

## DIRECT AND INDIRECT EFFECTS OF MORPHOPHYSIOLOGICAL TRAITS ON KERNEL PROTEIN CONTENT OF CONFECTIONARY SUNFLOWER

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The most important criterion for introducing new confectionary sunflower hybrids into production is high protein yield. In the breeding process it is important to identify traits which could be used as selection criteria for increased kernel protein content. Increase of kernel protein content results in increased protein yield. This research was conducted during three vegetation seasons on 22 NS high-protein two-line confectionary sunflower hybrids produced within the breeding program at IFVCNS, Novi Sad, Serbia. Strong and very strong correlations were found among the largest number of examined traits. Based on the analysis of simple correlation coefficients, strong negative correlation was determined between kernel protein content and kernel ratio (-0.516\*). A weak negative interdependence was determined between head diameter, seed protein content, and kernel protein content. Positive but weak correlation was determined between kernel protein content and thickness of seed, length of seed, width of seed, and 1000 seed weight. Path coefficient analysis for kernel protein content at phenotypic level showed that the thickness of seed had a strong positive direct effect on kernel protein content (DE=382\*). Kernel ratio and width of seed had a very strong direct negative effect on kernel protein content (DE=-0.990\*\*; DE=0.600\*\*). A weak direct positive effect of head diameter, seed protein content and length of seed was established, whereas 1000 seed weight had a weak direct negative effect on kernel protein content. Path coefficient analysis indicates showed that the thickness of seed has high great influence on kernel protein content, and an important selection criterion for breeding for high protein yield.

*Key words:* confectionary sunflower, correlations, kernel protein content, path coefficient analysis, quantitative traits.

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## INTRODUCTION

Sunflower is mainly grown for the production of vegetable oils in the world, there are many countries that prefer confectionary sunflower hybrids and varieties (landraces). Confectionary sunflower breeding is characterized by the fact that different markets have different demands when it comes to the seed size, hull color and other traits, which makes this process more difficult and costly (HLADNI, 2016). Around 10% of the world's annual production of sunflower seed is used for non-oil purposes, mainly for confectionary and snack food, as well as for bird and pet food (National Sunflower Association, 2011). Confectionary seed is usually black, white, black with white stripes, colorful and notably larger than the seed of oil type sunflower. It has high hull percentage, with thicker hull loosely connected to the kernel, as well as variable seed shape. The hull is easily separated from the kernel and allows the seed to be dehulled as a whole (FERNANDEZ-MARTINEZ *et al.*, 2009; HLADNI *et al.*, 2012). The most important criteria for introducing new confectionary hybrids into production are: protein and seed yield, plant height, head diameter, seed protein content, seed oil content, number of seeds per head, 1000 seed weight, seed size, color of seed, hull kernel ratio (HLADNI *et al.*, 2016; PEKCAN *et al.*, 2015). Seed protein content is important for seed quality since there is generally a negative correlation between oil and protein content (VEAR, 2016). There has not been done enough research in genetics concerning protein content (DAUGET *et al.*, 2016). Therefore, selection efforts to increase the protein content in sunflower seeds are focused in two directions: confectionary types and oil types. Furthermore, the genetic variability of confectionary types is considerably less than that of oil types, so it should be taken into account during the selection process (KAYA, 2016). According to HLADNI (2010) protein content in sunflower seed is a variable trait, depending on the genotype, agro-ecological conditions and the interaction of the genotype and environment conditions. Breeding for increase of kernel protein content results in increased protein yield. The crude protein ratio had the greatest direct effect on crude protein yields, the direct effect of crude protein ratio on crude protein yield was 56.9% (SINCIK and GOSKOY, 2014). Traits such as seed yield, seed protein content, kernel ratio, 1000 seed weight have a very strong positive direct effect with protein yield and, that breeding for these traits simultaneously breeding for protein yield (SINCIK and GOSKOY, 2014; HLADNI, 2015).

The aim of this study was to determine the interdependence between kernel protein content and 1000 seed weight, head diameter, seed protein content, kernel ratio, length of seed, width of seed and thickness of seed.

## MATERIALS AND METHODS

The research was conducted during three vegetation seasons on 22 confectionary sunflower hybrids, produced within the breeding program at the Institute of Field and Vegetable Crops, Novi Sad, Serbia (IFVCNS). Twenty-two high protein two-line confectionary hybrids: NS-H-1, NS-H-2, NS-H-3, NS-H-4, NS-H-5, NS-H-6, NS-H-7, NS-H-8, NS-H-9, NS-H-10, NS-H-11, NS-H-12, NS-H-13, NS-H-14, NS-H-15, NS-H-16, NS-H-17, NS-H-18, NS-H-19, NS-H-20, NS-H-21, NS-H-22, created by crossing cytoplasmic male sterile female line and male line with a fertility restorer genotype, were examined during three vegetation seasons (2008, 2009 and 2010), at three locations: Rimski Šančevi, Erdevik in the Vojvodina region and Kula in central Serbia. The following traits were examined: 1000 seed weight (TSW), head diameter (HD), seed protein content (SPC), kernel protein content (KPC), kernel ratio (KR), length of seed (LS), width of seed (WS) and thickness of seed (TS). The plot where the experiment was

conducted was 28 m<sup>2</sup> in size and 70cm x 28cm plant density was used. Seeds were planted by hand in 4 rows in April and all plants from the two middle rows were harvested in September except for the first plants on each plot. The experiment was done as a randomized complete block design with 3 replications. Mass of 1000 seeds (g) was measured on a random sample of absolutely clean and air-dried seed in laboratory. Seed protein content and kernel protein content was determined by a conventional micro Kjeldahl method. Kernel ratio was determined by dehulling the seeds and their separation into kernel and hull. Length of seed, width of seed and thickness of seed (mm) was measured using a Vernier caliper.

