

## Influence of irrigation regime on the chemical composition of soybean grains

### Влияние на поливния режим върху компоненти от химичния състав на семената при соята

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#### ABSTRACT

The development aims to study the influence of the irrigation regime on the chemical composition of soybeans, the yield of protein, fats, and carbohydrates per unit area, as well as some relationships between them. The treatments are the following: 1) without irrigation; 2) irrigation with 30% of the calculated maximum irrigation rate (30% of m); 3) irrigation with a rate of 50% of m; 4) irrigation with a rate of 70% of m; 5) full (optimum) irrigation with a rate of 100% of m. The experiment is based on the block method in four replications with the size of the experimental plots 30 m<sup>2</sup> and the harvest plots – 10 m<sup>2</sup>. The chemical composition of the grain was determined according to approved methods and standards. The results of the experiment make us believe that the increase in yield of valuable components (protein and fat) in soybeans should be achieved by increasing the yield of grains by applying an appropriate irrigation regime, rather than by looking for an option to increase their content in grains.

**Keywords:** Soya (*Glycine max*), irrigation, yield of seeds, chemical composition, crude protein, crude fat

#### РЕЗЮМЕ

Целта на разработката е да се проучи влиянието на поливния режим върху химичния състав на семената на соята, добива от протеин, мазнини и въглехидрати от единица площ, както и някои зависимости между тях. Вариантите на опита са: 1) без напояване, 2) напояване с 30% от изчислената максимална поливна норма (30% m), 3) напояване с норма 50% m, 4) напояване с норма 70% m, 5) оптимално напояване с норма 100% m. Използван е метода на гравитачното напояване. Опитът е залаган по блоковия метод в четири повторения при големина на опитните парцели 30 m<sup>2</sup>, а на реколтната – 10 m<sup>2</sup>. Химичният състав на зърното е определен по утвърдени методики и стандарти. Резултатите от експеримента дават основание да се счита, че увеличаването на добива от ценните компоненти (протеин и мазнини) при соята трябва да се постига посредством увеличаване добива от семена, чрез прилагането на подходящ поливен режим, а не чрез търсене на вариант за повишаване на тяхното съдържание в семената.

**Ключови думи:** Соя (*Glycine max*), напояване, добив, химичен състав, суров протеин, сурови мазнини

## INTRODUCTION

Soybeans are one of the most important crops in the world's food balance, ranking third after wheat and rice. It is the most significant protein and oil crop, according to Malikov and Vasilchenko (2014), Peshkova et al. (2016), the soybean contains 37-42% protein and 19-22% fat, as well as many vitamins. These two components are crucial for the constant growth of soybean areas worldwide, and economically justified increase in yields would lead to lower production costs, including these of protein and fat. The results could be achieved by application of appropriate irrigation, given the fact that there is an inverse relationship between the content of these two components (Adamen, 1984; Kirnak et al., 2010). Nevertheless, irrigation increases the yield, as Pritoni et al. (1989) found out that the positive effect in dry years is more significant. Later, this opinion is confirmed by Seren (2013), according to which the yield of soybeans is between 90 and 200 kg/da. In extremely dry climate and poor soils in the territory of Tuva, obtained between 50 and 70 kg/da protein. These results are possible only with optimal irrigation, by maintaining pre-irrigation soil moisture above 70-80% of FC (field capacity). Nematov (2017) reported that for the conditions of the Fergana valley in Uzbekistan, the application of an irrigation scheme 70-80-80% of FC provides 210-310 kg/da seed yield and 42-48 kg/da protein yield. Applying a biologically optimal irrigation regime, Kresović et al. (2017a) reported for significantly higher yield values (protein 109.2 kg/da fat and 56.3kg/da). The authors found out a very close square relationship between the size of the irrigation rate and the protein yield, as well as between the irrigation rate and the fat yield, respectively at  $R^2=0.93$  and  $R^2=0.97$ .

Studies related to the influence of irrigation on the chemical composition of soybeans (including fat and protein content and yield) have been going on for several decades under different soil and climatic conditions and a large number of varieties with different qualities and length of the growing season. Furthermore, there are different criteria for setting up the trials and of irrigation optimization. As a result, the scientific literature presented

quite a contradictory information about the influence of irrigation (respectively water deficiency) on the fat and protein content of soybean.

An experiment conducted with three soya varieties irrigated at different size of irrigation rate, Goreti and Rossini (1992) found that the content of protein and fat in the soybean were not significantly affected by irrigation. Restuccia et al. (1992) present similar information applicable for Sicily conditions. According to the authors, the irrigation does not affect the percentage of fat in the seeds. However, as the soil water capacity improves, the seed yield increases, along with that, the yield of fat and protein increases. It is noted that the IWUE (irrigation water use efficiency) is significantly higher in terms of fat compared to protein, especially in irrigation with higher irrigation rates. Maintaining different pre-irrigation soil moisture by extending the duration of the inter-irrigation period also does not lead to a change in the protein and fat content of soybeans (Uppal et al., 1997). The authors state, the application of phosphorus fertilizer under irrigation conditions could have a positive effect on the amount of protein. The data reported by Sani et al. (2014) are also divergent regarding the impact of the length of the inter-irrigation period on these two indicators. Mahmoud and El Far (1994) reported the influence of irrigation only about the percentage of crude protein, indicating the importance of irrigation performed until the beginning of seed development. Studies conducted by Kozyrev (2014) show that the reduction of pre-irrigation soil moisture from 80% FC to 70% of FC has little effect on the fat and protein content of seeds, changing it by less than 1%.

There are studies that show that irrigation increases the protein content and reduces the fat in soybeans. Klik and Cepuder (1991) came to this conclusion, as the authors found out that the content of fatty acids is not affected by irrigation. Investigating the influence of different soybean irrigation rates, D'Andria et al. (1990) came to the same conclusion, according to which as the increase of the irrigation rate, the amount of protein in the grain increases, and the fat content tends to decrease. The amount of these two components

changes in the same direction and increasing the level of pre-irrigation soil moisture according to the results presented by Acosta Rivera et al. (1988), i.e. the higher it is (80-90% FC), the greater the amount of protein in the seeds and less fat. When maintaining low pre-irrigation soil moisture (60% FC), the opposite trend is observed. It was found that the highest yield of protein and fat per unit area is obtained at high soil moisture. Kresović et al. (2017b) found that the irrigation regime has a statistically proven effect on the protein content, being the highest when applying the optimal soybean irrigation recommended by the authors. However, according to the same publication, the irrigation has no proven effect on the amount of fat. Ali et al. (2009) studied the effect of water deficiency in phases on the accumulation of fat in soybeans, finding emphatically that it is greatest under non-irrigated conditions. Irrigation, especially during the period of flowering and bean formation, lowers it. Based on the results of a two-year field trial, Kirnak et al. (2010) prove that the optimization of soil moisture provides an increase in the protein content of soybeans by more than 4% compared to non-irrigated conditions, as the values range within the limits characteristic of this crop.

The main part of the research related to the influence of the irrigation regime on the chemical composition of soybeans generally shows that with the improvement of the water supply of the plants, the protein content in the seeds decreases and the fat content increases, as the yield of both components increases due to an increase in total yield under irrigated conditions (Cucci et al., 1989; Martín de Santa Olalla et al., 1994; Shelkovkina 2004; Candoğan and Yazgan, 2016; Ghassemi- Golezani and Farshbaf-Jafari 2012; Morsy et al., 2018; Tolokonnikov et al., 2018a, 2018b; 2018c). For example, Balakay (2000) found that by lowering the content by 0.5-2.2%, irrigation even in humid years can increase protein yield by 1.5-2.5 times. A significant increase in protein yield (49-57%) and feed units (47-54%) was reported by Panchenko (2003), which is a result of the increase in pre-irrigation soil moisture from 70 to 80% FC only in the period of flowering and bean formation. A number of studies aimed at establishing the influence of the irrigation treatment

during the different phases of soybean vegetation on the protein and fat content in the seeds and their yield.

