Resistance to *Fusarium oxysporum* f.sp. *lilii* in *Lilium*

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Abstract

The soil-born fungus, *Fusarium oxysporum* f. sp. *lilii*, causes bulb rot in lilies, which endangers its bulb production worldwide. The resistance of 44 species against *Fusarium* was scored under standardized test conditions. The Asiatic cultivars 'Orlito' and 'Connecticut King' were highly resistant, while 'Pirate' was highly susceptible. The resistance level of the Oriental hybrids such as 'Acapulco' and 'Stargazer' and L. longiflorum such as 'Snow Queen' and 'Gelria' was susceptible as compared with that of Asiatic hybrid lilies. *L. regale* and *L. dauricum* among several species were highly resistant, while *L. davidii* and *L. hansonii* were highly susceptible. Three of the 10 LA-hybrids from NHRI accession were highly resistant while the others were observed to be moderately resistant, however the LO-hybrids were susceptible. The resistance level of the four interspecific hybrids from PRI varies from moderately resistant (LA and OA) to susceptible (2 OAs).

INTRODUCTION

The lily (*Lilium* spp.) is a perennial bulb that is cultivated worldwide as a cut flower, pot, and garden plant. Eleven lily species, including *L. hansonii*, are distributed in Korea. As lily cultivation for cut flowers has increased from 84 ha in 1990 to 245 ha in 2000, import of lily bulbs from the Netherlands was also increased.

The soil-born fungus *Fusarium oxysporum* f. sp. *lilii* Imle., causing bulb rot disease especially at the scale bulblet stage (Straathof and Loffler, 1994), is the most serious threat to bulb and flower production of lilies. An environmentally safe method to overcome this problem would be the cultivation of resistant cultivars. In the *Fusarium*-lily interaction, high resistance has been reported (Imle, 1942; Smith and Maginnes, 1969; Maginnes and Smith, 1971; Van Tuyl, 1980; Loffler and Mouris, 1989; Straathof and Van Tuyl, 1994).

The objective of this research was to evaluate the genetic variation of resistance againist *Fusarium* in some lily species, hybrids, and breeding lines. Information about the levels of *Fusarium* resistance in *Lilium* genotypes, in relation to the usefulness of these genotypes in interspecific hybridization programmes, is a prerequisite for future breeding strategies.

MATERIALS AND METHODS

Plant Material

Mature bulbs were used to induce scale bulblets from NHRI and PRI genebank. Scales from 12 Asiatic hybrid cultivars, 2 Oriental hybrid cultivars, 2 *L. longiflorum* cultivars, 4 *L. formolongi* cultivars, 6 species, 13 breeding lines from NHRI, and 5 breeding lines from PRI (Table 1) were placed in plastic bags filled with wet vermiculite. After eight weeks at 25° C, the temperature was reduced to 17° C for another four weeks, followed by eight weeks at 5° C. After the final incubation, newly formed scale bulblets were harvested and selected for uniformity within each genotype. The resistance tests were then performed with the selected scale bulblets. 'Orlito' and 'Connecticut King' were chosen as resistant cultivars, 'Pirate' and 'Stargazer' susceptible and 'Acapulco', 'Snow Queen' and 'Gelria' were moderately resistant (Straathof and Van Tuyl; 1994).

