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Cytogenetic Study of an Interspecific Cross of *Nicotiana debneyi* × *N. umbratica*

T. Gangadevi and P. N. Rao
Department of Botany, Andhra University, Waltair (India)

K. V. Satyanarayana
Central Tobacco Research Institute, Rajahmundry (India)

Summary. Interspecific F_1 hybrids of *Nicotiana debneyi* Domin (2n = 48) and *N. umbratica* Burbidge (2n = 46), both belonging to the section Suaveolentes, showed a high degree of meiotic chromosome pairing. Two of the five F_2 plants obtained exhibited chromosome mosaicism. The first colchiploid generation (C_1) had the expected chromosome number of 2n = 94 while C_2 showed 2n = 88, a loss of three pairs of chromosomes. This same chromosome number continued in further colchiploid generations, followed up to C_5, except for a few plants in C_3 which showed chromosome mosaicism. The F_1 phenotype was stable through C_1 to C_5 and fertility was normal in colchiploids through all generations in spite of the loss of three pairs of chromosomes and chromosome mosaicism. This stability and fertility apparently reflect the tolerance of the genomes to the genetic adjustment of chromosome complements which is believed to be associated with the originally polyploid nature of the parental species and the chromosome doubling brought about in the amphidiploids.

Key words: Cytogenetics – Interspecific hybrid – *Nicotiana debneyi* – *N. umbratica* – Colchiploids

Introduction

Section Suaveolentes of the genus *Nicotiana* comprises twenty species, five of which were described by Burbidge (1960). Among the five, *N. umbratica* is in interesting for its appearance and chromosome number (2n = 46). Unlike other species of Suaveolentes (except for *N. benthamiana*) it has petiolate leaves devoid of wings. Further, the plants are weak-stemmed with spreading branches and cordate leaves. According to Burbidge, *N. umbratica* and *N. cavicola* (2n = 46) "fill the gap in the series of from 16 to 24 pairs with an exception of 17 pairs" in the section. In the present investigation, morphological and cytological studies made on F_1 intrasectional hybrids of *N. debneyi* Domin (2n = 48) × *N. umbratica* B. (2n = 46), their F_2 progenies and amphidiploids from C_1 through C_5 generations, are reported. While this work was in progress, a publication on the same hybrid, along with a few others, appeared (Williams 1975) but the information given pertains to only meotic chromosome pairing in F_1 hybrids.

Materials and Methods

Crosses were made reciprocally, but were successful only when *N. umbratica* was used as male parent. Fifty F_1 hybrids along with five plants each of the parental species were grown in pots kept in greenhouse. Apical buds of two month old F_1 seedlings were treated with a 0.5% aqueous solution of colchicine for eight hours per day on three successive days for doubling the chromosome number. Flower buds were fixed in Carnoy's fluid and meiosis in F_1 hybrids and in the C_1 generation was studied from PMCs squashed in propionocarmine. Modified carbol-Fuchsin (Miller et al. 1971) was used in subsequent generations since this gave better preparations, longer lasting, with clear cytoplasmic backgrounds and chromosomes stained magenta leaving the nucleolus unstained. Pollen fertility was noted as stainability of pollen in 1% acetoorcein. Cytological data gathered from different plants were pooled in F_1 and C_2. For arriving at the number of bivalents, a trivalent was evaluated as one bivalent, and a quadrivalent or pentavalent as two bivalents, following Goodspeed (1954). In colchiploids (from C_2 to C_5), 10 to 30 plants were examined in each progeny to establish the chromosome numbers.

Results

F_1 Hybrids

Germination of the crossed seed was good. All the F_1 plants were morphologically alike. They resembled *N. umbratica* (Figs. 1, 2) in plant height, leaf-size and

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shape (petiolate and cordate) and petal shape (acute tip). Plant appearance was similar to that of *N. debneyi* in having a rosette of leaves, with cauline leaves appearing after the emergence of inflorescence. Pigmented bases of stems, nodes and dorsal faces of petals and inclined position of corolla limbs were also as in *N. debneyi.* Size of the flower was intermediate (Fig. 2). The disposition of stamens characteristic of Suaveolentes is seen in *N. umbratica* where four sub-sessile stamens reach the throat of the corolla and show subdidynamy. The fifth one, reaching only half the length of the corolla tube, has partially free filament. *N. debneyi* is an exception to this, in having all the five filaments partially free. This was realised in the hybrid.

