MOLECULAR MARKERS AS A TOOL FOR BREEDING FOR FLOWER LONGEVITY IN ASIATIC HYBRID LILIES

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Segregation of flower longevity in two lily populations was studied and the genetic linkage of morphological markers and RAPD markers with loci involved in flower longevity was investigated. A large variation in flower longevity was found within the two populations tested at individual plant level. Longevity values were well correlated between experiments at the individual plant level and at clonal level. One out of 278 RAPD markers and one morphological marker (male sterility) were significantly ($P<0.005$) linked to flower longevity in the clonal test. Linkage studies were hampered because of the low number of descendants evaluated in both longevity and marker analysis. The possible influence of male sterility on flower longevity of Asiatic hybrid lilies is discussed.

INTRODUCTION

At CPRO-DLO a research program has been initiated to investigate the possibilities for improving flower longevity of Asiatic hybrid lilies by breeding. Screening conditions for flower longevity in Asiatic hybrid lilies have been determined to reduce undesirable non-genetic variation (Van der Meulen-Muisers et al., 1992). The available genetic variation has been investigated within a group of 47 Asiatic hybrid lilies, and was found to vary between 4 and 9 days per flower (Van der Meulen-Muisers and Van Oeveren, 1993).

Initial studies to improve flower longevity by crossbreeding were performed with two lily populations. The use of genetic markers to obtain information about the inheritance of flower longevity was investigated within one of the populations. Advantages of molecular markers as a tool for indirect selection are the possibility of determining flower longevity independently from environmental variation, and selecting in an early stage of development, even before flowering (Gebhardt and Salamini, 1992). Randomly amplified polymorphic DNAs (RAPDs) (Williams et al., 1990) were used. RAPD markers are easy to obtain and have proven to be useful in lily for linkage studies of both qualitative traits (flower colour, petal spots and male sterility) and quantitative traits (*Fusarium* resistance) as reported by Straathof et al. (1994).

In this paper we describe the segregation of flower longevity within two lily populations and the preliminary results of a linkage analysis of RAPD markers and three morphological markers with loci involved in flower longevity of Asiatic hybrid lilies in one of those populations.

MATERIALS AND METHODS

Plant materials. Bulbs of two populations were obtained from the CPRO-DLO *Lilium* collection. The first population (CPRO-858) was a cross between the female parent 'Yellito' (longevity about 8 days), and the male parent 'Orlito' (longevity about 7 days). Flowers of 114 descendants of the population were tested using one bulb per genotype. The second population (CPRO-1338) was a cross between the female parent 'Connecticut King' (longevity about 7 days), and the male parent 'Orlito'. Flowers of 70 descendants of the population were tested in a first experiment using one bulb per genotype. In a second experiment flowers of 28 clones were tested, consisting of 19 genotypes already tested at individual plant level and 9 additional genotypes. Up to 10 plants were used per clone.

Methods. Standard conditions were used during cultivation (17°C, 60% RH, 24 W/m² for 16 h/day [HPI-T 400 W]), harvest (anthesis of the most mature floral bud) and postharvest (17°C, 60% RH, 3 W/m² for 12 h/day [TL-D84 35W]) after Van der Meulen-Muisers et al. (1992). Flower longevity was measured as the time between bud anthesis and deformation of the flower. Individual flower longevity calculated per stem, was used as a parameter for screening (Van der Meulen-Muisers and Van Oeveren, submitted). Three morphological markers (flower colour, spots on the petals and male sterility) were scored on all descendants of CPRO-1338. Flower colours were scored as yellow or orange, and flower spots and male sterility were scored as present or absent. The RAPD analysis (of 39 individual plants and 28 clones) was carried out according to Straathof et al. (1994), using primers obtained from Operon Technologies and the primers originally described by Williams et al. (1990). The latter primers are encoded EJO in Table 1.

Statistical analysis. Completely randomized designs were used with plants as experimental units. Data were analyzed by analysis of variance. The heritability of flower longevity in the clonal test was calculated as $h^2=\sigma^2_g /(\sigma^2_g+\sigma^2_e /n)$, with σ^2_g denotes genotypic variance, σ^2_e denotes environmental variance and n is number of plants per genotype. The calculation was carried out for $n=1$, representing the early stage of selection (individual plant level). To compare the ranking of the genotypes between experiments, the correlation coefficient (r) was calculated.

Genetic association of a segregating RAPD marker with either the longevity values of the individual plant test or the clonal test of CPRO-1338 was assessed by applying the Kruskal-Wallis rank-sum test (Lehman, 1975), as implemented in the MapQTL computer program (J.W. Van Ooijen, personal communication). Mapping of markers in linkage groups was performed with the JoinMap computer software package (Stam, 1993).