Fungus

Two highly aggressive monospore isolates of *Fusarium oxysporum* f. sp. *lilii* (CPRO-Fol 4 and CPRO-Fol 11) were used. Stock material, stored on Protect Bacterial Preservers at -80° C, was propagated on Czapek-Dox agar medium before experimental use. For soil infestation, the fungus was incubated for two weeks at 23° C in an autoclaved (120° C, 2 hr) oatmeal-soil mixture (1:4 w/w). The fully-grown cultures were ground and mixed with soil in a 1:100 ratio. In order to determine the number of propagules in soil, samples of the test-soil (5 g) were shaken vigorously in 50 mL of water 100 rpm for 1 hour. Soil particles were removed by filtering this suspension over cheesecloth. The remaining suspension (solution 1) was diluted 10× (solution 2), 100× (solution 3), and 100 µL each of these solutions was plated on a modified Komada medium (Komada, 1975). The medium consisted of K_2HPO_4 (1 g), KCl (0.5 g), $MgSO_4.7H_2O$ (0.5 g), Fe-Na-EDTA (0.01 g), L-asparagine (2 g), D-galactose (20 g), Solacol (2 g), and agar (15 g) in 1000mL bottle. The medium was sterilized for 20 minutes at 120° C and allowed to cool to 60° C. Then, quintozeen (1 g), $Na_2B_4O_7.10\ H_2O$ (1 g), and streptomycine sulfate (0.3 g) were added. The pH was brought to 3.8 with 10% H_3PO_4. Selective plates were incubated at 23° C for 10 days, and then the number of *Fusarium* colonies was counted. The samples were collected twice, immediately after mixing ($\pm$ $2.7x10^5$ propagules per gram of soil) and at the time of planting the scale bulblets, two weeks after infestation of the soil ($\pm$ $1.5\ x10^4$ propagules per gram of soil).

Experimental Design

Five scale bulblets were planted in an 1-liter container and placed in a temperature-controlled greenhouse at 18/14° C (16 hr day / 8 hr night). The experiment was arranged in 4 blocks each of an infested and one non-infested (control) pot of each genotype. Forty-four genotypes were randomly assigned to the pots. Observations were made 6-8 weeks after bulblets were planted.

Measurement of Disease Rating

Decay of the infested bulblets was rated visually according to an ordinal scale with six categories (Fig. 1): 1=healthy; 2=slightly rotten; 3=moderately rotten; 4=heavily rotten; 5=very heavily rotten; and 6=completely decayed.

RESULTS AND DISCUSSION

The average disease rating (ADR) values of each genotype, standard deviation and shoot emergence percentage are given in Table 1. Genotypes are arranged by origin and average disease rating values. A high shoot emergence percentage was obtained in the infested and control group, although some genotypes (e.g. *L. henryi*, *L. speciosum*, 'Acapulco', 'Stargazer') gave a very low percentage. A level of resistance of genotypes is indicated as highly resistant comparable with the control cultivars 'Connecticut King' and 'Orlito', as susceptible comparable with 'Pirate' and as a moderate between those control cultivars.

The variation in resistance level within seven control cultivars is given in Fig. 2. The level of the Asiatic cultivars 'Orlito' and 'Connecticut King' was the most resistant, whereas 'Pirate' was the most susceptible. The level of resistance of the Oriental hybrids ('Acapulco', 'Stargazer') and *L. longiflorum* ('Snow Queen', 'Gelria') was more susceptible as compared with the most resistant Asiatic hybrid lilies.

The variation in resistance level within the Asiatic hybrid lilies is given in Fig. 3. The level ranged from highly resistant to moderate resistant compared with 3 standard cultivars. The average disease rating of 'Chianti', 'Pepper', and 'Sunray' was between 'Orlito' and 'Connecticut King'.

The level of resistance within the cultivars of the *L. longiflorum* and the *L. formolongi* was susceptible (Table 1, Fig. 4) compared with the most resistant Asiatic hybrid lilies. The resistance level of those cultivars ranged from moderate resistance to susceptible.

In the Sinomartagon section, *L. dauricum* showed a high level of resistance, while *L. davidii* was susceptible (Fig. 5). *L. regale* of the Leucolirion section was highly resistant. *L. speciosum* of the Archelirion section, *L. hansonii* of the Martagon section, and *L. henryi* were susceptible.

The variation in resistance of the four interspecific hybrids from PRI ranged from moderate resistant (LA and OA) to susceptible (2 OA) (Table 1). Three of the 10 LA-hybrid lilies from NHRI were highly resistant (Fig. 6) while the others were moderately resistant (Table 1). The LO-hybrid lilies from NHRI were susceptible.

In this study, additional genetic variation (Straathof et al., 1993, 1994) to *Fusarium* resistance in lily was investigated. Knowledge of this variation within the cultivar groups in combination with screening tests can lead directly to resistance breeding programs. Interspecific hybridization can also be helpful to enrich cultivar groups with *Fusarium* resistance found in non-related species.