#### *In terms of protein*

The studies of Abbas et al. (2013) show that the negative impact of drought increases with the progression of vegetation, i.e. the protein content is lowest in soil drought during the seed growth period (R5-R6). It is slightly higher in drought through R3-R4 (bean formation), followed by the flowering phase R1-R2. The influence of water stress is the weakest during the vegetative period and more precisely in phase 4 leaves. These results confirm the assumptions made by Pritoni et al. (1990). Investigating the influence of the irrigation treatment in phases on the chemical composition of soybeans, Demirtas et al. (2010) report the opposite trend, namely that the protein content is the highest during drought during the period of their pouring. Ghassemi-Golezani and Lotfi (2013) came to the same conclusion, but they considered the entire reproductive period to be critical. The authors note that the seeds from the upper part of the plant have a higher content of both fat and protein than those from the middle and lower part of the plant. In their publication, Ashraf et al. (2013) found that the maximum protein content is reached under non-irrigated conditions (up to 41.2%). The values are high (over 40%) when watering is canceled in two of the phases. The authors explain the high values with the influence of water deficiency on metabolism, in the direction of increasing protein synthesis in seeds. The imposition of a moderate water deficit with the abolition of irrigation only during one of the phases further reduces the protein content in soybeans, as there is no significant difference between the different periods (between 39.2 and 39.6%). However, the values significantly exceed those - with optimal irrigation (37.2%), which is in line with the opinion of most authors.

#### *In terms of fat*

Abbas et al. (2013) prove that the lowest fat content (20.8%) in water stress during the period of seed pouring. The authors report an impact not only on the quantity but

also on their quality, while finding a significant positive correlation with the absolute seed mass ( $r=0.74$ ). This was suggested two decades earlier by Pritoni et al. (1990), using data on the change in fat yield per unit area. Exactly in the opposite position are Demirtas et al. (2010), who claim that the fat content of soybeans is the highest of drought during the period of their pouring. According to Ghassemi- Golezani and Lotfi (2013), drought during each part of the reproductive period has a negative effect on fat synthesis, which further reduces their yield per unit area. Detailed information on the influence of the phases is provided by Ashraf et al. (2013), referring to the results of 6 varieties - different in origin and duration of vegetation. According to the authors, the least negative effect on fat yield has the cancellation of irrigation during flowering or during the vegetative period. If there is a drought in both phases, the fat yield decreases significantly. The yield is also high of drought during the period of pouring the seeds. When canceling irrigation during these three phases, the fat content of soybeans is over 20%. In case of water deficiency during phases Vn and R1-R4, Vn and R5-R6, as well as during the whole reproductive period (R1-R6), the fat content falls below 20%, and under non-irrigated conditions, they decrease to 17%. The authors explain the obtained results mainly with the influence of the irrigation regime on the duration of the seed pouring period (R5-R6). Provision of favorable soil moisture increases the duration of this period, allowing for longer accumulation of fat. For the same reason, there is the least fat in the seeds of non-irrigated soybeans.

The aim of the development is to study the influence of the irrigation regime on the chemical composition of soybeans, the yield of protein, fats and carbohydrates per unit area, as well as some relationships between them.

## MATERIAL AND METHODS

Data from a long-term field experiment (with a duration of 7 years), conducted in the experimental field of Agricultural University – Plovdiv on alluvial-meadow soil, with "Biser" variety were used. The treatments are

the following: 1) without irrigation; 2) irrigation with 30% of the calculated maximum irrigation rate (30% of m); 3) irrigation with a rate of 50% of m; 4) irrigation with a rate of 70% of m; 5) full (optimum) irrigation with a rate of 100% of m. Irrigation was performed on the basis of data on soil moisture in the layer 0-60 cm for the treatment 5, which for the experimental conditions is 80% of FC (Field Capacity). For the other treatments, the irrigations were carried out together with those in treatment 5, but with a corresponding reduction of the irrigation rates. Irrigation is carried out by gravity on furrows. The experiment is based on the block method in four replications with the size of the experimental plots 30 m<sup>2</sup> and the harvest plots – 10 m<sup>2</sup> (Barov, 1982). The chemical composition of the grain was determined according to approved methods and standards, using 0.5 kg of seeds from each treatment in 4 repetitions, after taking into account the yield from the harvest plots.

The crude protein content was determined by determining the total nitrogen by mineralizing the plant samples with concentrated sulphuric acid and selenium catalyst by the method of Kjeldahl (1883). The mineralized sample was then distilled using a Parnassus Wagner apparatus, and the total nitrogen values obtained were multiplied by a coefficient of 6.25. The crude fat content was determined by the residual method of Soxhlet (1879) by extraction with petroleum ether. The crude fiber was determined by the method of Henverger and Stomann, by treating the plant material with solutions of acids and bases with a certain concentration. The crude ash was determined by moderate incineration of the samples at a temperature of 550-600 °C. Nitrogen-free extracts are obtained by the formula:

$$\text{NFE} = 100 - (\text{crude protein} + \text{crude fat} + \text{crude fiber} + \text{crude ash})$$

The results for the grain yields, protein, fats and carbohydrates were statistically processed using the software product ANOVA1, to establish the proof of the differences between the treatments of the experiment. Yield is presented in units of kg/da (1kg/da=10 kg/ha).

## RESULTS AND DISCUSSION

The influence of irrigation on yield and its quality (including chemical composition) depends largely on meteorological conditions during the growing season, with the greatest importance of precipitation, as their quantity and distribution determines the number of irrigations and the time for their implementation. The trial period includes various years as meteorological conditions, which are grouped as follows: medium dry with drought during the period R2-R5 (mass flowering - seed filling), medium dry with drought during flowering (R2) and during the grain filling period (R5-R6), medium years, with drought during the period of bean formation and seed filling (R3-R6) and wet years, with short-term droughts during the reproductive period (mainly R4). During the medium dry years, three irrigations were carried out with irrigation rate at the full irrigation treatment 170-220 mm. During the medium rainfall years, the irrigations are two or three with a rate of 100-150 mm, and during the wet years - no more than one, with a rate of 60-70 mm.

### *Influence of the irrigation on the yield of crude protein and its content in soybean grain*

The results showing the influence of the irrigation regime on the content and yield of crude protein in different years are presented in Table 1. In medium dry years with pronounced drought during flowering (R2) and grain filling (R5-R6), the content of crude protein in non-irrigated soybeans is 42.5%, and in drought during the period of mass flowering - grain filling (R2-R5) it is 42.2%. This small difference gives reason to believe that the degree of water supply of plants during the period of bean formation (R3-R4) does not significantly affect the values of this indicator. This opinion is also confirmed in more meteorologically favourable (medium) years, when the drought covers the period of bean formation and grain filling (R3-R6). Relatively more favourable precipitation conditions in such years lead to a decrease in crude protein content by 1.0-1.3% in non-irrigated soybeans, with values reaching an average of 41.2%.

During humid years with short-term droughts during the reproductive period, the crude protein content of non-irrigated soybean grains decreased to 35.3%, which largely confirms the theory of the negative impact of high soil moisture on the values of this indicator. The application of full irrigation also creates preconditions for a decrease in the protein content, which is clearly expressed in years with continuous summer droughts. The difference compared to non-irrigated soybean varies depending on the characteristics of the year and is in the range between 1.7 and 4.0%. The most significant is the difference in the deficit of precipitation during the period of mass flowering - grain filling (R2-R5), which is actually decisive for the amount of yield. The difference (over 2.6%) is also significant in the absence of precipitation during the period of bean formation and grain filling. According to the data in Table 1, irrigation with reduced irrigation rates does not lead to a definite one-way change in the crude protein content of soybean grains, despite the fact that there is still a tendency to a slight decrease in the values at higher rates. For this reason, it is more logical to look for an increase in protein yield by increasing grain yield, rather than looking for ways to increase its seed content. A successful way to achieve this aim is to irrigate soybeans, and as discussed at the introduction of this article, all researchers are convinced that applying the suitable irrigation regime ensures higher protein yields per unit area. The results of the experiment allow to determine the influence of the irrigation regime on the yield of crude protein. The data are presented in Table 1. In medium dry years, even the smallest irrigation rate leads to a significant and statistically proven increase in yield (by over 60%). During medium (about rainfalls) years, the additional yield is lower (15%) but the difference compared to the non-irrigated variant is statistically proven. As the size of the irrigation norms increases, the positive effect on the yield increases, as in the range of 70-100% of  $m$  in average dry years, it is 2.3-2.6 times higher than in non-irrigated conditions and reaches 110-140 kg/da. In more favourable (medium) years the increase amounts to 50-60% and is statistically proven.

