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SCREENING FOR RESISTANCE TO FUSARIUM WILT IN INDUCED MUTANTS AND WORLD COLLECTION OF SESAME UNDER INTENSIVE MANAGEMENT

R. Soner SİLME*[1] M. İlhan ÇAĞIRGAN[1]

[1]*Akdeniz University, Faculty of Agriculture ,Dept. of Field Crops, Antalya-Turkey*
**Corresponding Author: ragipsoner@akdeniz.edu.tr*

ABSTRACT

Resistance levels of 25 sesame genotypes were evaluated for their reaction to *Fusarium oxysporium* f.sp. *sesami*. The evaluation was carried out during two successive seasons (2007 and 2008) in two soil conditions of an area known contaminated by a virulent isolate of the pathogen. All healthy and diseased plants for each row was counted and according to a formula reactions of the plants were recorded on a scale from 1 (≤ 10% resistant) to 9 (≥ 51% highly susceptible). Highly significant differences of susceptibility and resistance to the wilt pathogen were observed among genotypes. The level of infection varied in the genotypes. According to the mean of combined results, "Birkan", Çamdibi, WS-143, WS-313 were classified as resistant (R) while WS-131 were categorized as moderately susceptible (MS). These genotypes continued their resistance and susceptibility in both soil types and years, respectively. It was concluded that the genotypes in the resistant category and specifically "Birkan", a recently released cultivar for large seed and high yield, seems to possess also the resistance to the *Fusarium* wilt disease. This genotype could be used as the sources of resistance to the wilt in the breeding programmes aiming to develop sesame suitable to intensive management.

Key words: Sesame world collection, mutants, sesame wilting, resistance levels

INTRODUCTION

Sesame (*Sesamum indicum* L.) is known as the most ancient oil seed according to some archaeological findings (Nayar, 1984; Bedigian and Harlan, 1986). Its cultivation goes back to 2130 B.C. (Weiss, 1983). It is well suited to different crop rotations and is mostly grown under moisture stress without- or with low-inputs by small holders. Plant architecture of sesame is poorly adapted to modern farming systems due to its indeterminate growth habit, seed shattering at maturity and sensitivity to wilting under intensive management (Çağırgan, 2001). Although it is a low input requiring crop, it responds well to additional inputs of irrigation and fertilizer at least by doubling the yield capacity and thus has an important role in intensive management systems including sequential multiple cropping (Çağırgan, 2006).

One of the important biotic factor for intensive management of sesame crop, causing to wilting is *Fusarium oxysporum* f. sp. *sesami* (Fos) which limits production of sesame, was reported for the first time in America (Armstrong and Armstrong, 1950).

To overcome this problem, resistance sources of genotypes of sesame are developed by mutagenesis (Çağırgan 2001; Pathirana 1992) or populations/collections are screened against pathogens under controlled or field conditions by various methods (Matchett, 1995; El-Shazly et al., 1999; El-Bramawy et al., 2001; 2006; Silme and Çağırgan, 2005; Kavak and Boydak, 2006).

The available germplasm should be continously evaluated at intervals for resistance, as new races of Fos may arise. Mutation breeding has also been used to broaden genetic pool in disease-resistance breeding of sesame. In previous studies the use of induced mutations in sesame breeding has produced several useful mutants (Pathirina, 1992; Çağırgan, 2001). Consequently, this research concentrated on the screening and evaluation of resistance to Fos and, we report here the result of screening of the mutants developed and a world collection of sesame maintained at Akdeniz University.

MATERIALS AND METHODS

Genetic material and growing conditions

A total of 25 genotypes consist of 19 genotypes selected from the world sesame collection maintained at the Akdeniz University, 4 mutants (Çağırgan, 2001, Boureima et.al., 2009) and 2 cultivars from breeding program were used in the study (Table 1). The experiments for screening resistance levels were conducted in the fields known as contaminated with Fos at the campus of Akdeniz University, Antalya, Turkey; in 2007 and 2008 using Randomised Complete Blocks Design with three replicates in two different soil conditions. Soil 1 is clay and rich in organic compounds and soil 2 is calcareous and poor in organic compounds (Table 2).