The levels of *Fusarium* resistance of seven standard cultivars used as control in this study coincided with earlier tests (Straathof et al., 1993; Straathof et al., 1994). The resistant level of the Asiatic hybrid lilies, which were used as the male plants for the breeding of LA-hybrids were highly or moderately resistant. According to Straathof et al. (1994), although highly resistant Asiatic hybrid lilies were again found, none of the cultivars tested was significantly better than 'Connecticut King'. In this study, ADR value of 3 cultivars ('Chianti', 'Pepper', and 'Sunray') showed between 'Orlito' and 'Connecticut King' and we classified them as highly resistant.

Within cultivars of *L. longiflorum*, variation in resistance was found, although the level of resistance was lower than in the most resistant Asiatic hybrid lilies. The level of resistance of the *L. formolongi* cultivars 'Raizan' and 'F_1 August' which were used mainly as the female plants for the NHRI LA-hybrids were very susceptible. The *L. formolongi* was originated from *L. formosanum*, which is also very susceptible to virus. The virus susceptibility could be a problematic in the future selection program.

Interspecific crosses between *L. longiflorum* and Asiatic hybrid lilies gave rise to the so called, LA-hybrid lilies (Van Tuyl et al., 1988). Resistant LA-hybrid lilies against *Fusarium* can be made in case of crossing between resistant Asiatic hybrid lilies and cultivars of *L. longiflorum*. In this study, the progeny of the resistant Asiatic hybrid lilies 'Connecticut King', 'Prominence', and 'Sunray' were crossed with cultivars of *L. formolongi* showed high resistance levels.

The Oriental hybrid lilies were susceptible and showed only variation in *Fusarium* resistance (Straathof et al., 1994).

It was already known that *L. speciosum* of the Archelirion section are susceptible, however it is resistant among the Oriental hybrids.

It was reported that *L. hansonii* (Imle, 1942a; 1942b; Straathof et al., 1994) and *L. henryi* (Straathof, et al., 1994) were resistant. But, in this study, opposite results were found, possibly by differences among germplasm materials. We assume that several sources of resistance are found in the investigated 44 genotypes of the genus *Lilium*. Some of them can be used directly to produce new resistant cultivars. Based on existing interspecific hybridization protocols and the new hybridization techniques, which are being developed, it is expected that *Fusarium* resistance from some of *Lilium* species can be exploited to the Asiatic hybrids.

ACKNOWLEDGEMENTS

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Tables

Table 1. Average disease rating (ADR), standard deviation (SD) and shoot emergence percentage (SEP) of 44 *Lilium* genotypes after planting scale bulblets in *Fusarium*-infested soil.

