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Biochemical and physical kernel properties of a standard maize hybrid in different TopCross™ Blends

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ABSTRACT: A pilot experiment was undertaken in order to examine high oil populations of maize (*Zea mays* L.) to be used as pollinators in TopCross blends with commercial ZP341 standard hybrid. Five high oil populations (HOPs) from the Maize Research Institute (MRI) gene bank were chosen for this research, according to their high grain oil content, synchrony between silking of ZP341 and anthesis of the populations and good agronomic performances in 2012. Selfing of ZP341 and HOPs, as well as crosses of ZP341 *cmsS* sterile × HOPs were carried out in 2013. Oil content, fatty acid composition, protein and tryptophan content, and physical characteristics of the obtained kernels were measured. Four HOPs showed significant positive influence on the oil content in the TopCrosses (TC), 16.85 g kg^{-1} on average. Oleic acid, which is the principal monounsaturated fatty acid, was significantly lower in all HOPs and all TCs, while selfed ZP341 had almost twice the average value typical for standard maize. However, this decrease in TCs was in a narrow range from 1 % (in TC-3) to 5 % (in TC-4) and the oleic content of TCs was on average higher by 60 % compared to the typical standard maize. Different favorable and unfavorable significant changes were detected in fatty acid compositions, protein and tryptophan contents and physical kernel properties for each potential TC combination. Results indicate differences in gene effects present in different TC combinations and underscore the need to examine each potential TC blend by conducting similar simple experiments.

Keywords: *Zea mays* L., high oil, pollinators

Introduction

Maize kernel is slightly higher in oil content as compared to other cereals. Standard maize hybrids contain 4 % oil by weight, accumulated in the embryo and in the aleurone (Saoussem et al., 2009). Maize with oil content over 6 % is denoted as High Oil Corn (HOC; Alexander, 1988). The same author showed that feeding trials with HOC improved feed efficiency and increased the rate of weight gain. It was found that as well as high oil content, HOC also had higher protein content and greater concentration of several essential amino acids than conventional hybrids (Engelke, 1997; Cromwell, 2000; Gaspar, 2000). It also had higher oleic acid and more mineral components than conventional maize (Kahrıman et al., 2015).

In human nutrition, refined maize oil is one of the most appropriate edible oils used internationally, with various health benefits. Maize kernel contains an average fatty acid composition of 11 % palmitic (16:0), 2 % stearic (18:0), 24 % oleic (18:1), 62 % linoleic (18:2) and 1 % linolenic (18:3) acids (Weber, 1987). Unsaturated fatty acids are considered healthier because they have a positive influence on reducing serum cholesterol and low density lipoproteins (Mattson and Grundy, 1985), as well as on preventing degenerative diseases and cancer (Mattson and Grundy, 1985; Zhang et al., 2012). On the other hand, increased saturated (palmitic and stearic) fatty acids can enable production of margarines without hydrogenation and the subsequent formation of undesirable *trans* fatty acids (Duvick et al., 2006).

The major problem in developing high oil maize hybrids is a high negative genetic correlation between kernel oil content and grain yield (Lambert, 2001). The procedure named TopCross™ that exploits the xenia effect has been developed to allow production of high-oil maize while maintaining productivity. It consists of sowing a mixture of 90-92 % of sterile high-yielding hybrid and 8-10 % high-oil pollinator. Standard hybrids pollinated by high oil pollinators display an increase in germ weight and germ oil content without compromising yield (Berquist et al., 1998; Tanaka et al., 2009). This system was used successfully on large areas, under a variety of environmental conditions and a number of growing seasons and it has been essential to the commercial acceptance of high-oil maize (Mazur et al., 1999).

A standard drought tolerant commercial hybrid was crossed with five high-oil pollinators, obtained from populations in the MRI gene bank. The aim was to estimate changes in biochemical and physical properties of hybrid kernels under the influence of high-oil pollinators and thus evaluate the potential for their utilization in TC Blends.

Materials and Methods

ZP341 is a drought tolerant commercial hybrid, widely grown in Serbia, south-eastern and eastern Europe. Since commercial seed production of ZP341 is based on *cmsS* cytoplasmic male sterility, production of its sterile version for possible TopCross™ Blends is very feasible. Moreover, this sterile hybrid does not display late break of sterility.

All populations from the MRI gene bank (a total of 3443 landraces, open-pollinated varieties, composites and synthetics) were screened for protein, oil and starch contents using Near Infrared Spectroscopy (NIR) analysis with ANN calibrations. Accessions identified as the high oil populations (HOPs), with oil content over 6 %, were chosen as potential pollinators for TopCross™.