Mutual relationships between the examined characteristics and direct and indirect effects on kernel protein content were analyzed using path coefficient analysis (WRIGHT, 1921; DEWEY and LU, 1952; IVANOVIĆ and ROSIĆ, 1985). Statistical analysis was performed using R (2014): A language and environment for statistical computing. R Foundation for Statistical computing.

## RESULTS AND DISCUSSION

In confectionary sunflower breeding process it is important to be familiar with relationships between quantitative traits and kernel protein content. The analysis of simple correlation coefficients (Table 1) showed a negative strong correlation between KR and KPC (-0.516\*). A weak negative correlation was found when comparing KPC the HD and SPC. A weak positive correlation was found between LS, TSW, TS, WS and KPC. The analysis of simple correlation coefficient shows a very strong negative correlation between TSW and KR, which is consistent with the research of LI *et al.* (2010). A very strong positive correlation was observed between TS and LS, whereas TSW exhibited a strong positive correlation with both SPC and TS, and a strong negative correlation with KR. This result is in agreement with JOCKOVIĆ *et al.* (2015), who found a strong positive correlation between TSW and SPC. The analysis of the simple correlation coefficient showed a strong positive correlation between HD and KR. A very strong positive correlation was determined between SPC and KR and negative strong correlation between SPC and LS (Table 1). KR demonstrated a very strong negative interdependence with LS, WS and strong negative correlation with TS. A very strong positive correlation was observed between WS and TS (Table 1).

Table 1. Simple correlation coefficients of quantitative traits and kernel protein content

Trait		HD	SPC	KR	LS	WS	TS	KPC
		X <sub>2</sub>	X <sub>3</sub>	X <sub>4</sub>	X <sub>5</sub>	X <sub>6</sub>	X <sub>7</sub>	Y
TSW	X <sub>1</sub>	-0.295	0.538*	-0.615*	0.717**	0.463	0.549*	0.285
HD	X <sub>2</sub>		0.309	0.504*	-0.322	-0.329	-0.295	-0.179
SPC	X <sub>3</sub>			0.693**	-0.607*	-0.293	-0.374	-0.355
KR	X <sub>4</sub>				-0.731**	-0.675**	-0.506*	-0.516*
LS	X <sub>5</sub>					0.424	0.175	0.323
WS	X <sub>6</sub>						0.730**	0.197
TS	X <sub>7</sub>							0.252

X<sub>1</sub>-1000 seed weight (TSW); X<sub>2</sub>- head diameter (HD); X<sub>3</sub>- seed protein content (SPC); X<sub>4</sub>- kernel ratio (KR); X<sub>5</sub>- length of seed (LS); X<sub>6</sub>- width of seed (WS); X<sub>7</sub>- thickness of seed (TS); Y- kernel protein content (KPC).

Table 2. Path coefficient analysis of kernel protein content

Components	DE (P)	IE(Pxr)	CC (r)	Components	DE (P)	IE(Pxr)	CC (r)
Weight 1000 seed (TSW)	-0.148			Length of seed (LS)	0.072		
Indirect effect HD		-0.044		Indirect effect TSW		-0.106	
Indirect effect SPC		-0.117		Indirect effect HD		-0.048	
Indirect effect KR		0.609		Indirect effect SPC		-0.132	
Indirect effect LS		0.052		Indirect effect KR		0.724	
Indirect effect WS		-0.278		Indirect effect WS		-0.254	
Indirect effect TS		0.210		Indirect effect TS		-0.067	
Total			0.285	Total			0.323
Head diameter (HD)	0.148			Width seed (WS)	<b>-0.600**</b>		
Indirect effect WTS		0.444		Indirect effect TSW		-0.068	
Indirect effect SPC		0.067		Indirect effect HDC		0.049	
Indirect effect KR		-0.499		Indirect effect SPC		-0.064	
Indirect effect LS		-0.023		Indirect effect KR		0.668	
Indirect effect WS		0.197		Indirect effect LS		0.031	
Indirect effect TS		0.113		Indirect effect TS		0.279	
Total			-0.179	Total			0.197
Seed protein content (SPC)	0.217			Thickness of the seed (TS)	0.382*		
Indirect effect WTS		0.080		Indirect effect TSW		-0.081	
Indirect effect HD		0.046		Indirect effect HD		-0.044	
Indirect effect SKR		-0.686		Indirect effect SPC		-0.081	
Indirect effect LS		-0.044		Indirect effect KR		0.501	
Indirect effect WS		0.176		Indirect effect LS		0.013	
Indirect effect TS		-0.143		Indirect effect WS		-0.438	
Total			-0.355	Total			0.252
Kernel ratio (KR)	<b>-0.990**</b>						
Indirect effect TSW		0.091					
Indirect effect HD		0.074					
Indirect effect SPC		0.150					
Indirect effect LS		-0.053					
Indirect effect WS		0.405					
Indirect effect TSW		-0.193					
Total			-0.516*				

