820 Ca**	714 Cb**	714 Ca*	597 Bb*
9086RSF IPRO	941 Ba**	733 Cb*	808 Ba*	651 Bb*
M8644 IPRO	851 Ca*	849 Ba*	816 Ba*	764 Aa*
M9144 RR	905 Ba**	676 Cb*	625 Ca*	656 Ba*
Average	893 a*	763 b*	819 a*	656 b*
Average location	828 a*		738 a*	
F (C × L × T) = 0.001	CV = 9.34		Average = 783	

Capital letters in the same column and lowercase in the same row for each location do not differ statistically from one another by the Scott Knott test at 5% probability; *, ** comparison of the same times between sites, being **> *.

Table 4. Average of protein content (%) of seven soybean cultivars at two sowing times in trials conducted in Porto Nacional and Santa Rosa - TO, in the 2014/15 and 2015/16 harvests.

Cultivars	Porto Nacional		Santa Rosa	
	03/11	17/11	15/11	05/12
8576 RSF	34.1 Ba*	35.3 Ba**	35.4 Aa*	32.3 Bb*
8579RSF IPRO	31.5 Ca*	32.7 Ca*	31.7 Ba*	33.4 Ba*
ST 820 RR	32.2 Ca*	33.7 Ca*	34.6 Aa**	33.7 Ba*
TMG 132 RR	35.5 Ba*	35.6 Ba*	34.2 Aa*	35.4 Aa*
9086RSF IPRO	32.7 Ca*	34.2 Ca*	34.5 Aa**	33.9 Ba*
M8644 IPRO	33.5 Bb*	36.1 Ba**	35.5 Aa**	33.2 Bb*
M9144 RR	35.9 Ab*	37.6 Aa*	35.3 Aa*	36.0 Aa*
Average	33.6 a*	35.0 a*	34.5 a*	34.8 a*
Average location	34.3 a*		34.6 a*	
F (C × L × T) = 0.001	CV = 4.3		Average = 34.3	

Capital letters in the same column and lowercase in the same row for each location do not differ statistically from one another by the Scott Knott test at 5% probability; *, ** comparison of the same times between sites, being **> *.

Table 5. Average of protein yields (kg ha^{-1}) of seven soybean cultivars at two sowing times in trials conducted in Porto Nacional and Santa Rosa - TO, in the 2014/15 and 2015/16 harvests.

Cultivars	Porto Nacional		Santa Rosa	
	03/11	17/11	15/11	05/12
8576 RSF	1,119 Aa*	974 Bb*	1,474 Aa**	922 Bb*
8579RSF IPRO	1,265 Aa*	1,233 Aa**	1,311 Ba*	1,048 Ab*
ST 820 RR	1,237 Aa**	1,116 Ab**	1,073 Da*	910 Bb*
TMG 132 RR	1,267 Aa**	1,046 Bb*	1,108 Da*	991 Ab*
9086RSF IPRO	1,201 Aa*	1,018 Bb*	1,114 Da*	1,020 Aa*
M8644 IPRO	1,225 Aa*	1,141 Aa*	1,208 Ca*	1,093 Ab*
M9144 RR	1,222 Aa**	1,129 Aa*	1,035 Da*	1,049 Aa*
Average	1,219 a**	1,094 b*	1,189 a**	1,005 b*
Average location	1,157 a*		1,097 a*	
F (C × L × T) = 0.001	CV = 7.95		Average = 1,127	

Capital letters in the same column and lowercase in the same row for each location do not differ statistically from one another by the Scott Knott test at 5% probability; *, ** comparison of the same times between sites, being **> *.

The results are similar with those found by Albrecht et al. (2008), which verified higher protein content in the grains when the sowing season was prolonged, a fact related to climatic conditions (Freiria et al., 2016). In Santa Rosa, at sowing in 11/15 except for the cultivar 8579RSF IPRO, which presented the lowest mean, the other cultivars presented higher and similar means between them. At sowing in 11/17, the cultivar 8579RSF IPRO obtained similar performance to most of the cultivars except TMG 132 RR and M9144 RR cultivars that presented the best means.

The climatic variations in the period of dry matter transfer in the grain can alter the physiology of the cultivar and alter the concentrations of protein in the grain (Pípolo, 2002; Albrecht et al., 2008, Freiria et al., 2016) promoting acceleration in maturation by reducing the plant's accumulation period (Smiderle et al., 2009).

When the difference in grain protein content between sowing times is not a consequence of temperature fluctuation, it may be due to the poor distribution or even absence of rain during the grain filling period and the availability of nitrogen (Pípolo, 2002).

The cultivar M9144 RR presented the best results for protein content in grains at both sites and sowing times.

The data of the yield of the cultivars, according to the location and time of sampling are presented in Table 5. The average of the environments for the characteristic protein yield showed significant differences between them and ranged from 1,000 to 1,236 kg ha^{-1}, to those found by Maehler et al. (2003), whose values ranged from 1,102 to 1,314 kg ha^{-1}.

For sowing in 11/03 in Porto Nacional, the average protein yield of the cultivars did not show statistical differences between them. However, when sowed in 11/17, this difference was noticed, with the best means being the cultivars 8579RSF IPRO, ST 820 RR, M8644 IPRO, M9144 RR, with emphasis on cultivar M9144 RR with the best means in both seasons.

For sowing in 11/15 in Santa Rosa, the cultivar 8576 RSF stood out with higher average protein yield. When sown on 12/05 the best means were of the cultivars 8579RSF IPRO, TMG132 RR, IP9086RSF IPRO, M8644 IPRO and M9144 RR (Table 5).

The averages presented for sowing on November 17 and 12/05 (season 1) were lower than those sown in 11/03 and 11/15 (season 2) at both sites (Table 5).

This reduction in protein yield in most cultivars is associated with a decrease in water availability due to low rainfall from the end of January, coinciding with the beginning of the grain filling phase (Figure 1), since the deficit is one of the most damaging factors in grain yield and quality (Smiderle et al., 2009).

In a study evaluating the chemical composition of soybean as a function of water availability, Maehler et al. (2003) obtained similar results concluding that the lack of water in the grain filling phase promotes losses between 30 and 40% in protein yield.

The results obtained for the oil content (Table 2) and protein (Table 4), characterize well the negative correlation in the chemical composition of the grains, in agreement with Albrecht et al. (2008), where the time that presented the best values for oil content, presented inverse results for the protein content in the grain.

Conclusions

The concentrations of oil and protein varied according to the different sowing times. Thus, the later sowing reduced yields and oil yields and protein and higher protein contents in the grain.

The cultivar 8579RSF IPRO had the best averages for the oil content at all times of sowing.

The cultivar 8579RSF IPRO presented the best means for the oil yield in the first sowing season in Porto Nacional and Santa Rosa.

Only the cultivar M9144 RR presented the best averages for protein content in all sowing seasons.

The cultivars 8579RSF IPRO, M8644 IPRO, and M9144 RR presented the best means for protein yield for second sowing season in Porto Nacional and Santa Rosa.

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