Cytogenetic Studies in the Genus *Narcissus* L.
V. Cytology of a *N. pseudonarcissus* L. $\times$ *N. poeticus*
cultivar 'Whitewell'

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Extensive karyomorphological studies in *Narcissus* (see for example Nagao 1933, Fernandes 1968a) have revealed in good detail the pattern of chromosome evolution in the genus. Regarding an extinct form with $x=7$ as one of the ancestral groups Fernandes (1951, 1975) has envisaged origin of several species with chromosome numbers in multiples of 7. Interspecific relationships among many of these species have, however, remained obscure indicating the limitations of karyotype for such inferences. The acid test for phylogenetic kinship is provided by chromosome behaviour during hybrid meiosis. Such reports on narcissi are rather scanty (Nagao 1933, Fernandes 1950, Fernandes and de Almeida 1968, Karihaloo and Koul 1980) primarily because of difficulties posed by concealment of buds, undergoing meiosis deep inside the bulb.

Among the garden narcissi one popular group is represented by interspecific hybrids between *N. poeticus* and *N. pseudonarcissus*. The present studies on somatic complement and meiosis of variety 'Whitewell', an established *poeticus* $\times$ *pseudonarcissus* hybrid, were carried out in order to understand the genetic relationship between the two species.

Materials and methods

Bulbs of 'Whitewell' were collected from a private garden in Srinagar, Kashmir. Actively growing root tips were prefixed in 0.002M aqueous hydroxyquinoline solution for 4 hr at 10°C and fixed in 1:3 acetic alcohol for 24 hr. Squashes were prepared in 1% acetoorcein after hydrolysis in a mixture of 9 parts acetoorcein and 1 part 1N HCl. Anthers obtained by dissecting open the bulbs were fixed in 1:3 acetic alcohol and smeared in 1% acetoorcein. Terminology of Battaglia (1955) has been used for classification of somatic chromosomes.

Observations

Karyotype

All root tip cells were found to bear 21 chromosomes (Fig. 1) which can be grouped into 7 triplets; 6 long and 1 short (Fig. 2, Table 1). Four triplets: first,

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third, fourth and seventh are submetacentric; sixth is acrocentric. Chromosomes constituting the second triplet range between submetacentric – acrocentric and those of the fifth range between meta – submetacentric. The three chromosomes of the smallest triplet bear satellites on the short arms. Close scrutiny of the karyotype reveals that in six of the seven triplets one chromosome is somewhat dissimilar from the other two in terms of length and arm ratio (Table 1).

Figs. 1–2. Somatic chromosomes (Fig. 1) and idiogram (Fig. 2) of 'Whitewell'. Heteromorphic chromosomes are shown in outline. Scale 10 μ .

Male meiosis

Early stages of meiosis are not good enough for scoring chromosome associations. At metaphase I, when chromosomes are condensed, majority is involved in trivalent associations (Table 2). Of the 108 metaphase plates scored, 48 bear 7 III each. In others, trivalents range from 3 to 6 per plate. The average chromosome association per cell is 6.22 III, 0.78 II, 0.78 I.

In 8 of the 23 cells scored at anaphase I, chromosome distribution is 10:11; the extreme variation of 8:13 is obtained in 2 cells (Table 3). One or two lagging chromosomes are usually observed at this stage. Majority of these resolve into chromatids. Bridges with and without fragments are frequently noticed (Figs. 7, 8). Anaphase II is also characterized by lagging chromatids. Dicentric chromosomes stretched across the two poles of a dyad are quite frequent (Fig. 10). Ensuing pollen grains are highly variable in size.

Discussion

Botanical classification of *Narcissus* is based on foliage and flower shape. In

N. poeticus and *N. pseudonarcissus* the leaf is alike, being flat and keeled, but the shape of flowers is dissimilar. *N. pseudonarcissus* flowers are large with trumpet shaped corona exceeding perianth lobes in length. *N. poeticus* on the other hand bears relatively small flowers with saucer or small cup shaped corona. For reasons of this difference, the two have been referred to separate sections, *Pseudonarcissus* and *Narcissus* (Fernandes 1968b).

N. pseudonarcissus and *N. poeticus* bear identical chromosome complements.

Figs. 3-8. Stages in male meiosis of 'Whitewell'. 3-5, metaphase I showing 7 III (Fig. 3), 6 III, 1 II, 1 I (Fig. 4), 3 III, 4 II, 4 I (Fig. 5). 6-8, anaphase I showing 8: 13 (Fig. 6); 8: 12 and 2 chromatids (Fig. 7), 9: 12 (Fig. 8) distribution. Note bridge-fragment configurations in Figs. 7 and 8. Scale 10 μ .