**Table 1.** Content and yield of crude protein (CP) from soybeans, depending on the conditions of the year and the irrigation regime

Treatment	CP content %	CP Yield kg/da	to dry		proof	to 100% of m		proof	GD kg/da
			± kg/da	%		± kg/da	%		
Medium dry years with drought in R2 and R5-R6 (m=170 mm)									
dry	42.52	55.5	St.	100.0	St.	-83.7	39.9	C	
30% of m	43.34	92.6	37.1	166.8	C	-46.6	66.5	C	5%=11.3
50% of m	43.04	120.0	64.5	216.2	C	-19.2	86.2	B	1%=15.1
70% of m	42.87	131.8	76.3	237.6	C	-7.4	94.7	n.s.	0.1%=20.0
100% of m	40.79	139.2	83.7	250.9	C	St.	100.0	St.	
Medium dry years with drought in R2-R5 period (m=220 mm)									
dry	42.22	46.0	St.	100.0	St.	-75.4	37.9	C	
30% of m	41.27	75.1	29.1	163.1	B	-46.3	61.8	C	5%=12.1
50% of m	35.35	80.3	34.3	174.4	C	-41.1	66.1	C	1%=16.2
70% of m	44.47	113.9	67.9	247.5	C	-7.5	93.8	n.s.	0.1%=21.5
100% of m	38.20	121.4	75.4	263.8	C	0.0	100.0	St.	
Medium years with drought in R3-R6 period (m=150 mm)									
dry	41.18	88.5	St.	100.0	St.	-51.0	63.5	C	
30% of m	41.75	102.2	13.7	115.4	B	-37.3	73.3	C	5%=9.8
50% of m	38.29	119.8	31.3	135.3	C	-19.7	85.9	C	1%=13.1
70% of m	40.67	130.9	42.4	147.9	C	-8.6	93.8	n.s.	0.1%=17.4
100% of m	38.55	139.5	51.0	157.6	C	St.	100.0	St.	
Wet years with short drought in R4 (m=65 mm)									
dry	35.31	69.2	St.	100.0	St.	-14.7	82.5	n.s.	
30% of m	38.76	78.7	9.4	113.6	n.s.	-5.2	93.8	n.s.	5%=25.6
50% of m	37.86	89.8	20.6	129.7	n.s.	5.9	107.0	n.s.	1%=35.4
70% of m	38.16	79.3	10.0	114.5	n.s.	-4.7	94.4	n.s.	0.1%=48.9
100% of m	37.60	83.9	14.7	121.2	n.s.	0.0	100.0	St.	
Average data (m=150 mm)									
dry	40.31	65.6	St.	100.0	St.	-55.1	54.3	C	
30% of m	41.28	87.0	21.4	132.7	C	-33.7	72.1	C	5%=10.5
50% of m	38.64	102.0	36.4	155.5	C	-18.7	84.5	B	1%=14.6
70% of m	41.54	113.5	47.9	173.1	C	-7.2	94.1	n.s.	0.1%=20.2
100% of m	38.79	120.7	55.1	184.0	C	St.	100.0	St.	
m – full irrigation rate									

During such years the yield varies between 130-140 kg/da. Irrigation in humid years, regardless of the size of the irrigation rate, does not lead to a statistically proven change in crude protein yield. According to the results of statistical data processing, the reduction of irrigation rates by up to 30% does not negatively affect the yield of crude protein. The difference compared to the full irrigated treatment is in the range of 5-6%, but it is not proven. The larger reduction of irrigation rates in medium and medium-dry years leads to a statistically proven and significant reduction in the yield of this component.

#### ***Influence of the irrigation regime on the yield of crude fat and its content in soybean grains***

The results showing the influence of the irrigation regime on the fat content and yield in different years are presented in Table 2. With regard to the fat content of soybean grains, there is a slight influence of the conditions of the year, as in medium dry years with prolonged droughts during the reproductive period the values are lower compared to the more favourable average and humid years. Here, too, the insignificant influence of the water supply of the plants through R3 on the fat content in the grains is distinguished. For the conditions of the experiment, the values of this indicator do not change significantly and in one direction when irrigated with different irrigation rates, but this does not apply to the yield. It is influenced by the degree of plants water supply, which react positively even when irrigated with low irrigation rates (30% of *m*). During medium dry years, this irrigation regime increases the yield compared to non-irrigated soybeans by 54-57%, and at higher irrigation rates (between 50 and 100% of *m*) it increases by more than 2 times, reaching 65-70 kg/da with full irrigation. All these differences are statistically proven. In average years with drought during the period of bean formation-filling of grains (R3-R6) the degree of influence is a little less pronounced, but still at irrigation with norms in the range of 50-100%, the fat yield increases by 50-70%, reaching 70-80 kg/da, and the differences are statistically proven. In contrast to crude protein, irrigation with 70% of the maximum irrigation rate leads to more significant losses

of fat yield (between 10 and 24%), which are statistically proven compared to the optimal variant. Irrigation in humid years, regardless of the irrigation rate, does not affect fat yield. In the different variants its values are close (41-52 kg/da), and the differences are ambiguous and statistically unproven.

Using data on the content of crude protein and fat in soybean grains, in all variants of the experiment, a relationship was sought between them. The results show that such ones exist. It is square, negative and is graphically represented by a convex parabola approximating the experimental points at  $R=-0.66$ . The relationship is illustrated in Figure 1 (A).

According to the graph, the change of the two components is opposite, and is not proportional, i.e. when the crude protein content increases, a decrease in the fat content of the grain observed, and the step of this decrease becomes larger. As the range of this dependence expands, it is found that it is possible for the protein content to increase as the fat content increases. This hypothesis is reflected by the left half of the parabola of Figure 1(B).

#### ***Influence of the irrigation regime on the yield of nitrogen-free extracts (NFE) and its content in soybean grains***

Compared to fats, the content of NFE in soybean grains is higher, as a result of which the yield is higher, i.e. quantitatively, they rank second in importance after crude protein. The data are presented in Table 3. In general, there is an increase in the content of NFE during the more favorable years in terms of precipitation, and here the irrigation regime has an indefinite impact. The highest content of NFE was found in humid years, followed by the medium ones and the lowest in the medium-dry years. As the irrigation regime does not have a clear impact, it can be assumed that this trend is due to other environmental factors, which are characteristic of the different years as meteorological conditions.

Increasing the size of irrigation rates usually leads to an increase in seed yield, resulting in an increase in NFE per unit area.

**Table 2.** Content and yield of crude fat (CF) from soybeans, depending on the conditions of the year and the irrigation regime

Treatment	CF content %	CF Yield kg/da	to dry		proof	to 100% of m		proof	GD kg/da
			± kg/da	%		± kg/da	%		
Medium dry years with drought in R2 and R5-R6 (m=170 mm)									
dry	20.21	26.4	St.	100.0	St.	-43.4	37.8	C	
30% of m	19.40	41.4	15.1	157.1	C	-28.3	59.4	C	5%=5.4
50% of m	20.02	55.8	29.4	211.7	C	-14.0	80.0	C	1%=7.3
70% of m	20.45	62.9	36.5	238.5	C	-6.9	90.1	A	0.1%=9.6
100% of m	20.45	69.8	43.4	264.6	C	St.	100.0	St.	
Medium dry years with drought in R2-R5 period (m=220 mm)									
dry	21.48	23.4	St.	100.0	St.	-41.9	35.8	C	
30% of m	19.80	36.0	12.6	153.8	C	-29.3	55.1	C	5%=6.9
50% of m	21.88	49.7	26.3	212.3	C	-15.6	76.1	C	1%=9.2
70% of m	19.41	49.7	26.3	212.3	C	-15.6	76.1	C	0.1%=12.2
100% of m	20.55	65.3	41.9	279.0	C	0.0	100.0	St.	
Medium years with drought in R3-R6 period (m=150 mm)									
dry	21.81	46.9	St.	100.0	St.	-32.6	59.0	C	
30% of m	22.02	53.9	7.0	115.0	A	-25.6	67.8	C	5%=5.4
50% of m	22.82	71.4	24.5	152.3	C	-8.1	89.8	B	1%=7.3
70% of m	22.09	71.1	24.2	151.7	C	-8.4	89.5	B	0.1%=9.6
100% of m	21.96	79.5	32.6	169.5	C	St.	100.0	St.	
Humid years with short drought in R4 (m=65 mm)									
dry	20.72	40.6	St.	100.0	St.	-8.0	83.6	n.s.	
30% of m	20.90	42.4	1.8	104.4	n.s.	-6.2	87.3	n.s.	5%=14.2
50% of m	22.11	52.4	11.8	129.1	n.s.	3.8	107.9	n.s.	1%=19.6
70% of m	21.45	44.5	3.9	109.7	n.s.	-4.1	91.6	n.s.	0.1%=27.1
100% of m	21.78	48.6	8.0	119.7	n.s.	0.0	100.0	St.	
Average data (m=150 mm)									
dry	21.05	34.2	St.	100.0	St.	-30.4	53.0	C	
30% of m	20.77	43.3	9.0	126.4	B	-21.3	67.0	C	5%=5.4
50% of m	20.39	57.3	23.1	167.3	C	-7.3	88.7	A	1%=7.5
70% of m	21.71	55.7	21.5	162.7	C	-8.9	86.2	B	0.1%=10.3
100% of m	20.53	64.6	30.4	188.7	C	St.	100.0	St.	
m – full irrigation rate									