The synchrony between silking of ZP341*cmsS* and anthesis of HOPs for possible TC blends, as well as agronomic performances of HOPs were determined in field trials at Zemun Polje, Serbia (44°51′59′′ N, 20°18′60′′ E, 80 masl), in 2012. The experiment with thirty populations with the highest oil content and ZP341*cmsS* was set up according to the Randomized Complete Block Design (RCBD) in two replications, with each genotype being represented by one row with 10 hills. ZP341*cmsS* was repeatedly sown after every five populations. Conventional weed control and fertilization practices for optimum maize production were applied. The dates of silking and anthesis were noted when at least 50 % of the plants per plot had visible silks and extruded anthers with visible pollen. The populations were selected for the following important agronomic traits: plant emergence and early growth, plant and ear height, root and stalk lodging, tolerance to abiotic (drought) and biotic (diseases and pests) stresses, plant stand at harvest, pollen production and anthesis duration. Apart from the adequate pollen shed and compatible anthesis-silking interval with ZP341*cmsS*, the selection criteria was that the value of the population traits should be at least 70 % of the same trait for ZP341*cmsS*. Five out of 30 populations met this criterion (Table 1).

The five selected HOPs (three of which were landraces, and two synthetics) were crossed in hand pollination with ZP341*cmsS* in Zemun Polje, in 2013. At the same time, the fertile ZP341 was selfed, simulating commercial production. Both trials were set up with the Randomized Complete Block Design in two replications. Kernels from the mid-section of at least 10 pollinated ears were collected for further analysis.

Table 1 – High oil populations (HOPs) with good agronomic performances used as pollinators in the TopCross system.

Accession	Population type	Origin	Grain type	Grain color
L 982	Landrace	Serbia	Flint	Yellow
IP 3486	Synthetic	USA-USDA	Dent	Yellow
IP 3487	Synthetic	USA-USDA	Dent	Yellow
IP 4949	Landrace	Georgia	Flint	White
IP 6450	Landrace	Gene bank Bari - Italy	Flint	Yellow

L = landrace from Western Balkan; IP = introduced population.

Determination of oil content and fatty acid composition

Each sample consisted of 50 g of maize kernels. Oil content (OC) was determined by the standard Soxhlet method. Dried kernels were ground in a Cyclotec 1093 lab mill with a particle size < 500 μm and oil was extracted with hexane in a Soxhlet extractor. Fatty acid (FA) composition of the oil samples was determined as per Ignjatovic-Micic et al. (2015).

Determination of protein and tryptophan content

Each sample was represented by 60 randomly chosen kernels, divided into two subsamples of 30 kernels. Kernels were dried in a controlled oven at 65 °C, overnight (16-18 h), and ground in a Cyclotec 1093 lab mill with a particle size < 500 μm. The flour was defatted by hexane treatment for 4 h in a Soxhlet extractor.

Protein content (PC) was determined by the standard Kjeldahl method using a 2200 Kjeltec Auto Distillation Unit, based on nitrogen determination as explained in Vivek et al. (2008). It was estimated from the nitrogen value as: % protein = % nitrogen × 6.25 (conversion factor for maize).

Tryptophan content (TrC) was determined by the colorimetric method of Nurit et al. (2009). Tryptophan content was calculated using a standard calibration curve, developed with known amounts of tryptophan, ranging from zero to 30 μg mL^{-1}.

Kernel physical characteristics

Hundred-kernel weights (100KW) were measured by weighing 5 × 20 undamaged kernels per sample. The flotation index (FI) was measured by soaking 100 randomly selected kernels into an aqueous sodium nitrate solution with a specific weight of 1.25 g cm^{-3} at 35 °C and values were expressed as the percentage of floating kernels. Kernel dimensions were obtained by measuring thickness (KT), width (KW) and length (KL) of a 100 kernel sample using a digital micrometer. The hull (H), endosperm (E) and germ (G) were manually dissected after soaking 50 g of kernels for 2 min in 100 mL water. After draining the water, the wet kernels were first manually separated into hull, endosperm and germ, the resulting anatomical parts dried in an oven set at 60 °C and weighed in order to calculate relative percentages. The kernel hardness was measured by a modified version of the Stenvert-Pomeranz method, by grinding 20 g of kernels in the micro hammer-mill (3600 rpm, 2 mm sieve). Results were expressed as milling response (MR) and hard fraction portion (%HFP). The MR index presents the time (s) necessary for grain grinding until the top level of the material collected in a glass cylinder (125 × 25 mm) reached the level of 17 mL.

TopCross simulation for oil and fatty acid content

Grain yield of ZP341 from the large strip trials in Serbia in 2013 served as the base for calculating the average content of oil, fatty acids, protein and tryptophan (kg ha^{-1} of dry matter). Average yield of ZP341 over ten locations was 8092.5 kg ha^{-1} with 16 % grain moisture. This value was adjusted to 0 % grain moisture (kg ha^{-1} of dry matter) and multiplied by 0.92

(92 % of grain parent in a potential TC blend). The value obtained was multiplied by the percentages of oil and fatty acids for five TC blends. The same calculations were performed for ZP341 *per se* but without multiplication by 0.92, in order to compare the levels of these nutrients between the selfed hybrid (simulating commercial production) and the hybrid after topcross (used as grain parent).