Each genotype was sown in a two-row plot. The rows were 5 m long and 0.7 m apart. Border rows were also included to eliminate border effects. All rows in plots were irrigated by sprinklers as the need of plants.

Table 1. Characteristics of the germplasm used in the field studies for reaction to wilting.

Genotypes	Origin	Branching	Physiological maturity (days)	Seed colour	1000- seed Weight(g)
WS[1]-10	Mexico	+	84.0	yellowish light brown	2.7
WS-131	India	+	94.0	cream	2.8
WS-141	India	+	94.0	yellowish light brown	3.0
WS-166	India	+	89.3	light brown/black	3.2
WS-19	India	+	86.7	dark brown/black	2.6
WS-2	Venezuela	+	90.7	light brown	3.0
WS-606	Kore	+	85.3	white/cream	2.6
WS-616	Kore	+	85.0	cream/light brown	2.4
WS-638	Kore	+	91.3	dark brown	2.9
WS-65	China	+	88.0	cream/dark brown	2.4
WS-230	Pakistan	+	99.0	yellowish light brown	3.3
WS-262	Irak	+	89.7	yellowish light brown	3.2
WS-416	Irak	+	94.7	yellowish light brown	3.6
WS-564	Pakistan	+	102.0	cream/light brown	3.3
WS-565	Pakistan	+	99.0	dark brown	3.2
WS-571	Pakistan	+	101.0	yellowish light brown	3.2
WS-313	Turkey	+	81.0	light brown	3.3
WS-143	Turkey	+	74.3	cream	3.4
WS-189	India	+	99.3	dark brown/black	3.2
wt[2]-3	Turkey	+	73.0	light brown	3.9
wt-5	Turkey	+	80.0	dark brown	3.3
wt-8	Turkey	+	73.0	light brown	3.4
Birkan	Turkey	+	86.0	yellowish light brown	4.2
Çamdibi	Turkey	+	73.0	yellowish light brown	3.7
Muganlı-57	Turkey	+	74.0	yellowish light brown	3.8

[1]WS: world sesame; [2]wt: wilting tolerant mutant (Çağırgan 2001); "Birkan" a recently released cultivar with large seed and tolerant to Fos, Çamdibi unregistered local cultivar; Muganlı-57 widely grown local cultivar and parent to the mutant cultivar "Birkan".

The experimental site has an arid climate in which summer is hot and dry and the winter is generally warm. Almost all rainfall occurs during winter period (Table 3). The altitude of the research field from sea level is approximate 51 m and is located at 30° 44' E and 36° 52' N.

Table 2. Some physical and chemical properties of soils in the experimental field in the years 2007-2008.

Characteristics	Unit	Soil 1 2007	Soil 1 2008	Soil 2 2007	Soil 2 2008
pH		8.01	8.27	8.08	8.12
EC (Salt)	mmhos/cm	1.9	1.7	1.7	1.9
$CaCO_3$	%	17.3	11.0	25.0	42.7
Organic Matter	%	2.8	2.9	1.0	1.4
Total N	%	0.08	0.08	0.04	0.03
Available P	%	0.0174	0.0163	0.0170	0.0181
Available K	%	0.0230	0.0080	0.0242	0.0245
Available Ca	%	0.260	0.0609	0.280	0.270
Available Mg	%	0.0251	0.0053	0.0239	0.0233
Available Fe	ppm	6.6	7.6	9.1	8.1
Available Mn	ppm	14.65	13.00	15.24	15.56
Available Zn	ppm	0.5	0.5	1.1	0.61
Available Cu	ppm	1.5	1.3	3.0	1.5

Isolation and identification of the pathogen

Fos was identified according to macroscopic and microscopic symptoms. Isolations were made from main and lateral root pieces of infected plants. The roots were washed in tap water to remove attached soil particles, surface-sterilized by immersion for 3 min in 5% sodium hypochlorite followed by 70% ethyl alcohol for 1 min, then rinsed several times with sterile water. The roots were dried by sterile paper and cut into pieces, these placed on potato dextrose agar (PDA) and incubated at 28°C in the dark. If fungal growth developed it was transferred onto PDA plates for identification.

