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SEED YIELD AND PROTEIN CONTENT IN SUNFLOWER DEPENDING ON STAND DENSITY

ABSTRACT: The aim of this research was to investigate the effect of stand density on seed yield and protein content in sunflower hybrids. The field experiment was carried out at Rimski Šančevi location. Six NS sunflower hybrids were examined. Five hybrids are confectionery (NS Goliat, NS Slatki, NS Gricko, Vranac and Cepko), and one is used for bird food (NS-H-6485). The trial was arranged as randomized complete block design (RCBD) with four replications. Sowing was done with six different densities (from 20,000 to 70,000 plants per hectare, with an increment of 10,000 plants per hectare). Analysis of variance (ANOVA) showed that the effect of hybrid, stand density and hybrid × stand density interaction were highly significant for seed yield and protein content. The highest seed yield, on the basis of average for all densities, was found in NS-H-6485 (4.77 t ha⁻¹) and in NS Gricko (4.43 t ha⁻¹). Average seed yield of hybrids significantly increased up to 50,000 plants per ha⁻¹, when it reached the value of 4.50 t ha⁻¹, and then decreased. Significantly higher protein content, taking into account all stand densities, showed hybrid Cepko (16.94%). Protein content, above the overall average value, was achieved in hybrid Vranac (16.11%). The highest protein content in the average for all six hybrids was at the lowest stand density (20,000 plants per ha⁻¹), and then decreased up to higher densities. The results showed that stand density had significant effect on seed yield and protein content in sunflower hybrids.

KEYWORDS: hybrid, interaction, oil content, seed yield, stand density, sunflower

INTRODUCTION

One of the most important annual plant species, whose seeds are used for extraction of edible oil, is sunflower (*Helianthus annuus* L.). Except in oil, sunflower seeds are rich in protein, and minerals such as calcium and phosphorus. There are two main types of sunflower: oilseed and non-oil seed sunflower [Salunkhe *et al.*, 1991; Jocić *et al.*, 2015]. Non-oil seed type *Helianthus*

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annuus L. var. *macrocarpus* (DC) Ckll is also called confectionery, protein, or big seed sunflower. Demand for confectionery sunflower is increasing both in the world and in Serbia, considering the rising possibility of using protein from the seeds in the food and confectionery industry. In the human diet it is used as seed with shell and as hulled kernels. The sunflower seed is used for preparing over 100 different food products: special types of bread, semi-prepared and ready meals, cakes, ice cream, chocolate, peanut butter, mixtures with butter, honey and salt as a spread, and as a substitute for nuts in cakes, addition to salads, and yogurt supplement [Jovanović 2001; Dijanović *et al.*, 2003; Cold *et al.*, 2012; Jocić *et al.*, 2012]. The protein content in 17 hybrids of confectionery type sunflower ranged from 17.3% to 21.1% according to the results reported by Jovanović *et al.* [2008]. A number of authors found a significant correlation between protein content and other seed traits in sunflower [Joksimović *et al.*, 1999; Radić *et al.*, 2009, Cold *et al.*, 2012; Hassan *et al.*, 2013; Ramzan *et al.*, 2015, Hladni *et al.*, 2015]. The protein content was correlated with seed yield, weight of 1,000 seeds, dry matter content, oil content and other traits, communicated these authors.

Stand density affects plant architecture, and it is one of the most important cultural practices which determines seed yield, as well as other agronomic attributes of the crop. It is very important to determine the optimum stand density in order to get high seed yield. According to Villalobos *et al.* [1994], as well as Diepenbrock *et al.* [2001], optimal sowing density in sunflower is influenced by several factors, such as temperature, soil fertility, availability of moisture and genotype.

The aim of this study was to investigate the seed yield and protein content in sunflower hybrids depending on the stand density.

MATERIAL AND METHODS

In the experiment conducted in 2012 at Rimski Šančevi location, seed yield and protein content in six NS sunflower hybrids were examined. Five hybrids are confectionery (NS Goliat, NS Slatki, NS Gricko, Vranac and Cepko), and one is used for bird food (NS-H-6485).