Statistical analysis

One-way analysis of variance (ANOVA) was performed for the traits analyzed. The significance of differences between ZP341 selfed and HOPs selfed, as well as between ZP341 selfed and the crosses of ZP341*cmsS* × HOPs (TCs), were tested by Fisher's least significant difference (LSD) test at 0.05 level. Also, the significance of differences between average values of ZP341 from large strip trials in 2013 and appropriate simulated TCs for all traits were estimated by the LSD test at 0.05 level. All statistical analyses were carried out using the MSTAT-C software program.

Results

The flowering synchrony of the potential TC blends was confirmed, whereas the anthesis of the HOPs occurred 2 to 3 days after the silking of ZP341 sterile hybrid. Changes in the kernels' biochemical properties of the commercial maize hybrid ZP341 influenced by pollination with the five HOPs are shown in Table 2. One-way ANOVA revealed that all of the HOPs had significantly higher oil contents than the conventional hybrid. However, only four HOPs showed significant positive impact on the oil content in the TopCross blends, on average 16.85 g kg^{-1}. In TCs where the pollinators were synthetics the increase was 22.65 g kg^{-1} and in TCs with landraces as pollinators it was 10.15 g kg^{-1}. Population IP 6450 (HOP-5) caused a significant negative impact on the oil content in the Top-Cross (51.8 g kg^{-1} oil for ZP341 selfed in comparison with 46.6 g kg^{-1} in TC-5).

Significant changes were detected among TC blends for total fatty acid contents (Table 2). Saturated FA (SFA) content increased significantly in TC-1, TC-2 and TC-4, while it decreased significantly in TC-3 and no significant changes occurred in TC-5. All HOPs had a lower content of monounsaturated FA (MUFA) compared to ZP341 selfed. However, significant decreases were found in TC-1, TC-2, TC-4 and TC-5. Regarding polyunsaturated FA (PUFA) content, significant increases were found in TC-2, TC-3 and TC-5, a significant decrease in TC-1 but no significant changes in TC-4. All HOPs had significantly higher PUFA content compared to ZP341 selfed. ω-6/3 ratio was high in all analyzed genotypes, but this relationship was significantly lower in all TCs compared to ZP341 selfed.

Fatty acid composition of the analyzed genotypes is presented in Table 3. ZP341 selfed had average contents of palmitic (16:0) and stearic acids (18:0), higher contents of oleic (18:1) and lower contents of linoleic (18:2) and linolenic (18:3) acids compared to typical standard maize values (11 %, 2 %, 24 %, 62 % and 1 %, respectively). All the other FAs were present in traces in all analyzed geno-

Table 2 – Oil and protein parameters of ZP341 selfed, five high oil populations (HOP) selfed, and crosses of ZP341 *cmsS* with HOPs (TCs).

Genotype		Oil				Protein		
		OC	Total fatty acids (g kg^{-1})			ω-6/3	PC	TrC
			SFA	MUFA	PUFA			
		g kg^{-1}				%	g kg^{-1}	
Original	ZP341 selfed	51.8 g[†]	141.0 f	415.1 a	443.9 e	98 b	117.2 g	0.63 b
HOP-1	L 982 selfed	▲56.8 f	143.6 ef	▼361.0 g	▲499.0 a	94 b	▲126.9 e	0.65 b
TC-1	ZP341*cmsS*xL 982	▲63.0 e	▲190.7 a	▼381.2 d	▼428.2 f	▼69 d	▼111.6 i	0.64 b
HOP-2	IP 3486 selfed	▲110.2 a	▲148.3 de	▼352.7 i	▲499.2 a	▼64 d	▲142.9 b	▲0·79 a
TC-2	ZP341*cmsS*xIP 3486	▲74.2 c	▲153.7 cd	▼368.4 f	▲478.0 c	▼78 c	▼113.1 h	0.65 b
HOP-3	IP 3487 selfed	▲91.4 b	▲157.2 c	▼362.2 g	▲480.7 c	▲126 a	▲137.8 c	▲0.79 a
TC-3	ZP341*cmsS*xIP 3487	▲73.9 c	▼131.7 g	402.3 a	▲466.0 d	▼71 cd	▲119.0 f	0.63 b
HOP-4	IP 4949 selfed	▲69.6 d	▲151.9 cd	▼357.6 h	▲490.5 b	▼64 d	▲127.8 d	0.67 b
TC-4	ZP341*cmsS*xIP 4949	▲60.9 e	▲165.2 b	▼394.7 c	440.1 e	▼65 d	▼96.7 k	▼0.55 c
HOP-5	IP 6450 selfed	▲75.2 c	143.7 ef	▼377.2 e	▲480.7 c	▼69 d	▲144.0 a	▲0.74 a
TC-5	ZP341*cmsS*xIP 6450	▼46.6 h	142.3 ef	▼393.0 c	▲464.8 d	▼90 d	▼108.7 j	0.63 b
Mean		68.0	151.7	378.7	470.1	81	122.3	0.67
MS		6.186**	4.956**	8.339**	11.543**	749.025**	4.524**	0.00001**
SD		1.69	1.51	1.95	2.30	18.63	1.43	0.01
CV		1.63	1.86	0.31	0.51	4.32	0.29	2.69
LSD$_{0.05}$		0.2387	0.6263	0.254	0.5366	7.779	0.07046	0.007046

