SEGREGATION OF *FUSARIUM* RESISTANCE IN AN INTERSPECIFIC CROSS BETWEEN *LILIUM LONGIFLORUM* AND *LILIUM DAURICUM*

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Abstract

Using interspecific hybridisation techniques, a resistant accession of *L. dauricum* (section *Sinomartagon),* was crossed with the susceptible *L. longiflorum* cultivars 'Gelria' and 'Flevo' (section *Leucolirion).* The progeny was tested for *Fusarium* resistance in a standardised scale-bulblet assay. Both resistant and susceptible descendants were found, indicating that the resistance was passed from the resistant parent to the progeny and that the resistance segregates. The level of the most resistant descendants equalled that of the resistant parent. It is concluded that *L. dauricum* can be used as a source of *Fusarium* resistance in interspecific crosses with *L. longiflorum.*

1. Introduction

Fusarium oxysporum is a soil-borne fungus which causes serious diseases in many crops (Armstrong and Armstrong, 1981). Based on its host-range, this fungal species is further divided in *formae speciales.* In lily, the forma specialis *lilii,* and to some extent isolates from other formae speciales (Löffler and Mouris, 1993), causes basal rot. This disease is characterised by a brownish rot of scales at the basal plate. In a later stage, the rot can spread and eventually destroy the whole bulb (Imle, 1942; Linderman, 1981; McRae, 1988). Basal rot is the most important disease in lily and threatens the cultivation of both bulbs and flowers. Dipping the bulbs in fungicides before planting and soil disinfection are the predominant ways of control. Chemical disinfection, however, is not always adequate and pollutes the environment. Moreover, the fungus can develop resistance to fungicides (Bollen, 1972; Duineveld and Beijersbergen, 1975; Hornok, 1983). The most sustainable and obvious alternative way of control is the cultivation of resistant lilies.

Presently three economically important groups of lilies are cultivated: Asiatic hybrids, Oriental hybrids and cultivars belonging to *L. longiflorum.* A high level of *Fusarium* resistance is already present in some lily cultivars, especially in Asiatic hybrid lilies (Straathof and Löffler, 1994a). Only a low level of resistance, however, is found in *L. longiflorum* and almost none in Oriental hybrids (Straathof and Van Tuyl, 1994). Interspecific hybridisation is necessary to obtain level of resistance in both latter groups suitable for practical cultivation. In an extended survey for resistance at CPRO-DLO, an accession of *L. dauricum* was found to be highly resistant to *Fusarium* (Straathof and Van Tuyl, 1994). This *Lilium* species belongs to the section *Sinomartagon* and is one of the ancestors of the Asiatic hybrid lilies. Besides its *Fusarium* resistance, the species is an important genitor for early flowering. Using pollination and embryo-rescue techniques, the *L. dauricum* was crossed with different *L. longiflorum* cultivars, belonging to the section *Leucolirion* (Van Tuyl et al., 1991). The *Fusarium* resistance of 36 hybrids, originating from a resistant *L. dauricum* accession and the susceptible *L. longiflorum* cultivars 'Gelria' and 'Flevo', is subject of this article.

Proc. Int. Sym. on *Lilium*
Ed. J.S. Lee, M.S. Roh
Acta Hort. 414. ISHS 1996

2. Materials and methods

2.1. Fungi

The isolates 'Fol-11' and 'Fol-4' of *F. oxysporum* f.sp. *lilii* were kindly provided by G. Bollen (Department of Plant Pathology, Agricultural University Wageningen, The Netherlands) and E.J.A. Roebroeck (Bulb Research Centre, Lisse, The Netherlands) respectively. Both isolates originate from lily and are highly aggressive (Löffler and Rumine, 1991). Isolates were single-spored and stored on PROTECT bacterial preserves (Technical Service Consultants) at -80 °C. Prior to use the isolates were revitalised on Czapek Dox agar plates (CDA, OXOID).

2.2. Plant Material

The susceptible *L. longiflorum* cultivars 'Gelria' and 'Flevo' (Straathof and Van Tuyl, 1994) were pollinated with a resistant accession of *L. dauricum* (Van Tuyl et al., 1991). About half of the progeny originates from pollination with the cut-style method (CSM) whereas the other half was obtained using the grafted style method (GSM). Ovary-ovule culture was applied to allow the ovules to germinate and to form bulblets (Van Tuyl et al., 1991). The in-vitro grown bulblets were transferred to the greenhouse and grown to maturity in two years. From the progeny, 36 individuals (27 from the 'Flevo' *x L. dauricum* cross and 9 from the 'Gelria' x *L. dauricum* cross) were randomly chosen for the assessment of *Fusarium* resistance. As controls, the resistant *L. dauricum,* the resistant Asiatic hybrid 'Connecticut King' and the susceptible Asiatic hybrid 'Pirate' were included. Before the *Fusarium* test, bulbs were propagated. Scales were broken loose from the bulbs and allowed to form scale bulblets in wet vermiculite for 10 weeks at 25 °C. Subsequently the bulblets were treated for 4 weeks at 17 °C and 9 weeks at 5 °C. From each genotype, 20 uniform, healthy bulblets were selected and used for resistance screening.

