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Determination of Yield Performances of Oleic Type Sunflower (*Helianthus annuus* L.) Hybrids Resistant to Broomrape and Downy Mildew

Goksel Evci[1] **Veli Pekcan**[1] **Ibrahim M. Yilmaz**[1] **Nesrin Cıtak**[2] **Nilüfer Tuna**[2] **Onur Ay**[2] **Altug Pilaslı**[2] **Yalcin Kaya**[3*]

[1] Trakya Agricultural Research Institute, Edirne, Turkey.
[2] Trakya Oilseeds Grower Cooperatives, Edirne, Turkey.
[3] Trakya University, Plant Breeding Research Center, Edirne, Turkey.

[*] Corresponding author e-mail: yalcinkaya22@gmail.com

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ABSTRACT

Sunflower (*Helianthus annuus* L.) is the most important edible oil crop in Turkey. Downy mildew and broomrape are the most important limiting factors for yield production in sunflower areas both in Turkey and also Eastern Europe and Black Sea countries. High or mid oleic type sunflower have recently started to gain importance year by year in the world because of that high oleic acid sunflower oil is more appropriate for frying as it is more beneficial to health. Therefore, higher oleic varieties will be demanded frequently in the future both in Turkey and also in the world. The study is involved the oleic type sunflower hybrids developed in Hybrid Sunflower Breeding Project conducted by Trakya Agricultural Research Institute, Edirne, Turkey. The measured values were evaluated in oleic type hybrids evaluated in regional yield trials in dry conditions at Edirne and Luleburgaz in the project during 2011 and2012. Oleic type hybrids resistant to broomrape and downy mildew were tested in yield trials. Some experimental hybrids exhibited higher performance than controls in some locations both for seed yield and seed weight and promising candidate hybrids were selected to promote in registration trials. Based on yield trials in the study, some oleic type candidate hybrids exhibited higher performance in terms of grain yield than other varieties. These hybrids were also resistant to broomrape and downy mildew and their oleic acid contents were measured as over 80% which were characterized as high oleic varieties while some of them were categorized mid oleic acid type as having 60-75% oleic acid content.

Keywords: sunflower, oleic acid, hybrid, yield, broomrape, downy mildew, resistance

Introduction

Downy mildew (*Plasmopara halstedii* (Farl.) Berl. et de Toni) and broomrape (*Orobanche cumana* Wallr) are the most important limiting factors for yield production in sunflower areas both in Turkey, Spain, and also Eastern Europe and Black Sea countries which have more than 60% of world sunflower production (Kaya *et al*. 2012).

Downy mildew occurs depending on the climatic conditions during the sunflower growing season and mildew increases especially in rainy season when sunflower seeds stay longer than ten days under the soil due to low temperature during the sowing time. The mildew infection type is calling as primary (systemic) or secondary infection and the primary infection causes substantial yield reductions up to 100%, whereas secondary infection has non-significant importance on the production of sunflower (Spring *et al*. 1991; Fernandez-Martinez *et al*. 2009; Kulkarni *et al*. 2009; Jocic *et al.* 2010; Vear 2010; Kaya *et al*. 2012).

The metalaxyl seed treatment is the most effective way for chemical control of the primary infection of downy mildew at early stages of development of sunflower. However, the chemical control lost the effectiveness due to appeared high virulent new races in recent years. Therefore; genetic control seems to be the best solution for downy mildew nowadays. Downy mildew has more than determined twenty races in sunflower and resistance is controlled by several single dominant genes called *Pl*-genes providing vertical resistance. Genetic resistance to new races of downy mildew has been determined in wild sunflowers and has been transferred into cultivated sunflower genotypes (Tan *et al*. 1992; Shindrova *et al*. 1994; Kulkarni *et al.* 2009; Jocic *et al.* 2010; Vear 2010; Kaya *et al*. 2012).