[†] Means followed by the same letter(s) within the same columns are not significantly different ($p < 0.05$); HOP = high-oil population; TC = TopCross; **statistically significant at 0.01 level; OC = oil content; SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids; PC = protein content; TrC = tryptophan content; ▲ and ▼= values that are significantly higher and lower than for ZP341 selfed; MS = mean square of genotypes from one-way ANOVA; CV = coefficient of variation; SD = standard deviation (in units of measurement); LSD$_{0.05}$ = least significant difference value at 0.05 level.

Table 3 – Fatty acid composition (g kg^{-1} in total oil) of ZP341 selfed, five high oil populations (HOP) selfed, and crosses of ZP341 *cmsS* with HOPs (TCs).

	Fatty acids (g kg^{-1})											
Genotype	16:0	16:1	17:0	18:0	18:1	18:2	18:3	20:0	20:1	20:3	22:0	24:0
Original	110.1 g[†]	0.65 de	0.60 ab	22.2 g	412.7 a	436.1 f	4.5 fg	5.9 defg	1.8 abc	2.9 a	1.1 d	1.2 bc
HOP-1	107.5 g	0.5 e	0.60 ab	▲22.8 e	▼358.9 i	▲491.1 a	▲5.3 e	6.5 cde	1.5 bc	2.6 a	1.2 cd	1.5 abc
TC-1	▲139.6 a	▲0.95 bc	0.6.5 ab	▲39.4 a	▼378.5 e	419.6 h	▲6.2 cd	▲7.7 ab	1.7 bc	2.4 ab	▲1.6 a	1.8 abc
HOP-2	▲116.1 ef	▲0.11 ab	0.65 ab	▼22.5 f	▼354.8 j	▲488.4 a	▲7.7 a	5.8 efg	1.8 abc	2.7 a	▲1.4 abc	1.9 ab
TC-2	▲118.1 de	0.65 de	0.65 ab	▲25.0 c	▼366.2 g	▲468.9 d	▲6.1 d	▲7.1 bc	1.5 bc	2.9 a	▲1.4 abc	1.4 abc
HOP-3	▲125.1 b	0.55 e	0.75 a	▲22.9 e	▼360.4 h	▲474.9 c	3.8 g	6.3 def	1.3 c	▼1.8 bc	1.2 d	1.1 c
TC-3	▲103.4 h	▲0.95 bc	0.55 ab	▼18.7 i	▼399.4 b	▲457.9 e	▲6.5 cd	5.7 fg	2.0 ab	▼1.7 c	▲1.5 ab	▲2.0 a
HOP-4	▲120.8 c	0.85 cd	0.55 ab	▼20.5 h	▼354.8 j	▲479.9 b	▲7.5 ab	6.6 cd	2.0 ab	2.7 a	▲1.5 ab	▲2.1 a
TC-4	▲115.2 f	0.50 e	0.75 a	▲38.4 b	▼392.5 c	▼431.0 g	▲6.7 cd	▲8.2 a	1.8 abc	2.3 abc	1.3 bcd	1.4 abc
HOP-5	▲120.4 cd	▲0.13 a	0.50 b	▼14.2 j	▼372.0 f	▲472.0 cd	▲6.9 bc	5.3 g	2.5 a	▼1.7 bc	▲1.4 abc	1.9 ab
TC-5	109.5 g	0.65 de	0.60 ab	▲23.5 d	▼390.8 d	▲456.6 e	▲5.1 ef	6.2 def	1.6 bc	2.8 a	1.2 d	1.4 abc
Mean	116.9	0.79	0.62	24.5	376.4	461.5	6.0	6.5	1.8	2.4	1.3	1.6
MS	1.973**	0.001**	0.0001*	1.178**	7.862**	11.289**	0.030**	0.016**	0.002**	0.004**	0.001*	0.002**
SD	0.95	0.03	0.008	0.73	1.89	2.27	0.12	0.09	0.03	0.05	0.02	0.03
CV	1.02	9.39	8.99	1.67	0.51	0.46	4.09	5.43	8.71	5.04	7.96	6.16
LSD$_{0.05}$	0.2636	0.02228	0.02228	0.02228	0.09965	0.4286	0.05732	0.07046	0.07046	0.07046	0.02228	0.07046

[†] Means followed by the same letter(s) within the same columns are not significantly different ($p < 0.05$); *,**statistically significant at 0.05 and 0.01 level, respectively; 16:0 = palmitic acid; 16:1 = palmitoleic acid; 17:0 = heptadecanoic acid; 18:0 = stearic acid; 18:1 = oleic acid; 18:2 = linoleic acid; 18:3 = linolenic acid; 20:0 = arachidic acid; 20:1 = eicosenoic acid; 20:3 = mead acid; 22:0 = behenic acid; 24:0 = lignoceric acid; ▲ and ▼ = values that are significantly higher and lower than for ZP341 selfed; MS = mean square of genotypes from one-way ANOVA; CV = coefficient of variation; SD = standard deviation (in units of measurement); LSD$_{0.05}$ = least significant difference value at 0.05 level.

types. Arachidic acid (20:0) was the most abundant trace FA, with an average value of 6.2 g kg^{-1}.

