Indian Journal of Agricultural Sciences **81** (9): 812–15, September 2011

Genetic analysis of resistance to wilt caused by Fusarium (*Fusarium oxysporum melongenae*) in eggplant (*Solanum melongena*)

HATICE FILIZ BOYACI[1], ABDULLAH UNLU[2] and KAZIM ABAK[3]

Bati Akdeniz Agricultural Research Institute, Antalya, Turkey 07800

Received: 23 November 2010; Revised accepted: 30 May 2011

ABSTRACT

Inheritance of the resistance to *Fusarium oxysporum melongenae* in eggplant was investigated. LS1934 and LS2436, two resistant genotypes from Malaysia, were reciprocally crossed with the susceptible local inbred line NSB99 and seeds of F_1, F_2 and BC_1 generations were obtained. Resistance tests were carried out by artificial inoculations in segregating populations, using a highly virulent isolate (AF) originated from Turkey. The root-dip inoculation method was used as the resistance test. Results showed that the inheritance of resistance in both LS1934 and LS2436 are controlled by a single dominant gene. Goodness-of-fit test (c^2 analysis) was performed for the segregation. The segregations fitted a '3 resistant and 1 susceptible' ratio in χ^2 populations, and '1 resistant and 1 susceptible' ratio in BC_1 populations. Maternal effect was not found in the inheritance of the resistance.

Key words: Artificial inoculation, *Fusarium oxysporum*, Reciprocal crosses, Segregation, *Solanum melongena*

Eggplant (*Solanum melongena* L.) is an important vegetable crop grown in various tropical, subtropical and temperate parts of the world. Its cultivation is especially concentrated in Asia and Africa, but also in the Mediterranean Basin and Europe as well as in America (Mutlu *et al.* 2008). Cultivated varieties of eggplant are susceptible to a wide array of pests and pathogens as well as to various abiotic stress conditions which limit crop productivity significantly (Kashyap *et al.* 2003; Rajam and Kumar 2007). A main factor affecting the eggplant production is its susceptibility to soil-borne diseases. *Fusarium* wilt is one of the most serious diseases in eggplant cultivation (Rotino *et al.* 2004). *Fusarium oxysporum melongenae* induces vascular wilt disease in eggplant and causes heavy yield losses, especially in Asian countries. This disease also occurs in the Mediterranean basin and European countries including Turkey, both in greenhouse and in the open-field cultivations (Altinok 2005). The symptoms of *Fusarium* wilt are often confused with those of *Verticillium* (Rizza *et al.* 2002) and because of this, its importance is generally underestimated. The fungus penetrates through the roots and proliferates in the vascular tissues. Therefore, control of the disease is difficult and cumbersome. Presently, the disease is partially controlled by fungicide treatments and soil sterilization; and in greenhouses using grafted seedling. Chemical use is restricted due to various adversities (Yücel *et al.* 2001). The use of resistant cultivars is a desirable approach to control the disease. It was previously shown in a screening experiment that almost all commercial eggplant cultivars are susceptible to *Fusarium* wilt (Stravato and Cappelli 2000, Boyaci et al. 2010). However, resistance to *F. oxysporum melongenae* has been identified in some non-commercial *S. melongena* forms and in related eggplant species, i e *Solanum indicum*, *S. aethiopicum*, *aculeatum* Group (also found in literature as *S. integrifolium* Poir.), *S. aethiopicum* Gilo Group, *S. torvum*, *S. incanum*, *S. violaceum* and *S. sisymbriifolium* (Rizza *et al.* 2002, Gousset *et al.* 2005, Boyaci *et al.* 2010).

Previously, it was reported that inheritance of resistance to *F. oxysporum melongenae* in some wild Solanum species relatives of eggplant are determined by monogenic dominant genes (Rotino *et al.* 2001; Toppino *et al.* 2008). The aim of the study was to characterize inheritance of *Fusarium* resistance present in two cultivated *S. melongena* genotypes LS1934 and LS2436.

MATERIALS AND METHODS

This study was conducted at Bati Akdeniz Agricultural Research Institute, Antalya, Turkey in 2004–07. Two resistant

[1]Vegetable Breeder (e mail: filiz_boyaci@yahoo.com), Department of Vegetable Crops, [2]Director (abunlu@hotmail.com), Department of Plant Protection;

[3]Professor of Horticulture (e mail: kazimabak@gmail.com), European University of Lefke, Faculty of Agricultural Sciences and Technologies, Department of Horticultural Production and Marketing, Gemigonagi-Lefke, TRNC, Mersin 10, Turkey

eggplant genotypes, LS1934 and LS2436 from Malaysia and a susceptible inbred line NSFB99 from BATEM (Bati Akdeniz Agricultural Research Institute, Antalya, Turkey) were used as initial plant material.