2.3. Soil infestation

Inoculum was prepared by infecting a sterilised oatmeal-soil mixture (20% w/w oatmeal) in 100-ml glass jars with five 5-mm agar plugs taken from CDA-plates Fol-11 or Fol-4 and incubating the mixture for 2 weeks at 23 °C. Subsequently, the mixture was ground by using a food processor. Potting soil was mixed with the ground cultures of both fungal isolates (0.05% v/v Fol-11 and 0.05% v/v Fol-4) and incubated for 2 weeks prior to use to allow the fungal population to stabilise (Löffler and Mouris, 1989). After this time, the fungal density was approximately 6,000 propagules per gram soil.

2.4. Resistance screening

In total 156 1-litre pots were filled with one litre of the infested soil. Twenty scale bulblets of each of the 39 genotypes were planted in four pots (five bulblets per pot). The experiment was set up in a split-plot design with four randomised blocks, each block containing one pot of each genotype. After 9 weeks of incubation in the greenhouse at 18/14 °C (day/night) temperature, the bulblets were harvested. The infection was evaluated visually on a disease-index scale running from '1' (healthy) to '6' (completely decayed).

2.5. Statistical analysis

The disease-index data were analysed using a threshold model for ordinal categorical data according to Straathof et al. (1993). With this generalized linear model (GLM), the non-linear disease index data are transformed to the linear disease severity scores (DSS).

3. Results

After 9 weeks, all individual bulblets were scored 1 to 6 based on their *Fusarium* infection. For each genotype, the Disease Severity Score (DSS) and the standard error of the difference (sed) relative to the resistant parent *L. dauricum* was calculated (table 1). Since the DSS scale is arbitrary, the DSS of *L. dauricum* was set to zero. Analysis of deviance showed no block effects but a large genotype effect (deviance=283.4; df=38; significant at $p<0.001$). Any genotype differing 2*sed from *L. dauricum is* significantly more or less resistant than *L. dauricum.*

At harvest, the susceptible control 'Pirate' was heavily affected (DSS=1.59), whereas the partial resistant control 'Connecticut King' (DSS=-0.41) as well as the resistant accession of *L. dauricum* (DSS=0) were only slightly affected. The DSS of the progeny ranged from -0.46 to 2.73, while most genotypes scored intermediately. No descendants were found with a higher resistance than the resistant parent. The resistance of the progeny of both crosses was roughly equally distributed (table 1) and are therefore combined. A histogram of DSS of all progeny genotypes shows a continuous distribution without separate groups (fig. 1).

4. Discussion

In this study, the *Fusarium* resistance level of progeny of crosses between *L. longiflorum* and *L. dauricum* has been assessed. The rate of affection was recorded on a disease index (DI) scale. Disease indices, however, are qualitative and ordinal rather than quantitative and linear. Therefore care must be taken in applying statistics. The calculation of average DI might not be appropriate since bulblets scoring DI=4 are not necessarily twice as affected as bulblets scoring DI=2. In using a threshold model, the non-linear DI data can be transformed to linear DSS values (Straathof et al, 1993). Therefore this model is applied in the current research.

Both the resistant 'Connecticut King' and *L. dauricum* were slightly affected, indicating that the resistance is not absolute. This has been shown before by Straathof and Van Tuyl (1994). Apart of the resistance level, the affection of lily bulbs depends on the infection pressure (Straathof and Inggamer, 1992) and on the developmental stage of the bulbs (Straathof and Löffler, 1994a). Since a high infection pressure is used in the trials, and since the bulbs used in commercial cultivation are more sturdy than the tested small bulblets, the resistance level of both Connecticut King and *L. dauricum* probably will protect the plant sufficiently against *Fusarium* in practice.

As expected, the susceptible cultivar 'Pirate' was heavily affected. Although 'Gelria' and 'Flevo', the susceptible parents of the cross, were not included in the experiment, it is known that the resistance of these cultivars is very similar to that of 'Pirate' (Straathof and Van Tuyl, 1994). Since resistant individuals are found in the progeny of the tested cross, the resistance of *L. dauricum* apparently is passed to its progeny (table 1). Moreover, significant differences are found between individuals (table 1), indicating that the resistance segregates. The distribution of the resistance level of the progeny is continuous with only one peak. Therefore the resistance is probably polygenic. Identical results were found for the resistance of 'Connecticut King' (Straathof and Löffler, 1994b). Since *L. dauricum is* one of the ancestors of the Asiatic hybrids (the lily group to which 'Connecticut King' belongs), the resistance of 'Connecticut King' may originate from this species.

