Evaluation of Resistance to *Fusarium oxysporum* f.sp. *sesami* in Hybrid Lines of Sesame (*Sesamum indicum* L.) under Greenhouse Conditions

M. A. EL-BRAMAWY[1], KAREL VEVERKA[2], SLAVOJ VAVERKA[2], M. S. EL-SHAZLY[1], M. A. EL-SATTAR[3], M. A. EL-ASHARY[1] and S. E. AMMAR[1]

Suez Canal University – Faculty of Agriculture, [1]Agronomy Department and [3]Plant Protection Department, Ismailia, Egypt; [2]Mendel University of Agriculture and Forestry Brno – Plant Protection Institute, Brno, Czech Republic

Abstract

EL-BRAMAWY M.A., VEVERKA K., VAVERKA S., EL-SHAZLY M.S., EL-SATTAR M.A., EL-ASHARY M.A., AMMAR S.E. (2001): **Evaluation of resistance to** *Fusarium oxysporum* f.sp. *sesami* **in hybrid lines of sesame** (*Sesamum indicum* L.) **under greenhouse conditions**. Plant Protect. Sci., **37**: 74–79.

Thirty-six samples of sesame (15 F_1 and 15 F_2 generations and their 6 parents) were evaluated for their reaction to *Fusarium oxysporum* f.sp. *sesami* (Zaprometoff) Castellani after artificial inoculation in greenhouse tests. The experimental soil was analysed mechanically and microbiologically. Reactions of the plants were recorded on a scale from 0 = no visible infection (immune) up to 6 ≥70% infected plants (highly susceptible). Highly significant differences of susceptibility and resistance to the wilt pathogen were observed among hybrids and their parents. The level of infection in the parental generation varied from 2.22 to 63.77% (mean at 33.74%), in the F_1 from 13.46 to 73.78% (mean at 32.09%), and in the F_2 populations from 0.71 to 59.4.5% No population was immune. Nine lines of the F_1 were classified as resistant (R) and three of them had the same rank in the F_2. Only one parent (P3) and three lines from the F_2 (13, 9 and 12) showed infection below 10% and were identified as highly resistant (HR) by infection rates of 2.22%, 0.71%, 6.08% and 9.57%, respectively. They can thus be considered as promising parents for breeding programs.

Keyword: sesame; Fusarium wilt; wilt resistance, disease resistance

Sesame (*Sesamum indicum* L.) is one of the oldest oilseed crops known, and its use probably dates back to 2130 BC (WEISS 1983). Africa is considered to be the primary centre of origin of sesame because of the presence of several wild species there. Sesame is an annual oil crop and grown mainly in the developing tropical and subtropical areas of Asia, Africa, South and Central America. However, it is well adapted to diverse growing conditions, particularly in developing countries in southern latitudes, where small holders usually grow it on small plots (ODOERDO & NERLEY 1982). Practically 100% of the world's sesame area is found in developing countries, with the largest areas in India, Myanmar, China, Sudan, Nigeria and Uganda (ASHRI 1998). Recently, sesame has attracted increasing interest as a source of good quality vegetable oil with antioxidative constituents, i.e. sesamol, sesamolinol, sesaminol, α-tocopherol (NAMIKI & KOBAYASHI 1989) and as an inexpensive source of protein, because it is rich in sulphur amino acids, especially methionine (BRITO & NUMEZ 1982). Wherever sesame is grown it is liable to attack by at least eight economically important fungal diseases (KOLTE 1985) and by 65 species of insect pests in different stages of its plant growth (AHUJA & BKHEITE 1995), causing considerable yield losses. Of the fungi, *F. oxysporum* f. sp. *sesami* is a serious pathogen that limits production of sesame. It was reported for the first time from America in 1950 (ARMSTRONG & ARMSTRONG 1950). Many investigations have concentrated on the study and evaluation of resistance to Fusarium wilt disease within sesame germplasm (GAIKWAD & PACHPANDE 1992; XIAO *et al.* 1992a,b; ZIEDAN 1993; RAGHUWANSHI 1995a,b; EL-BRAMAWY 1997; EL-SHAKHESS 1998; EL-SHAZLY *et al.* 1999; HYUN *et al.* 1999). Meanwhile, the screening for resistance against wilt disease will help to identify the genotypes with resistance, which can be used directly for large-scale cultivation and

also in hybridization programs to incor-porate resistance in economically suitable cultivars.