Genotype	Origin	ADR	SD	SEP
Orlito(2x)	Asiatic hybrid lily	1.33	0.13	95
Orlito(4x)	Asiatic hybrid lily	1.60	0.32	90
Chianti	Asiatic hybrid lily	1.60	0.28	100
Pepper	Asiatic hybrid lily	1.65	0.38	100
Sunray	Asiatic hybrid lily	1.65	0.38	100
Connecticut king	Asiatic hybrid lily	1.80	0.47	100
Kinks	Asiatic hybrid lily	2.35	0.30	100
Nove Cento	Asiatic hybrid lily	2.70	0.33	100
Sanzio	Asiatic hybrid lily	2.75	0.50	90
Beatrix	Asiatic hybrid lily	3.05	0.55	100
Kansas	Asiatic hybrid lily	3.10	0.33	100
Solemio	Asiatic hybrid lily	3.20	1.10	100
Pirate	Asiatic hybrid lily	5.83	0.30	65
Acapulco	Oriental hybrid lily	4.43	1.28	50
Stargazer	Oriental hybrid lily	5.45	0.46	50
Snow Queen	*L. longiflorum* Thunb.	3.63	0.80	95
Gelria	*L. longiflorum* Thunb.	5.13	0.55	95
Raizan # 1	*L. formolongi*	3.38	0.86	95
Raizan # 3	*L. formolongi*	4.05	0.46	85
Raizan # 2	*L. formolongi*	4.13	0.33	90
F_1 August	*L. formolongi*	4.48	0.36	60
72254-3	*L. regale* Wilson	1.83	0.60	70
73139	*L. dauricum* Ker-Gawler	1.91	0.47	100
77017-6	*L. speciosum* Thunb.	3.75	0.75	15
73275-1	*L. davidii* Duchartrei	4.35	0.36	95
86262	*L. hansonii* Leichtlinii	4.98	0.72	65
72113-6	*L. henryi* Baker	5.38	0.65	50
952016-4	LA	2.75	0.30	100
951301-5	OA	3.4	0.42	100
951914-1	OA	4.63	0.84	80
951934-1	OA	5.48	0.31	80
NHRI-LA96/25	*L. formolongi* ‘Raizan' x Asiatic (‘Cote d'Azur’ x ‘Prominence’)	1.5	0.10	100
NHRI-LA96/18	*L. formolongi* 'Silky White' x Asiatic 'Sunray'	1.85	0.26	100
NHRI-LA96/16	*L. formolongi* 'Silky White' x Asiatic 'Connecticut King'	1.90	0.41	100

Genotype	Origin	ADR	SD	SEP
NHRI-LA97/4	*L. formolongi* 'Eunha' x Asiatic 'Corina'	2.05	0.30	100
NHRI-LA96/10	*L. formolongi*' 'Raizan' x Asiatic ('Cote d'Azur' x 'Prominence')	2.15	0.17	100
NHRI-LA96/1	*L. formolongi* x Asiatic 'Anabelle'	2.80	0.68	100
NHRI-LA96/26	*L. formolongi*' 'Raizan' x Asiatic ('Cote d'Azur' x 'Prominence')	2.88	0.80	90
NHRI-LA97/15	*L. formolongi* 'Silky White' x Asiatic 'Connecticut King'	3.13	0.60	85
NHRI-LA96/9	*L. formolongi*' 'Raizan' x Asiatic ('Cote d'Azur' x 'Prominence')	3.40	0.28	90
NHRI-LA97/6	*L. formolongi* 'Eunha' x Asiatic 'A64'	3.40	0.65	100
NHRI-LO97/8	*L. formolongi* 'Raizan' x Oriental 'O54'	3.68	0.78	95
NHRI-LO97/11	*L. formolongi* 'Raizan' x Oriental 'O54'	4.45	0.90	75
NHRI-LO97/1	*L. formolongi* 'Raizan' x Oriental 'O54'	5.75	0.22	80

Figures

Fig. 1. The index of disease severity (left to right, 1=healthy; 2=slightly rotten; 3=moderately rotten; 4=heavily rotten; 5=very heavily rotten; and 6=completely decayed).

