

## A preliminary study for determination of the possibility of simultaneous selection for oil content and grain yield in maize

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### Abstract

We investigated the genetic potential of six maize families for simultaneous selection of oil content and grain yield. Six generations of six families were generated in 2011 and 2012. These genotypes were tested in a field experiment, conducted in Dardanos Research and Application Center, Çanakkale, Turkey, in 2013. Data were collected on oil content and grain yield per plant and then analyzed by using Generation Mean Analysis method to determine the appropriate families for simultaneous selection studies. We also calculated genetic effect estimations for these traits in the investigated genotypes.

Results showed that there were significant differences among the families and generations for oil content and grain yield. The variation in oil content in the families was mainly controlled by additive gene actions. Simultaneous selection did not seem feasible in the tested families, though there was a good chance for considerable enhancement in some genotypes if the investigated traits were taken into account singlehandedly. Three families (A680x-IHO, IHOxB73 and IHOxHYA) showed potential for selection to enhance oil content, while two others (IHOxMo17 and Mo17xIHO) were promising for grain yield. Estimated genetic gains were in the range of 0.4% to 4.1% per cycle for oil content, and 17.7 g to 60.7 g per cycle for grain yield.

**Keywords:** additive gene action, dominance gene action, selection response, Zea mays

### Introduction

Maize is a giant crop with a production value of over 1 billion tonnes in the world. It is widely used in industrial areas as well as feed and food uses (Nuss and Tanumihadrjo, 2010). Despite not considered as an oil plant, maize yields oil in the order of major oil crops (e.g., palm, canola, soybean, peanut, and sunflower). Maize oil consumption is remarkably high in some countries such as Turkey and India (Yayar and Bal, 2007; Langade et al, 2013). Maize oil has a major food use as cooking/salad oil, margarines and spreads. Nonfood uses in cosmetics, biodiesel and other industrial areas are not less important (Moreau, 2005). Also, it is traditionally considered as a premium vegetable oil worldwide because of its lower price for production (Moreau, 2005) as well as its high stability (Pollak and Scot, 2005). Maize oil also has been advocated as a replacement for more expensive oil seeds such as cottonseed and whole soybean (Dado, 1999). Thus, development of high oil maize genotypes is of importance for many countries.

Normal maize genotypes have 3-5.5% oil in their kernels, while «high oil maize» has 6% or over (Lambert, 2001; Langade et al, 2013). The possibility of enhancing oil concentration in maize kernel has been well studied. One of the most famous experiments in plant science, «Illinois Long Term Selection» was launched by Hopkins in 1896, and managed to in-

crease oil level to over 20% in about 100 generations (Dudley and Lambert, 2004). Oil content in «Beijing High Oil» population was increased from 4,71% to 15,5% in 18 cycles of selection (Song and Chen, 2004). Misevic and Alexander (1989) carried out a selection experiment and they achieved to reach 17% level in 24 cycles of selection, from an original 5% oil content. Another high oil maize population KYHO was derived from 14 different Chinese inbred lines after 10 generations of selection (Wang et al, 2012). These results suggest that increasing of oil content in maize need a long time. Breeders need to make a detailed plan before starting selection for oil content and grain yield. To achieve maximum genetic gain with limited time and resources, choice of starting material as well as selection procedures would be extremely important. Otherwise, long-term studies will fail or do not reach the intended destination. For this purpose, uncovering the gene actions and heritability values in starting material gives valuable information to breeders (Robinson et al, 1949; Kumar and Wehner, 2013). The knowledge of type of the gene action makes it possible to choose an effective selection procedure in breeding (Shahrokhi et al, 2013). In fact, determination of gene actions by different analyses such as North Carolina Designs and Generation Mean Analysis (GMA) may provide valuable information before launching a breeding program.

