1248 Simultaneous breeding of extra-long staple cotton for Fusarium and Alternaria resistance, yield and fiber quality: length, strength, perimeter and maturity

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ABBREVIATIONS: ELS (extra-long staple); FOV3 (*Fusarium oxysporum* f.sp. *vasinfectum* race 3); AM (*Alternaria macrospora*)

ABSTRACT

Fusarium oxysporum f.sp*. vasinfectum* race 3 (FOV3), which appeared in the late seventies, threatened to destroy Pima cotton (*Gossypium barbadense* L.) in Israel. Resistant cultivars, which are superior in yield and quality compared to the susceptible ones, even in noninfested soil, were bred from resistant escapees, using the forward-pedigree method. Since then, only resistant cultivars have been planted in Israel. The main cultivars and year of release were F-27 (1984), F-177 (1988), PF-15 (1997), P00-8 (2002) and E2 (2007). The mean improvement per year was: 7 kg ha^{-1} lint, 0.4 cN tex^{-1} fiber strength, and 0.09 mm staple length. The fiber perimeter was reduced and the maturity increased. In parallel, breeding for resistance to *Alternaria macrospora* (AM) was established. Several applications of fungicides in an AM-infested field increased the yield of the AM-susceptible cultivar PF-15 by 11.4%, the partially AM-tolerant commercial cultivar P00-8 by 6.5% and the new highly AM-tolerant cultivar E2 by 2.8%.

Keywords: *Alternaria, fiber maturity, fiber perimeter, Fusarium, resistant cultivars*

Extra-long staple (ELS) cotton (*Gossypium barbadense* L.) constitutes only 3% of the world cotton production. ELS cotton has long and strong fibers that make fine yarns and fabrics and is generally sold for a high price. There are two main groups of ELS cotton: Egyptian cotton, for hand-picking, and Pima ELS cotton from the US, for mechanical picking. Israel's Pima growers depended on American Pima cultivars until the *Fusarium oxysporum* f.sp*. vasinfectum* race 3 (FOV3) outbreak in the late seventies.

Dishon and Nevo (1970) were the first to describe cotton Fusarium wilt in Israel caused by *Fusarium oxysporum* f.sp*. vasinfectum* and Netzer et al. (1980) identified it as race 3 using differential cultivars. The populations of FOV3 in Israel belong to a single vegetative-compatibility group, indicating that they have a single source (Katan and Katan, 1988). FOV3 is virulent to Pima cultivars but not to the upland cultivars (*G. hirsutum* L.). It exists in Asia and Africa but was not reported, until 2005, in America and Australia. Kim et al. (2005) identified two samples of FOV3 from California using molecular tools, but neither field-disease assessment nor pathogenicity tests were reported. FOV3 is a soilborne pathogen that attacks the plant at all growing stages. In highly infested fields, most of the plants are destroyed at the seedling stage and the damage can be total. Leaf wilt and desiccation, vascular discoloration and stunting are common symptoms of FOV3. A unique symptom of FOV3 is yellow leaf veins, especially in the cotyledons.

Infested soil, debris, and irrigation water spread the pathogen within and among fields (Grinstein et al., 1983). Infected seeds can spread the pathogen as well: Halfon-Meiri (1981) found 0.04% infected seeds among seeds of diseased plants which is a rate that is difficult to detect but is, nevertheless, sufficient to infest a field. Methyl bromide soil fumigation failed to control FOV3. On the other hand, soil solarization was very effective (Katan et al., 1983), but too expensive for cotton.

Alternaria macrospora (AM) is known worldwide as the cause of leaf spot disease on both *G. hirsutum* and *G. barbadense* (Ellis and Holliday, 1970). In Israel it does not cause commercial damage to Acala cultivars (*G. hirsutum*) but does damage Pima which, in severe epidemics, may become completely defoliated at the end of the season. Treating the AM-infested field with fungicides increased the yield by 23% (Sachs et al., 1979) and even by 32% (Bashi et al., 1983). Therefore, fungicide treatments became common practice in Israel in Pima fields. In other countries, AM may defoliate *G. hirsutum* as well (Hillocks, 1991), and experiments were performed to control it under field conditions (Colson-Hanks et al., 2000). Cotty (1987a) compared Pima and upland cotton (*G. hirsutum*) cultivars under artificial inoculation. The Pima cultivars were more susceptible than the upland cultivars but differences in resistance were found only among the latter. No reports on AM resistance in Pima cultivars or attempts to breed resistant Pima are available.

Cotton-fiber perimeter and maturity are very important quality traits for yarn, fabric, and garment production. Finer fibers with small perimeter and high maturity make better products. Those two traits, which are presumably controlled by different genes (John and Crow, 1992), are difficult to measure and are usually replaced by the micronaire, which measures a combination of fiber fineness and maturity (Thibodeaux and Rajasenkaran, 1999; Montalvo, 2005). However, using the micronaire as a fineness criterion in breeding programs might be misleading, as the same micronaire value may indicate either coarse immature fibers or fine mature ones (Montalvo and Von-Hoven, 2005). The objectives of our studies were to develop Pima cultivars with improved traits which are also resistant to FOV3 and AM.