Table 3. Monthly temperature (°C), rainfall (Kg/m^2) and humidity (%) in the years 2007-2008.

Months	Temperature (°C)		Rainfall (Kg/m^2)		Humidity (%)	
	2007	2008	2007	2008	2007	2008
May	21.7	21.1	5.2	5.2	69.4	62.7
June	27.2	27.1	1.4	0.6	55.7	57.3
July	29.7	29.5	0.2	0.0	54.2	56.4
August	29.0	30.2	1.0	20.4	68.1	60.8
September	26.3	26.0	0.0	6.6	52.0	64.2
October	22.8	22.1	16.6	13.0	55.2	52.0

Source: Antalya Meteorology Bulletin.

Screening process for resistance

Ninety-seven days after sowing, all healthy and diseased plants for each row was counted. The results were expressed as percentage by using the formula described. The reaction of the entries to the wilt pathogen was categorised as indicated in table 4, using the scale proposed by Dinakaran and

Mohammed (2001). Percentages were compared using Duncan's Multiple Range Test at 0.05 level of probability (Duncan, 1955)

Table 4. Scale of disease ratings used.

Score	Infection (%)	Category
1	1-10	Resistant (R)
3	11-20	Moderately resistant (MR)
5	21-30	Moderately susceptible (MS)
7	31-50	Susceptible (S)
9	51-100	Highly susceptible (HS)

RESULTS AND DISCUSSION

Plants are constantly challenged by a wide variety of biotic factors including viruses, bacteria, fungi, and nematodes. Where such a biotic factor is one of the important limiting factors for crop cultivation, the evaluation of the reaction of plant germplasm to infection is an important goal for plant breeding programs. Therefore, the use of resistant varieties becomes part of integrated disease management, and is the ideal way for preventing damage to crops by diseases.

According to the analysis of variance (ANOVA) of the data, the reactions of the genotypes to the fungal pathogen differed statistically significant (Table 3 and 4). In 2007, The mutant cultivar, Birkan, released in 2010 with a production permission, was the most resistant genotype with average 3.0% infection rate and the 1st scale value. Genotypes, viz., WS-143, WS-313, Muganlı-57 and Çamdibi were the other resistant sesame genotypes. Their infection rates were lower than 9% and categorized in the 1st scale value.

In 2008, WS-143 was the most resistant genotype with average 1.9% infection rate and got the 1st scale value. Genotypes, viz., Birkan, WS-313, wt-3, WS-565, Çamdibi, WS-2, WS-564 and WS-416 were the other resistant sesame genotypes. Their infection rates were lower than 11% and grouped in the 1st scale value.

Comparison of the two soil types indicate a stability of resistance among genotypes; Birkan, Çamdibi, WS-143 and WS-313. These are the genotypes which are stable in their resistance in all environments. Previous studies have been reported for the phenomenon of changing the reaction to wilt pathogen with years (Abd El-Ghany et al., 1974; Bakheit et al., 1988). These results from previous studies may be attributed to the degree of stability of the resistance character within the genotypes. This is a clue that some genotypes were more stable than the others. The stability of resistance shown by some genotypes may indicate a prediction of they

Table 5. Mean infection rates of 2007 results. Scale values and resistance levels of sesame germplasm.

