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Protein, Oil and Fatty Acid Contents of Hybrids and Their Parents of Sunflower (*Helianthus annuus* L.)

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This research was carried out to determine the contents of protein, oil, fatty acids and the correlation coefficients and heterosis effects for sunflower parents and hybrids. Field trials were conducted during 2004-06, using randomized complete block design with four replications in Mustafa Kemal Pasa, Bursa, Turkey. Three cytoplasmic male sterile lines (CMS01, CMS10 and CMS23) and 2 restorer lines (RHA03 and RHA10) were crossed and obtained 6 hybrid lines (CMS01 × RHA03, CMS10 × RHA03, CMS23 × RHA03, CMS01 × RHA10, CMS10 × RHA10 and CMS23 × RHA10). Protein contents varied among 15.92-23.59 %, oil contents 36.33-50.15 %, oleic acid rates 27.91-49.71 %, linoleic acid 43.25-60.38 %, palmitic acid 3.32-6.70 % and stearic acid rates 3.30-6.48 %. Heterosis rates were found -2.6 to +27.07 % for protein content, -9.62 to +19.88 % for oil, -10.54 to +30.88 % for oleic acid, -24.81 to +5.20 % for linoleic acid, -41.92 to +16.11 % for palmitic acid and -4.62 to +69.81 % for stearic acid rates. Protein content was negatively correlated with oil (r = -0.468**), linoleic acid (r = -0.495**) and palmitic acid (r = -0.349*) and positively correlated with oleic acid (r = 0.482**) and stearic acid (r = 0.544**). It was recorded that there was negative and the highest correlation coefficient between oleic acid and linoleic acid (r = -0.958**), whereas the high positive correlation was fixed between oleic acid and stearic acid (r = 0.576**).

Key Words: Sunflower (*Helianthus annuus* L.), Protein, Oil, Fatty acids, Heterosis and Correlation coefficients.

INTRODUCTION

Sunflower (*Helianthus annuus* L.) is the fourth largest oilcrop, after soybean, oil palm and rapeseed[1]. In Turkey, sunflower is the main crop for production of edible oil. Sunflower farmers use hybrids in more than 90 % of the sunflower production in Turkey[2]. It is grown around 0.5 M ha per year with average seed yields of 1.2-1.6 t/ha^{-1}, a production insufficient for national seed and oil requirements[3].

Oil quality is associated with fatty acid composition[4,5], mainly with percentage of oleic and linoleic acids. However, oils with different fatty acid composition are required depending on their use in industry or for human consumption. Oils with a

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high proportion of oleic acid are more stable than others and contribute to reduction in cardiovascular diseases in humans[6]. On the other hand, linoleic acid is a essential fatty acid for humans and is preferred by industries when oil hydrogenation is required[4]. From a nutritional point of view, saturated fatty acids, especially palmitic acid are regarded as undesirable for human consumption because they have a detrimental atherogenic effect mainly by rising serum cholesterol levels as compared with mono and polyunsaturated fatty acids. Conversely, oleic and linoleic acid are hypocholesterolemic but, although linoleic acid is an essential fatty acid, oils rich in oleic acid are preferred as it combines the hypocholesterolemic effect[7].

Sunflower hybrids are often classified according to the potential oleic acid percentage in their oil. Oleic acid percentage in oil is 10-50 % in traditional hybrids, 50-70 % in mid oleic hybrids and more than 70 % in high oleic hybrids[8]. In all cases, oleic and linoleic acids represent nearly 90 % of the fatty acid content, with stearic and palmitic acids making up most of the remainder. Sunflower oil belongs to the group of high quality edible oils. This is based on its nutritional value considering its high level of biological and energy values[9]. Sunflower oil contains[10] 14-43 % oleic acid and 44-75 % linoleic acid. Chemical and physical properties of oils vary with their fatty acid kinds and composition. Fatty acid composition of vegetable oils is not constant. There is a big different among species in terms of fatty acid composition and is changeable to a large scale depending on many factors. Oil quality depends largely on nourishment value, fatty acid composition and the processing manner for crude oil[11]. Quality of sunflower oil is judged on the basis of the ratio of oleic/linoleic acid. The most frequent fatty acid composition in sunflower oil is: 55-65 % of linoleic acid, 20-30 of oleic acid and remainder including other fatty acid primarily palmitic and stearic acids. It has been determined that there exists a negative correlation between the contents of oleic and linoleic acid, that their contents are genetically controlled[12].

Maximum heterosis over the better parents was 30.08 % for oil content, 13.17 % for palmitic acid, 8.94 % for stearic acid and 77.43 % for oleic acid. The correlation between oleic acid was negative and highly significant[13]. Magnitude of heterosis for oil content was 31.19 % in direct crosses[14].