Due to negative relationships between oil content

and grain yield in maize, enhancing oil usually result in decreasing grain yield in selection studies (Hammes, 1997). Oil yield per area is related to not only oil content in kernel but also kernel yield per plant. In fact, these traits should be improved together in a maize breeding program targeted to obtain high oil genotypes. The possibility of simultaneous selection for oil content and grain yield was rarely addressed in the previous studies. Also, there were different findings in this regard, because genetic variability and selection potential varied in the used materials in different studies. The results of some previous studies showed that there was no possibility for the simultaneous selection of oil content and grain yield, due to the negative correlation between these two traits (Misevic and Alexander, 1989; Dudley and Lambert, 1992). However, some others, such as Mittelman et al (2003) and Môro et al (2012), speculated that there was a possibility of increasing of oil content and grain yield together. These results demand a thorough analysis of the issue for newly developed maize genetic resources.

This study aims to evaluate the chances of simultaneous selection for oil content and grain yield in different families which were generated by crossing high oil and normal maize genotypes. Also, we intend to determine the theoretical gain from selection for oil content and grain yield in the families under investigation.

## Materials and Methods

### Plant Material and Experimental Details

In this study, seven parental lines were used to obtain 6 generations of 6 families. In two successive years (2011 and 2012), F1, F2 and backcrosses of families were generated by a seed increasing trials. To develop F1 generations of families, parental lines crossed in 2011, while F2s and backcrosses were made in 2012. F2s were generated by selfing the F1 plants and backcrosses were obtained by crossing F1s with their each of respective parental lines. The information about the families and their generations are summarized in Table 1.

The field experiment to evaluate the families was carried out in Dardanos Research and Application Unit of Çanakkale Onsekiz Mart University in Turkey (long 26.4°N; lat 40.1°E). Each generation of families was planted on May 2013. Experimental design was a randomized complete block design with three replicates. Each plot consisted of two rows, 2 meter long, with 0.7 m spacing. Over-planted plots were thinned

to uniform plant density of approximately 71,400 plants ha<sup>-1</sup>. The plots were fertilized with a total of 180 kg ha<sup>-1</sup> nitrogen and 80 kg ha<sup>-1</sup> phosphorus. Nitrogen application was made in 2 occasions (i.e., before planting and before flowering). Plots were irrigated by drip irrigation on a weekly basis throughout the season. To prevent the pollen contamination among the genotypes, 5-8 plants were hand pollinated in each rows. Harvesting of ear samples were made by hand after physiological maturity.

### Determination of Yield and Oil Content

At least 10 bagged ears were harvested for parents and hybrids, 20 random competitive ears were taken from F2's and 15 ears for backcrosses for oil analysis. For yield determination, same number of ears was randomly taken from open pollinated plants in the same rows. Harvested ear samples were bagged and marked for future analysis. Grain yield per plant were measured by weighing all kernels for each ear samples. For this purpose, all ear samples (840 ears) were shelled by hand and weighed. Next, kernel samples were cleaned and grinded in a laboratory mill (Fritsch pulverisette 14, Germany) with a 0.5 mm sieve. Oil content of each sample was measured by a NIR spectroscopy (Spectrastar 2400D, USA). For this purpose, grinded samples (n = 420) were put into a ground samples cup of the NIR instrument than they scanned in 1,200 - 2,400 nm interval with 1 nm scanning density. Oil content of the samples were determined by Infostar™ software (Unity Scientific, USA) using a local calibration model.

### Statistical Analysis

The variance analysis was done using proc GLM command of SAS V8 software (SAS Institute, 1999). Mean separation test (LSD) was applied to determine the significant differences for grain yield and oil content among the generations of same family and same generation of different families. The genetic analysis for the measured traits was performed using SASQuant macro in SAS (Gusmini et al, 2007). Generation means and variances computed on 5 samples for each parents (P1 and P2), and F1s, 15 to 18 samples for each F2s and backcrosses (BcP1 and BcP2). Data were combined to estimate the genetic effects (Mather and Jinks, 1977; Foolad and Lin, 2001). Additive, dominance and epistatic gene actions were partitioned according to Hayman's procedure (Gamble, 1962; Hayman, 1958). Two main components (additive variance [a] and dominance variance [d]) and three interaction components,