Both principal saturated palmitic and stearic FA contents were significantly higher in TC-1, TC-2 and TC-4, while significantly lower in TC-3 compared to ZP341 selfed. In TC-5, palmitic acid was not significantly different and stearic acid was significantly higher than in the original hybrid. Additionally, palmitic acid was significantly higher in all HOPs except HOP-1 (not significantly different), while stearic acid was significantly higher in HOP-1 and HOP-3 and significantly lower in the other three HOPs compared to ZP341 selfed.

Oleic acid, which is the principal monounsaturated fatty acid was significantly lower in all HOPs and all TCs, while ZP341 selfed had almost twice the average value typically found in standard maize. However, this decrease in TCs was in a narrow range from 1 % (in TC-3) to 5 % (in TC-4) and the oleic content of TCs was on average higher by 60 % compared to the typical standard maize (data not shown).

ZP341 selfed hybrid was poor in both principal polyunsaturated FA (linoleic 18:2 and linolenic 18:3), with 436.1 and 4.5 g kg^{-1}, respectively. Moreover, all five HOPs had low linoleic acid content, while HOP-1 and HOP-3 had low and HOP-2, HOP-4 and HOP-5 average linolenic acid contents in comparison with the average values for standard maize. Significant increases in linoleic acid content was achieved in TC-2, TC-3 and TC-5, while in the other two TCs the content of this FA significantly decreased. Linolenic acid content was significantly increased in all five TCs.

Protein content was significantly higher in all HOPs and TC-3 compared to ZP341 selfed (Table 2). All the other TCs had significantly lower protein contents than the original hybrid. Tryptophan content (TrC) was significantly higher in HOP-2, HOP-3 and HOP-5 than in ZP341 selfed and the values were characteristic for *opaque2* genotypes. Nevertheless, all TCs had lower tryptophan contents compared to HOPs and among Top-Crosses TC-4 had significantly lower value compared to ZP341 selfed.

Physical properties of the kernels are given in Table 4. Considering 100KW, all HOPs had significantly lower values compared with ZP341 selfed, from 33 % (HOP-4) up to 54 % (HOP-3). However, a decrease of 100KW was found only in TC-1 and TC-4. Significant differences in the flotation index (FI) were not found in TCs, except for TC-3, where it increased significantly compared to the original hybrid. Flotation index was significantly higher in HOP-2 and HOP-3. Kernel dimensions were significantly changed only in TC-4, where kernel width and length decreased significantly. Kernel proportions of HOP-1 and TC-1 were at the level of ZP341 selfed. However, hull proportion was significantly lower in all other HOPs and TCs, except HOP-3. Endosperm proportion was significantly lower and germ proportion was significantly higher in HOP-2, HOP-3, TC-2 and TC-3. A significantly higher germ proportion was also found in HOP-5. Among TCs, the two kernel hardness parameters decreased significantly only in TC-1. Both parameters were significantly higher in HOP-1, HOP-2 and HOP-3 compared to ZP341 selfed.

The results of TopCross simulation (kg ha^{-1}) for oil content and fatty acid composition are shown in Table 5. Oil content, 16:0, 18:2, SFA and PUFA increased in comparison to standard hybrid ZP341 for TC-1 to TC-4, while in TC-5 it decreased. Values of 18:0 were at the same level as for the check hybrid for TC-3 and TC-5, while for TC-1, TC-2 and TC-4 these values were higher. For 18:1 and MUFA TC-5 values were lower, for TC-4 and TC-1 at the same level, while the remaining TCs had values higher than ZP341. Regarding 18:3, only TC-5 was at the same level as ZP341, while the remaining TCs had higher values. ZP341, however, was higher in both PC and TrC in comparison with all the TCs.

Discussion

Maize grain oil content showed a low level or absence of genotype by environment interaction and was influenced by the xenia effect (Lambert et al., 2004; Laurie et al., 2004; Mittelman et al., 2003). Thomison et al. (2003) found that weather conditions limiting grain yield did not strongly affect the increased oil content in kernels of TC Blends. They also found insignificant environment × maize type and environment × grain parent interactions for oil content. In previous studies, year, location and hybrid effects on maize fatty acids content were present, but year and location effects were relatively small compared to the hybrid differences (Jel-

Table 4 – Kernel physical characteristics of ZP341 selfed, five high oil populations (HOP) selfed, and crosses of ZP341 *cmsS* with HOPs (TCs).