MATERIALS AND METHODS

This study was carried out in the greenhouse of the Plant Protection Institute of Mendel University of Agriculture and Forestry in Brno (MZLU) in the Czech Republic (CR) in the year 2000. Its purpose was to test and evaluate the reaction of two generations of sesame, F_1 and F_2, and their respective parents to artificial infection by *Fusarium oxysporum* f.sp. *sesami*. The two generations resulted from a diallel crossing program (one-way) among the six parents (Table 1). All possible crosses, without reciprocals, were made in 1998 using selfed seed to obtain F_1 seeds. In 1999, the seeds of the F_1 from each cross were planted, the flowers were covered with bags to prevent insect pollination, and selfed F_2 seed was obtained. At the same time, the parents were again crossed to obtain an adequate amount of seeds of F_1 hybrids. The crossing technique used in 1999 involved removal of the corolla tube with attached anthers, but leaving the pistil in its normal position. Ripe anthers from the pollen parent were slipped over the style next morning. Table 1 shows names and sources of the parents used in the study, and Table 2 the crosses between them.

Table 1. Names and sources of sesame parents used

No.	Parent	Source
1	Hybrid 38	Agriculture Research Center, Giza, Egypt
2	Local line 14	U.C.R. × Giza 25, Egypt
3	Local line 1	El Tal kabir district, Ismailia Governorate, Egypt
4	Local line 2	Mina El kamih district, El Sharkia Governorate, Egypt
5	Local line 3	Abou Hammad district, Ismailia Governorate, Egypt
6	Local line 4	Abou Hammad district, Ismailia Governorate, Egypt

Pathogen Isolation and Pathogenicity Test

Wilted plants of sesame collected from a field in Egypt were transferred in tightly closed polyethylene bags to the laboratory. 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 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. Subcultures were taken in test-tubes from Egypt to the Czech Republic. The fungus was then cultured on PDA in Petri dishes for increasing. Pathogenicity tests were carried out using the variety Giza 25 in 4-kg capacity pots. This cultivar was known to be moderately susceptible to *F. oxysporum* f.sp. *sesami* (SERRY & SATOUR 1981; BAKHEIT *et al.* 1988; EL-BAROUGY 1990; EL-BRAMAWY 1997 and EL-SHAZLY *et al.* 1999). The pots were inoculated artificially and plants observed daily for wilt symptoms. To confirm the pathogenicity of the isolates to sesame, the pathogen was reisolated from wilted plants to fulfil Koch's postulates.

Inoculum Preparation

Discs (15-mm diameter) were cut out from the growing edges of 10-day old cultures using a sterile stainless cork borer. Discs were put into glass bottles containing 65 g autoclaved barley grain. The bottles were closed with aluminium foil and incubated at room temperature for 2 weeks. Then their content was mixed with potting soil at a ratio of 3:6 w/w.

The soil was a loamy soil (7.40% medium sand, 20.84% fine sand, 35.64% coarse silt, 16.52% fine and medium silt and 19.60% clay), analyzed according to the method described by ROBINSON (1922). Microbiological analysis was also done by using the soil dilution method to estimate the content of soil fungi. Nutrient agar (Chapek-Dox agar) was inoculated by soil suspension (dilution 10^{-4}) and fungi were enumerated after 6 d culture at 28°C. Results were recalculated per 1 g of dry soil (DUNGER & FIEDLER 1989).