RESULTS

Longevity values for the descendants of CPRO-858 and CPRO-1338 tested, at individual plant level were approximately normally distributed (Fig.1 and 2, respectively). The average longevity of both populations (7.3 and 6.8 days respectively) was in between or conform to the longevity levels of the parents. A large segregation of longevity was found within both F_1-populations. The correlation coefficient (r) calculated between longevity values of the 19 genotypes of CPRO-1338 tested both at individual plant level and at clonal level was 0.82 (Fig. 3). The longevity level of genotypes with a short longevity at individual plant level slightly increased after being tested at clonal level. The heritability of flower longevity in the clonal test amounted to 0.67. In both the individual plant test and the clonal test of CPRO-1338 18 percent of the descendants tested were male sterile. The mean longevity of the male sterile flowers exceeded the mean longevity of the whole population in both tests.

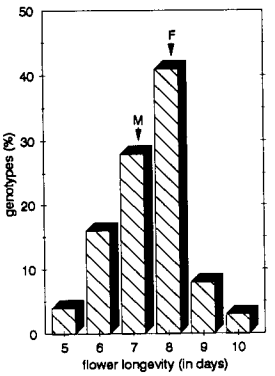

Fig.1. Distribution of flower longevity values of 114 descendants of the lily population CPRO-858, a cross between the female parent 'Yellito' (F) and the male parent 'Orlito' (M). The longevity values of the parents are indicated with arrows.

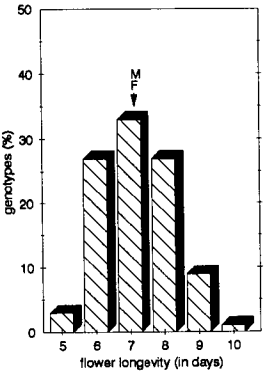

Fig.2. Distribution of flower longevity values of 70 descendants of the lily population CPRO-1338, a cross between the female parent 'Connecticut King' (F) and the male parent 'Orlito' (M). The longevity values of the parents are indicated with arrows.

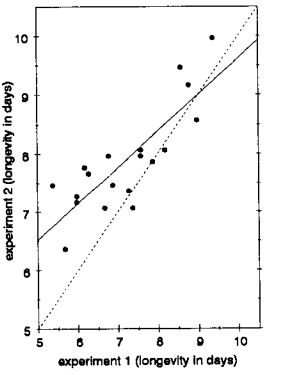

Fig.3. Correlation diagram between flower longevity values of an individual plant test (expt. 1) and a clonal test (expt. 2) of the lily population CPRO-1338, a cross between 'Connecticut King' and 'Orlito'; (r = 0.82).

The three morphological markers (flower colour; petal spots; male sterility) together with 278 segregating RAPD markers were tested for association with loci involved in flower longevity using the Kruskal-Wallis test. These tests were carried out both at individual plant level (using 39 plants) and clonal level (using 28 clones). The Kruskal-Wallis test resulted in 10 significantly ($P<0.05$) associated markers in the individual plant test and 10 markers in the clonal test, with 3 markers significant at $P<0.05$ in both experiments (Table 1). One marker (V-03-02) in the individual plant test and two markers (AP-06-05; male sterility) in the clonal test were significantly linked to the longevity data with $P<0.005$.

Table 1. List of Markers Linked significantly ($P<0.05$) to Longevity data of descendants of the lily population CPRO-1338 in either Expt.1 (39 individual plants) or Expt.2 (28 clones).

Marker	Expt.1	Expt.2	Marker	Expt.1	Expt.2	Marker	Expt.1	Expt.2
AE-03-01	***	-	EJO-04-03	**	**	U-02-01	-	**
AK-15-03	**	-	EJO-05-01	**	**	U-02-02	**	-
AO-16-05	-	**	EJO-10-04	-	**	V-03-02	****	**
AP-06-05	-	****	P-20-03	-	**	X-05-01	**	*
B-13-02	*	**	P-20-07	**	-	Male Sterile	-	****
E-12-02	**	-	Q-20-02	**	*			

-,,**,****, Nonsignificant or significant at P = 0.1, 0.05, or 0.005, respectively, by Kruskal-Wallis test.

The three markers calculated to be linked to flower longevity, were used to determine linkage to other markers. Linkage analysis with a significance threshold for linkage of 3.0 LOD (^{10}log of odds) showed linkage between male sterility and 6 RAPD markers. For two of those markers (P-20-03; P-20-08) significant linkage (P=0.05, 0.1 respectively) with flower longevity in the clonal test was found. However, the markers were not ordered according to linkage with longevity. With a significance threshold for linkage of 3.0 LOD marker AP-06-05 belongs to a linkage group together with three other markers. Male sterility and AP-06-05 are also linked with a threshold of 2.0 LOD. This is a relatively low probability but it implicates that both markers possibly are linked to the same longevity locus. No linkage at all was found between V-03-02 and any other marker.