**Table 1 -** The plant materials used in this study.

Family Number	P1	P2	F1	F2	BCa	MBCa
1	A680	IHO	A680xIHO	A680xIHO	(A680xIHO)xA680	(A680xIHO)xIHO
2	IHO	B73	IHOxB73	IHOxB73	(IHOxB73)xB73	(IHOxB73)xIHO
3	IHO	HYA	IHOxHYA	IHOxHYA	(IHOxHYA)xHYA	(IHOxHYA)xIHO
4	IHO	MO17	IHOxMO17	IHOxMO17	(IHOxMO17)xMO17	(IHOxMO17)xIHO
5	IHP	IHO	IHPxIHO	IHPxIHO	(IHPxIHO)xIHO	(IHPxIHO)xIHO
6	MO17	IHO	MO17xIHO	MO17xIHO	(MO17xIHO)xIHO	(MO17xIHO)xIHO

**Table 2** - Means of generations of the families for oil content and grain.

Family	Oil Content						P Values for Families
	MPa	MPb	MF1	MF2	MBCa	MBCb	
A680xIHO	4.0 e BC	13.1 a A	7.9 c B	7.9 c B	5.9 d D	10.1 b A	0.000
IHOxB73	13.1 a A	3.8 e C	8.3 bc B	8.2 bc B	9.9 b B	5.7 d B	0.000
IHOxHYA	13.1 a A	8.9 c B	11.1 b A	11.2 b A	11.5 ab A	9.8 bc A	0.000
IHOxMo17	13.1 a A	3.7 e C	7.7 c B	7.7 c B	10.8 b AB	5.5 d B	0.000
IHPxIHO	4.9 d B	13.1 a A	9.8 b A	8.3 bc B	7.4 c C	10.6 b A	0.000
Mo17xIHO	3.7 d C	13.1 a A	7.2 bc B	7.9 c B	6.1 c CD	9.9 b A	0.000
P Values for Generations	0.000	0.000	0.000	0.000	0.000	0.000	
Family	Grain Yield						P Values for Families
	MPa	MPb	MF1	MF2	MBCa	MBCb	
A680xIHO	77.8 b A	85.0 b A	159 a B	165 a A	145 a A	139 a B	0.000
IHOxB73	85.0 c A	99.7 c A	172 a B	150 b AB	121 bc A	161 ab A	0.000
IHOxHYA	85.0 bc A	77.5 c A	159 a B	124 ab B	136 a A	123 ab B	0.000
IHOxMo17	85.0 bc A	76.3 c A	152 a B	117 ab B	129 ab A	119 ab B	0.000
IHPxIHO	71.0 c A	85.0 bc A	106 ab B	125 a B	128 a A	120 ab B	0.000
Mo17xIHO	76.3 d A	85.0 d A	175 a A	166 ab A	124 c A	138 bc B	0.000
P Values for Generations	0.787	0.514	0.000	0.000	0.595	0.019	

Different lowercase letters in rows and uppercase letters in columns indicate statistically significant difference (LSD test,  $p < 0.05$ ). Mpa: Mean of P1, MPb: Mean of P2, MF1: Mean of F1, MF2: Mean of F2, MBCa: Mean of backcross with P1, MBCb: Mean of backcross with P2.

(additive+additive [aa], additive+dominance [ad], and dominance+dominance [dd]) were determined in genetic estimations. Heritability estimates were classified into three classes (low = 0-30%, moderate = 30.1-60%, and high >60%), as suggested by Robinson et al (1949). Theoretical gain from selection for investigated traits was calculated by Gusmini et al (2007), using selection differential (k) equal to 2.05 for 5% selection intensity.