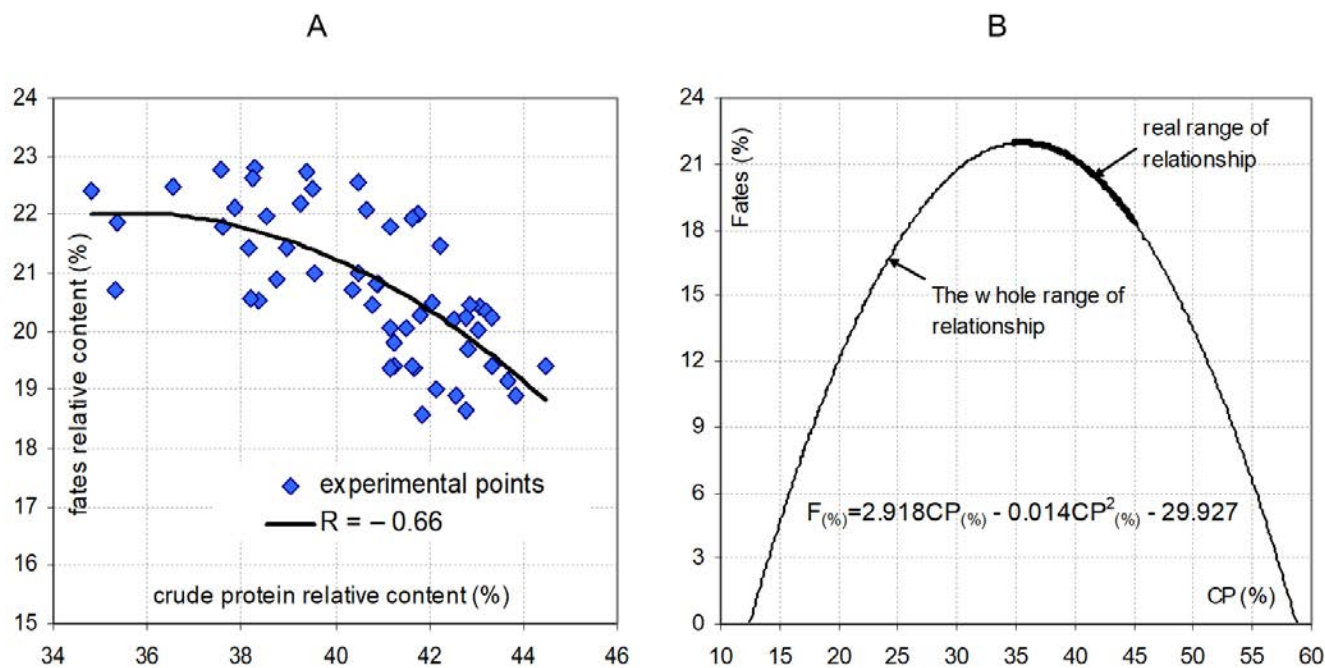


Figure 1. Relationship between crude protein and fat content in soybean grains

The influence of the irrigation regime is more pronounced during drier years, when the supply of 30% of the maximum irrigation rate increases the yield by between 60-70%, and at higher rates this increase is between 2 and 3 times, reaching about and over 80 kg/da. All differences compared to the non-irrigated treatment are statistically proven. In such years, irrigation with irrigation rates reduced by 30% reduces the statistically proven amount of NFE per unit area. During the average rainfall years, the influence of the irrigation regime is a little less pronounced, but as can be seen in the table, it is significant. In such years, irrigation with a norm in the range of 50-100% gives approximately the same results. In wet years, the irrigation regime has no statistically proven effect on the amount of NFE per unit area.

#### ***Influence of irrigation regime on the content of lysine, crude fiber and crude ash in soybean grains***

Lysine is an essential amino acid that is very important for soy when used for food or feed purposes. According to the results of the experiment, the content of lysine in soybeans is influenced by meteorological conditions during the growing season. It is higher in warm years with prolonged droughts during the reproductive period, reaching 0.9 to 1.0% of the dry weight of the grains. The

lack of precipitation in R2-R5, combined with high air temperature, leads to an increase in values up to 1.2%. In wet and cool years, the lysine content is lower and is in the range of 0.7-0.8%. Regarding the irrigation regime, there is a similar trend, as in non-irrigated conditions the values are higher than in full irrigation. The mild and moderate water deficit has a favorable effect on the values of this indicator, as on average for all experimental years' irrigation with a rate of 70% provides the best results (Figure 2). This also affects the yield results, as it becomes comparable to that of full irrigation, respectively 2.65 and 2.64 kg/da (Figure 3). At lower irrigation rates the values decrease and the differences compared to the optimal variant are statistically proven. The effect of irrigation on the lysine content of the grains is best expressed by the additional yield. The effect of irrigation on the lysine content of the grains is best expressed by the additional yield. Irrigation with 50% of the optimal irrigation rate further increases the yield, which can increase by more than 2 times in medium dry years and by more than 60% in middle years. Even more significantly increase the values at irrigation rates at the range of 70-100% of m. In humid years, the irrigation regime has no statistically proven effect on lysine yield.

**Table 3.** Content and yield of nitrogen-free extracts (NFE) from soybeans, depending on the conditions of the year and the irrigation regime

Treatment	NFE %	Yield NFE kg/da	to dry		proof	to 100% of m		proof	GD kg/da
			± kg/da	%		± kg/da	%		
Medium dry years with drought in R2 and R5-R6 (m=170 mm)									
dry	23.13	30.2	St.	100.0	St.	-47.3	39.0	C	
30% of m	24.05	51.4	21.2	170.2	C	-26.1	66.3	C	5%=6.3
50% of m	23.00	64.1	33.9	212.5	C	-13.3	82.8	C	1%=8.5
70% of m	22.68	69.7	39.5	231.0	C	-7.7	90.0	A	0.1%=11.2
100% of m	22.69	77.4	47.3	256.6	C	St.	100.0	St.	
Medium dry years with drought in R2-R5 period (m=220 mm)									
dry	20.91	22.8	St.	100.0	St.	-57.4	28.4	C	
30% of m	20.11	36.6	13.8	160.5	C	-43.6	45.6	C	5%=7.6
50% of m	25.84	58.7	35.9	257.4	C	-21.5	73.2	C	1%=10.2
70% of m	20.17	51.6	28.9	226.6	C	-28.5	64.4	C	0.1%=13.5
100% of m	25.22	80.1	57.4	351.7	C	0.0	100.0	St.	
Medium years with drought in R3-R6 period (m=150 mm)									
dry	20.78	44.7	St.	100.0	St.	-38.6	53.6	C	
30% of m	23.81	58.3	13.6	130.5	C	-25.0	70.0	C	5%=5.6
50% of m	26.46	82.8	38.1	185.3	C	-0.5	99.4	n.s.	1%=7.5
70% of m	23.90	76.9	32.3	172.2	C	-6.3	92.4	A	0.1%=9.9
100% of m	23.01	83.3	38.6	186.4	C	St.	100.0	St.	
Humid years with short drought in R4 (m=65 mm)									
dry	28.30	55.5	St.	100.0	St.	2.8	105.3	n.s.	
30% of m	26.40	53.6	-1.9	96.5	n.s.	0.9	101.6	n.s.	5%=18.0
50% of m	25.43	60.3	4.8	108.7	n.s.	7.6	114.4	n.s.	1%=24.8
70% of m	25.49	52.9	-2.6	95.4	n.s.	0.2	100.4	n.s.	0.1%=34.3
100% of m	23.62	52.7	-2.8	95.0	n.s.	0.0	100.0	St.	
Average data (m=150 mm)									
dry	28.28	46.0	St.	100.0	St.	-27.5	62.6	C	
30% of m	23.59	49.7	3.7	108.1	n.s.	-23.8	67.6	C	5%=6.2
50% of m	25.18	66.5	20.5	144.5	C	-7.1	90.4	A	1%=8.6
70% of m	23.06	63.0	17.0	137.0	C	-10.5	85.7	B	0.1%=11.9
100% of m	23.64	73.5	27.5	159.8	C	St.	100.0	St.	
m – full irrigation rate									