The isolate of *Fusarium oxysporum melongenae* used in the experiments was obtained from the vascular tissues of a diseased eggplant plant collected in Antalya province, Turkey. Fungal strain was purified by single spore isolation and stored at 4°C on the PDA. The pathogenicity of the isolate was checked before using in the tests. For this purpose, a preliminary study was carried out using four susceptible (NSFB99, NSFB203, NSFB204 and NSFB205) and one resistant (LS1934) lines. The resistant genotypes LS1934 and LS2436 were crossed with the susceptible line NSFB99. Crosses were made reciprocally to check if an eventual cytoplasmic inheritance is present. The F_1 plants of the two series of crosses using LS1934 and LS2436 were selfed and F_2 populations were raised. In addition, BC_1 progenies were produced by backcrossing F_1 with either susceptible or resistant parents. As the result of these crosses, 16 different populations of F_1, F_2 and BC_1 progenies were produced (Table 1).

Artificial inoculation tests were carried out in a climate-controlled greenhouse in BATEM Antalya, according to the root dip method. Parental seeds, F_1, F_2 and BC_1 populations were sown in plastic trays containing sterile peat medium, and the plants were grown until the second-third true leaf stage. Initially, use of ca. 50 plants from the parents and ca. 300 plants from the segregated hybrid generations (F_2 and BC_1) was planned in the tests. However, because of lack of seeds and low germination levels, only 100 plants in each of the F_2 of two series could be used.

Table 1 Parents and hybrid populations used in the tests

Parents and crosses		No. of plant
P[a]	LS1934 (R[e])	50
P	LS2436 (R)	50
P	NSFB99 (S[f])	50
F_1[b]	LS1934 × NSFB99	310
F_1	NSFB99 × LS1934	310
BC_1[c]	(LS1934 × NSFB-99) × LS1934	310
BC_1	(LS1934 × NSFB99) × NSFB99	310
BC_1	(NSFB99 × LS1934) × LS1934	310
BC_1	(NSFB99 × LS1934) × NSFB99	310
F_2[d]	LS1934 × NSFB99	100
F_2	NSFB99 × LS1934	100
F_1	LS2436 × NSFB99	300
F_1	NSFB99 × LS2436	300
BC_1	(LS2436 × NSFB99) × LS2436	300
BC_1	(LS2436 × NSFB99) × NSFB99	300
BC_1	(NSFB99 × LS2436) × LS2436	300
BC_1	(NSFB99 × LS2436) × NSFB99	300
F_2	LS2436 × NSFB99	100
F_2	NSFB99 × LS2436	100

a, Parents; b, selfed F_1; c, backcrossed; d, crossed parents; e, resistant; f, susceptible

The *Fusarium oxysporum melongenae* isolate, AF, was grown on the potato dextrose agar (PDA) at 24°C in dark for 10 days. Liquid medium were prepared from this culture. Liquid cultures were shaken at 50 rpm in a rotary shaker for 8 days at 24 to 25°C. The suspensions were filtered through cheesecloth. The spores were resuspended and spore density was adjusted to 1×10^6 conidia/ml. For inoculation, seedlings were removed from the trays; roots were first washed with tap water and then wounded by trimming the tips. The roots were submerged in the inoculums for 5 min. Roots of the control plants were immersed in sterile tap water. After inoculations, seedlings were immediately transplanted into pots containing a mixture of sterile peat and perlite in a ratio of 1:1 (v/v), and maintained in a greenhouse at 20°C night and 27°C day temperatures. Disease symptoms were scored after five weeks from inoculation; where 1= no disease symptoms; 2= plants lacking one or two cotyledons; 3= reduced growth of plants with yellowing of the leaves; 4= heavy stunting; 5= plants dead. Seedlings with ratings of 1 and 2 were considered resistant, while 3, 4 and 5 were classified as susceptible. Segregation ratios obtained by phenotypic observations (resistant or susceptiple) were compared with theoretical segregation ratios using χ^2 analysis. Goodness-of-fit test was performed using Microsoft Excel spreadsheet software for the segregation ratios 3:1 (resistant : susceptible) for the F_2, and 1:1 (resistant : susceptible) for the BC_1.