Broomrape which is angiosperm parasite reduces up to 100% of sunflower seed yield. Like downy mildew, it results into appearance of new more virulent races of the parasite overcoming the existing sources of resistance (Vranceanu *et al*. 1980). New races other than 5 known races are observed recently that infested sunflower production areas largely in many countries such as Turkey, Spain, Bulgaria, Romania and Russia (Shindrova *et al*. 1998; Kaya *et al*. 2009; Evci *et al*. 2011b; Fernandez-Martinez *et al*. 2013). There has been no chemical control for broomrape until the last ten years so the genetic control was only solution. However, Clearfield system which combines Imidazilonone (IMI) herbicides and IMI-tolerant hybrids designing two technologies working together is also effective chemical way to control both weeds and broomrape in sunflower. On the other hand, new resistant genes were also developed by sunflower breeders against those new races and these resistant hybrids are used widely in the market. Both IMI and genetic resistance is effective solution for broomrape control in sunflower currently and IMI types mostly preferred in intensive weed problem areas (Kaya *et al*. 2009 and 2012; Fernandez-Martinez *et al*. 2013).

Classical sunflower oil fatty acid composition is saturated acids 11% (stearic, palmitic), oleic 20% and linoleic acid 69% and it has a large utilization for cooking or margarine (Baydar and Erbas, 2005). However, high (over 80%) or mid oleic type (60-70%) sunflower oil have recently started to gain importance year by year in the world because of that high oleic acid sunflower oil is more appropriate for frying and is more beneficial for health. Oleic type sunflower production reached over 50% in Spain and France, almost 100% in US, but it was just started recently in Turkey and some Eastern European countries too (Kaya *et al*. 2008; 2010; 2012).

High oleic content is firstly discovered in Russia by Soldatov utilizing from chemical mutations in Pervenent population (Demurin and Borisenko 2011). Oleic acid content is determined by Ol genes and it is determined by genetic factors which is also highly influenced by environmental factors (mainly night temperatures during grain filling period) (Pacureanu-Joita *et al*. 1999 and 2005; Baydar and Erbas, 2005; Izquierdo *et al*. 2006; Evci *et al*. 2009; Fernandez-Martinez *et al*. 2009; Demurin and Borisenko 2011). Today, mostly Pervenent mutation sources are using widely in sunflower breeding programs. Due to this recent trend, higher oleic varieties will be demanded frequently in the future both in Turkey and also in the world (Kaya *et al*. 2012).

Use of genetically resistant hybrids is definitely the most effective, economically feasible and environmentally friendly solution for controlling both broomrape and downy mildew in sunflower (Vear 2010). Therefore, most of the sunflower breeding program is designed as primary goal to develop resistant inbred lines and hybrids to both broomrape and downy mildew together as well as having high yielding and quality performances (Pacureanu-Joita *et al*. 1999; Fernandez-Martinez *et al*. 2009; Kaya *et al*. 2012).

National Sunflower Research Project being conducted by Trakya Agricultural Research Institute (TARI) in Edirne is primary public breeding program in Turkey. Many sunflower hybrids and inbred lines have been developed until today in National project. The yield trials in this project have been set up with the objective of developing sunflower genotypes genetically resistant to dominant races of downy mildew and new races of broomrape in both oleic and linoleic types in Trakya Region which covers almost 60% of sunflower production in Turkey. The study covered 2011-12 cycles of that National project with joint project of Trakya Birlik and the aim of study was to determine yield and quality performance of high oleic type sunflower hybrids resistant to broomrape and downy mildew in different locations.

Materials and methods

The experiments involving candidate sunflower hybrids and lines developed by National Sunflower Research Project conducting by TARI were conducted in 2011 and 2012 in the present study. Two regional yield trials in 2011 in Lüleburgaz and Edirne locations and one in Edirne in 2012 were also conducted in the study. Total 22 candidate hybrids existed in 2009 and 13 candidates in 2010 with four controls. Commercial hybrids such as linoleic types Tunca belonging to Limagrain and Bosfora belonging to Syngenta Seed Company and as high oleic types LG 5400 belonging to Limagrain Seed, P64H34 belonging to Pioneer Seed Co, Oliva from May Seed Co and Oleko belonging to Syngenta Seed Company were used as controls in the yield trials.