	100 kernel weight	flotation index	kernel dimensions			kernel proportions			kernel hardness	
			width	length	thickness	hull	endosperm	germ	hard fraction portion	milling response
	g	%	mm			$g\ 100\ g^{-1}$			%	s
Original	43.00 a†	48 cd	4.9 a	12.3 a	9.4 ab	8.00 ab	80.76 ab	11.24 def	66 d	10.88 cd
HOP-1	▼26.50 d	39 d	5.1 a	▼8.5 d	▼8.8 c	8.02 a	82.08 a	9.90 f	▲72 b	▲14.15 a
TC-1	▼39.00 b	43 cd	5.0 a	12.4 a	9.1 b	7.55 bc	81.54 a	10.92 ef	▼63 e	▼9.58 e
HOP-2	▼22.50 e	▲57 b	▼4.4 b	▼10.9 b	▼7.6 d	▼7.50 c	▼75.86 d	▲16.64 a	▲71 b	▲13.35 a
TC-2	44.50 a	43 cd	5.2 a	12.3 a	9.5 a	▼7.50 c	▼78.71 c	▲13.80 bc	67 d	10.08 de
HOP-3	▼20.00 e	▲80 a	▼4.2 b	▼9.8 c	▼7.5 d	7.73 abc	▼78.06 c	▲14.210 b	▲69 c	▲11.90 b
TC-3	43.50 a	▲58 b	5.1 a	12.3 a	9.6 a	▼7.45 c	▼78.20 c	▲14.35 b	66 d	11.18 bc
HOP-4	▼29.00 d	mv	5.1 a	▼8.6 d	9.4 ab	▼5.89 e	81.52 a	12.59 cd	mv	mv
TC-4	▼34.00 c	50 bc	▼4.5 b	▼11.3 b	9.4 ab	▼7.28 cd	81.06 a	11.66 de	65 d	10.13 de
HOP-5	▼27.00 d	46 cd	5.0 a	▼8.5 d	9.1 b	▼6.24 e	79.39 bc	▲4.17 b	74 a	14.10 a
TC-5	42.50 a	52 bc	4.9 a	12.4 a	9.3 ab	▼6.87 d	81.10 a	12.04 de	66 d	10.48 cde
Mean	33.7303	52	4.8	10.8	9.0	7.28	79.84	12.85	68	11.58
MS	167.336**	265.808**	0.228**	5.635**	1.131**	0.931**	7.561**	7.65**	23.731**	5.883**
SD	8.76	11.39	0.34	1.61	0.73	0.67	1.92	1.91	3.28	1.67
CV	3.67	7.99	3.03	2.31	1.61	2.90	0.92	4.71	0.59	3.44
$LSD_{0.05}$	2.761	9.325	0.3305	0.5548	0.3229	0.4727	1.636	1.352	0.9077	0.902

†Means followed by the same letter(s) within the same columns are not significantly different ($p < 0.05$); **Statistically significant at 0.01 level; ▲ and ▼ = values that are significantly higher and lower than for ZP 341 selfed; MS = mean square of genotypes from one-way ANOVA; CV = coefficient of variation; SD = standard deviation (in units of measurement); $LSD_{0.05}$ = least significant difference value at 0.05 level; mv = missing value.

Table 5 – TopCross simulation for oil, fatty acids, protein and tryptophan contents for ZP341 (kg ha^{-1} of dry matter).

	Oil content	Fatty acids					SFA	MUFA	PUFA	PC	TrC
		16:0	18:0	18:1	18:2	18:3					
TC-1	393.83 b†	55.00 a	15.52 a	149.07 c	165.25 b	2.42 c	75.08 a	150.13 c	168.64 b	696.79 cd	4.00 bc
TC-2	463.17 a	54.68 a	11.58 b	169.61 b	217.18 a	2.81 b	71.17 b	170.61 b	221.40 a	706.47 c	4.06 b
TC-3	461.61 a	47.73 b	8.61 c	184.34 a	211.37 a	3.00 a	60.79 c	185.70 a	215.11 a	743.32 b	3.94 c
TC-4	380.41 b	43.82 c	14.61 a	149.29 c	163.95 b	2.53 c	62.85 c	150.14 c	167.42 b	603.71 e	3.43 d
TC-5	290.78 d	31.84 e	6.82 d	113.64 d	132.76 d	1.48 d	41.38 e	114.23 d	135.14 d	678.99 d	3.94 c
ZP341	351.70 c	38.69 d	7.81 cd	148.31 c	153.38 c	1.58 d	49.58 d	145.99 c	156.12 c	795.74 a	4.28 a
Mean	390.25	45.29	10.83	152.38	173.98	2.30	60.14	152.8	177.31	704.17	3.94
MS	39452.25**	762.55**	133.31**	5424.78**	9885.48**	3.808**	1532.63**	5527.32**	10257.68**	41504.81**	0.842**
SD	137.21	16.58	4.89	52.93	62.67	0.94	22.40	53.30	63.86	232.46	1.29
CV	5.43	6.50	11.36	5.16	6.08	9.03	6.94	5.18	6.09	3.06	2.46
$LSD_{0.05}$	18.960	2.635	1.101	7.015	9.472	0.187	3.737	7.096	9.667	19.450	0.085