Table 2. Crosses between sesame parents used in the study

No.	Combinations ♂ × ♀	No.	Combinations ♂ × ♀	No.	Combinations ♂ × ♀
1	Hybrid 38 × Local line 14	6	Local line 14 × Local line 1	11	Local line 1 × Local line 3
2	Hybrid 38 × Local line 1	7	Local line 14 × Local line 2	12	Local line 1 × Local line 4
3	Hybrid 38 × Local line 2	8	Local line 14 × Local line 3	13	Local line 2 × Local line 3
4	Hybrid 38 × Local line 3	9	Local line 14 × Local line 4	14	Local line 2 × Local line 4
5	Hybrid 38 × Local line 4	10	Local line 1 × Local line 2	15	Local line 3 × Local line 4

Evaluation of Sesame Populations

Pots (4-kg capacity) were used in the experiments, and each pot received about 4 kg of mixed inoculated soil and 15 sesame seeds were planted into it. Three replicates were prepared for each entry and arranged in a Randomized Complete Block Design (R.C.B.D). Pots were watered every 3–5 d according to need. Uninoculated pots served as control. All plants were inspected for wilt symptoms every day, and those with clear symptoms of wilting were regarded as infected. The data were recorded 2 weeks after planting. The pathogen was re-isolated from the wilted plants confirm that the symptoms were due to *F. oxysporum* f.sp. *sesami*.

Disease Scale and Statistical Analysis

The wilted plants were counted and scored according to the scale in Table 3.

Table 3. Scale used to rate the reaction of the entries

Score	Infection (%)	Category
0	no infection	Immune (I)
1	0.1–10	Highly resistant (HR)
2	10.1–20	Resistant (R)
3	20.1–40	Moderately resistant (MR)
4	40.1–60	Moderately susceptible (MS)
5	60.1–70	Susceptible (S)
6	> 70	Highly susceptible (HS)

Many scales can be used to evaluate the reaction of sesame germplasm to Fusarium wilt disease. However, we used our own scale according to previous results. This scale ranks from 0 (no visible symptoms) to 6 (over 70% diseased plants). For statistical analysis Arcsine value tables were used to transform percentage into numerical values (FEDERER 1963). Analysis of variance for randomized complete block design was used to evaluate the significance of data (COCHRAN & COX 1957). Hybrids and parents were compared by testing the least significant difference (LSD) at 5%.

RESULTS AND DISCUSSION

According to the analysis of variance (Anova) of the data, the reactions of the populations (parents, F_1's and F_2's) to the fungal pathogen differed highly significantly (Table 4).

None of the evaluated genotypes was immune. The absence of immune plants in sesame genotypes had been reported earlier by LI *et al.* (1991) during their evaluation of 2229 accessions of sesame from six ecological regions in China and of 116 accessions from 14 other countries. Yet, immune plants were reported by HASSAN (1973) and RAGHUWANSHI *et al.* (1992a).

The level of infection of the parents varied from 2.22% (P3), scored as highly resistant (HR), to 63.77% (P1) and scored as susceptible (S) (Table 3 and Fig.1). These two parents had the same reaction in a previous evaluation (EL-BRAMAWY 1997 and EL-SHAZLY *et al.* 1999) under Egyptian conditions, which confirmed our results under

Table 4. Reaction* of sesame populations (P, F_1 and F_2) to the wilt pathogen (*F. oxysporum* f.sp. *sesami*)

P No.	Mean	Category	F_1 No.	Mean	Category	F_2 No.	Mean	Category
1	63.77	S	1	15.58	R	1	14.28	R
2	25.93	MS	2	70.50	HS	2	14.18	R
3	2.22	HR	3	13.46	R	3	58.89	MS
4	46.09	MS	4	62.65	S	4	58.39	MS
5	34.13	MS	5	73.78	HS	5	59.45	MS
6	30.32	MS	6	40.06	MS	6	15.30	R
LSD	11.26		7	16.66	R	7	15.81	R
			8	19.88	R	8	54.04	MS
			9	13.68	R	9	6.08	HR
			10	13.65	R	10	15.24	R
			11	16.55	R	11	53.28	MS
			12	16.90	R	12	9.57	HR
			13	42.51	MS	13	0.71	HR
			14	17.46	R	14	57.94	MS
			15	60.19	S	15	58.00	MS
			LSD	8.31		LSD	9.56	

* mean % infection; HR – highly resistant, R – resistant, MR – moderately resistant, MS – moderately susceptible, S – susceptible, HS – highly susceptible