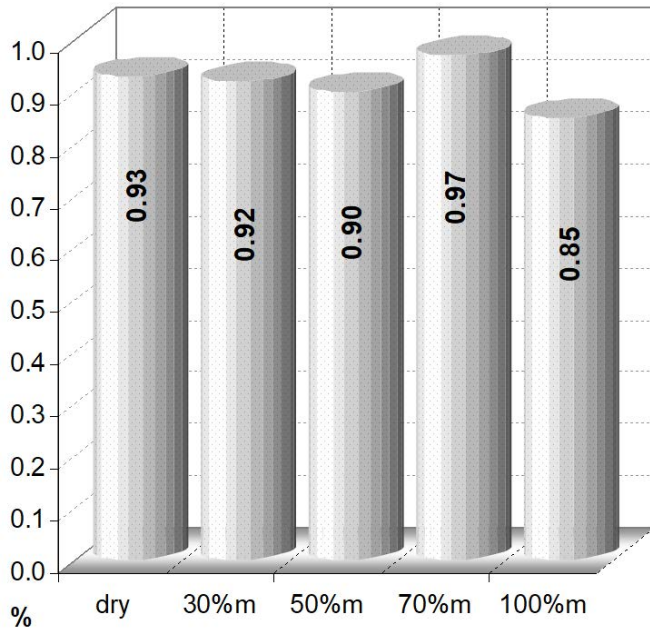


Figure 2. Lysine content of soybean seeds (average)

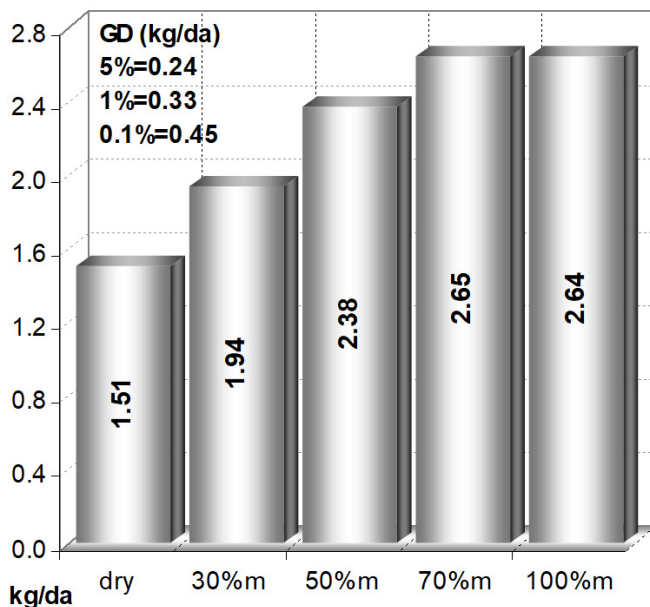


Figure 3. Lysine yield (average)

On average for the experimental period the small irrigation norms (30% of *m*) increase the yield of lysine by 28%, and with irrigation with a norm of 50% *m* it increases by 57%. Irrigation with irrigation norms in the range of 70-100% of *m* increase by 75%, and all differences compared to the non-irrigated variant are statistically proven.

According to the results of the biochemical analysis of the grains, no influence of the conditions of the year and

of the irrigation regime on the content of crude fibers is established, as the values vary between 8 and 12%, but without a clear trend. However, the amount of crude fiber per unit area varies, depending on the conditions of the year and the irrigation regime. In cool and humid years, the values are lower and vary between 21 and 26 kg/da (respectively under non-irrigated conditions and under full irrigation). The averaged data are presented in Figure 4.

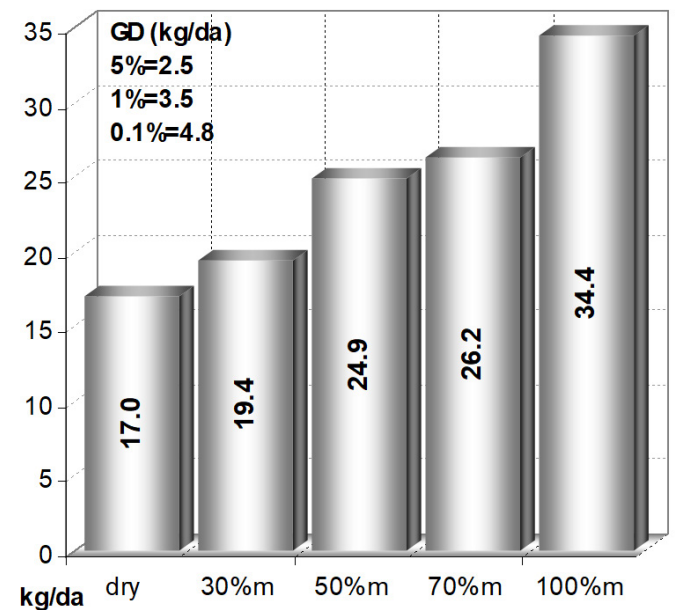


Figure 4. Crude fiber (kg/da) on average

Irrigation with low irrigation rates (30% of *m*) increases fiber yield by an average of 14%, but this difference is not statistically proven. Increasing the irrigation norms to 50% of *m* makes the difference compared to the dry variant 46%, and the same is statistically proven. In contrast to the components presented above, the difference between the rate of 70% and the rate of 100% is very significant (24%), which means that the formation of fibers is significantly affected by mild water stress. As can be seen in the graph, with full irrigation, the fibers obtained per unit area are on average twice as much as those obtained under non-irrigated conditions.

The content of crude ash in soybean grain is also not affected by the conditions of the year and the irrigation regime, as like crude fiber, there is no clear trend. Values

range from 4.5 to 5.7% and rarely over 6.0%. The amount of crude ash per unit is less in wet and cool years (between 9 and 12 kg/da), compared to that found in warm years with longer summer droughts, when with full water supply it can reach up to 19 kg/da. The average data for the influence of the irrigation regime on this indicator are presented in Figure 5.

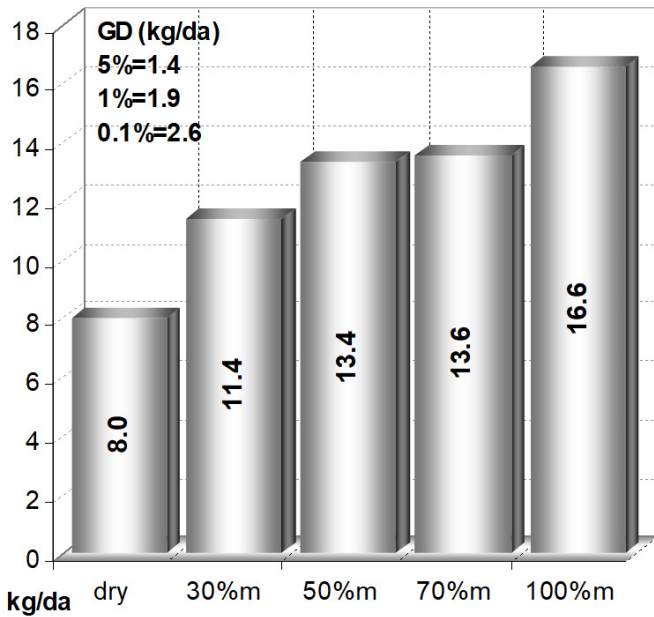


Figure 5. Crude ash (kg/da) on average

Small irrigation rates increase the statistically proven values by 43%, and when irrigated with rates in the range of 50-70% of *m*, they increase by up to 70%. With full irrigation, the crude ash per unit area is on average twice as much as that under non-irrigated conditions.

The average data about relative distribution of the chemical components in the soybean grains depending on the irrigation regime are presented in figure 6. In none of them there is a one-way change in the content in the conditions of different water supply of the plants. All values are within the limits typical for this crop, with the highest percentage of crude protein, followed by that of NFE and crude fat. The crude fiber is significantly less and the crude ash content is the lowest.