All thirty plants examined had the expected chromosome number of 2n=47 (Fig. 3). Meiotic pairing behaviour (Table 1) approximated "Drosera Scheme" (see Goodspeed 1954). Mean bivalent frequency was 21.82. This is in agreement with the mean univalent frequency of 2.48 reported by Williams (1975). Multivalent formation was prevalent as seen in 66.26% of the PMCs analysed, with a mean of 0.98 per cell (Table 1). Post MI divisions were irregular with laggards and bridges occurring in 80.6% of the PMCs analysed, leading to the formation of tetrads with micronuclei and polyads. Pollen fertility was low (1–10%). However, a few capsules, 25 from 30 F_1 plants, were formed, each with a very low seed-set of 8 to 30 seeds (false and real).

Table 1. Chromosome associations at diakinesis and MI in F_1 hybrids (2n=47) of *N. debneyi* × *N. umbratica*

Chromosome associations					No. of cells
V	IV	III	II	I	
			23	1	9
			22	3	10
		1	22		1
			21	5	6
		1	21	2	2
	1		21	1	8
1			21		1
			20	7	3
	1	1	20		1
	1		20	3	17
		2	20	1	4
		1	20	4	1
	1		19	5	2
		2	19	3	3
	1	1	19	2	3
		1	19	6	2
	2		18	3	1
	1	2	18	1	1
	1		18	7	1
1		1	18	3	1
	2	1	18		1
		2	18	5	1
		2	17	7	1
	2	2	16	1	1
	2		15	9	1
	1	3	14	6	1
Total					83

Table 2. Intraplant variation of chromosome numbers in F_2 generation of *N. debneyi* × *N. umbratica*

Number of chromosomes	No. of cells	
	Plant no. 1	Plant no. 4
44		1
45	3	1
46	1	5
47	64	18
48	1	2
49	3	8
51		1
Total number of cells	72	36