MATERIALS AND METHODS

The resistance of cotton lines to FOV3 was assessed using two approaches: (1) planting in a field with highly infested soil in which 95 to 100% of the plants of the reference susceptible cultivars became diseased and (2) inoculation tests of seedlings under greenhouse conditions using the root-dip method (Katan et al., 1983).

Three FOV3-resistance sources were used in the first stages of the breeding program: Acala cultivars, resistant Egyptian cultivars, and escapees showing apparent resistance in a highly infested field planted with the susceptible Pima S4 cultivar at Hamadia in 1977. Among the escapees there were a few resistant plants whose progenies did not show any symptoms when tested in various severely infested fields. None of these plants resembled Pima S4, they had many shortcomings and therefore could not serve as commercial cultivars. However, they could be easily crossed with Pima cultivars and the resistance was transferred. Therefore, this source was used exclusively during the rest of the program. In the first years, the selection for resistance was performed both in the greenhouse using the aforementioned seedling-root inoculation and by planting in a naturally infested field. Only the first method is currently being used.

Selection for AM resistance is usually very difficult because the levels of infection and the symptoms are highly affected by physiological and environmental factors including: plant

and leaf age, air and soil temperature and moisture (Rotem el al., 1990; Cotty, 1987b), the number of flowers and fruits on the plant (Rotem et al., 1988) and plant nutrition (Hillocks and Chinodia, 1989). The cotyledons are very sensitive and defoliate as the disease develops (Shtienberg, 1991); however, infecting them induces some resistance in the subsequent leaves (Brock et al., 1994). Therefore, we based our screening for resistance and breeding selection on natural infection of the cotyledons under field conditions or artificial inoculation under field and greenhouse conditions. Inoculation of AM was carried out by placing two drops of a conidial suspension containing 100 to 200 conidia per drop on each cotyledon. A conidial suspension was obtained from a 5% V8 agar culture of the pathogen previously incubated at 27^{o}C for 7 to 14 days under partial daylight. First disease symptoms were visible 2 to 4 days after inoculation. The experimental design was randomized blocks with 25 to 100 replications of a single plant. Leaf-spot lesion diameter was used to measure susceptibility (Cotty, 1987a) along with relative lesion area and rate of cotyledon abscission. Each experiment and each block within an experiment had different environments, and the results were therefore computed and calibrated into a disease index where the most susceptible entry was assigned a value of 1 and the least susceptible, 0. GENSTAT statistical software (VSN international Ltd., GB.) was used to analyze the data.

Pedigree forward breeding was used to simultaneously improve disease resistance, yield, and quality. Fiber length and strength were measured using a fibrograph and stelometer, respectively, with fiber maturity measured with a fiber maturity tester (FMT, Shirley Laboratories, GB.) Fiber perimeter was computed from the resultant data using equations from Montalvo (2005) and Montalvo and Von-Hoven (2005). To show the significance of the results they were standardized to LSD units (least significant differences at $P = 0.05$ as computed by GENSTAT from replicated experiments) by dividing each measurement by its corresponding LSD.

RESULTS

Out of six Pima plants showing resistance to FOV3 in a severely infested field,

a resistant escapee was chosen and crossed with the FOV3-susceptible cultivar Pima S5. After a few generations of screening using greenhouse and field studies, the first FOV3-resistant cultivar F-27 was released for commercial use in 1984. F-27 was of the same quality as Pima S5 and outyielded it, even in noninfested fields. Four more generations of resistant cultivars have been released since then (Table 1). The fiber quality and yield have improved significantly, the fiber perimeter has been reduced, and the maturity increased. E1, E2, and P00-8 have about the same micronaire, 4.2, but the fibers of E1 and E2 are much finer and more mature (Fig. 1). The mean progress per year of the other traits has been: 7 kg ha^{-1} lint, 0.4 cN tex^{-1} fiber strength, and 0.09 mm staple length (Table 1).

Resistance to AM was improved as well; the FOV3-resistant cultivar P00-8, which in 2006 became the main Pima cultivar in Israel, is more tolerant than the old inferior cultivar PF-15, while the new improved cultivar E2 is more tolerant to AM than P00-8, at both the cotyledon stage (Table 2) and at the end of the season (Table 3). Moreover, the ranking of AM resistance over two years followed a similar trend (Table 2). Several applications of fungicides at Acre field station in 2006 increased the yield of the AM-susceptible cultivar PF-15 by 11.4%, of the partially tolerant P00-8 by 6.5% and of the new tolerant cultivar E2 by 2.8%.

DISCUSSION AND CONCLUSIONS

From the start of the FOV3-resistance breeding program, it was clear that in the long run, and for long-lasting cotton production, the resistant cultivars must show better performance than the susceptible ones, even in noninfested fields. Therefore, a forward-pedigree, rather than backcross program, was used. The first FOV3-resistant cultivar F-27 already performed better than the susceptible Pima S5, and it was therefore used to replace the latter throughout the country. Consequently, FOV3 buildup was prevented as expressed in a very low incidence of disease in previously infested fields that had been planted with susceptible cultivars. This indicated a reduction in inoculum density of the pathogen. We assumed that this reduction in pathogen population in the soil would reduce the chances of appearance of a new virulent strain which could attack these resistant cultivars. Indeed, we have monitored the fields with resistant cultivars for more than 20 years and there has been no evidence of outbreak of a new virulent strain. To maintain this achievement, the FOV3-resistance breeding program must continue while avoiding the growing of susceptible cultivars in order to prevent a buildup of FOV3 populations. In our country, cotton was grown for a certain period in the 19th century and FOV3 was, presumably, introduced during that time.