Some individuals of the progeny have a resistance level equalling that of the resistant parent. Therefore the resistance of *L. dauricum* can efficiently be used in breeding programs. It might be difficult, however, to apply this resistance source for developing resistant cultivars of *L. longiflorum* It is still unknown what the introgression of the

Fusarium resistance in the genome of *L. longiflorum* is. Moreover, since the latter is a pure species, more back crosses, followed by testing the progenies, probably are necessary to introduce the resistance without affecting the specificity of *L. longiflorum.* To speed up this process, efficient selection techniques are necessary. Molecular markers, based on Restriction Fragment Length Polymorphism (RFLP) or Random Amplified Polymorphic DNA (RAPD) techniques may provide a tool for such an efficient selection. Presently these techniques are being developed at CPRO-DLO for Asiatic hybrids (Straathof et al., 1994). At a later stage, these techniques may be applied to interspecific crosses, thus accelerating the breeding programs and enhancing the possibilities to introduce desirable traits like resistance from one lily group into another.

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Table 1. Disease Severity Scores (DSS) and standard error of the difference (sed) relative to *L. dauricum* of descendants of the 'Gelria' x *L. dauricum* (G*Ld) and the 'Flevo' x *L. dauricum* (F*Ld) cross, of the resistant controls 'Connecticut King' (CK) and *L. dauricum* (Ld) and of the susceptible control 'Pirate' (Pl).

Geno	DSS	sed	Geno	DSS	sed	Geno	DSSsed
F*Ld	-0.46	0.34	F*Ld	0.53	0.34	F*Ld	1.080.34
CK	-0.41	0.34	F*Ld	0.57	0.34	G*Ld	1.080.34
F*Ld	-0.32	0.34	F*Ld	0.59	0.34	F*Ld	1.090.34
F*Ld	-0.17	0.34	F*Ld	0.64	0.34	G*Ld	1.250.34
G*Ld	-0.17	0.34	G*Ld	0.65	0.34	F*Ld	1.350.34
Ld	0.00	-	F*Ld	0.67	0.34	F*Ld	1.400.34
F*Ld	0.12	0.34	G*Ld	0.68	0.34	F*Ld	1.490.34
F*Ld	0.18	0.34	F*Ld	0.76	0.34	Pl	1.590.34
G*Ld	0.23	0.34	F*Ld	0.78	0.34	F*Ld	1.850.35
G*Ld	0.37	0.34	G*Ld	0.84	0.34	F*Ld	1.860.35
F*Ld	0.45	0.34	F*Ld	0.90	0.34	F*Ld	1.890.35
F*Ld	0.49	0.34	F*Ld	0.95	0.34	F*Ld	2.160.36
F*Ld	0.49	0.34	F*Ld	1.07	0.34	G*Ld	2.730.40

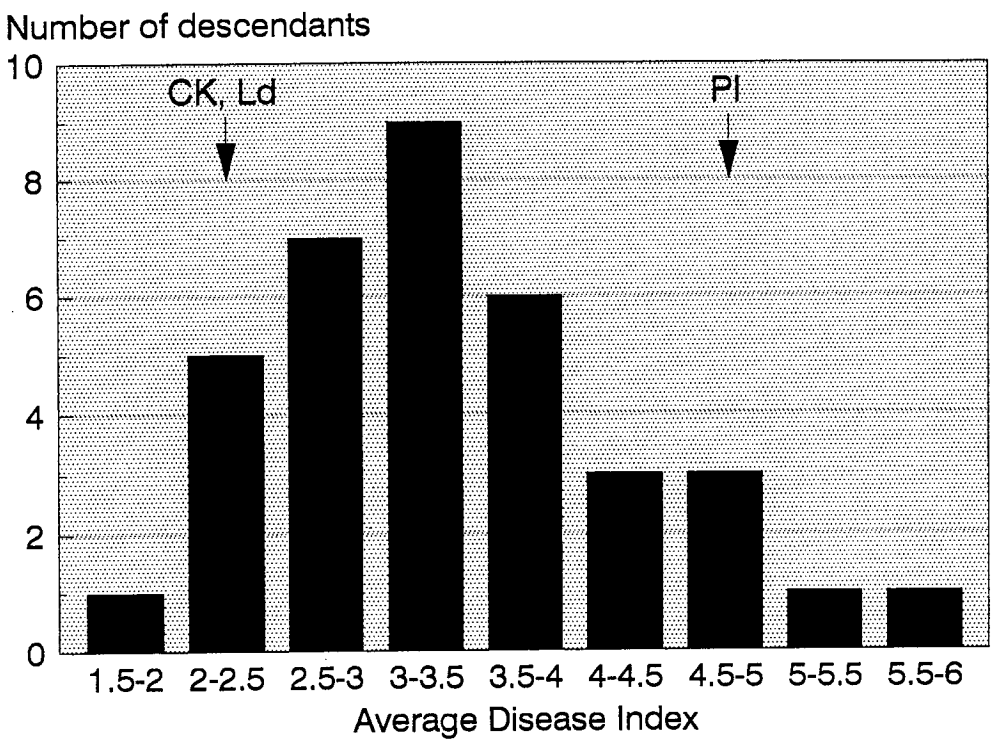

Fig 1. Distribution of the *Fusarium* resistance level of descendants of *L. longiflorum* x *L. dauricum* crosses. Arrows indicate the resistance level of the resistant 'Connecticut King' (CK), *L. dauricum* (Ld) and the susceptible 'Pirate' (Pl).