The primary objective of this study was to estimate the percentages and the effects of heterosis for oil, protein and fatty acids percentage in original sunflower hybrid seeds.

EXPERIMENTAL

Field studies were conducted in 2005 and 2006 at the experimental field of Mustafa Kemal Pasa Vocational School, Uludag University, Mustafa Kemal Pasa, Bursa, Turkey (40º02' M, 28º24' E and altitude 25 m above sea level) on a clay soil having 0.1 % total nitrogen content (Kjeldhal method), 0.41 kg ha^{-1} phosphorus, 7.70 kg ha^{-1} exchangeable potassium and 3.0 % organic matter.

The local climate is temperate, summers are hot and dry, winters are mild and rainy. Mustafa Kemal Pasa is located in the southern Marmara region of Turkey, with average annual rainfall 703 mm and 14.6 ºC mean monthly temperature. Total rainfall from March to August were 236 and 147 mm in 2005 and 2006, respectively. This correspond to 33.5 and 21 %, respectively of the annual precipitation. Mean air temperature during the flowering of sunflower was *ca.* 14.6 ºC in the both experimental years. Mean air temperature during flowering period of the plants was 21-22 ºC. The second year of the study was very dry with 113 mm below average. In 2005, precipitation during the growing period of sunflower was 24 mm below normal but with favourable distribution.

Three cytoplasmic male sterile lines, *viz.*, 'CMS 01', 'CMS 10' and 'CMS 23' and fertility restorer lines; *viz.*, 'RHA 03' and 'RHA 10' using as the parent in the study were improved from certain germplasm sources by the Uludag University, Bursa, Turkey.

The experiments were in a split-split plot arragement of randomized complete block design with four replications. Different three female lines (CMS lines) and one male line (restorer line) were sown in planting ratio of 1 restorer : 3 CMS (as two rows for each CMS line) in the each male cage. Row spacing 60 cm while plant to plant distance 30 cm. Each cage was 21.6 m^2 (5.4 m × 4.0 m). Male and female lines were crossed by honeybees. The cages were surrounded with wooden of 4 m × 5 m × 2.5 m. To prevent bees from escaping and to impede the entrance of insects from out, the cages were covered by 2.5 mm plastic material with holes.

Plantings were done on 18 April 2005 and 21 April 2006. 60 Kg of nitrogen per hectare as diammonium phosphate (DAP, 18-46-0) composed fertilizer was applied prior to sowing and a further 60 kg N ha^{-1} was added when the plants were 30-40 cm in height. After planting, Linuron was sprayed at a rate of 20 cm^3 m^{-2} for weed control. Hand hoeing was done when necessary. Previous crop of the trial field was soybean in the both years. Irrigations were applied as far as reach to FC level the soil moisture deficit at three critical growth periods of sunflower: heading, flowering and milking. In prior to flowering of the plants, small bee hives were put the cages with bee.

The following single cross hybrids and their parents were evaluated in this study.

Parents (cytoplasmic male sterile = CMS)	Parents (restorers)
CMS01	RHA03
CMS10	RHA10
CMS23	

Hybrids

CMS01 × RHA03	CMS01 × RHA10
CMS10 × RHA03	CMS10 × RHA10
CMS23 × RHA03	CMS23 × RHA10

Oil, protein and fatty acids percentages measured in this experiment. Protein content was determined in the Kjeldahl digestor and oil content at The Food Laboratory and fatty acid compositions were determined through gas choromotography in the Oilseeds Agricultural Sales Cooperatives Union Thrace (Trakya Birlik) Laboratory, Corlu, Tekirdag, Turkey. The fatty acid methyl esters were analyzed in an Agilent 6890 N gas chromatography equipped with a flame ionization dedector. The components were separated in an Agilent DB 23 capillary column (60 m with internal diameter of 250 mm and film thickness of 0.25 mm). The following chromatographic conditions were observed: column temperature program, 130 °C for 1 min, 130-170 °C at 6.5 °C/min, 170-215 °C at 2.75 °C/min, 215 °C for 12 min, 215-230 °C at 4 °C/min, 230 °C for 3 min; injector temperature: 250 °C; detector temperature: 250 °C; carrier gas: helium; gas linear speed: 45 mL/min; air linear speed: 300 mL/min; split mode:1:150; volume of injected sample: 1 mL; internal standard: Supelco 37 component FAME mix 10.000 mg/mL. Fatty acids were identified on the basis of pure fatty acid methyl ester and expressed as percentage of total fatty acids (area/area), including minor fatty acids[15]. The contents of oleic (18:1), linoleic (18:2), palmitic (16:0) and stearic (18:0) acids were only evaluated.