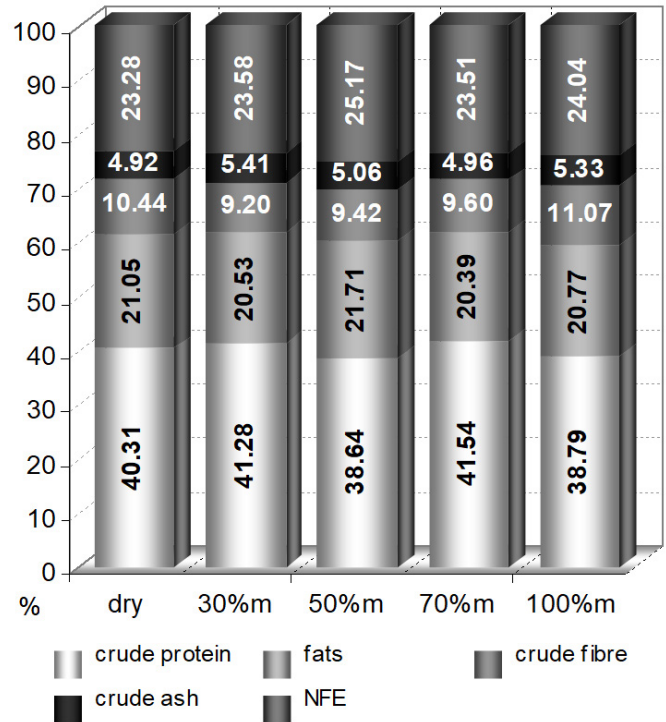


Figure 6. Relative distribution of the chemical components in the soybean grains depending on the irrigation regime (average for the experimental period)

#### Water use efficiency in terms of chemical composition of soybean grains

The efficiency of water can be determined in two directions - in terms of irrigation rate and in terms of evapotranspiration. The first case is the additional yield for 1mm of irrigation rate, and the second - the yield for 1 mm of evapotranspiration. The results for both directions are presented in Table 4.

#### Irrigation water use efficiency (IWUE)

The efficiency of the irrigation rate is determined by the disproportionate increase in grain yield, as a result of increasing the size of the irrigation rate. This, in most cases, leads to an increase in the values at lower irrigation rates compared to those at full irrigation. Thus, in medium dry years, the IWUE for seeds is highest at a rate of 50% *m*, followed by that at a rate of 30% of *m* and 70% of *m*. In medium years, the highest additional yield of 1 mm of irrigation water was found again at a rate of 50% *m*, but the values are high at irrigation with a rate of 70% *m*.

**Table 4.** Water use efficiency depending on the conditions of the year and the irrigation regime

Treatment	M (mm)	ET (mm)	IWUE (kg/da.mm)				WUE (kg/da.mm)			
			S	CP	CF	NFE	S	CP	CF	NFE
Medium dry years with drought in R2 and R5-R6 (m=170 mm)										
dry	0	292	0.000	0.000	0.000	0.000	0.445	0.190	0.090	0.103
30% of m	51	337	1.556	0.722	0.294	0.412	0.624	0.275	0.123	0.153
50% of m	86	356	1.752	0.754	0.343	0.396	0.786	0.337	0.157	0.180
70% of m	120	378	1.503	0.637	0.305	0.330	0.819	0.348	0.166	0.184
100% of m	171	434	1.227	0.489	0.254	0.276	0.784	0.321	0.161	0.178
Medium dry years with drought in R2-R5 period (m=220 mm)										
dry	0	346	0.000	0.000	0.000	0.000	0.318	0.133	0.068	0.066
30% of m	65	353	1.075	0.447	0.194	0.212	0.510	0.213	0.102	0.104
50% of m	109	410	1.097	0.314	0.240	0.328	0.562	0.196	0.121	0.143
70% of m	153	465	0.980	0.444	0.172	0.189	0.560	0.245	0.107	0.111
100% of m	219	501	0.961	0.345	0.192	0.263	0.639	0.242	0.130	0.160
Medium years with drought in R3-R6 period (m=150 mm)										
dry	0	319	0.000	0.000	0.000	0.000	0.691	0.278	0.147	0.140
30% of m	45	320	0.667	0.304	0.156	0.302	0.781	0.319	0.168	0.182
50% of m	75	369	1.200	0.417	0.327	0.508	0.839	0.324	0.193	0.224
70% of m	105	409	0.952	0.404	0.230	0.308	0.782	0.320	0.174	0.188
100% of m	150	434	0.933	0.340	0.217	0.257	0.829	0.321	0.183	0.192
Wet years with short drought in R4 (m=65 mm)										
dry	0	399	0.000	0.000	0.000	0.000	0.476	0.173	0.102	0.139
30% of m	20	411	0.513	0.482	0.092	-0.097	0.487	0.192	0.103	0.131
50% of m	33	427	1.538	0.634	0.363	0.148	0.562	0.210	0.123	0.141
70% of m	46	428	0.440	0.220	0.086	-0.057	0.491	0.185	0.104	0.124
100% of m	65	347	0.462	0.226	0.123	-0.043	0.634	0.242	0.140	0.152
Average data (m=150 mm)										
dry	0	339	0.000	0.000	0.000	0.000	0.481	0.193	0.101	0.136
30% of m	45	355	1.038	0.472	0.199	0.082	0.591	0.245	0.122	0.140
50% of m	76	391	1.349	0.481	0.306	0.271	0.678	0.261	0.147	0.170
70% of m	106	420	1.059	0.453	0.203	0.161	0.655	0.270	0.133	0.150
100% of m	151	429	0.972	0.364	0.201	0.182	0.723	0.281	0.151	0.171

IWUE – irrigation water use efficiency; WUE – water use (ET) efficiency; CP – crude protein; CF – crude fates; NFE – nitrogen-free extract; M – seasonal irrigation rate; ET – seasonal evapotranspiration; S – seeds

This is explained by the lower irrigation rates in all variants and the lower additional yield due to the higher yield under non-irrigated conditions. During humid years, the high rates have a weak effect on the yield, due to which the values are highest at the rate of 50% of *m*. As the irrigation regime does not lead to one-way changes in the content of CP, CF and NFE, the efficiency of the irrigation rate for these three components should be similar to that for grains. The IWUE for CP reaches a maximum when applying rates in the range of 30-70% of *m*, and regardless of the conditions of the year, the values for full irrigation treatment are the lowest. On average for the experimental period, the additional yield of crude protein per 1mm of irrigation water at full irrigation is 75% of that - at irrigation with a rate of 50%*m*, which is indicative. The IWUE for CF is also the highest for irrigation with a rate of 50%*m*. It is lower and comparable with the norms of 30 and 70% of *m*, as at the lower norm this is due to the lower additional yield compared to the non-irrigated variant, and at the higher norm - to the decreasing effect of the increase of the norms, arising from the nature of the connection "Irrigation rate-yield". On average for the experimental period, the additional yield of CF per 1 mm of irrigation water with full irrigation is 66% of that - with irrigation rate of 50%*m*, i.e. in the case of CF, the IWUE is more strongly influenced by the irrigation regime and the related level of water supply of the plants. In the case of NFE the irrigation regime influences in a similar way, as higher values are reported in the case of deficient irrigation, with a maximum again at the norm of 50% of *m*. In humid years, the values of this indicator are very low in irrigation with ½ rate, and in the other treatments they are even negative, i.e. for every 1mm of irrigation water NFE per unit area decreases. On average for the experimental period, the additional NFE per 1 mm of irrigation water with full irrigation represent 67% of those - with irrigation with a rate of 50%*m*.