Genotypes	2007 Soil-1 Mean		Scale value and levels	2007 Soil-2 Mean		Scale value and levels	Mean combined 2007/2008		Scale value and levels
WS[1]-10	64.4	ab	9-HS	32.6	bcdef	7-S	48.5	bcd	7-S
WS-131	26.0	defg	5-MS	27.8	cdefgh	5-MS	26.9	efghi	5-MS
WS-141	54.3	bc	9-HS	82.5	a	9-HS	68.4	a	9-HS
WS-166	31.6	cdef	7-S	30.8	bcdefg	5-MS	31.2	defg	7-S
WS-19	66.4	ab	9-HS	52.0	b	9-HS	59.2	abc	9-HS
WS-2	37.6	cde	7-S	33.9	bcdef	7-S	35.7	def	7-S
WS-606	68.6	ab	9-HS	46.6	bcd	7-S	57.6	abc	9-HS
WS-616	64.2	ab	9-HS	22.7	defghi	5-MS	43.4	cde	7-S
WS-638	49.4	bcd	7-S	47.5	bc	7-S	48.5	bcd	7-S
WS-65	82.3	a	9-HS	46.3	bcd	7-S	64.3	ab	9-HS
WS-230	6.7	fg	1-R	19.1	efghi	3-MR	12.9	ghij	3-MR
WS-262	20.1	efg	3-MR	41.5	bcde	7-S	30.8	defgh	5-MS
WS-416	18.9	efg	3-MR	22.6	defghi	5-MS	20.7	fghij	3-MR
WS-564	3.6	g	1-R	25.5	cdefghi	5-MS	14.5	ghij	3-MR
WS-565	9.8	fg	1-R	14.0	fghi	3-MR	11.9	hij	3-MR
WS-571	3.4	g	1-R	49.4	bc	7-S	26.4	efghi	5-MS
WS-313	3.6	g	1-R	8.2	ghi	1-R	5.9	j	1-R
WS-143	3.7	g	1-R	2.7	i	1-R	3.2	j	1-R
WS-189	24.5	defg	5-MS	27.2	cdefgh	5-MS	25.9	efghi	5-MS
wt[2]-3	20.1	efg	3-MR	15.3	fghi	3-MR	17.7	fghij	3-MR
wt-5	15.9	efg	3-MR	16.5	fghi	3-MR	16.2	ghij	3-MR
wt-8	17.9	efg	3-MR	25.7	cdefghi	5-MS	21.8	fghij	5-MS
Birkan	2.0	g	1-R	4.0	hi	1-R	3.0	j	1-R
Çamdibi	12.1	efg	3-MR	5.6	hi	1-R	8.8	ij	1-R
Muganlı-57	9.0	fg	1-R	4.8	hi	1-R	6.9	j	1-R
F value	9.4**			7.0**			12.3**		

1 = Resistant (R), 2 = Moderately resistant (MR), 3 = Moderately susceptible (MS), 4 = Susceptible (S), Highly susceptible (HS).
**Significant at the p<0.01 level.

Table 6. Mean infection rates of 2008 results. Scale values and resistance levels of sesame germplasm.