RESULTS AND DISCUSSION

In the susceptible genotype NSFB99, first disease symptoms on the leaves started to appear two weeks after inoculation and all plants died between the third and the fourth week (Fig 1). However, the inoculated plants of the two resistant genotypes showed no symptom at the end of a five-week period (Fig 2). All the F_1 progenies were resistant regardless whether susceptible or resistant genotypes was used as either female or male parent (Table 2). In all the F_2 populations, segregation ratio of resistant and susceptible plants fitted to the 3:1 ratio as confirmed by the chi-square analyses. Accordingly, segregation ratios of resistant and susceptible plants in the backcross populations fitted to the dominant monogenic inheritance model. All the plants of the backcross progenies obtained by crossing the F_1 with LS1934 and LS2436 were resistant, whereas, plants obtained by crossing the F_1 with the susceptible genotype NSFB99 segregated according to 1:1 ratio, as confirmed by the chi-square analyses at 1% confidence level (Table 2). Additionally, these genotypes show various advantages for their potential exploitation in breeding programmes, they are members of the cultivated species of eggplant (*S. melongena*)

Fig 1 Beginning of disease symptoms on infected seedlings after four weeks following inoculation (*a, b*)

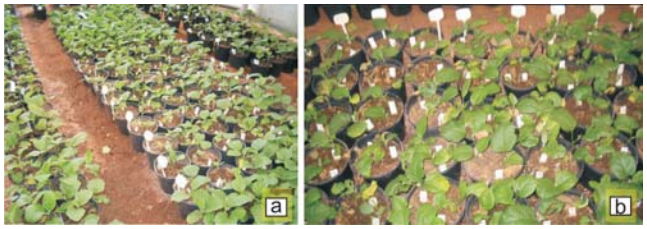

Fig 2 Fruit shapes and colours in resistant LS1934 and susceptible NSFB99 genotypes, and in their F_1 hybrid progeny (*a*) and in the backcross progeny to the susceptible genotype (*b*).

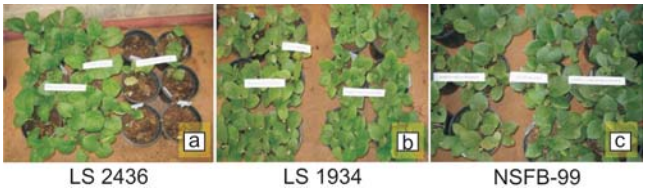

Fig 3 Control (*left*) and inoculated (*right*) plants of the susceptible NSFB99 (*a*) resistant LS2436 (*b*) and LS1934 (*c*) parents five weeks after artificial inoculation

and can be easily crossed with cultivated varieties producing fertile progeny; although the resistant genotypes give small fruited plants, they display plant characteristics indistinguishable from those of cultivated eggplant (Fig 3).

Results have clearly shown that the resistance character, of LS1934 and LS2436 followed a simple inheritance pattern of a monogenic dominant trait. These findings are in line with previous reports concerning the results obtained in wild *Solanum* species showing also that the resistance to *Fusarium* wilt was governed by a single dominant gene (Rotino *et al.* 2001). Also, Toppino *et al.* (2008) working on wild *Solanum* genotypes, determined that the inheritance of resistance they found in *S. aethiopicum* gr. Gilo and *S. integrifolium* was governed by a monogenic dominant locus named *Rfo-sa1*. However, crossing eggplant with wild relatives is difficult due to sexual incompatibilities (found in LS1934 and LS2436 can easily be used in the breeding of *Fusarium*-resistant eggplant cultivars.

This study provides a better understanding of the genetic control of resistance to *Fusarium oxysporum melongenae* by a single dominant gene in eggplant. Although *Fusarium* is controlled by nuclear factors, cytoplasmic factors are not effective.

There are two main aspects we have to look at when using LS1934 and LS2436 in a breeding program, ie These genotypes belongs to *Solanum melongena* and are crossed easily with cultivated types, and resistant gene is dominance and resistance gene must be introduced into only one parent when looking for a commercial F_1 hybrid.

Table 2 Results of chi-square tests for segregation reactions of parents, F_1, F_2 and BC_1F_1 progenies against *Fusarium oxysporum melongenae*

Parents or cross population	Resistant		Susceptible		χ^2	P value
	Expected	Observed	Expected	Observed		
LS1934 (R^a)	50	50				
LS2436 (R)	50	50				
NSFB99 (S^b)			50	50		
LS1934 × NSFB99	75	82	25	18	2.61	0.11
NSFB99 × LS1934	75	79	25	21	0.85	0.36
(LS1934 × NSFB99) × LS 1934	310	310				
(LS1934 × NSFB99) × NSFB99	155	150	155	160	0.32	0.57
(NSFB99 × LS1934) × LS 1934	310	310				
(NSFB99 × LS1934) × NSFB99	150	138	150	162	1.92	0.17
LS1934 × NSFB99	310	310				
NSFB99 × LS1934	310	310				
LS2436 × NSFB99	75	74	25	26	0.05	0.82
NSFB99 × LS2436	75	81	25	19	1.92	0.17
(LS2436 × NSFB99) × LS 2436	300	300				
(LS2436 × NSFB99) × NSFB99	150	144	150	156	0.48	0.49
(NSFB99 × LS2436) × LS 2436	310	310				
(NSFB99 × LS2436) × NSFB99	150	163	150	137	2.24	0.13
LS 2436 × NSFB99	300	300				
NSFB99 × LS2436	300	300				