Comparison of karyotypes of the two species (Fig. 11), detailed elsewhere (Karihaloo 1977), reveals that the pattern of gradation in chromosome size as well as arm ratio is quite similar. Only minor differences distinguish the two karyotypes. This difference manifests itself in the hybrid, 'Whitewell'.

Table 1. Details of the karyotype of 'Whitewell'

Chromosome number	Triplet number	Chromosome length (μ)	Relative length value*	Arm ratio	Index
1	I	12.3	5.767	2.24	0.447
2		12.0	5.626	2.53	0.395
3**		11.4	5.204	1.85	0.541
4**	II	12.0	5.626	3.19	0.313
5		11.6	5.438	2.87	0.349
6		11.6	5.438	2.52	0.398
7	III	11.3	5.298	2.32	0.430
8		11.2	5.251	2.29	0.436
9**		10.0	4.923	2.09	0.479
10**	IV	11.9	5.579	1.75	0.571
11		10.7	5.016	1.89	0.529
12		10.4	4.876	1.81	0.552
13	V	10.0	4.688	1.33	0.754
14		10.0	4.688	1.50	0.667
15**		9.6	4.500	1.53	0.655
16	VI	10.5	4.927	3.77	0.265
17		10.3	4.829	3.90	0.256
18**		9.4	4.407	3.27	0.306
19	VII	5.8	2.719	1.90	0.526
20		5.5	2.579	2.06	0.486
21		5.2	2.484	1.79	0.559

* Relative length value: length of the chromosome as percent of the total complement length.

** Chromosome which stands out markedly from other two partners of the triplet.

Table 2. Chromosome configurations at metaphase I in 'Whitewell'

S. no.	Configurations			No. cells	% of total cells	Fig. no.
	III	II	I			
1	7	—	—	48	44.44	3
2	6	1	1	45	41.67	4
3	5	2	2	8	7.41	
4	4	3	3	5	4.63	
5	3	4	4	2	1.85	5
Total	672	84	84	108	100.00	

Meiotic events of 'Whitewell' testify the genomic kinship of the two species. In 48 of the 108 metaphase I cells all chromosomes pair as trivalents. Average tri-valent frequency per cell is 6.22 which is quite close to the frequency expected in an autotriploid. In fact, the autotriploid of *N. poeticus* has 6.32 III per cell (Karihaloo

1977). Such a similarity in the trivalent frequency of two varieties, one of which is a hybrid and the other an autotriploid, suggests that there is nearly as much homology between *poeticus* and *pseudonarcissus* genomes as within different *poeticus* genomes. Obviously, genomic differentiation in the two species has not kept pace with their morphological divergence. Free gene flow between the two species resulting from perfect chromosome synapsis leads to extreme variation among hybrids. Hybrid varieties ranging in morphology all the way from one parent to another have been raised, making this group the largest and most varied among cultivated narcissi.

Table 3. Anaphase I chromosome distribution in 'Whitewell'

	Distribution pattern					
	8	8	8	9	9	10
		:	:		:	:
		1	1*		2	1*
	:	:	:	:	:	:
		12	12	12	10	10
No. cells	2	2	1	5	2	10
Fig. no.	6		7	8		

* Chromatids.

Figs. 9-10. Anaphase II of 'Whitewell'. 9, chromatid showing centromeric misdivision with the two arms going to opposite poles. 10, chromatin bridge. Scale 10 μ .

Presence of bridge-fragment configuration at meiotic anaphase of 'Whitewell' suggests the involvement of paracentric inversion heterozygosity. At anaphase I such configurations result from formation of chiasmata within the inversion loop whereas at anaphase II they are a result of more than one chiasma located both

within and outside the inversion loop. There are two possible sources of the inversion. First, that *N. pseudonarcissus* and *N. poeticus* have differentiated through a paracentric inversion and second, that the inversion has secondary origin in the hybrid variety. Involvement of inversion has been suggested in the differentiation of some other species of the genus such as *N. concolor* and *N. bulbocodium* (Fernandes and de Almeida 1968).

Fig. 11. Graph showing chromosome length and arm ratio of basikaryotypes of *N. poeticus* and *N. pseudonarcissus*.

Summary

N. pseudonarcissus × *N. poeticus* hybrid variety 'Whitewell' is triploid with 21 chromosomes. Except for minor differences in chromosome length and arm ratio, the three genomes of the variety are quite similar. Male meiosis reveals preponderance of trivalents; the average association per cell at metaphase I being 6.22 III, 0.78 II, 0.78 I. Close genomic homology of the two species in the face of morphological divergence suggests that their genomic differentiation has not kept pace with phenotypic differentiation. Free gene exchange between the two species accounts for extreme variation in their garden hybrids. Bridge-fragment configurations suggest the involvement of paracentric inversion.

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