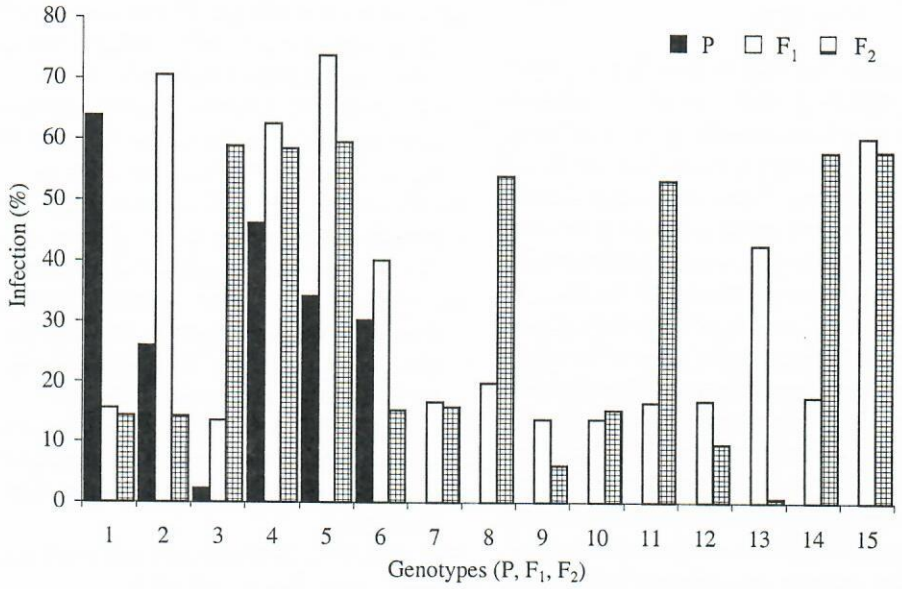

Fig. 1. Percentage infection (*Fu-sarium oxysporum* f.sp. *sesami*) on parents and lines in this study

the conditions in the Czech Republic. The other parents were ranked as moderately resistant (MR), except P4 that was moderately susceptible (MS) with 46.09% infection.

The reactions in the two hybrid generations ranged from resistance (R) of line 3 (13.46% infection) to high susceptibility (HS) of line 5 (73.78% infection) in the F_1. In the F_2 the reaction ranged from high resistance (HR) of line 13 (0.71% infection) to high susceptibility (HS) of line 5 (59.49% infection) – Table 4 and Fig. 1.

The highest infection among all parents was found on P1 (Hybrid 38) and P4 (Local line 2) and they were ranked as highly susceptible (HS, with 63.77% infection) and moderately susceptible (MS, with 46.09% infection).

Three hybrids (1, 7 and 10) gave approximately the same reaction in both F_1 and F_2; they were ranked resistant (R) with only small differences in level of infection (Table 4). This indicates that some genotypes are more stable than others. Such stability of resistance of some genotypes suggests that they could be valuable for breeding programs. This finding agrees with earlier studies (EL-MARZOKY 1982), where six crosses and their progenies were exposed to the wilt pathogen (*F. oxysporum* f.sp. *sesami.*).

The highest infection levels among the hybrids were shown in the F_1 by lines 2 (HS, at 70.50%), 4 (S, at 62.65%), 5 (HS, at 73.78%) and 15 (S, at 60.19%). However, in the F_2 the infection level on these lines was lower, i.e. 2 (R, at 14.18%), 4 (MS, at 58.89%), 5 (MS, at 59.45%) and 15 (MS, at 58.00%) – Table 4. Conversely, while in the F_1 the infection levels of lines 9, 12 and 13 were below 10% infection with 6.08%, 9.57% and 0.71%, respectively, and all were classified as highly resistant, these lines had 13.68%, 16.90 % and 42.51% infection in the F_2 and were then classified as resistant (R), resistant (R) and moderately susceptible (MS), respectively. In the same trend, in the F_1 the nine lines 1, 3, 7, 8, 9, 10, 11, 12 and 14) were ranked as resistant (R), with small differences in level of infection (under 20% infection). In the F_2 some of these lines (1, 7 and 10) had the same rank of (R), but the others had a higher infection and were classified as moderately susceptible (lines 3, 8, 11 and 14) or highly susceptible (lines 9 and 12). These changes in level of infection among F_1's and F_2's and their parents were likely due to segregation in the second generation (F_2). For example, some of them were positive (F_2 – lines 6, 9, 12 and 13) and others were negative (F_2 – lines 3, 8, 11, 14 and 15). Our results are fairly comparable to those reported by EL-MARZOKY (1982) who found no complete resistance to *Fusarium* and also that the F_2 offspring showed a hybrid ratio of 9 tolerant:7 susceptible.