Genotypes	2008 Soil-1 Mean	Scale value and levels	2008 Soil-2 Mean	Scale value and levels	Mean combined 2007/2008	Scale value and levels
WS[1]-10	12.0 bcdef	3-MR	24.5 ab	5-MS	18.3 abcdef	3-MR
WS-131	37.8 a	7-S	16.0 ab	3-MR	26.9 ab	5-MS
WS-141	25.3 abcd	5-MS	11.3 ab	3-MR	18.3 abcdef	3-MR
WS-166	27.2 abc	5-MS	23.5 ab	5-MS	25.4 abc	5-MS
WS-19	21.6 abcde	5-MS	22.0 ab	5-MS	21.8 abcd	5-MS
WS-2	10.4 cdef	1-R	9.5 b	1-R	10.0 cdefg	1-R
WS-606	29.3 ab	5-MS	35.6 a	7-S	32.4 a	7-S
WS-616	18.9 bcdef	3-MR	25.6 ab	5-MS	22.3 abcd	5-MS
WS-638	16.2 bcdef	3-MR	12.3 ab	3-MR	14.3 bcdefg	3-MR
WS-65	19.9 bcdef	3-MR	18.3 ab	3-MR	19.1 abcde	3-MR
WS-230	10.7 cdef	1-R	17.3 ab	3-MR	14.0 bcdefg	3-MR
WS-262	15.9 bcdef	3-MR	15.1 ab	3-MR	15.5 bcdefg	3-MR
WS-416	8.8 def	1-R	12.6 ab	3-MR	10.7 cdefg	1-R
WS-564	2.2 f	1-R	18.9 ab	3-MR	10.6 cdefg	1-R
WS-565	10.1 cdef	1-R	6.8 b	1-R	8.4 defg	1-R
WS-571	16.0 bcdef	3-MR	7.1 b	1-R	11.5 bcdefg	3-MR
WS-313	6.1 ef	1-R	3.6 b	1-R	4.9 efg	1-R
WS-143	3.1 ef	1-R	1.1 b	1-R	2.1 g	1-R
WS-189	5.1 ef	1-R	23.1 ab	5-MS	14.1 bcdefg	3-MR
wt[2]-3	5.1 ef	1-R	5.1 b	1-R	5.1 efg	1-R
wt-5	15.2 bcdef	3-MR	6.0 b	1-R	10.6 cdefg	1-R
wt-8	7.9 def	1-R	17.2 ab	3-MR	12.5 bcdefg	3-MR
Birkan	2.7 f	1-R	3.2 b	1-R	3.0 fg	1-R
Çamdibi	9.1 cdef	1-R	10.6 b	1-R	9.8 cdefg	1-R
Muganlı-57	15.7 bcdef	3-MR	15.5 ab	3-MR	15.6 bcdefg	3-MR
F value	2.9 **		1.4^{ns}		2.7**	

1 = Resistant (R), 2 = Moderately resistant (MR), 3 = Moderately susceptible (MS), 4 = Susceptible (S), Highly susceptible (HS). **Significant, ns= not significant at the p<0.01 level.

are good parents for breeding programmes. Although no evaluation for wilting was made for Birkan's breeding history, it was the best entry in resistance to Fos, which may be related with its changed flowering habit to a lateness and with the level of desirable resistance in the original genetic background cultivar, i.e. Muganlı-57.

Also the susceptible genotypes must be used in screening studies to understand and confirm the level of resistance. Results from 2007 experiments showed that, the maximum infection rate is 68.4% and displayed by the WS-141. Results from 2008 experiments showed that, the maximum infection rate is 32.4% and displayed by the WS-606. In both soils WS-131, showed a continuous moderately susceptible reaction with 5th scale value.

Crop plants are particularly vulnerable because they are usually grown as genetically uniform monocultures; when disease strikes, losses can be severe. Sesame improvement programme heavily dependents on the magnitude of genetic variability. Searching for sesame varieties or collections having a high resistance level to Fusarium wilt is a time consuming work which needs a great effort and patience. Use of Gamma irradiation generated a useful genetic variation to wilting in Turkish sesame genetic pool in previous studies (Çağırgan, 1996) and the first fungal pathogen tolerance was also described in Phytophtora blight (Pathirana, 1992) induced by mutagenesis.

In the studies of Pathirana (1992), radiation doses; 450 Gy and 600 Gy resulted more tolerant varieties than the others. Also, Birkan is a high yielding mutant cultivar that derived from 400 Gy gamma radiation. These results confirm the possibility to find more tolerant mutants in these doses.

Although germplasm having perfect resistance (without symptom) to wilt disease, was reported previously (El-Shazly et al., 1999), in this study, there was no line that has the same results as above. However, the 1st scale genotypes may carry the resistance gene of the sesame against the infection but further investigations should be done in different environments and these genotypes should be taken to breeding programs and the heritage of resistance must be understood in order to broaden the resistance against other fungal diseases.

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