The experimental design was a Randomized Complete Block Design with four replicates. The four rows plots were 7,5 m long with the 70x35 cm plant spacing. The middle 2 rows were harvested and the border rows were discarded, and plot size was 4.16 m² at harvest. Trials were planted mostly in mid April and they were harvested manually in mid September in each year. Oleic acid content (%), seed yield (kg ha^{-1}), 1000 seed weight (g), flowering and physiological maturity (days), plant height (cm), head diameter (cm), oil content (%) were measured. Oil contents of the hybrids were determined using Nuclear Magnetic Resonance (NMR) analysis at TARI institute lab whereas fatty acid contents including oleic acid were measured by Gas Chromatography (GC) in Trakya Birlik lab using 122-2361 DB-223 type colon. Briefly, samples from sunflower genetic material were ground, at least 1 g ground materials was treated with N-Heptan solution followed by 0.5 Ml, Methanol KOH solution. Finally, 100 microliters clarified solution was analyzed in GC after waiting for at least one hour. The data were analyzed statistically in JUMP program.

Broomrape tolerance of hybrids was determined under artificial and natural conditions. To use in artificial tests in growth chamber during winter, seeds were harvested from the broomrape plants collected from different infested fields of Trakya Region. For artificial screening, broomrape seeds in the pots (1-2 g/pots) were mixed with soil (sand, turf and normal soil). Broomrape shoots were counted on the root system of the sunflower plants of each pot after 45 days from planting (Kaya *et al*. 2009). In the natural infested fields in the Malkara, Tekirdag, *Orobanche* test plots were 5 m length, 14-15 plants existed in each row with 35 cm x 1 m plant density in two replications. Susceptible Sanbro hybrid was used as one control after every 40 rows. On the basis of observations, broomrape resistant, tolerant and susceptible hybrids were determined.

The resistance to downy mildew of sunflower hybrids was determined in artificial downy mildew tests in this study. In the tolerance tests, firstly sunflower seeds were germinated in the dark room until seedlings root elongated up to 0,5-1 cm. Then germinated seeds were soaked with spore solution in petri dishes to infest to downy mildew diseases. These germinated seeds after waiting at +16 °C planted into plastic glasses or pots filling with sterilized sand + perlite in growth chamber. Temperature +24 °C was maintained as 12 hours day and 12 hours dark in the chamber under controlled conditions then plants were grown in that conditions (Spring *et al*. 1991; Kulkarni *et al*. 2009; Evci *et al*. 2011a)

When first true leaves reached to 2-3 mm, the pots or glasses were covered with plastic bags strictly without allowing any air to appear 100% humidity. These plants have been staying at +16-17 °C temperature until 24-48 hrs then while susceptible ones were infested with disease and white spores appeared over cotyledons, resistant ones were immune (Viranyi 1985; Spring *et al*. 1991; Evci *et al*. 2011a).

Results

In the study, almost all candidate hybrids exhibited broomrape and downy mildew resistance in the both artificial tests in the lab. There was no broomrape and downy mildew in the hybrids in the field trial observations too. Some experimental hybrids exhibited higher performance than controls in some locations both for seed yield and seed weight and promising candidate hybrids were selected to send registration trials. Based on yield trials in 2011, 10 TR 048, 11 TR 068, 11 TR 076 and 11 TR 072 oleic type candidate hybrids exhibited higher performance in terms of grain and oil yield than other varieties in both locations. Among these high oleic varieties, 11 TR 072, and 11 TR 066 varieties were high oleic acid type, 11 TR 068, 10 TR 048 and 11 TR 076 varieties were mid oleic acid type in 2011 (Table 1-2).

Only 12 TR 013 was observed as promising candidate hybrid resistant to broomrape and downy mildew in seed and oil yield among candidate hybrids existed in 2012 regional yield trial (Table 3). Its oil content was higher than controls (46,4%) and its oleic acid content was 74.72% so that hybrid was categorized as mid oleic type.