Coefficient of R<sup>2</sup> determination 0.367

Since the values of simple correlation coefficients did not provide clear connections between the examined characteristics on one hand and KPC on the other, their correlations were further analyzed by using path coefficient analysis, including the involvement of correlation coefficients in a direct and indirect effect on a specific trait (Table 2). Path coefficient analysis for KPC at the phenotypic level showed that the KR and WS had a very strong direct negative effect on KPC (DE=-0.990\*\*; DE=-0.600\*\*), which is in accordance with the simple correlation coefficient of KR. The TS had a strong positive direct effect on KPC (DE=0.382\*), which is in discordance with the simple correlation coefficient as the simple correlation coefficient is weak and of the positive direction. The effect of the simple correlation coefficient was masked with the indirect effect of the TS through KR and WS. The TSW had a weak direct negative effect on KPC. The direct effect of TSW was masked by its positive indirect effect through the KR (IE=0.609) and TS (IE=0.210), and by the negative indirect effect through the WS (IE=-0.279). The HD had a weak positive direct effect on KPC (DE=0.148), while the simple correlation coefficient is weak and of the negative direction. The existence of a weak negative simple correlation coefficient between KPC and HD is the result of the indirect positive effect of HD through TSW (IE=0.444) and indirect negative effect of HD through KR (IE=-0.499). The SPC had a weak positive direct effect (DE=0.217) on KPC, while the simple correlation coefficient is weak and of the negative direction. This correlation was masked with the negative indirect effect of SPC through KR (IE=0.686) and TS (IE=-0.143), as well as the positive indirect effect of SPC through WS (IE=0.176). The LS demonstrated a weak positive direct effect (DE=0.072) on KPC, which is in accordance with the simple correlation coefficient. The focus should be placed on traits with a very strong positive direct effect on KPC. Presence or absence of correlations can contribute to the right choice of examined traits, so as to enhance the efficiency of some selection criteria.

#### CONCLUSION

Within the development of new high-protein hybrids for confectionary use, it is important to find the traits that can be easily determined and at the same time show their interdependence with kernel protein content. The applied path coefficient analysis gave a somewhat different picture than the one given by the correlation analysis. Path coefficient analysis has partitioned the direct and indirect effects of the quantitative traits on kernel protein content of sunflower. The data obtained in this research, indicates that the characteristic such as thickness of seed (0.382\*\*) is the main kernel protein content component which should be used as selection criterion in sunflower breeding.

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**DIREKTNI I INDIREKTNI EFEKTI MORFOFIZIOLOŠKIH SVOJSTAVA NA  
SADRŽAJ PROTEINA U JEZGRU KONZUMNOG SUNCOKRETA**

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**Izvod**

Najvažniji kriterijum za uvođenje novih konzumnih hibrida u proizvodnji je visok prinos proteina. U procesu oplemenjivanja je važno identifikovati svojstva koja utiču na povećanje sadržaja proteina u jezgru. Povećanjem sadržaja proteina u jezgru dolazi do povećanja prinosa proteina. Istraživanje je sprovedeno tokom tri vegetacione sezone sa 22 eksperimentalna konzumna hibrida suncokreta, stvorena u oplemenjivačkom programu Instituta za ratarstvo i povrtarstvo. Između najvećeg broja ispitivanih svojstava postojale su jake i vrlo jake korelacije. Analiza prostih koeficijenata korelacije je pokazala da postoji jaka negativna korelacija između sadržaja proteina u jezgru i udela jezgra (-0.516 \*). Slaba negativna međuzavisnost je ustanovljena između prečnika glave, sadržaja proteina u semenu i sadržaja proteina u jezgru. Utvrđena je pozitivna, ali slaba korelacija sadržaja proteina u jezgru sa debljinom semena, dužinom semena, širinom semena, i težinom 1000 semena. Path koeficijent analiza za sadržaj proteina u jezgru na fenotipskom nivou je pokazala da je debljina semena imala jak pozitivan direktan uticaj na sadržaj proteina u jezgru (DE = 382 \*). Udeo jezgra i širina semena su imali veoma jak direktan negativan uticaj na sadržaj proteina u jezgru (DE = -0,990 \*\*; DE = -0.600 \*\*). Na sadržaj proteina u jezgru ustanovljen je slab direktan pozitivan efekat prečnika glave, sadržaj proteina semena i dužine semena, dok je težina 1000 semena imala slab direktan negativan uticaj. Path koeficijent analiza je pokazala da debljina semena ima veliki uticaj na sadržaj proteina u jezgru i predstavljaju važan selekциони kriterijum kod oplemenjivanja na visok prinos proteina.

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