## Results

### Changes in Means and Variances

The variance analysis showed that there were significant differences for oil content and grain yield in most of the genotypes (Table 2). Oil content ranged from 4% to 13.1% in parents, while it varied from 7.2% to 11.1% in F1s, from 7.7% to 11.2% in F2s, and 5.5% to 11.5% in backcrosses. All F1s had over 7% oil and IHOxHYA cross had the highest value (11.1%). Mean of oil content in backcrosses varied between 5.5% and 11.5%. Backcrosses with high oil recurrent parent showed the higher oil content than the other backcrosses (Table 2). Grain yield per plant was in the range of 71.0 - 99.7 g in parents, with considerable variation (7.2 g and 14.7 g) between parental pairs. The F1 crosses had highest values for grain yield per plant among the generations except the IHPxIHO cross. The yield mean of F2 generations varied from 117 g to 166 g per plant. Backcrosses also showed a high variation (from 119 g to 145 g) for grain yield per plant in the tested materials.

Variance component estimations were summarized in Table 3. Low phenotypic variance was observed in IHOxMo17 and Mo17xIHO crosses compared to other families. Low values for genotypic variance and negative estimates of additive variance observed in these four families (Table 3). Dominance variance for oil content was positive in evaluated ma-

terials except for the IHOxB73. This cross had also higher genetic and additive variance for oil content compared with the others (Table 3). Variance estimations for grain yield showed that environmental variance was higher in three families (A680xIHO, IHOxB73, IHOxHYA) than the others. In these families, negative genetic and additive variance was observed for grain yield. IHOxMo17 and its reciprocal cross had highest genetic and additive variance for grain yield per plant.

### Gene Actions, Heritability and Selection Response

The mean effects (m) in all families found to be significantly different from zero for both investigated traits. The positive and significant additive effects (a) for oil content were found in two families, IHOxB73 and IHOxMo17. Other effects, dominance (d) and interaction components, were found to be nonsignificant for oil content. Heritability estimates for oil content showed that A680xIHO and IHOxB73 crosses had high values, while others had moderate values. Narrow sense heritability was higher in the same families (over 60%) than the others. This case resulted in a higher predicted selection response in A680xIHO and IHOxB73 crosses. It was showed that the oil content could be increased to 1.9% per cycle in A680xIHO cross and 4.1% per cycle in IHOxB73 cross. Despite having a low theoretical gain from selection value (0.4%), IHOxHYA cross should also be consider in selection experiment because of its F2 generation had a 11.2% per cent oil. It seems that IHOxMo17 and its reciprocal cross were not suitable for using in selection targeting to increase the kernel oil content (Table 4).

For grain yield, the significant but negative additive effect was found in only one family (IHOxB73). Interestingly, all of other genetic effects for grain yield were found as nonsignificant in all of remaining families. The most of heritability estimates were negative

**Table 3** - Variance component estimations for investigated traits.

Family	VarP	VarE	Oil Content		
			VarG	VarA	VarD
A680xIHO	2.39	0.47	1.92	1.45	0.47
IHOxB73	2.53	0.41	2.12	3.18	-1.05
IHOxHYA	3.01	1.77	1.24	0.33	0.91
IHOxMo17	1.82	0.85	0.97	-0.49	1.46
IHPxIHO	3.13	1.42	1.71	0.49	1.21
Mo17xIHO	0.78	0.41	0.37	-4.25	4.62
Family	VarP	VarE	Grain Yield		
			VarG	VarA	VarD
A680xIHO	765.47	1329.2	-563.7	-1305	741.7
IHOxB73	963.29	1926.1	-962.8	-1598	635.61
IHOxHYA	1319	1621.5	-302.5	-1326	1023.8
IHOxMo17	1842.1	1509.1	333	368.72	-35.72
IHPxIHO	916.33	886.55	29.78	-1476	1505.7
Mo17xIHO	1752.3	754.43	997.84	1228	-230.2

VarP: Phenotypic variance, VarE: Environmental variance, VarG: Genotypic variance, VarA: Additive variance, VarD: Dominance variance. of backcross with P2.

in the tested materials, excluding IHOxMo17 and its reciprocal cross. These two crosses had a potential to increase grain yield per plant, 17.7 g and 60.4 g per cycle, respectively (Table 4). Considering that Mo17xIHO cross had the highest F2 mean (165 g), considerable yield improvement (225.4 g) could be obtained with selection in this cross.