Heterosis was computed using mid-parent values.

$$\text{Heterosis} = \frac{\text{Hybrid} - \text{Mid parent}}{\text{Mid parent}} \times 100$$

RESULTS AND DISCUSSION

Results pertaining to different chemical characters of 5 sunflower parents and their 6 hybrids are discussed. In the analysis of variance, the mean squares for genotypes were significant for all traits examined, indicating the presence of variability among the hybrids and their parents.

Protein contents: Analysis of variance revealed significant genetic differences among parents and hybrids. Mean data for protein contents among the parents and hybrids ranged 15.92 to 23.59 %. Among the parents, maximum protein content of 22.08 % was observed for CMS23, while minimum (15.92 %) value observed for RHA03 (Table-1). Among the hybrids CMS23 × RHA03 showed maximum protein content (23.59 %), whereas CMS01 × RHA03 has the minimum value (17.65 %). The works of Maia *et al.*[16], Jasso de Rodriguez *et al.*[17], Roche *et al.*[18] and Lahaye *et al.*[19] who have reported different contents of protein, also support the present study.

Oil contents: Significant genetic differences were observed among the means of parents and hybrids. Oil contents were varied among 36.13-50.15 %. The parental means indicated that CMS01 yielded all other inbred lines with a content of 42.33 %, whereas RHA10 gave the lowest content of 41.18 % (Table-1). These findings were also in accordance with results of previous studies[3,18,20,21].

Fatty acid composition: The data clearly show that there are significant differences in fatty acid composition between sunflower parents and hybrids. Statistical analysis showed that the differences are significant for each of the four fatty acids.

TABLE-1
PROTEIN, OIL AND FATTY ACID CONTENTS OF PARENTS AND HYBRIDS

Genotypes	Protein	Oil	18:1	18:2	16:0	18:0
CMS01	20.34 c	42.33 d	34.02 cd	54.34 b	3.32 g	5.99 b
CMS10	18.93 e	41.84 de	34.83 cd	53.69 b	5.74 d	3.41 e
CMS23	22.08 b	38.78 g	43.35 b	43.25 c	4.88 e	5.85 b
RHA03	15.92 g	41.35 e	32.62 d	54.19 b	6.52 ab	4.22 d
RHA10	18.14 f	41.18 e	27.94 e	60.38 a	6.05 c	3.51 e
CMS01 × RHA03	17.65 f	50.15 a	41.76 b	46.17 c	4.06 f	4.94 b
CMS10 × RHA03	22.15 b	39.83 f	36.72 c	51.19 b	3.56 g	6.47 a
CMS23 × RHA03	23.59 a	38.62 g	49.71 a	36.63 d	5.53 d	5.63 b
CMS01 × RHA10	22.38 b	44.56 c	36.69 c	51.13 b	3.58 g	6.48 a
CMS10 × RHA10	19.55 d	47.01 b	27.93 e	60.00 a	6.70 a	3.30 e
CMS23 × RHA10	21.81 b	36.13 h	31.88 de	54.45 b	6.34 b	4.67 c
LSD (0.05)	0.6152	0.6773	4.0110	3.9820	0.2782	0.4297

The rates of the four main fatty acids are given in Table-1. Oleic, linoleic, palmitic and stearic acids comprised over 97.7% of total fatty acids on the average and of these oleic and linoleic acids comprised over 87.76 % of total fatty acids.

Oleic acid rates: As shown in Table-1, the oleic acid rates varied between 27.93-49.71 % in parents and hybrids. The highest oleic acid rate was obtained in CMS23 × RHA03 hybrid, minimum value from CMS10 × RHA10 hybrid. Average oleic acid rates were evaluated 34.52 % in parents and 37.44 % in hybrids. Previous studies[1,22-24] indicated that the oleic acid rates ranged from 40.90 to 60.01 % and results are in agreement with these studies.

Linoleic acid rates: Mean data for this fatty acid among the parent lines altered from 43.25 to 60.38 % and among the hybrids from 36.63-60.00 %. However, mean values of parents were measured higher from average of hybrids (53.17 and 49.92 %, respectively). Increased linoleic acid rate is positively affect the oil quality. Earlier researchers[1,10,17,21,24] have reported similiar results.

Palmitic acid rates: Average of palmitic acid rate measured as 5.11 %. The parent lines (CMS01, CMS10, CMS23, RHA03 and RHA10) gave higher palmitic acid than the hybrid lines (5.30 and 4.96 %). CMS10 × RHA10 happened first (6.70 %), CMS01 latter (3.32 %) in point of the palmitic acid rates. These findings were also confirmed by several researchers[1,17,21,22,25,26].

Stearic acid rates: Average of hybrids (5.24 %) measured higher more than parent lines (4.59 %). The highest stearic acid rates observed among all lines were 6.48 and 6.47 % for CMS01 × RHA10 and CMS10 × RHA03 (Table-1). The average values for stearic acid was similar to previous reports: 3.0 % by Fernandez-Martinez *et al.*[1], 4.6 % by Jasso de Rodriguez *et al.*[17], 5.1 % by Baydar and Erbas[21] and 5.62-6.82 by Ahmad and Hassan[22].