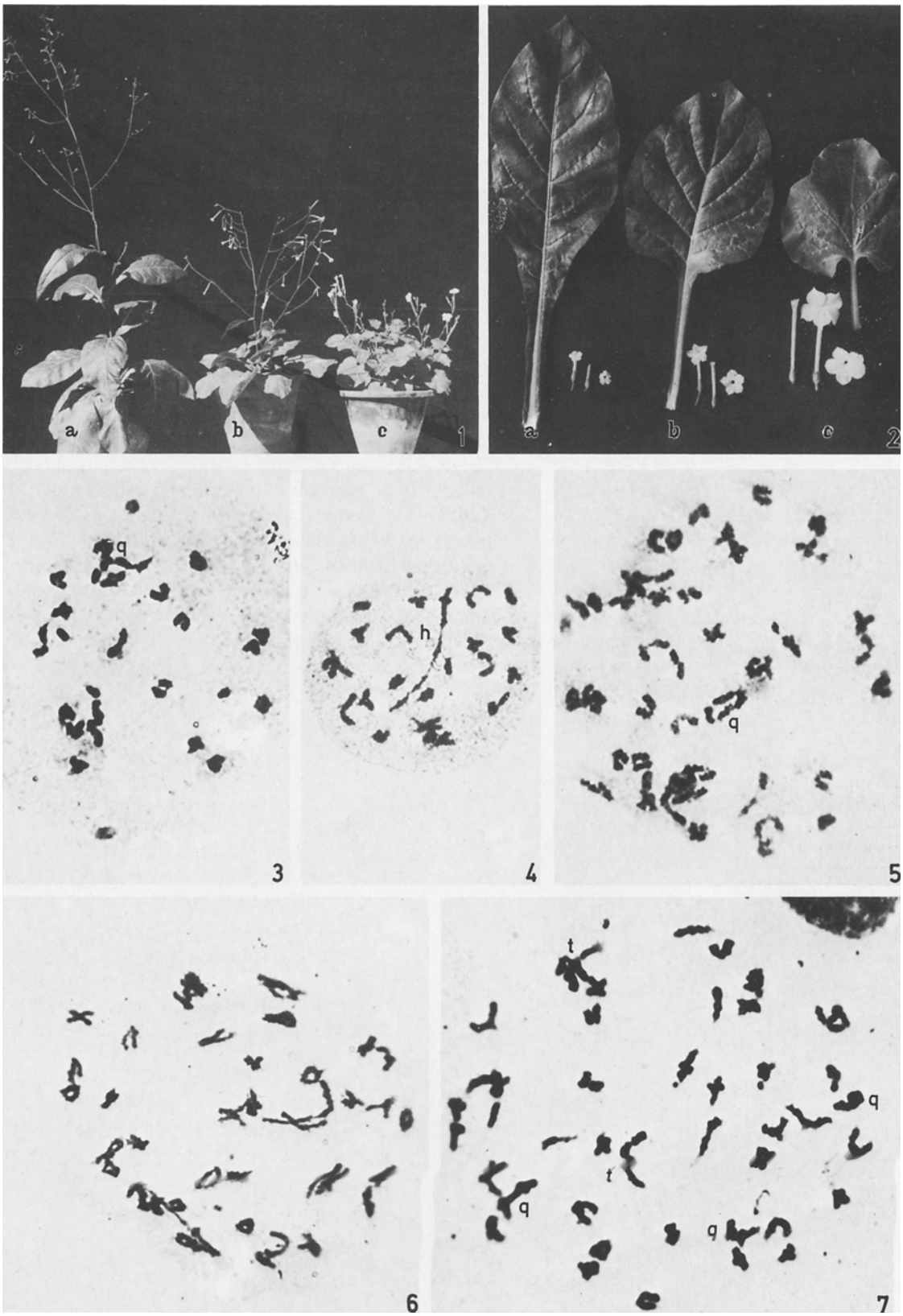
F_2 generation

The entire seed collected from F_1 plants was sown. Only five germinated and all reached maturity. Plants were morphologically similar to F_1 hybrids except for a few minor differences, like lighter green and thinner leaves and slightly larger flowers.

Cytological data were obtained from two plants only. Chromosome numbers varied even within the same anther (Fig. 8, Table 2). The most frequent configuration, 23 bivalents and 1 univalent, is suggestive of the probable presence of 24 chromosomes of *N. debneyi* and 23 of *N. umbratica,* a chromosome constitution same as that of the F_1. Multivalents were frequently formed (Fig. 4), occurring in 51.39% of the PMCs in plant No. 1 with a mean of 0.65 per cell and 41.67% of the PMCs in plant No. 4 with a mean of 0.47 per cell. Plant No. 4 showed a pollen fertility of about 40% while others showed a meagre fertility. There was false capsule formation on this plant and ten capsules with as low as 7 to 32 seeds from each were collected. The seeds however failed to germinate.

Colchiploids

The colchiploids and their chromosome numbers studied in different generations are summarised in Fig. 8. Out of 20 F_1 plants treated with colchicine, four were doubled sectorially and all these had the expected chromosome number of 2n=94. A limited number of PMCs available for study showed 47 bivalents or one



Figs. 1–7. Morphology and cytology of parents, interspecific hybrids and colchiploids of *N. debneyi* × *N. umbratica:* **1** Plants of (a) *N. debneyi,* (b) F_1 hybrid and (c) *N. umbratica;* **2** Leaf, flower, flower bud and corolla-limb-spread in (a) *N. debneyi,* (b) F_1 hybrid and (c) *N. umbratica;* **3** Diakinesis in F_1 hybrid: 1 IV(q), 20II, 3I (×750); **4** Diakinesis in F_2 generation: 1 VI(h), 21II, 1I (×750); **5** Diakinesis in C_1 generation: 1 IV(q), 45II (× 750); **6** Diakinesis in C_2 generation: 44II (× 750); **7** Diakinesis in C_3 generation: 3 IV(q), 2III(t), 36II, 4I (× 750). Note: h = hexavalent, q = quadrivalent, t = trivalent

Table 3. Chromosome associations at diakinesis and MI, in C_2 generation (2n = 88) of *N. debneyi – umbratica*

Chromosome associations						No. of cells
VI	V	IV	III	II	I	
				44		87
				43	2	25
		1		42		22
			1	42	1	2
				42	4	1
		1		41	2	22
		1	1	40	1	2
		1		40	4	1
	1			40	3	1
		2		40		2
1		1		39		1
	1	1		39	1	1
		2		39	2	1
1		1		38	2	2
		3		38		1
		2	1	38	1	1
		1	1	38	5	2
1		1	1	37	1	1
		3		37	2	1
		4		36		1
Total						177

Table 4. Intraplant variation of chromosomes in C_3 generation of *N. debneyi – umbratica*

Number of chromosomes	Number of cells			
	Plant no. 1	Plant no. 2	Plant no. 5	Plant no. 9
78			2	1
79			1	
82				4
84		3	8	1
85			1	
86		1	9	1
87	2		3	
88	2	18	9	4
89			7	
90	12	6	11	3
91	2		6	2
92	15	10	16	2
93	12	1	4	1
94	5	5	2	2
95			2	
96	4	4	3	2
97	3		1	
98	4	1	1	
100	2			
102	1			
Total number of cells	64	49	86	23

quadrivalent and 45 bivalents (Fig. 5). Doubled sectors gave capsules with full seed-set and germination of the seed was good.