On the other hand, some changes in infection levels may have been brought about by the number of other organisms in the soil used in our study. For example, the number of fungi was 8.75×10^4 per 1 g of dry soil (D.S.).

Our results were in accordance with the findings reported by other workers where most of the trials had been carried out under both artificial and natural infection. The researchers found no immune strains but in some cases resistant or tolerant, moderately resistant or susceptible genotypes were identified (BULDEO & RANE 1978; GOYAL *et al.* 1980; JEYARAJAN *et al.* 1980; DESAI & GOYAL 1981; KIRK & GEMAWAT 1981, MAZZANI *et al.* 1981; RAGHUWANSHI *et al.* 1992b; EL-BRAMAWY 1997 and EL-SHAZLY *et al.* 1999). Thus, our results indicate that some of the hybrids tested were highly resistant to infection with *F. oxysporum* and could be considered as a source of resistance in breeding programs.

Conclusion

This work was done to identify the reaction of parents and their F_1 and F_2 offspring of sesame to *F. oxysporum* f.sp. *sesami*. The trial was intentionaly performed in the region not suitable for sesame growing. Despite that of growing in the glashouse, the plants were exposed to the stress, so that their reaction under extreme conditions could be revealed. External environmental factors increased variability and decreased the expression of resistance genes especially in F_1 and F_2 of variants 9, 12 and 13. Highly significant differences for range of susceptibility and resistance were observed among all populations and their progenies. Only one parent and three lines of the F_2 were identified as highly resistant (HR) by a level of infection below 10% and could be considered as promising parents for breeding programs. The resistance to wilt disease was not complete, and there were not only positive, but also negative segregations between both generations.

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EL-BRAMAWY M.A., VEVERKA K., VAVERKA S., EL-SHAZLY M.S., EL-SATTAR M.A., EL-ASHARY M.A., AMMAR S.E. (2001): **Hodnocení rezistence hybridních linií sezamu** (*Sesamum indicum*) **vůči** *Fusarium oxysporum* f.sp. *sesami* **ve skleníkových podmínkách**. Plant Protect. Sci., **37**: 73–79.

Ve skleníkových testech za pomoci umělé infekce bylo hodnoceno 36 vzorků sezamu (15 F_1, 15 F_2 a 6 rodičovských odrůd na odolnost vůči *Fusarium oxysporum* f.sp. *sesami* (Zaprometoff) Castellani. Použitá půda byla analyzována mechanicky a mikrobiologicky. Reakce rostlin na infekci byly hodnoceny dle stupnice 0 = žádné viditelné symptomy až 6 ≥ 70% infikovaných rostlin (vysoce náchylné). Mezi rodičovskými odrůdami a hybridy byly zjištěny vysoce významné rozdíly v náchylnosti a odolnosti k původci vadnutí. Stupeň napadení v rodičovské generaci kolísal mezi 2,22 a 63,77% (průměr 33,74%), v F_1 mezi 13,46 a 73,78 % (průměr 32,09%) a v F_2 populaci mezi 0,71 a 59,45%. Žádná z populací nebyla imunní. Devět linií F_1 bylo hodnoceno jako rezistentní (R) a tři z nich měly stejné zařazení i v generaci F_2. Pouze jeden z rodičů (P3) a tři z linií F_2 (13, 9, 12) vykázaly napadení nižší než 10% a byly hodnoceny jako vysoce rezistentní (HR) se stupni napadení 2,22, 0,71, 6,08 a 9,57%. Tyto lze považovat za slibné výchozí materiály pro další šlechtění.

Klíčová slova: sezam; fusariové vadnutí; odolnost vůči vadnutí; rezistence vůči chorobám

Corresponding author:

Dr. M. A. El-BRAMAWY, Suez Canal University, Faculty of Agriculture, Agronomy Department, 41522 Ismailia, Egypt, e-mail: el.bramawy@hotmail.com