VARIATION IN DNA-CONTENT IN THE GENUS *Lilium*

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Abstract

Variation in DNA-content between species or cultivars within a cultivated crop are known and can be caused by differences in ploidy level or, at similar levels of ploidy, by taxonomic differences between genotypes. The first phenomenon is well-known in lilies. Differences in DNA content can be measured efficiently by using flow cytometry. Only a few milligrammes of plant material is sufficient for these DNA-determinations. Using flow cytometry it was possible to determine reproducibly the DNA-content of lilies with high precision. A large variation in DNA content between diploid lily species and cultivars was observed, up to a difference of 34% in DNA content between *L. henryi* and *L. canadense*. Variation between cultivars was less pronounced. Interspecific hybrids had the intermediate DNA content of both parents. Therefore the technique is useful for very early identification of interspecific hybrids.

1. Introduction

Variation in DNA-content between species or cultivars within a cultivated crop is known and can be caused by differences in ploidy level or, at the same level of ploidy by taxonomic differences between genotypes. The first phenomenon is well-known in lilies (Van Tuyl *et al.*, 1989). In this paper we have analysed the second type of variation by using flow cytometry. Flow cytometry enables the efficient measurement of small differences in DNA content. Only a few milligrammes of plant material is sufficient for these DNA-determinations. Variation in DNA content on the diploid level within the genus *Lilium* can be used for taxonomic classification or for the identification of the hybrid character of interspecific hybrids.

2. Plant Material

Plant material from the CPRO-DLO *Lilium* collection was used. Species of six different sections (Archelirion, Leucolirion, Lilium, Martagon, Pseudolirium, Sinomartagon) and commercial varieties of the Asiatic-hybrid group, the Oriental-hybrid group and the *L. longiflorum* group were analysed.

To determine the inheritance of nuclear DNA-content, interspecific *Lilium* hybrids developed at CPRO-DLO (Van Tuyl *et al.*, 1996) as well as their parents, originating from different sections were analysed:

The interspecific hybrids were: *L. longiflorum* x *L. bulbiferum*, *L. longiflorum* x *L. dauricum*, *L. longiflorum* x *L. henryi*, *L. longiflorum* x Oriental hybrids (LO),

L. longiflorum x L. canadense, Oriental x Asiatic hybrids (OA) and *L. henryi x L. candidum.*

3. Methods

3.1 Nuclei isolation

For determination of relative nuclear DNA-content 0.5 cm^2 of *Lilium* leaf was mixed together with 0.5 cm^2 of reference leaf of *Tulipa* before isolation. *Tulipa* was used because of the small difference in DNA-content between *Tulipa* and *Lilium*. In each series of analyses the *Lilium* cultivars 'Connecticut King' and 'Stargazer' were used as standard. *Vivo* as well as *vitro* material was used. Nuclei samples were prepared by chopping the leaf tissue with a sharp razor blade in 0,5 ml isolation buffer (High resolution DNA kit Partec, Münster, Germany). The suspension of released nuclei was filtered through a 50 1µm nylon filter. After 5-1 0 minutes 2 ml of staining solution of the kit was added. Samples were analysed within one hour.

3.2 Flow cytometry

All samples were analysed with a Partec CA-II cell analyser equipped with a HBO 100W/2 lamp. KG1, BG38 and UG1 filters were used for excitation, TK-420 as dichroic mirror and GG-415 as barrier filter. In each sample 1000-5000 nuclei were analysed at a rate of 10-30 nuclei/s. Peak positions and coefficients of variation were calculated with the software program DPAC (Partec). Relative DNA content was calculated by dividing the peak position of *Lilium* by the peak position of *Tulipa*. The relative DNA content of *Tulipa* was first assessed by comparing *Tulipa* with *Allium cepa* (DNA content = 33,5 pg, Bennet and Smith, 1976).

4. Results and Discussion

Flow cytometry of suspensions of nuclei of both *Lilium* and *Tulipa* yielded histograms with one G0/G1 peak (Fig. 1) and only small number of nuclei from S-phase or G2 nuclei. The coefficient of variation of both *Tulipa* as *Lilium* varied between 1.0 and 2.5%. In all cases the maximum deviation from average value was less then 1% in DNA-content.