†Means followed by the same letter(s) within the same columns are not significantly different ($p < 0.05$); SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids; PC = protein content; TrC = tryptophan content; **Statistically significant at 0.01 level; MS = mean square of genotypes from one-way ANOVA; CV = coefficient of variation; SD = standard deviation (in units of measurement); $LSD_{0.05}$ = least significant difference value at 0.05 level.

lum and Marion, 1966; Weber, 1987). Consequently, our results obtained in one year and at one location could be considered valid from the genetic point of view.

The TopCross system was developed for production of high oil maize to maintain high yields. Usually, pollinators used in a TC blend are hybrids or synthetics. In our research, pollinators were five high oil accessions from the MRI gene bank. Two of them were synthetics and three were landraces. Oil content increase in TCs with synthetics was twice the increase found in TCs when the pollinators were landraces. These results could have been expected because synthetic HOPs had, on average, higher oil contents for 37.6 g kg^{-1} compared to the landraces, most probably due to selection for high oil content previously performed with them. Another reason may be the fact that landraces are more heterogeneous than synthetics.

The oil content change cannot be directly compared with other TopCross experiments, because our results do not refer to TC Blends but to the grain parent alone. Surprisingly, the grain parent ZP341 had almost two times higher to oleic acid content than typical standard maize, as well as some other differences from standard maize. Since this hybrid was selected primarily for its high grain yield under drought conditions, and we are just starting high oil breeding programs, ZP341 has never been checked for oil content and fatty acid composition. According to the literature, differences in oil content between TC Blends and conventional hybrids have averaged approximately 30 g kg^{-1} (Cromwell, 2000; Strachan and Kaplan, 2001; Thomison et al., 2003) and in Thomison et al. (2003) these differences ranged from 26 to 37 g kg^{-1}. Compared to Thomison et al. (2003), our extrapolated values were three times lower when landraces were pollinators, but approximately 20 % lower when synthetics were pollinators. These differences could be explained by the fact that our grain parent showed relatively high oil content (51.8 g kg^{-1}), while in Thomison et al. (2003) it was lower, between 38 g kg^{-1} and 43 g kg^{-1}.

Fatty acid composition was changed in different ways, depending on the pollinator. SFA increased in three out of four TCs with increased oil content and the most significant change was found in TC-1 (29.5 g kg^{-1} for palmitic acid and 17.2 g kg^{-1} for stearic acid). In several published papers it has been stated that oleic acid was slightly higher and linoleic acid slightly lower in HOC compared to normal maize (Cromwell, 2000; Lambert, 2001; Thomison et al., 2003). In our research, oleic acid content decreased in all TCs. This could be explained by the fact that the grain parent ZP341 had a high oleic content itself, which was significantly higher compared to HOPs (on average for 44.9 g kg^{-1}). However, oleic acid was still high in TCs, on average higher for 123.9 g kg^{-1} compared to normal maize (Weber, 1987). PUFA increased in three TCs, but decreased and was unchanged in the remaining two TCs. Considering individual fatty acids, linoleic acid decreased significantly in some of the TCs and increased in others, while linolenic acid significantly increased in all TCs. This is contrary to the decrease in PUFA found in Thomison et al. (2003). Nevertheless, all TCs can be considered as low PUFA genotypes because linoleic acid was on average lower for 252.6 g kg^{-1} and linolenic acid for 0.6 g kg^{-1} compared to standard maize contents of 619.0 g kg^{-1} and 7.0 g kg^{-1}, respectively (Weber, 1987). These changes lowered ω-6/3 ratios, from 20 % in TC-2 to 32 % in TC-4. Although the increase in linolenic acid content could be interesting considering that it offers better health benefits than linoleic acid, it is more prone to oxidation and flavor reversion (Preciado-Ortiza et al., 2013), and its content in TCs is still low compared to standard maize genotypes.

The lower level of MUFA in all TCs and higher level of SFA and PUFA in some TCs compared to ZP341 could be the result of a special effect of pollen parent on the grain parent. The complex process of fatty acids synthesis has not yet been completely clarified. However, it has been shown that enzyme activity of desaturases is responsible for synthesizing the majority of polyunsaturated fatty acids. The *fad2* gene encoding enzyme desaturase responsible for oleic to linoleic acid conversion is considered a major locus for natural variation in oleic acid content (Belo et al., 2008). It was suggested that differences in oleic acid content could result from differential temporal regulation of alleles for low and high oleic content of *fad2* gene during the embryo development of maize and it might be that temporal regulation moved in favor of alleles for low oleic acid content in TCs. Additionally, genes other than *fad2* are involved in the regulation of fatty acid content and it is evident that different pathways were activated in different TCs.