Discussion

After evaluating yield performance and other yield traits of candidate hybrids, 9982-R, 9987-R, 9979-R and 99791-R sunflower restorer lines of these promising sunflower hybrids having higher seed and oil yield performance as well as resistance to broomrape and downy mildew resistant , these were also sent to registration trials too. These male lines also depicted broomrape and downy resistance as well as high and mid oleic content in the tests. As a result, National Sunflower project reached the target for developing both higher seed and oil yielding inbred lines and also being broomrape and downy mildew resistance which are the biggest problems in Turkish sunflower production areas.

Table 1. List of morpho-physiological traits and adaptation mechanism under heat/drought stress.

Hybrids	S Yield (kg ha^{-1})	Rk	Rate to Std(%)	Oil Y (kg ha^{-1})	Rk	Oil C (%)	Oleic A %	TSW (g)	Flower (Day)	PM (day)	PH (cm)	HD (cm)
11 TR 077	227	4	101,3	116	1	51,1	58,03	40,9	63	98	152	16
P64H34 (C)	239	2	106,7	105	2	44,1	85,45	44,0	62	102	133	16
LG 5400 (C)	242	1	108,0	104	3	42,8	86,31	46,8	63	99	138	15
11 TR 072	229	3	102,2	103	4	44,8	84,42	40,9	63	101	127	13
11 TR 068	224	7	100,2	103	5	45,7	72,53	40,8	62	99	138	14
10 TR 048	225	5	100,5	102	6	45,2	58,70	42,2	62	102	133	15
11 TR 076	224	8	99,8	101	7	45,3	68,16	41,6	61	100	138	14
11 TR 066	224	6	99,9	98	8	43,6	83,21	41,7	62	102	135	15
OLEKO (C)	216	9	96,4	92	9	42,7	89,15	40,6	60	98	134	17
11 TR 070	196	16	87,4	92	10	47,1	57,83	38,5	62	101	129	15
11 TR 075	199	13	89,0	91	11	45,5	83,44	41,6	62	99	133	15
11 TR 064	210	10	93,7	90	12	42,9	73,01	44,2	63	99	136	14
OLİVA (C)	199	11	88,9	90	13	45,0	86,73	37,9	62	102	118	15
11 TR 065	199	12	89,0	90	14	45,3	72,92	38,5	62	103	133	14
11 TR 069	182	21	81,3	90	15	49,6	57,44	40,4	65	-	128	15
11 TR 071	198	14	88,4	88	16	44,5	86,78	40,5	63	101	120	14
11 TR 063	196	15	87,6	86	17	43,7	85,37	37,8	65	102	133	14
11 TR 074	185	20	82,7	86	18	46,2	77,70	35,8	64	101	140	13
11 TR 062	194	17	86,5	85	19	43,8	81,99	40,9	64	103	143	15
11 TR 073	185	19	82,6	83	20	45,1	78,87	38,4	64	103	136	13
11 TR 067	190	18	84,8	82	21	43,4	75,16	40,5	62	103	132	15

Rk: Rank; Oil Y: Oil Yield; Oil C: Oil Content; Oleic A: Oleic Acid; TSW: Total Seed Weight; Flowr: Flowering Time; PM: Physiological Maturity; PH: Plant Height; HD: Head Diameter

CV (%) =8,6 LSD=253,2 kg ha^{-1} for seed yield,
CV (%) =8,6 LSD=114,3 kg ha^{-1} for oil yield,