NS Goliat is a confectionery sunflower hybrid used for nutrition and peeling. It belongs to the group of medium early hybrids. The stem is solid, average plant height is 190 to 195 cm. The genetic potential for seed yield is higher than 4.5 t ha^{-1} . The oil content is less than 33%, and protein content in the kernel is greater than 23%. It has a low content of the shell. It is resistant to rust and sunflower moth, and tolerant to *Phomopsis*. NS Goliat is attractive to pollinators, and is also well adaptable to different environmental conditions and soil types.

NS Slatki is a confectionery sunflower hybrid used for nutrition and peeling. It belongs to the group of medium early hybrids. The stem is solid, average plant height is 185 to 195 cm. The genetic potential for seed yield is higher than 4.5 t ha^{-1} . The oil content in the seed is less than 35%; protein content in the kernel is higher than 25%. It has a low content of the shell. This hybrid is resistant to

rust and sunflower moth, and tolerant to *Phomopsis*. It is attractive to pollinators, and is also well adaptable to different environmental conditions and soil types.

NS Gricko, a confectionery sunflower hybrid, is used for nutrition and peeling. It belongs to the group of medium early hybrids. The stem is solid, average plant height is 185 to 195 cm. The genetic potential for seed yield is higher than 4,5 t ha⁻¹. The oil content in the seed is less than 35%; protein content in the kernel is higher than 25%. It has a low content of the shell. It is resistant to rust and sunflower moth, and tolerant to *Phomopsis*. NS Gricko is attractive to pollinators, and is also well adaptable to different environmental conditions and soil types.

NS-H-6485 is a sunflower hybrid for bird feed. It belongs to the group of medium early hybrids. The stem is solid, average plant height is 180 to 190 cm. The genetic potential for seed yield is higher than 4.5 t ha⁻¹. Seed oil content is 37-40% and the protein content in the kernel is about 25%. The seed is black with gray lines marked at the ends. It is resistant to rust.

Vranac is a confectionery sunflower hybrid for food and peeling. It belongs to the group of medium early hybrids. The stem is solid, average plant height is 175 to 180 cm. The genetic potential for seed yield is higher than 4 t ha⁻¹. The oil content is from 44 to 48%. It has a low content of the shell. It is resistant to rust and sunflower moth, and tolerant to *Phomopsis*. Vranac is attractive to pollinators, and is also well adaptable to different environmental conditions and soil types.

Cepko, a confectionery sunflower hybrid, is used for peeling and bird food. It belongs to the group of medium early hybrids. The stem is solid, average plant height is 180 to 185 cm. The genetic potential for seed yield is higher than 4.5 t ha⁻¹. The oil content is less than 42%, and seed protein content is more than 16%, and therefore this hybrid is suitable for bird food. It is resistant to rust and sunflower moth and tolerant to *Phomopsis*. This hybrid is attractive to pollinators, and is also well adaptable to different environmental conditions and soil types.

In hybrids Vranac and Cepko the genes responsible for high genetic potential for seed yield and good technical-technological traits of the seeds are successfully combined. Hybrids Vranac and Cepko are suitable for peeling and production of kernels. In addition, Cepko is suitable for bird food [Jovanović and Škorić 2006].

The experiment was set up in a randomized complete block design (RCBD) with four replications. Sowing was done with six different densities (from 20,000 to 70,000 plants per ha⁻¹, with an increment of 10,000 plants per ha⁻¹). Seed was sown in four rows at spacing of 70 cm between the rows. Row length was 10 m. The first and fourth row served as protection, and two inner rows were used for analysis. Seed yield was measured after the harvest from two inner rows, without first and last plants in a row. The seed yield is calculated in t ha⁻¹ and corrected to 11% moisture. Seed protein content (%) was determined by Kjeldahl method (VAP-50-Gerhardt).

Statistical analysis (two factorial ANOVA) was performed using the program STATISTICA 12.0. Differences between the treatments were determined by LSD range test at 0.05 and 0.01 level.

RESULTS AND DISCUSSION

Seed yield

Sunflower seed yield is a complex trait and it is strongly under the influence of environmental factors. Yield depends on the genetic potential of hybrids for yield, and yield stability depends on the ability of hybrids response to the environmental conditions [Jocić 2003; Škorić 2012].