#### **Water use efficiency (WUE)**

As the total yield (including non-irrigated yield) is included here, the differences between the different experimental treatments are smaller. For the experimental

conditions, the improvement of the water supply conditions increased the values of ET, but at the same time the grain yield also increased. As a result, the maximum WUE is reached in the optimal variant (Table 4). It exceeds that in non-irrigated conditions by an average of 50%, as in dry years the difference reaches between 75 and 100%, and in medium and wet years - between 20 and 30%. With regard to CP, there is a positive effect of irrigation on the WUE, as it increases, but between the different irrigation treatments the differences are insignificant and ambiguous. Therefore, it cannot be considered that the amount of irrigation rates significantly affects the values of this indicator. These results stem from the fact that the increase in irrigation rate does not change the content of CP in soybean seeds in one direction, affecting the yield of this component, but at the same time, increases the values of ET. However, on average for the experimental period, the highest ET efficiency in terms of crude protein was found in the full irrigation treatment. The trend in CF is the same, but in addition to the non-irrigated variant, lower efficiency of ET was found in irrigation with a rate of 30%*m*, and the highest values are obtained with full irrigation. Regardless of the irrigation regime and the conditions of the year, 1mm ET produces significantly less CF than CP, and these results are derived from the percentage of the two components in the seeds. For example, the WUE in relation to CF under non-irrigated conditions represents on average 52% of that of CP, ranging between 47 and 59%. With full irrigation, the average difference is also 52% in favor of CP, with variation over the years in a slightly narrower range (between 50 and 58%). With regard to NFE, the trend is maintained, as the efficiency of ET is slightly higher than that of crude fats. This is due to the higher content of NFE in the grains and, respectively, their quantity per unit area. The difference is more significant in non-irrigated conditions (average 34%), while in irrigated variants it is in the range of 13-15%, decreasing with increasing irrigation rate.

Using water efficiency data, linear relationships were found between grain values and those of crude protein and crude fat. The derived dependencies include both

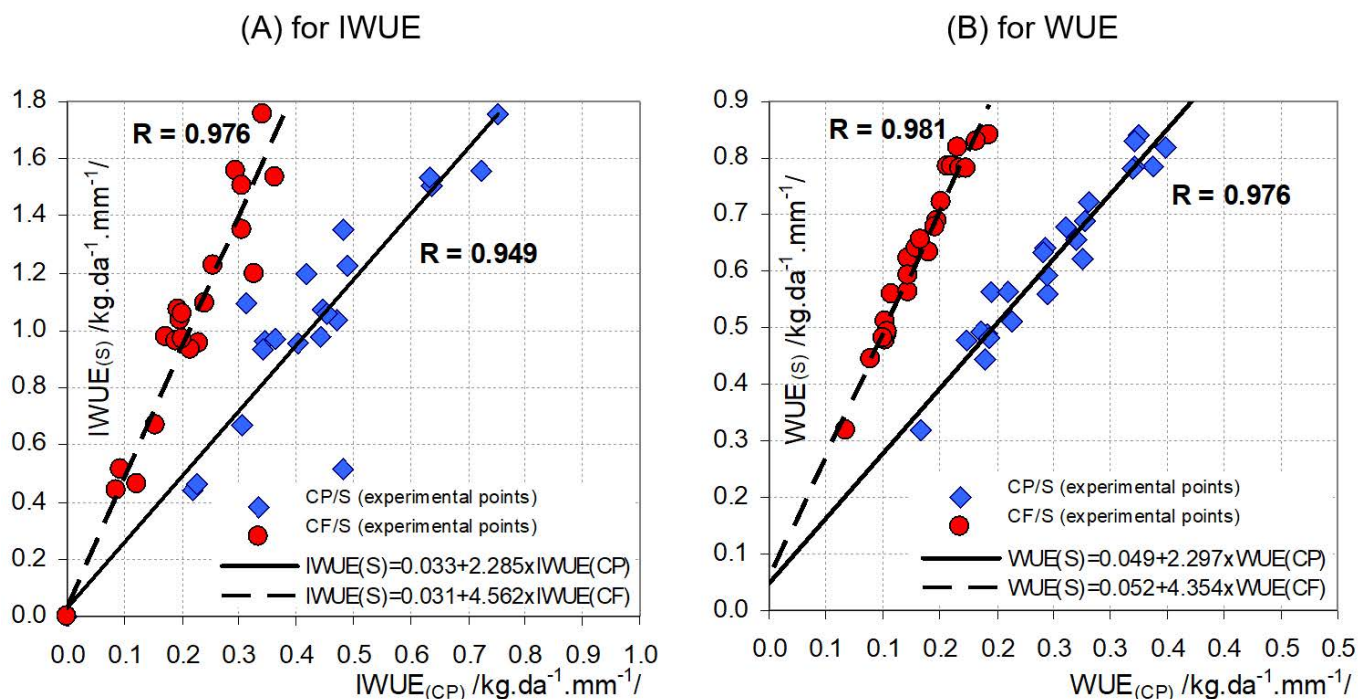


Figure 7. Relationship between water efficiency in terms of seeds and that in crude protein and crude fat

directions - IWUE and WUE. The results are presented visually in Figure 7. The lines describing them approximate the experimental points at a very high correlation coefficient ( $R \geq 0.95$ ), with the equations describing each of the dependencies being placed within the graph. Slightly higher accuracy is found in the dependence using the data on the WUE. When compared between the two components, greater accuracy demonstrates the dependence associated with crude fat. This can also be seen when comparing the scattering of the experimental points. The established dependencies have no significant practical application, but clearly show that the efficiency of water in terms of seed yield is very closely related to the efficiency of water, related to the biochemical components of yield and mainly CP and CF. There is such a dependence with regard to NFE, but it is less pronounced at the irrigation rate ( $R=0.782$ ), but very well expressed in terms of ET ( $R=0.927$ ).

#### "Yield-Water" relationship

The "Yield - water" relationship has been studied in relation to crude protein and crude fat in two directions: "Yield - irrigation rate" and "Yield - ET". For this purpose,

existing formulas were used, the parameters of which were established on the basis of experimental data.

#### "Yield - irrigation rate" relationship

To establish the parameters of this dependence, the data on the relative irrigation rate and the relative yield of CP and CF (total and additional) for all experimental years were used. A power formula was used, which was developed to interpret the relationship in terms of both total and additional yield (Davidov, 1982; 1994; 1998; 2004).

The relationship "Yield-irrigation rate" is expressed by an equation of the form:

$$Y = 1 - (1 - Y_c) (1 - x)^n$$

The relationship "Additional yield-irrigation rate" is expressed by an equation of the form:

$$Y = 1 - (1 - x)^n$$

where:  $Y$  is the required yield, and  $Y_c$  - the relative yield in non-irrigated conditions compared to that in full irrigation.  $X$  indicates the relative irrigation rate, and  $n$  is a variable exponent.

The relationship related to the crude protein is illustrated in Figure 8.

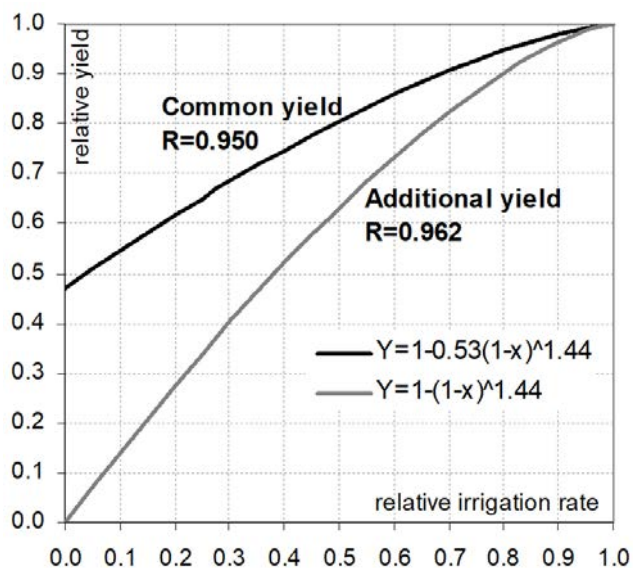


Figure 8. The relationship "Yield of CP – irrigation rate"

It is expressed by convex parabolas, which approximate the experimental data with very high accuracy ( $R=0.95$  for the total yield and  $R=0.96$  for the additional yield), and the power in both cases is  $n=1.44$ . With regard to crude fats, the dependence is also represented by a parabola. It is slightly more convex even at  $n=1.26$ . This is an indicator of less positive impact of reduced irrigation rates on yield. The accuracy of the approximation is high, as  $R=0.93$  for the total yield, and for the additional yield  $R=0.95$  (Figure 9).