## Discussion

Both oil content and grain yield are quantitatively inherited traits in maize (Dudley and Lambert, 1992; Wang et al, 2012). These traits are highly affected by genetic and environmental factors. Therefore, a high variation can be seen in the results of different studies where different genetic materials were used. In this study, there was also a notable variation for both traits in all tested families.

Oil content is predominantly controlled by female parent characteristics (Mittelman et al, 2003). Nevertheless, pollen parents may also have significant role on the changes in kernel oil content in maize (Letcworth and Lambert, 1998). In this study, higher values for oil content were obtained in the hybrids, including hi-oil genotypes as female parent. When the female parent had low oil content, some of the crosses, such as IHPxIHO, also gave high values for oil content. When the hi-oil line was used as female parent and it was crossed with a moderate oil parent (IHOxHYA), the superior results were obtained for oil content in its F1 and F2 families. The mean performance of the most F1s were higher than their lower parents, while only two crosses had higher oil content than their parent means. In terms of grain yield, all of the F1 values were higher than their parental lines. This finding indicates that there were heterosis for grain yield in all crosses, and some of them had also positive heterosis for oil content. This result is in agreement with the previous studies where grain yield (Abou-Deif, 2007) and oil content (Werle et al, 2013) showed positive

heterosis. The high, moderate or low oil genotypes used as female parent had no effect on the grain yield of certain crosses (Table 2). For example, IHOxB73 and Mo17xIHO had the highest F1 yield. Transgressive segregation in F2 generations was recorded earlier for these traits (Yadav and Singh, 2011). The loss of yield in the F2 generation of one cross (A680xIHO) was not observed in A680xIHO, indicating a tolerance by this cross to inbreeding. Most of the backcrosses showed their recurrent parent characteristics for both oil content and grain yield. This is an expected result, because backcrossing increases the frequency of the alleles of the recurrent parent in a certain cross (Gonzalez et al, 2014).

Variance component estimations varied by the statistical method, tested materials and experimental design adopted. Thus, there were a high variability for variance component and genetic effect estimations for the investigated traits in earlier studies (Rosulj et al, 2002; Wang et al, 2012). However, most of studies concluded that kernel oil content had higher genotypic variance than the environmental variance (Dudley and Lambert, 1992). Our results are in agreement with this. In GMA, environmental variance is estimated on variances of parental lines and F1s (Kumar and Wehner, 2013). Higher environmental variance for grain yield was observed due to high variability for parental and F1 variance (Table 2).

The variation in the kernel oil content was predominantly attributed to additive gene effects in previous studies (Dudley, 1997). Our results also showed that additive effect was the main genetic factor in most of the crosses (Table 4). Additive effect was found also as positive and significant in the crosses that high oil parent (IHO) was used as female parent. Dominance gene actions play more important role on the changes of grain yield in maize (Zdunic et al, 2008). We observed positive and high dominance effects in the plant material was tested, however, it was



**Table 4** - Genetic effect estimations, heritabilities and selection responses of used families.

Family	Oil Content								
	m	a	d	aa	ad	dd	H2	h2	GS05
A680xIHO	7.89**	-4.17**	-0.26	0.36	0.35	0.64	0.80	0.61	1.9
IHOxB73	8.24**	4.24**	-1.91	-1.81	-0.38	4.24	0.84	1.25	4.1
IHOxHYA	11.17**	1.66	-1.87	-2.0	-0.41	3.59	0.41	0.11	0.4
IHOxMo17	7.73**	5.29**	0.98	1.71	0.62	-2.19	0.53	-0.27	-0.7
IHPxIHO	8.27**	-3.19**	3.54	2.77	0.90	-1.17	0.55	0.16	0.6
Mo17xIHO	7.88**	-3.8**	-0.49	0.87	-1.61	0.47	-5.48	-9.9	
Family	Grain Yield								
	m	a	d	aa	ad	dd	H2	h2	GS05
A680xIHO	164.95**	6.23	-13.1	-91.02	9.86	3.58	-0.74	-1.71	-97.2
IHOxB73	150.26**	-40.1*	41.64	-38.24	-32.74	4.72	-1.00	-1.66	-106
IHOxHYA	123.85**	12.4	99.2	21.89	8.64	-59.55	-0.23	-1.01	-75.2
IHOxMo17	117.18**	9.94	98.46	27.04	5.57	-57.4	0.18	0.20	17.7
IHPxIHO	124.78**	7.60	25.96	-2.15	14.6	-126.6	0.03	-1.61	-100
Mo17xIHO	165.55**	-14.13	-44.87	-139	-9.76	126.5	0.57	0.70	60.4