Observing the results of the experiment conducted at Rimski Šančevi location, sunflower seed yield depended on hybrid, stand density and hybrid \times stand density interaction. All sources of variation were significant. Hybrids had the largest impact on seed yield (50.07%), while the stand density and hybrid \times stand density interaction almost equally contributed to seed yield (Table 1). The stand density showed a relatively large effect on seed yield of confectionery sunflower seed, and had a smaller effect on oil content and seed size, according to Zubriski and Zimmermann [1974].

Table 1. ANOVA for seed yield in sunflower hybrids

Source of variation	df	SS	SS (%)	<i>P</i>
Hybrid (H)	5	10.86	50.07	< 0.001
Stand density (SD)	5	4.88	22.50	< 0.001
H \times SD	25	5.95	27.43	0.044
Error (E)	105			

P* < 0.05; *P* < 0.01

On the basis of average for all densities, the highest seed yield was found in NS-H-6485 (4.77 t ha⁻¹) and in NS Gricko (4.43 t ha⁻¹), while the lowest yield was in Cepko (Table 2). According to Balalić *et al.* [2013], in 2012 the average yield for all hybrids and densities was significantly higher (4.29 t ha⁻¹) than in 2011 (3.72 t ha⁻¹). This could be explained by favorable weather conditions during the growing season of 2012. Based on the test results of 97 confectionery sunflower hybrids in Turkey, Kaya *et al.* [2008] reported that the two-year average yield was 2.24 t ha⁻¹, which is significantly lower than the yield achieved in our experiment. Average seed yield of hybrids significantly increased up to 50,000 plants per ha⁻¹, when it reached the value of 4.50 t ha⁻¹, and then decreased (Table 2). In the average, for three years and all hybrids (NS-Dukat, NS-H-111, and NS-H-103), seed yield in oil sunflower type grew up to 60,000 plants per hectare, significantly up to 50,000 plants per hectare, while the regression maximum was at 55,000 plants per hectare [Dušanić *et al.*, 2004]. Crnobarac *et al.* [2006] reported that an average seed yield for four oil sunflower hybrids in 2005 increased to the maximum density. Seed yield at densities of 60,000 and 70,000 plants per hectare was significantly higher than at lower densities, while in 2004 only the lowest (30,000) and the highest density (80,000) had

significantly low yield. According to Barros *et al.* [2004], in Mediterranean region optimum stand density for achieving the maximum sunflower yield was between 3 and 4 plants per m².

Variability of seed yield was 8.9%.

Table 2. Seed yield (t ha⁻¹) in sunflower hybrids

Hybrid	Stand density (plants/ha ⁻¹)						Mean
	20,000	30,000	40,000	50,000	60,000	70,000	
NS Goliat	3.49	3.60	4.21	4.24	4.33	4.26	4.02
NS Slatki	3.79	4.19	4.53	4.56	4.57	4.41	4.34
NS Griccko	4.31	4.48	4.24	4.84	4.38	4.31	4.43
NS-H-6485	3.94	4.69	4.88	5.16	4.93	5.05	4.77
Vranac	4.18	4.42	4.39	3.99	4.10	4.14	4.20
Cepko	3.75	4.12	3.88	4.19	4.03	3.71	3.95
Mean	3.91	4.25	4.35	4.50	4.39	4.31	4.29
LSD	H	SD	H × SD				
0.05	0.22	0.22	0.53				
0.01	0.29	0.29	0.71				

CV= 8.9%

Protein content

One of the indicators of sunflower seed quality is protein content. This is a quantitative trait, determined poligenically. The protein content varies depending on the genotype, agro-ecological conditions, as well as on the interaction between genotype and environmental conditions. Confectionery or protein sunflower type is characterized, among other traits, by lower oil content and increased seed protein content. Radić [2006] reported that, in the process of maturing, protein synthesis previously stabilized in relation to biosynthesis of oil in sunflower seed.