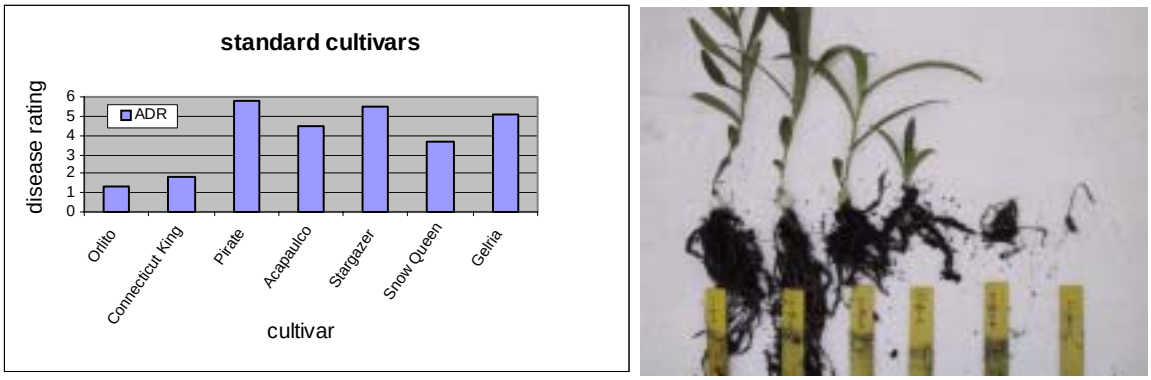

Fig. 2. Average disease rating of the standard cultivars. Photo: Disease infection of 6 standard cultivars planted in *Fusarium*-infested soil (Left to right 1. 'Orlito', 2. 'Connecticut King', 3. 'Snow Queen', 4. 'Acapulco', 5. 'Gelria', 6. 'Pirate').

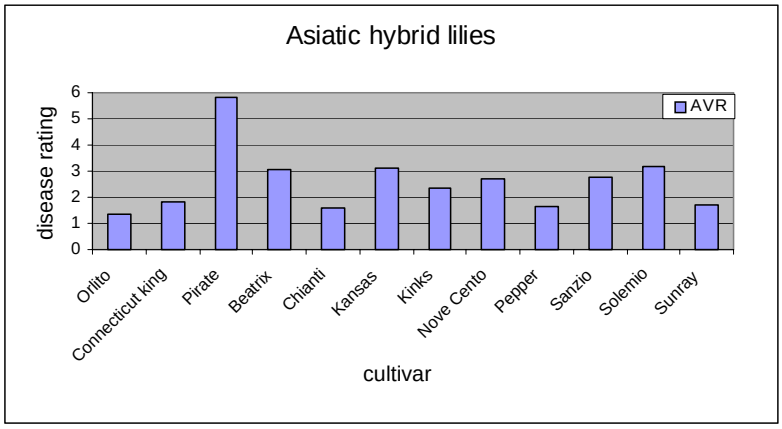

Fig. 3. Average disease rating of the Asiatic hybrid lilies planted in *Fusarium*-infested soil.

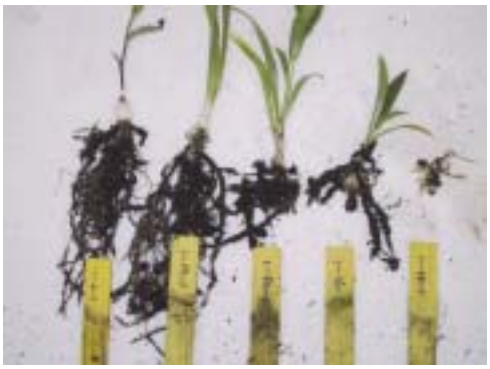

Fig. 4. The level of resistance to *Fusarium* of the *L. longiflorum* and the *L. formolongi* cultivars (From left: 1. 'Orlito', 2. 'Snow Queen', 3. 'Raizan #1', 4. 'Gelria', 5. 'F_1 August').

Fig. 5. Infection of lily species (From left: 1. ‘Orlito’, 2. *L. henryi*, 3. *L. regale*, 4. *L. dauricum*, 5. *L. davidii*, left to right).

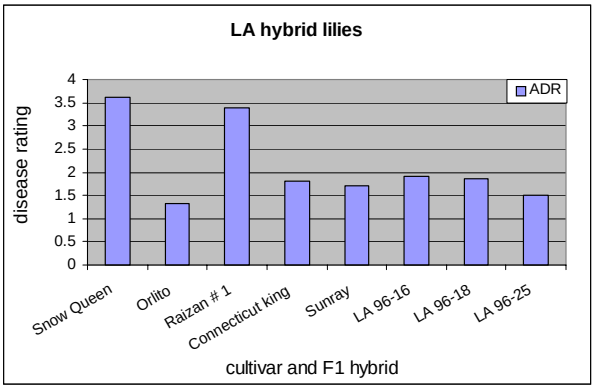

Fig. 6. Average disease rating of the LA-hybrid lilies planted in *Fusarium*-infested soil. Photo: LA breeding lines from NHRI as a resistant (1. ‘Orlito’, 2. ‘CK’, 3. ‘LA96-18’, 4. ‘LA96-25’).