Table 2. Sunflower hybrids in Yield Trial-4 at Lüleburgaz in 2011

Hybrids	S Yield (kg ha^{-1})	Rk	Rate to Std(%)	Oil Y (kg ha^{-1})	Rk	Oil C (%)	TSW (g)	Flower (Day)	PM (day)	PH (cm)	HD (cm)
11 TR 076	247	1	127,8	111	1	44,8	39,8	59	99	143	18
11 TR 077	226	2	116,7	100	2	44,4	48,4	61	97	155	21
OLEKO (C)	219	3	113,5	100	3	45,4	48,8	62	97	148	17
10 TR 048	214	4	110,8	96	4	44,7	45,0	60	100	155	18
11 TR 072	207	5	107,1	95	5	45,8	51,7	61	101	149	21
11 TR 065	198	6	102,4	89	9	44,8	44,5	61	102	156	17
11 TR 071	197	7	101,9	87	10	44,1	53,8	63	100	151	22
11 TR 068	194	8	100,2	95	6	49,2	42,8	61	98	159	21
11 TR 066	193	9	99,6	83	14	43,1	42,9	62	102	154	21
OLİVA (C)	191	10	99,0	93	7	48,7	49,0	62	101	162	20
LG 5400 (C)	189	11	97,7	93	8	49,0	49,1	62	99	130	17

Continuing table 2

Hybrids	S Yield (kg ha^{-1})	Rk	Rate to Std(%)	Oil Y (kg ha^{-1})	Rk	Oil C (%)	TSW (g)	Flower (Day)	PM (day)	PH (cm)	HD (cm)
11 TR 067	187	12	96,9	86	11	46,1	52,7	62	102	163	19
11 TR 074	186	13	96,3	86	12	46,4	36,3	62	99	154	15
11 TR 075	183	14	94,8	83	15	45,5	43,6	61	98	150	18
11 TR 073	181	15	93,7	80	17	44,0	51,8	62	101	160	17
11 TR 062	179	16	92,7	86	13	48,0	42,7	61	101	146	19
P64H34 (C)	174	17	90,1	83	16	47,4	42,2	64	100	123	15
11 TR 063	164	18	84,7	73	18	44,8	40,2	61	102	142	18
11 TR 070	149	19	77,3	73	19	48,6	53,1	63	100	146	19
11 TR 064	145	20	74,9	66	20	45,6	49,1	61	98	156	17

CV (%) =8,6 LSD=253,2 kg ha^{-1} for seed yield,
CV (%) =8,6 LSD=114,3 kg ha^{-1} for oil yield,

Table 3. Sunflower hybrids in Yield Trial-1 at Edirne in 2012

Hybrids	S Yield (kg ha^{-1})	Rk	Rate to Std(%)	Oil Y (kg ha^{-1})	Rk	Oil C (%)	Oleic A %	TSW (g)	Flower (Day)	PM (day)	PH (cm)	HD (cm)
BOSFORA(C)	253	1	107	110	1	43,6	37,01	44,88	64	100	167	17
P 64 H 34 (C)	243	2	103	105	3	43,3	86,90	38,44	63	105	148	16
12-TR-013	235	3	99	109	2	46,4	74,72	36,55	66	100	146	21
12-TR-001	229	4	97	87	15	38,0	61,66	34,04	65	105	138	14
TUNCA (C)	227	5	96	101	4	44,4	42,22	35,10	67	102	151	14
12-TR-003	226	6	96	101	5	44,8	83,66	27,65	67	102	154	14
12-TR-008	225	7	95	99	6	43,8	59,96	35,72	65	105	165	17
LG 5400 (C)	223	8	94	97	8	43,3	85,36	38,64	65	100	147	14
12-TR-012	221	9	94	97	9	44,0	83,36	42,46	66	106	161	17
12-TR-009	216	10	91	97	10	44,7	86,79	31,45	66	107	156	15
12-TR-012	212	12	90	90	14	42,5	80,88	34,12	67	106	174	14
12-TR-005	209	13	89	93	13	44,5	-	32,24	64	106	161	17
12-TR-007	206	14	87	86	16	41,9	60,48	32,24	64	105	159	16
12-TR-004	203	15	86	97	11	47,8	78,90	28,48	67	105	154	16
12-TR-002	203	16	86	86	17	42,5	82,94	34,68	66	102	152	15
12-TR-006	202	17	86	96	12	47,3	82,41	40,85	65	108	152	18
12-TR-014	170	18	72	75	18	44,0	54,36	34,25	66	99	145	15

CV (%) =9,30 LSD=287,3 kg ha^{-1} for seed yield,
CV (%) =9,31 LSD=126,7 kg ha^{-1} for oil yield,

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