Table 3. ANOVA for protein content in sunflower hybrids

Source of variation	df	SS	SS (%)	P
Hybrid (H)	5	243.46	70.07	< 0.001
Stand density (SD)	5	60.77	18.74	< 0.001
H × SD	25	20.08	6.19	< 0.001
Error (E)	105			

P* < 0.05; *P* < 0.01

For seed protein content additive (hybrid, stand density) and non-additive (hybrid \times stand density interaction) sources of variation showed high significance. Hybrids showed the highest impact on protein content at Rimski Šančevi location (70.07%), followed by stand density (18.74%), while the lowest proportion was observed in hybrid \times stand density interaction (Table 3). According to Dijanović *et al.* [2004], genotype (26.42%) and genotype \times year \times location interaction (20.32%) almost equally contributed to protein content. In addition to genotype, environmental factors have a large impact on seed protein content, as reported by some authors [Merriam *et al.*, 1988; Dušanić 1994]. Stanojević *et al.* [1998] stated that protein content in sunflower depended on environmental factors and locations. Kandil *et al.* [1990], examining the content of protein and oil in five sunflower hybrids and varieties at two locations (Germany and Egypt), came to the conclusion that the protein content was strongly influenced by the location. Dijanović *et al.* [2004] also noted that the protein content in the lines of confectionery sunflower seed also varied depending on the location. Radić *et al.* [2009] examined the seed protein content in two lines (L2 and L4) grown in two locations. The line L4 in the location 2 had significantly lower protein content (15.07%) compared with the location 1 (26.74%).

Table 4. Protein content (%) in sunflower hybrids

Hybrid	Stand density (plants/ha ⁻¹)						Mean
	20,000	30,000	40,000	50,000	60,000	70,000	
NS Goliat	14.08	13.11	13.33	13.03	12.76	12.91	13.20
NS Slatki	16.39	16.24	14.82	15.11	13.34	14.08	15.00
NS Gricko	16.98	15.47	15.46	14.57	14.38	14.61	15.24
NS-H-6485	15.37	14.31	13.42	12.72	13.10	12.93	13.64
Vranac	16.70	16.46	15.87	15.84	15.99	15.82	16.11
Cepko	17.92	17.10	16.92	16.55	16.77	16.40	16.94
Mean	16.24	15.45	14.97	14.64	14.39	14.46	15.02
LSD	H	SD	H \times SD				
0.05	0.29	0.29	0.71				
0.01	0.38	0.38	0.93				

CV = 3.3%

Significantly higher protein content, taking into account all stand densities, showed hybrid Cepko (16.94%). Above the overall average value, protein content is achieved in hybrid Vranac (16.11%). Other hybrids had significantly lower protein content compared with the overall average, which was 15.02% (Table 4). Observing the influence of stand density on protein content it can be seen that the highest content in the average for all six hybrids was at the lowest density (20,000 plants per ha⁻¹), and then decreased up to higher densities.

There were no significant differences in protein content between the last three stand densities, whereby the protein content was significantly lower than the overall average (Table 4). Crnobarac *et al.* [2013] suggested, based on two-year results of the protein content in three confectionery sunflower hybrids grown in eight sowing dates, that the protein content was higher in the medium terms compared with earlier planting dates. Taking into account three hybrids, protein content had the highest value (15.78%) at planting date on 20.04. Dijanović *et al.* [2004] examined the protein content in three inbred lines of confectionery sunflower at three locations (Zaječar, Leskovac and Požarevac) and in three generations (S3, S4 and S5). Cultivar Kolos was taken as a control. The highest protein content had the line Rs4I10-S3 (21.75% in Zaječar and Požarevac, and 22.24% in Leskovac). The minimum protein content showed line D4441-S5 (Zaječar 18.85%, 19.44% Leskovac, Požarevac 19.18%). Hladni *et al.* [2009] reported that the protein content in the seed of new confectionery hybrids ranged from 11.9% (NS-H-6309) to 14.0% (NS-H-6309), while by standards Cepko and Vranac it was 12.8% i.e. 13.2%. The results obtained in 2008 [Hladni *et al.* 2011a] at two locations (Rimski Šančevi, Vojvodina region and Kula, central Serbia) expressed higher seed yield in comparison with standards (Vranac and Cepko), though with a lower seed oil content. In the experiment were used 13 confectionary hybrids and Vranac and Cepko as the control. The protein content ranged from 10.7% (NS-H-17) to 14.2% (NS-H-04). Vranac had 13.1% and Cepko 13.7% protein content. The same authors reported a very strong positive correlation between seed yield and seed protein content, kernel content, and mass of 1,000 seeds. Hladni *et al.* [2011b], based on two-year results at Rimski Šančevi location with three confectionery sunflower hybrids, concluded that the seed protein content ranged from 13.6% (NS-H-6316) to 15.8% (NS-H-6320). According to the results of Hladni *et al.* [2012], the lowest mean value of seed protein content was found in NS-H-6487 (14.4%), and the highest in NS-H-1206 (20.1%). Vranac had 17.5%, and Cepko 20.0% seed protein content. The protein content in the seed ranged from 21.7% (NS-H-1206) to 27.5% (NS-H-6317). The average protein content in the seed of confectionery hybrid Proteinac 94 was 21.43%, according to the results of Dijanović *et al.* [2003]. Dimitrov [1990] talked about the impact of selection on high sunflower seed protein content. The resulting cultivar was Obitel which had an average seed protein content of 22.3%, oil content of 42.6% and seed yield of 2.8 tha^{-1} .