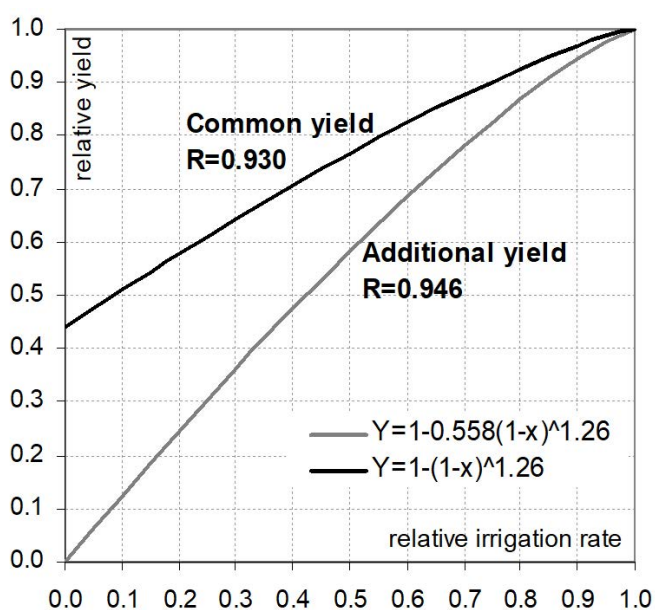


Figure 9. The relationship "Yield of CF – irrigation rate"

The dependences presented in this way can be used to predict the yield of crude protein and crude fat from soybeans, in the conditions of shortage of irrigation water and application of deficient irrigation.

### The "Yield- ET" relationship

The relationship between ET and CP and CF yields has been established, with experimental data for all treatments and years being processed by the method of least squares, using the FAO linear equation (Doorenbos and Kassam, 1979) and two-power equation (Davidov, 1994; 1998; 2004). The linear equation is of the form:

$$Y = 1 - Kc(1 - ET)$$

The two-power equation has the following form:

$$Y = [1 - (1 - ET)^n]^m$$

The components that make up the two equations have the following meaning: **Kc** is the yield coefficient, and **ET** is the relative evapotranspiration in deficient irrigation, compared to that in full irrigation. The powers **n** and **m** are variable.

The results of the application of the two equations are presented in parallel for both components. Figure 10 shows the graphs for the relationship "Yield of CP-ET" on the two equations.

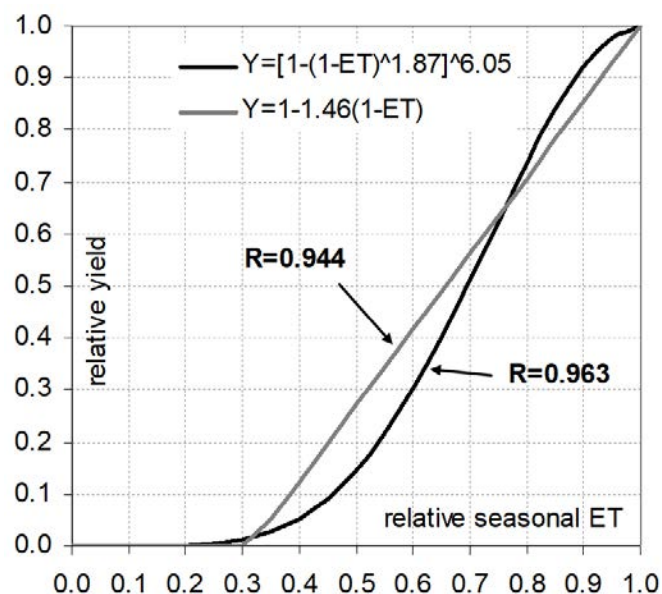


Figure 10. Relationship „Yield of CP - ET"



In order to obtain some crude protein yield (other than 0) it is necessary to consume a minimum of 30% of the maximum ET. This result is confirmed by both the linear and the two-power equation, and has a rather theoretical meaning, as the relative ET under non-irrigated conditions is significantly higher (0.5-0.6 and more). The FAO equation describes linearly the change in CP yield depending on ET, with high accuracy ( $R = 0.944$ ). The two-power equation demonstrates higher accuracy. It describes the dependence by means of an S-shaped curve, smoothly and with greater precision ( $R = 0.963$ ) the change in yield from CP. The most significant difference between the results of the two equations is in the range of the relative ET between 0.45 and 0.60, where the yields determined by the linear equation are over 10% higher than those established by the two-power. The two graphs intersect at 0.75 of  $ET_{(max)}$ , after which the trend is reversed and in the range 0.85-0.95 of  $ET_{(max)}$  the yield of the linear equation is 6-8% lower. These results give reason to recommend two-power relationship. Given the high accuracy, the linear equation can also be used, but according to the features described above. With regard to crude fats, the exact same trend is maintained (Figure 11).

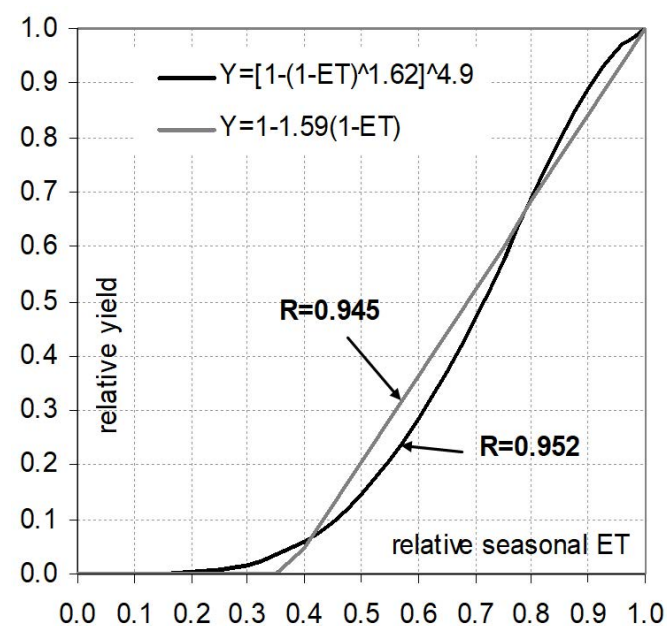


Figure 11. Relationship „Yield of CF - ET”

According to the linear relationship, a yield other than 0 can be obtained at 0.35 of  $ET_{(max)}$ , and according to the two-power relationship - at 0.25 of  $ET_{(max)}$ . Here again, the S-curve describes more smoothly the change in yield from CF, as a result of which the accuracy increases ( $R = 0.952$ ), compared to that achieved by the FAO equation ( $R = 0.945$ ). In terms of crude fat, the results of the two equations are closer. The most significant differences are in the range 0.5-0.6 of  $ET_{(max)}$  and 0.9 of  $ET_{(max)}$ , but they are smaller compared to those in crude protein. However, the use of the two-stage dependence is recommended due to the greater accuracy and precision in calculating the crude fat yield depending on the relative total ET. The linear equation can also be used, taking into account the larger deviations from the actual yield that it generates.

## CONCLUSIONS

The content of crude protein in soybean grains is more strongly influenced by the conditions of the year, as in warmer and drier years it is between 41 and 42%. In cool and humid years, the values decrease to 35-38%. The irrigation regime does not affect this indicator in one direction. For the conditions of the experiment, the content of crude fat is in the range that is typical for soybeans (20-23%), and is not significantly and unilaterally influenced by the conditions of the year and the irrigation regime. There is an inversely proportional square relationship between the content of crude protein and crude fat at  $R=0.66$ .

The irrigation regime does not have a significant and one-way effect on the content of NFE, crude fiber and crude ash. There is a tendency for lysine to decrease with increasing irrigation rates.

IWUE is highest in irrigation with a rate of 50%, which is due to the peculiarities of the relationship "Irrigation rate-yield". WUE is highest in irrigation with rates in the range of 70-100%. There is a close linear relationship between WUE in seeds and that in crude fat and crude protein at  $R \geq 0.98$ . This dependence on IWUE is also very well expressed at  $R \geq 0.95$ .

The relationship between the yield of CP and the irrigation rate is power ( $n=1.44$ ) at  $R=0.96$ . With regard to crude fats, the dependence is also graded with a power of  $n=1.26$  and  $R=0.95$ . The relationship between yield of CP and ET is a two-power function ( $n=1.87$ ;  $m=6.05$  and  $R=0.96$ ). With regard to CF, it is also two-stage with the following parameters:  $n=1.62$ ,  $m=4.9$  and  $R=0.95$ . The dependence is represented graphically by an S-shaped curve.

The results of the experiment make us believe that the increase in yield of valuable components (protein and fat) in soybeans should be achieved by increasing the yield of grains by applying an appropriate irrigation regime, rather than by looking for an option to increase their content in grains. Increasing the size of irrigation rates is guaranteed to increase the yield of these two components.

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