Pollen mother cells from C_2 plants revealed only 2n = 88 chromosomes (Fig. 6) showing a loss of three pairs of chromosomes. The phenotype was similar to that of the F_1 hybrids. Multivalents ranging from trivalents to hexavalents were found in 36.16% of the PMCs with a mean of 0.49 per cell (Table 3). Among these, quadrivalents were more frequent, occurring in 81.4% of the cases. In post MI stages laggards and bridges were found in 48.1% of the PMCs. Fertility was normal in spite of the occurrence of univalents, multivalents and meiotic irregularities.

A few plants in C_3 showed chromosome numerical mosaicism within the same anther (Fig. 7, Table 4). These plants did not differ from others in morphology except for the presence of heteromorphic pollen. Pollen fertility and seed-set were normal. Progenies of these plants as well as those with no traces of chromosome mosaicism, studied up to C_5 generation, remained stable at 2n = 88. In the hybrids and amphiploids, differential condensation of chromosomes was observed occasionally (eg: one of the trivalents in Fig. 7).

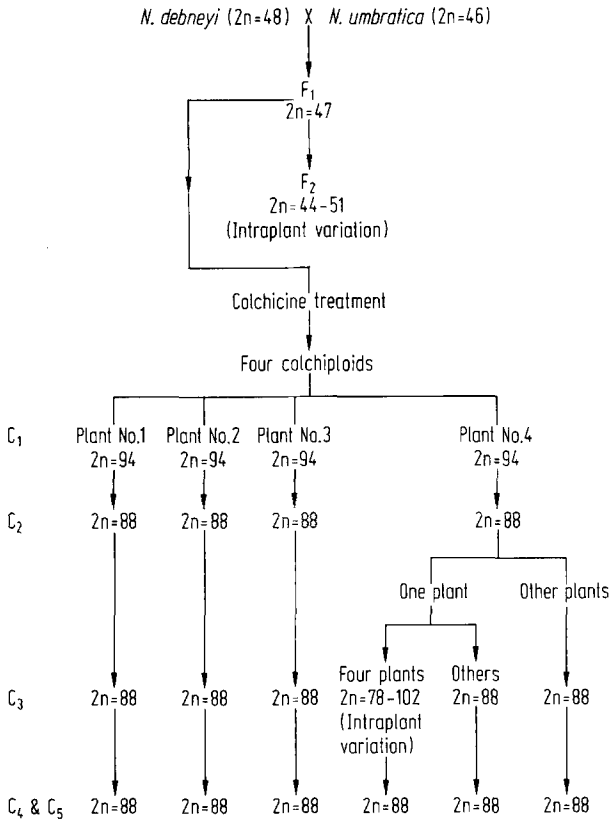


Fig. 8. Chromosome numbers in F_1, F_2 and C_2 through C_5 generations of *N. debneyi* × *N. umbratica*

Discussion

The high degree of chromosome pairing in the F_1 indicates a close homology of the parental genomes. Frequent multivalent formation indicates the presence of duplications of whole chromosomes or chromosome segments, established during the evolution of one or both genomes of the parental species or of homologies within the parental subgenomes (Goodspeed 1954). F_1 hybrid sterility is believed to be predominantly due to chromosomal causes.

Occurrence of univalents, even at diakinesis in colchiploids, might be due to the replacement of autosyndesis by allosyndesis (Goodspeed 1954) or due to the mechanics involved at higher polyploidy.

Chromosome mosaicism could be due to hybridity, colchicine effect, polyploidisation, genotype or a combination of two or more of these (Yang 1965). However, in the present study, it could not be due to hybridity since it was not found in F_1 hybrids. It might not be due to the other three factors since the bizarre behaviour of the PMCs was limited to a few plants only in one generation.

Phenotype was stabilised at the F_1 hybrid level. The unchanged phenotype of colchiploids even after a loss of three pairs of chromosomes might be due to the high level of polyploidy, the parents themselves having intra- and intergenomic homologies. Unaffected fertility of colchiploids in spite of loss of chromosomes and meiotic irregularities could be due to the functioning of gametes with an apparently balanced complement of 44 chromosomes. *N. hesperis* ($2n=42$) maintained its identity even after a spontaneous loss of five pairs of chromosomes (unpublished). In the present study, it is probable that the same three pairs were missing from all the four C_1 plants since all the progenies showed the same chromosome number and phenotype.

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Mrs. T. Gangadevi
Dr. Panuganti N. Rao
Department of Botany
Andhra University
Waltair-530 003 (India)

Dr. K. V. Satyanarayana
Division of Genetics and Cytogenetics
Central Tobacco Research Institute
Rajahmundry-533 105 (India)