Variability of protein content was rather low (3.3%).

CONCLUSION

According to the results obtained in this paper, the following conclusions can be reached:

- Results of ANOVA for seed yield showed highly significant differences for all sources of variation. Hybrids had the highest impact on seed yield (50.07%), while stand density (22.50%) and hybrid \times stand density interaction (27.43%) almost equally contributed to seed yield.

- The highest average seed yield for all stand densities were found in NS-H-6485 (4.77 t ha⁻¹) and NS Gricko (4.43 tha⁻¹).
- The average seed yield for all hybrids significantly increased up to 50,000 plants per ha⁻¹, when it reached a value of 4.50 tha⁻¹, and then decreased.
- The content of seed protein additive (hybrid, stand density) and non-additive (hybrid × stand density) sources of variation showed high significance. Hybrids have the highest impact on the protein content at Rimski Šančevi location (70.07%), followed by stand density (18.74%), while the lowest proportion showed hybrid × stand density (5.19%).
- Significantly higher protein content was obtained in hybrid Cepko (16.94%), taking into account all stand densities. Above the general average protein content was achieved in hybrid Vranac (16.11%). Other hybrids had significantly lower protein content compared with the overall average, which stood at 15.02%.
- The highest protein content in the average for all six hybrids was at the lowest stand density (20,000 plants ha⁻¹), and then decreased up to highest densities. Between the last three stand densities (50,000, 60,000, 70,000 plants per ha⁻¹) there were no significant differences in protein content, but it was significantly lower than the overall average.

Study results may be helpful in recommending optimal sunflower stand density in this region.

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ПРИНОС СЕМЕНА И САДРЖАЈ ПРОТЕИНА СУНЦОКРЕТА У ЗАВИСНОСТИ ОД ГУСТИНЕ СЕТВЕ

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САЖЕТАК: Циљ истраживања био је да се испита ефекат густине сетве на принос семена и садржај протеина код хибрида сунцокрета. Пољски оглед изведен је на локалитету Римски шанчеви. Испитивано је шест хибрида сунцокрета. Пет хибрида су конзумног типа (НС Голиат, НС Слатки, НС Грицко, Вранац и Цепко), а један је за исхрану птица (NS-H-6485). Оглед је постављен по потпуно случајном блок систему (RCBD), у четири понављања. Сетва је изведена са шест

густина (од 20.000 до 70.000 биљака по хектару, са кораком од 10.000 биљака по хектару). Анализа варијансе (ANOVA) је показала да су ефекти хибрида, густине сетве и интеракције хибрид \times густина сетве били високо значајни за принос семена и садржај протеина. Највећи принос семена, на основу просека свих густина сетве, показали су хибриди NS-H-6485 ($4,77 \text{ t ha}^{-1}$) и HC Гричко ($4,43 \text{ t ha}^{-1}$). Просечан принос хибрида значајно се повећавао до 50.000 биљака по хектару, када је постигнут принос од $4,50 \text{ t ha}^{-1}$, а затим је опадао. Узимајући у обзир све густине сетве, значајно највећи садржај протеина постигао је хибрид Цепко (16,94%). Садржај протеина изнад општег просека имао је и хибрид Вранац (16,11%). Највећи садржај протеина у просеку за свих шест хибрида био је код најмање густине сетве (20.000 биљака по хектару), а затим се смањивао до већих густина. Резултати су показали да густина сетва има значајан утицај на принос семена и садржај протеина код хибрида сунцокрета.

КЉУЧНЕ РЕЧИ: хибрид, интеракција, садржај уља, принос зрна, густина сетве, сунцокрет