Heterosis effects

Protein contents: Heterosis rates altered among 2.60 ± 27.07 % for protein contents. CMS01 × RHA03 except, in other hybrids determined positive heterosis

effects. The highest positive heterosis was measured in CMS10 × RHA03 (27.07 %). Ali *et al.*[27] reported that heterosis rates was ranged among 0.63-13.11 % in the protein contents.

Oil content: Heterotic effects for oil content character were positive except for CMS10 × RHA03, CMS23 × RHA03 and CMS23 × RHA10, which showed negative heterosis of -4.23, -3.61 and -9.62 %, respectively. The highest positive heterosis was found in CMS10 × RHA10 (13.27 %). Similar findings were also reported by several researchers[13,28-31].

TABLE-2
HETEROSIS RATES FOR HYBRIDS (%)

Hybrids	Protein	Oil	18:1	18:2	16:0	18:0
CMS01 × RHA03	-2.60	19.88	25.33	-14.90	-17.47	-3.13
CMS10 × RHA03	27.07	-4.23	8.89	-5.09	-41.92	69.81
CMS23 × RHA03	24.21	-3.61	30.88	-24.81	-2.98	11.92
CMS01 × RHA10	16.32	6.73	18.43	-10.86	-23.50	36.42
CMS10 × RHA10	5.44	13.27	-10.99	5.20	13.75	-4.62
CMS23 × RHA10	8.23	-9.62	-10.54	5.09	16.11	-0.21

Oleic acid: Maximum heterosis (30.88 %) was shown by CMS23 × RHA03 hybrid, followed by CMS01 × RHA03 with heterosis value of (25.33 %) for oleic acid. However, CMS10 × RHA10 and CMS23 × RHA10 hybrids gave negative heterosis for this character (-10.99 and -10.54 %, respectively). Singh *et al.*[13] explained 77.43 % heterosis for oleic acid.

Linoleic acid: Heterosis effects varied among 24.81 ± 5.20 for linoleic acids. CMS10 × RHA10 and CMS23 × RHA10 hybrids observed pozitive (5.20 and 5.09 %), whereas in other hybrids measured negative effect. Singh *et al.*[13] reported that none of the hybrids was found superior than parents.

Palmitic acid: Maximum positive heterosis rates of 16.11 and 13.75 % was recorded for CMS23 × RHA10 and CMS10 × RHA10, respectively. However, in other hybrids, heterosis counted as negatively. Some researhers worked with palmitic acid fixed heterosis effects for this character[13].

Stearic acid: The highest heterosis rates found only in stearic acid between fatty acids. Variation borders of stearic acids measured among 4.62 ± 69.81 %. The maximum positive heterosis (69.81 %) observed in CMS10 × RHA03 hybrid. Singh *et al.*[13] reported 8.94 % heterosis for stearic acid.

Correlation coefficients: Correlation coefficients between protein, oil and fatty acids are given Table-3. Protein content was negatively correlated with oil (r = -0.468**), linoleic acid (r = -0.495**) and palmitic acid (r = -0.349*) and positively correlated with oleic acid (r = 0.482**) and stearic acid (r = 0.544**). As oil content increased, protein content decreased as evidenced by a high negative correlation coefficient (r = -0.468**). It was recorded that there was negative and the highest correlation coefficient between with oleic acid and linoleic acid (r = -0.958***).

TABLE-3
CORRELATION COEFFICIENTS AMONG PROTEIN, OIL AND FATTY ACIDS COMPONENTS

Components	1	2	3	4	5	6
Protein (%)	1.000	-0.468**	0.482**	-0.495**	-0.349*	0.544*
Oil (%)	-0.468**	1.000	-0.114	0.175	-0.218	-0.015
Oleic acid (%)	0.482**	-0.114	1.000	-0.958**	-0.359*	0.576**
Linoleic acid (%)	-0.495**	0.175	-0.958**	1.000	0.305	-0.551**
Palmitic acid (%)	-0.349**	-0.218	-0.359*	0.305	1.000	-0.842**
Stearic acid (%)	0.544*	-0.015	0.576**	-0.551**	-0.842**	1.000

*Significant 0.05 and **Significant 0.01.

Whereas, the high positive correlation was fixed between oleic acid and stearic acid (r = 0.576**). The minimum correlation coefficient was determined among oil content and stearic acid (r = -0.015). Singh *et al.*[13], Solorzano Vega and Solorzano Vega[32], Jukic *et al.*[33] and Erdemoglu *et al.*[23] reported that there was an negative and highly significant correlation between oleic and linoleic acid rates. This finding similiar to present research results.

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