4.1 Variation in DNA-content

A large variation in DNA content existed amongst the *Lilium* species (Fig. 2). Between the largest (*L. canadense*) and smallest (*L. henryi*) genome the difference was 34 %. Such a variation within a genus is not uncommon (Bennet and Smith, 1976; Bennet *et al.*, 1982; Bennet and Leitch, 1995). Variation was not only present between species of different sections but also between species from the same section. Although per section a limited number of species has been analysed, the variation in DNA content in the section Sinomartagon seemed to be less, and the average DNA content of the section Pseudolirium seemed to be higher when compared with the other sections. The variation within the cultivar groups was much smaller than within the sections from which they were derived (Oriental hybrids - section Archelirion, Asiatic hybrids - section

Sinomartagon). There was no overlap between the Oriental hybrids and the Asiatic hybrids (Fig. 3). Between the *L. longiflorum* cultivars there were only small differences in DNA content. Since between the *L. longiflorum* cultivars, which are derived from one species, variation is present, it seems that also some intra-specific variation is present.

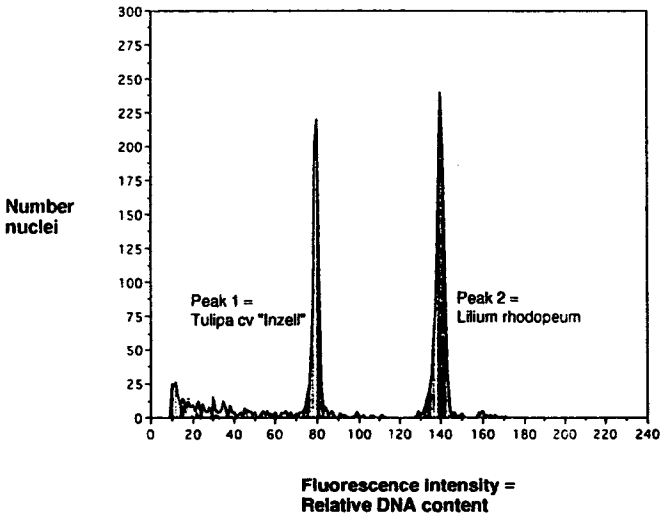

Fig. 1: Relative DNA content of mixed nuclei of *Tulipa* and *Lilium*.

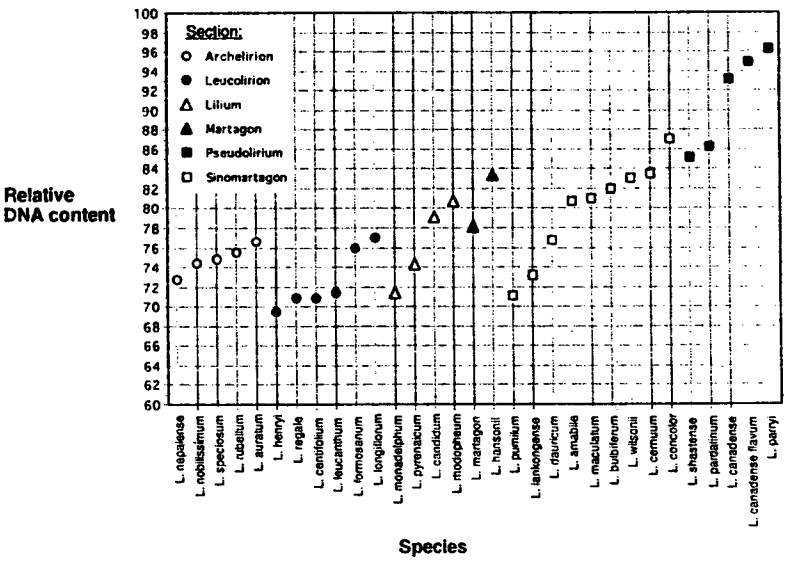

Fig. 2: Variation in the nuclear DNA content in the genus *Lilium*.

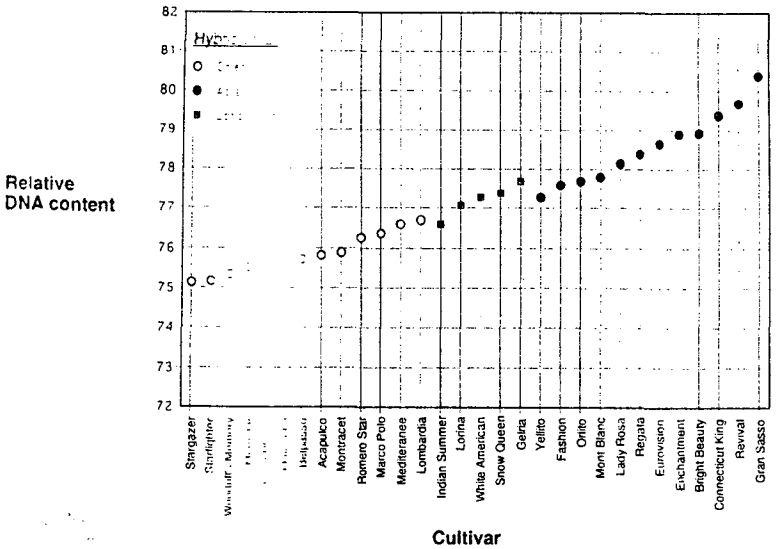

Fig. 3: Variation in the nuclear DNA content between cultivars of different hybrid lily groups