Several other unexpected results were obtained related to oil, protein and tryptophan contents. Oil content in one TC decreased compared to ZP341 selfed, although the corresponding HOP had a high oil content of 75.2 g kg^{-1}. Protein content was significantly lower in all TCs. On the contrary, Cromwell (2000) found that high oil maize contains up to 10 g kg^{-1} more protein than standard maize, while in some other research studies protein levels were similar for TC Blends and check hybrids (Lambert et al., 1998; Strachan and Kaplan, 2001; Thomison et al., 2003). The deviations from the expected results could indicate the complexity of trait inheritance. It has been shown that oil content and fatty acid composition are regulated by more than 40 QTL (Alrefai et al., 1995; Yang et al., 2010), while 22 QTL for protein content were found in Goldman et al. (1994). Discrepancies between our results and those obtained in other studies could also be due to differences in materials studied (relatively non-selected populations vs. elite material). Our accessions could harbor alleles not present in commercial germplasm, leading to gene actions involved in oil, fatty acid and protein metabolic pathways different from those present in elite germplasm.

Also, tryptophan content was at the level of the check hybrid in four TCs and significantly lower in one TC. Lysine and tryptophan are highly correlated with an approximate 3:1 ratio (Vivek et al., 2008). Higher lysine levels in TC Blends than in the check hybrids were found in Cromwell (2000) and Thomison et al. (2003). Significant positive correlation between tryptophan and oil content was also found in Ignjatovic-Micic et al. (2015). Lambert et al. (1998) attributed increased lysine content in TC Blends to the larger embryo associated with high oil maize, as most essential amino acids are located in the kernel embryo. Significant increase in the germ size was noted only in TC-2 and TC-3, but their tryptophan content was at the level of the ZP341 selfed. This result could be explained similarly to oil and protein discrepancies attributed to different genotypes used and the possible existence of alleles not present in elite materials.

Profiles of grain physical parameters are important for dry milling and food processing industries. Kernels with 100KW higher than 32 g and FI less than 20 % are desirable (Serna-Saldívar, 2010). All TCs had 100KW higher than 32 g, but none had FI below 20 %. Moreover, FI values were more than twice as high. According to the hardness classification based on the floatation index proposed by Salinas et al. (1992) all TCs had intermediary hard kernels.

Kernel dimensions are important for breakfast cereals and oil crushing (Serna-Saldívar, 2010). No significant changes were found in TCs compared to the original hybrid, except in TC-4 where KL and KT were significantly lower. Considering kernel proportions in high oil TCs, significant increases in germ size followed by significant decreases in hull and endosperm size were found only in TCs where pollinators were high oil synthetic populations, which is in agreement with the findings of Berquist et al. (1998).

The hardness of maize kernel is the most important physical property in dry milling and alkaline cooking industries, as well as for feed efficiency. Except in TC-1, in which both parameters significantly decreased, no significant changes were detected in TCs compared to ZP341. Moreover, these two parameters were comparable with commercial high oil hybrids analyzed in Radosavljevic et al. (2000).

Significances of the differences between the average values of parental genotypes and the appropriate TCs for each trait could be considered a counterpart of the heterosis. Absence of heterotic effect in all five TCs was noted for MUFA and 18:1, as well as for six kernel physical parameters. The presence of positive heterotic effect in all five TCs was detected for 16:1 and 18:0. However, for nine traits (OC, 16:0, 16:1, 18:0, 20:1, 20:3, SFA, 100KW and H) in some of the TCs there was a significantly positive, while in others TCs there was a significantly negative heterotic effect. This clearly indicates differences in gene actions present in different TC combinations for these traits. OC in two TCs did not show intermediate values between grain parent and HOP, unlike expectations assumed by Lambert (2001). These results could also be explained by the heritability of the traits analyzed, since traits with lower heritability tend to exhibit higher heterosis. Highly heritable traits are OC (Dudley et al., 2007; Mangolin et al., 2004; Môro et al., 2012; Zhang et al., 2008), 18:1 and 18:2 (Wassom et al., 2008). On the other hand, moderate heritability was found in 16:0, 18:0 and 18:3 (Wassom et al., 2008), as well as in PC and TrC (Lal and Singh, 2014).

Regarding that the grain OC and composition are not influenced by the environment (Berke and Rocheford, 1995; Goldman et al., 1994; Lambert et al., 2004; Laurie et al., 2004; Mittelman et al., 2003), we assumed that the grain yield of ZP341 from the large strip trials in Serbia in 2013 could serve as a basis for Top-Cross simulation. This is not the case with protein and tryptophan contents which are greatly determined by environmental conditions. The calculated values for oil and fatty acids yields should be viewed with caution, because they refer only to the grain parent of five potential TC blends, without high oil pollinators (we assume that given values should be higher if the actual TC blends were to be analyzed).

The results of this research point to the need to explore each future TC blend by conducting similar simple experiments. In this way, grain yield, oil content and fatty acid composition of new TC blends could be planned with more accuracy.

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