DISCUSSION

In this preliminary study on flower longevity in lily a large segregation of longevity within F_1-populations was found. Therefore, at least some loci involved in flower longevity seem to be heterozygous present in the parents. The approximately normally distributed patterns of the longevity levels of the descendants indicate quantitative inheritance of this character. Longevities of many descendants were as long as or even longer than both parents (Fig. 1 and 2). Even by using parents with a moderate longevity of about 7 days, longevity levels occurred which exceeded the highest longevity level (9 days) found within 47 lily genotypes (Van der Meulen-Muisers and Van Oeveren, 1993). Therefore, the prospects for improving flower longevity in lily by breeding and selection are promising.

The correlation between tests using individual plants or clones was high (r=0.82) indicating a high heritability of flower longevity. This was confirmed in the test at clonal level (h^2=0.67). A high heritability together with a large segregation of the desired character are of great benefit in linkage studies and future breeding programs. In this study the genetic linkage between loci involved in the encoding of flower longevity and 281 genetic markers was determined.

To link flower longevity in lily to the morphological markers and RAPD markers, one highly significant (P<0.005) linked marker in the individual plant test (V-03-02) was also found to be significantly linked in the clonal test (P<0.05). In the clonal test two highly significant (P<0.005) markers were found (AP-06-05, male sterility). However, no significant linkage of those markers was found in the individual plant test, although linkage to male sterility was almost significant at P<0.1. Many markers were found to be linked to flower longevity with lower significancies. This may be explained in several ways. First, these markers may be distantly linked to flower longevity loci. Second, the outcome of the Kruskal-Wallis test is sensitive to individual genotypes with extreme values. This effect is stronger when low numbers of individuals are tested.

An improvement of the flower longevity as found in male sterile flowers could be due to the absence of an ethylene peak which normally occurs in male fertile lily flowers at the end of the pollen meiosis (Van Meeteren and Slootweg, 1986) and is likely to be absent in male sterile flowers as discussed by Van Tuyl et al. (1985). Furthermore, in male sterile flowers a reduction of the ethylene precursor ACC, which is found in ripening pollen of many species (Spikman, 1987; Whitehead et al., 1983), can be expected. Influence of ethylene on longevity of lily flowers has been found by Woltering and Van Doorn (1988) and Van der Meulen-Muisers and Van Oeveren (1990). Also the beneficial effect of the ethylene retardant silver thiosulphate (STS) in Asiatic hybrid lilies is known (Swart, 1980; Van Meeteren and De Proft, 1982). Therefore, an indirect linkage between male sterility and flower longevity can be expected.

Two regions of 7 (male sterility) and 4 (AP-06-05) genetic markers respectively, were calculated to harbour loci involved in flower longevity. However, male sterility and AP-06-05 proved to be linked at 2.0 LOD, suggesting that all 11 markers might be linked to the same flower longevity locus. JoinMap was not able to determine this interlinkage for the following two reasons. First, linkage analysis was hampered by low numbers of individuals. Second, calculation of linkage of the dominant RAPD markers is hampered since they may segregate in the gametes of one parent of the cross, in the other, or in both as illustrated by Straathof et al. (1994). A marker segregating in one parent cannot be linked directly to a marker segregating in the other parent, but only through markers segregating in both parents. Dominant RAPD markers segregating in both parents, however, provide less information than co-dominant markers. To conclude definitely that only one flower longevity locus has been tagged, more individuals have to be tested on both flower longevity and markers. After confirmation of linkage, highly linked markers can be transformed to sequence characterized amplified regions (SCARs), (Paran and Michelmore, 1991) for application of RAPD markers in plant breeding.

Although no genetic map of lily is available yet, markers significantly linked to flower longevity were detected with the Kruskal-Wallis test. Therefore, this RAPD approach seems promising to obtain markers linked to loci involved in flower longevity. However, the limited number of descendants used in our

preliminary experiments and the sensitivity of the Kruskal-Wallis test to extreme data make linkage studies speculative in this stage. So longevity levels of more descendants have to be determined, preferably at clonal level. The use of a saturated genetic linkage map, consisting of polymorphic marker loci uniformly distributed throughout the genome, is an essential prerequisite to study the number of loci involved in flower longevity and to ensure that markers identified to be linked with the Kruskal-Wallis analysis, but which are from different segregation types are not linked. We are in the process of scoring more RAPD markers and developing other PCR based marker types which inherit co-dominantly to establish a saturated linkage map of this population.

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