a, Resistant; b, susceptible

REFERENCES

Altinok H H. 2005. First report of Fusarium wilt of eggplant caused by *Fusarium oxysporum* f. sp melongenae in Turkey. *Plant Pathology* **54**: 577–9.

Boyaci F, Unlu A and Abak K. 2010. Screening for resistance to fusarium wilt of some cultivated eggplants and wild *Solanum* Accessions. Presented in *28th International Horticultural Congress*, August 22–27 2010, Lisboa-Portugal and submitted to *Acta Horticulturea*.

Gousset C, Collonnier C, Mulya K, Mariska I, Rotino G L, Besse P, Servaes A and Sihachakr D. 2005. *Solanum torvum*, as a useful source of resistance against bacterial and fungal diseases for improvement of eggplant (*S. melongena* L.). *Plant Science* **168**: 319–327.

Kashyap V, Kumar SV, Collonier C, Fusari F, Haicour R, Rotino G L, Sihachakr D and Rajam M V. 2003. Biotechnology of eggplant. *Scientia Horticulturae* **97**: 1–25.

Mutlu N, Boyaci FH, Göçmen M and Abak K. 2008. Development of SRAP, SRAP-RGA, RAPD and SCAR markers linked with a *Fusarium* wilt resistance gene in eggplant. *Theoretical and Applied Genetics* **117**: 1303–12.

Rajam M V and Kumar SV. 2007. Eggplant. *Biotechnology in Agriculture and Forestry* **59**: 201–20 Springer-Verlag; ISSN: 0934–943X.

Rizza F, Mennella G, Collonnier C, Sihachakr D, Kashyap V, Rajam M V, Prestera M and Rotino G L. 2002. Androgenic dihapliods from somatic hybrids between *Solanum melongena* and *Solanum aethiopicum* group Gilo as a source of resistance to *Fusarium oxysporum* f.sp. *melongenae*. *Plant Cell Reports* **20**: 1022–32. DOI 10.1007/s00299–001–0429–5.

Rotino G L, Mennella G, Fusari F, Vitelli G, Tacconi M G, D'alessandro A, Acciarri N. 2001. Towards introgression of resistance to *Fusarium oxysporum* f. sp. *melongenae* from *Solanum integrifolium* into eggplant. *XIth EUCARPIA Meeting on Genetics and Breeding of Capsicum and Eggplant*, pp 303–7. Antalya, Turkey.

Rotino GL, Rizza F, Mennella G, Tacconi MG, Alberti P, D'alessandro A, Acciarri N and Toppino L. 2004. Production and Utilization of sexual 'double hybrid' between the somatic hybrids *S. melongena* (+) *S. integrifolium* and *S. melongena* (+) *S. aethiopicum* gr. gilo. *XIIth Eucarpia Meeting on Genetics and Breeding of Capsicum and Eggplant*, 17–19 May. Noordwijkerhout - Netherlands.

Sekara A, Cebula S and Kunicki E. 2007. Cultivated eggplants - origin, breeding objectives and genetic resources, a review. *Folia Horticulturae Ann* **19**(1): 97–114.

Stravato V M, Cappelli C. 2000. Behaviour of *Solanum* spp on inoculation with different isolates of *Fusarium oxysporum* f. sp. *melongenae*. *OEPPEPPO Bulletin* **30**: 247–49.

Toppino L, Vale G and Rotino G L. 2008. Inheritance of *Fusarium* wilt resistance introgressed from *Solanum aethiopicum* Gilo and Aculeatum groups into cultivated eggplant (*S. melongena*) and development of associated PCR-based markers. *Molecular Breeding* **22**: 237–50.

Yücel S, Elekçioglu I H, Uludag A, Can C, Sögüt M, Özarslandan A and AksoY E. 2001. The first year result of methyl bromide alternatives in strawberry, pepper and eggplant in the eastern mediterranean part of Turkey. *Annual International Research Conferance on Methyl Bromide Alternatives and Emissions Reductions*. 5–9 November, The Double Tree Hotel Mission Valley 7450 Hazard Center Drive San Diego, California 92108.