Breeding for AM resistance is feasible, though complicated and difficult. However, the cotyledon test is reliable and consistent, achieving a correlation of 0.94 between two consecutive years (Table 2) and good agreement between yield increase by fungicide application and recorded susceptibility. The tolerant cultivar E2 might enable a reduction in the number of fungicide applications and exhibits high yield and quality. Nevertheless, higher resistance is still needed.

Perimeter and maturity are not only two separate qualities for the textile mills but are also two different biological traits, as the fiber perimeter is defined during the first days of fiber development while the wall thickness is determined during the last days. Therefore, breeders must reconsider the common practice of breeding for micronaire which is a combination of fineness and maturity and breed separately for the two.

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the susceptible .resistant cultivars vs-(3FOV)Representatives of five generations of Fusarium wilt .1le Tab .cultivar that preceded them

Lint yield	Tenacity	Staple length	Year of	3FOV	Cultivar
(kg. ha^{-1})	(cN tex^{-1})	(mm)	release	resistance	
1790	33	33.5	Imported	Susceptible	5Pima S
1860	33	33.3	1984	Resistant	27-F
1890	34	34.8	1988	Resistant	177-F
1880	35	35.1	1997	Resistant	15-PF
1890	37	35.6	2002	Resistant	8-00P
1940	42	35.1	2007	Resistant	2E

Field .Shmuel over two years-Gan in a field experiment at *Alternaria macrospora* Resistance to .2Table .cotyledon inoculation test

2005Shmuel -Gan			2006Shmuel -Gan	
Index	Cultivar		[z]Index	Cultivar
a1.00	15-PF		[y]a1.00	15-PF
b0.64	4E		ab0.74	1E
b0.56	1E		bc0.60	4E
c0.38	8-00P		c0.40	5E
d0.18	3E		c0.39	8-00P
d0.18	5E		d0.07	3E
e0.00	2E		d0.00	2E

.most resistant cultivar = 0 ,most susceptible cultivar =1 :disease index =Index [z]

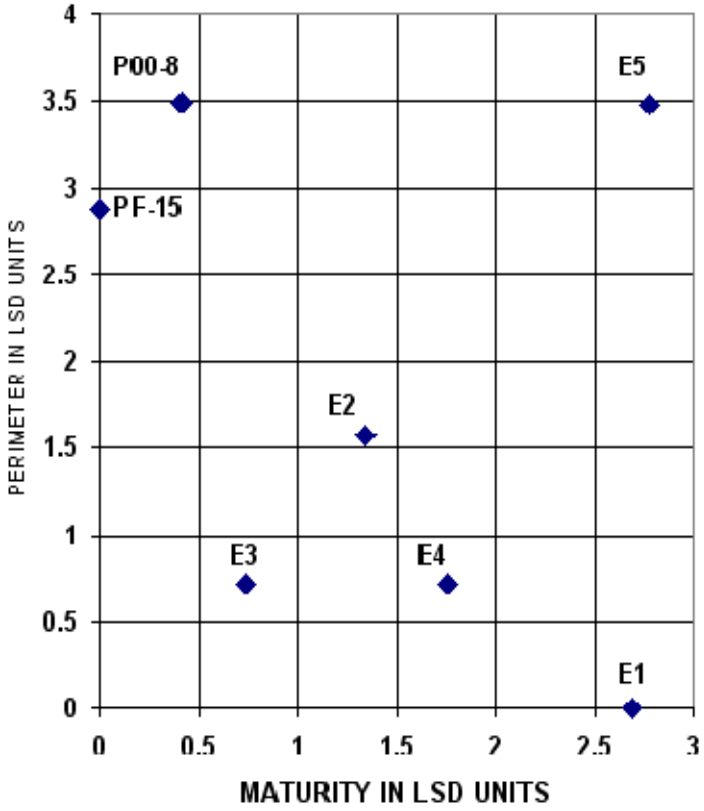
[y] Figures in a column followed by the same letter are not significantly different according to Duncan's multiple range test at *P* = 0.05.

Table 3. Resistance to *Alternaria macrospora* of mature cotton plants in a field experiment at Gan-Shmuel in 2006.

Cultivar	Defoliation[z] (%)
P00-8	58a[y]
E5	35b
E2	29b

[z] Rate of leaf defoliation due to *Alternaria macrospora* 12 days before harvest.

[y] Figures followed by the same letter are not significantly different according to Duncan's multiple range test at *P* = 0.05.



Caption to Figure

Each unit on the axes is a least .ivarscult (E)and new (15-PF ,8-00P)Perimeter and maturity of old .1 .Fig .0.05 = *P* at (LSD)significant difference