\*significant at 0.05; \*\*significant at 0.01; m: mean effect which is equal to F2 mean; a: additive effect, d: dominance effect, aa: additive+additive effect, ad: additive+dominance effect, dd: dominance+dominance effect, H2: broad sense heritability, h2: narrow sense heritability, GS05: selection response estimated at 5% selection intensity.

nonsignificant. This may be due to insufficient sample size or by higher order and complex gene interactions (Zdunic et al, 2008). The sign of genetic effect estimations in GMA shows the allelic dispersion in the parents used. If there is negative dominance effect, and the additive gene action is significant, then the favorable and unfavorable alleles for the investigated trait have been inherited from the different parents (Mather and Jinks, 1977). The parents of two crosses (IHOxB73 and IHPxIHO) had different types of alleles for oil content. When the additive+additive estimates have negative sign, gene pairs among the parental genotypes are in a dispersive form in terms of gene contribution from the parents (Mather and Jinks, 1977). Our results indicate this is the case in IHOxB73 cross. If the GMA analysis yields dominance and dominance+dominance effects with opposite signs, this suggests duplicate epistasis. Having the same sign, on the other hand, implies complementary epistasis. In case of the dominance effects had opposite sign of dominance+dominance effect which indicate a duplicate epistasis, while same sign indicate a complementary epistasis (Kearsey and Pooni, 1996). Our findings showed that there was duplicate epistasis in five of the families for both grain yield and oil content. Also, complementary epistasis was observed in Mo17xIHO for oil content and IHOxB73 for grain yield (Table 4). If heritability is high for a trait, genetic gain may be obtained by individual plant selection in early generations. Otherwise, replicated trials and multiple locations in more advanced generations are needed in selection programs (Kumar and Wehner, 2013). In this study, only two families (A680xIHO and IHOxB73) were found to be appropriate for increasing oil content and one family (Mo17xIHO) was suitable for increasing grain yield. In line with this, individual plant selection in early generations can be practiced in those families. In the other genotypes, backcrossing several generations or reciprocal recurrent selection would be appropriate for fixing allele frequencies.

Our estimations for genetic gain from selection per cycle (0.4% for IHOxHYA, 1.9% for A680xIHO, and 4.1% for IHOxB73) were in consistence with the results of previous studies for oil content (Mittelman et al, 2003; Song and Chen, 2004). In terms of grain yield per plant, Mo17xIHO showed higher values for genetic advance compared with the previous studies (El-badawy, 2012). In some families that have been calculated negatively additive and genetic variance, heritability of these families also has led to be negative. Negative heritability estimations in GMA are not unusual and they should be omitted (Gusmini et al, 2007). In fact, the possibility of encountering negative estimations is probably the weakest feature of Generation Mean Analysis Method, which is a highly demanded procedure in plant breeding (Piepho and Möhring, 2010).

In conclusion, it was found that none of the tested materials had potential for simultaneous increase of oil content and grain yield. However, some crosses such as IHOxHYA and IHOxB73 had a notable oil content and they may be suitable for hybrid breeding programs as well as new population development for higher oil content. IHOxMo17 and Mo17xIHO crosses had potential to be used in studies targeting to increase grain yield.

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