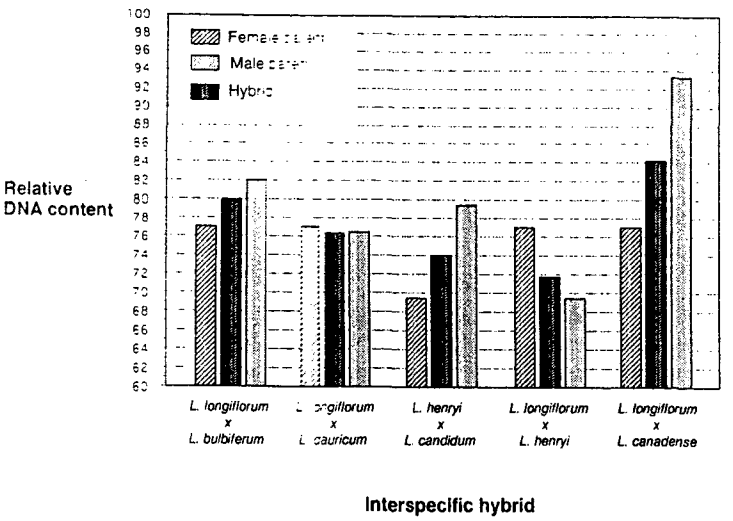

Fig. 4: Inheritance of nuclear DNA content in interspecific hybrids.

4.2 Inheritance of DNA content

In all cases the hybrids had intermediate DNA contents (Table 1, Fig. 4). Except for the combination *L. longiflorum x L. henryi* all hybrids differed 1 % or less from the midparent value. This means that the method can be used for detection of interspecific hybrids provided that the distance between parents is large enough. Some deviation from the midparent value can be expected when the parents themselves originate from parents with different DNA amounts because of segregation of homologous chromosomes with unequal DNA content. The obtained results so far assume that this deviation is only small.

4.3 Relative DNA-amount and absolute DNA-amount

The relative DNA-content is derived from indirect comparison with *Allium cepa* which DNA content is 33,5 pg (Bennet and Smith, 1976). This value of *Allium* was obtained with feulgen densitometry and not with flow cytometric analyses DAPI stained nuclei. DAPI is assumed to be a AT preferent dye. Therefore no direct correlation exists between relative fluorescence of DAPI and absolute DNA amount (Dolezel *et al.*, 1992, Godelle *et al.*, 1993). Propidium iodide is suggested as stain for absolute DNA measurements (Dolezel *et al.*, 1992). Literature data on DNA content of *Lilium* (*L. henryi* of 66 pg: Sentry and Smith, 1985 and 73,2 pg for *L. formosanum*: Bennet *et al.*, 1982) do not differ much from the data presented in this paper . Work with other genera in our lab (data not presented) showed only limited variation in the ratio of DAPI/Propidium fluorescence). Further research with DNA dyes like propidium iodide or ethidium bromide which are not basepare preferent should confirm this.

Although the data obtained may not reflect precisely absolute amounts of DNA the data are highly reproducibly. This reproducibility in combination with the large variation in the relative DNA-content between species makes it a powerful tool for applications such as detection of interspecific hybrids in all plant genera where interspecific variation in DNA content is present.

Table 1: Inheritance of nuclear DNA content in the genus *Lilium*.

Female parent	Male parent	DNA female (pg)	DNA male (pg)	Difference parents	Expected value	Measured value	% Difference measured-expected
L. henryi	*L. candidum*	69.5	79.5	-12.6%	74.5	74.0	0.7%
L. longiflorum	*L. bulbiferum*	77.0	82.0	- 6.1%	79.5	79.9	-0.5%
L. longiflorum	*L. dauricum*	77.0	76.4	0.8%	76.7	76.3	0.5%
L. longiflorum	*L. henryi*	77.0	69.5	10.8%	73.3	71.7	2.2%
L. longiflorum	*L. canadense*	77.0	93.1	-17.3%	85.1	84.2	1.0%
Oriental hybrid	Asiatic hybrid	75.4	79.5	- 5.2%	77.5	76.5	1.2%
Oriental hybrid	Asiatic hybrid	75.4	79.5	- 5.2%	77.5	77.2	0.3%
Oriental hybrid	Asiatic hybrid	75.4	79.5	- 5.2%	77.5	78.0	-0.7%
L. longiflorum	Oriental hybrid	77.0	75.4	2.1%	76.2	76.5	-0.4%

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