Theor Appl Genet (1985) 71:545–549

In vitro hybridization in an incompatible cross *Nicotiana glutinosa* × *Nicotiana megalosiphon*

U. Subhashini, T. Venkateswarlu, K. Anjani and G. S. R. Prasad
Central Tobacco Research Institute, Rajahmundry, A.P., India

Accepted July 25, 1985
Communicated by Hu Han

Summary. Using conventional techniques interspecific hybridization between *N. glutinosa* (2n = 24) and *N. megalosiphon* (2n = 40) results in failure while the reciprocal is successful. Attempts were made here to obtain an F_1 hybrid of *N. glutinosa* × *N. megalosiphon* by embryo rescue. These reciprocal hybrids resembled each other in their phenotypic and chromosomal behaviour, i.e. there is broad spectrum of phenotypic variation coupled to chromosomal range from 2n–28 to 32. This may be due to the functional disharmony between chromosome sets of two unrelated species resulting in the elimination of chromosomes.

Key words: In vitro hybridization – Phenotypic variation – Chromosomal range – Functional disharmony

Introduction

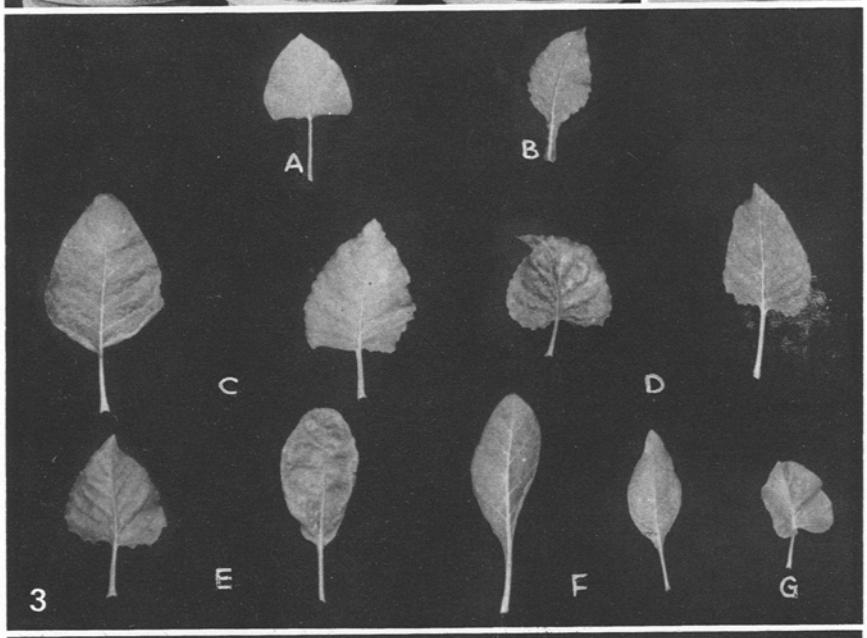
Interspecific hybridization by conventional techniques has been attempted successfully in the genus *Nicotiana* by several workers (Satyanarayana and Subhashini 1964; Subhashini 1976). *N. glutinosa* is a species from the section tomentosiformis, sub-genus *tabacum,* while *N. megalosiphon,* a species from the section suaveolentes falls under the sub-genus *petunioides.* Elimination of whole chromosomes and phenotypic variation has been observed in the F_1 population of the inter subgeneric cross *N. megalosiphon* × *N. glutinosa* (Satyanarayana and Subhashini 1964). To see whether such behaviour also occurred in the reciprocal, crosses were attempted but failed. To circumvent sexual incompatibility, in vitro hybridization was tried in *Arachis* (Bajaj et al. 1982), *Vigna* (Gosal and Bajaj 1983), *Gossypium* (Gill and Bajaj 1984), and in *Brassica* (Mahapatra and Bajaj 1984). In the present article, investigations on embryo rescue were conducted and cytological observations in the interspecific hybrid, i.e. *N. glutinosa* × *N. megalosiphon,* are reported.

Materials and methods

The flower buds of *N. glutinosa* (Fig. 1 A) were emasculated one day before anthesis and cross pollinated with pollen from *N. megalosiphon* (Fig. 1 B) on the following day. Immature ovules at the critical stage, 7 days after pollination, were aseptically excised and cultured on Nitsch (1969) medium. Developing ovules from 22 ovaries were inoculated. All manipulations were conducted under sterile conditions in a laminar flow chamber. For callusing, Murashige and Skoog's (1962) medium (M & S) supplimented with 6 mg of NAA and 2 mg of BAP/L was used. Shooting was induced on M & S supplimented with 2 mg of IAA and 2 mg of kinetin/L and rooting was done on M & S with 3–5 mg of IAA and 2 mg kinetin/L. Meiotic studies were made as per Swaminathan et al. (1954).

Results

The developing capsules of *N. glutinosa* × *N. megalosiphon* will wither away from the parent plant on the 7th day after pollination. Developing ovules from 22 capsules were inoculated at this critical stage. The embryo is at the globular stage of development and is microscopic. Only one seed germinated out of these ovules. Sprouting was observed 15 days after inoculation. As only one hybrid plant (Fig. 2) was obtained, explants from hypocotyl and cotyledens were kept for callusing and plants were regenerated. Cytological studies of callus confirmed that it was a hybrid.



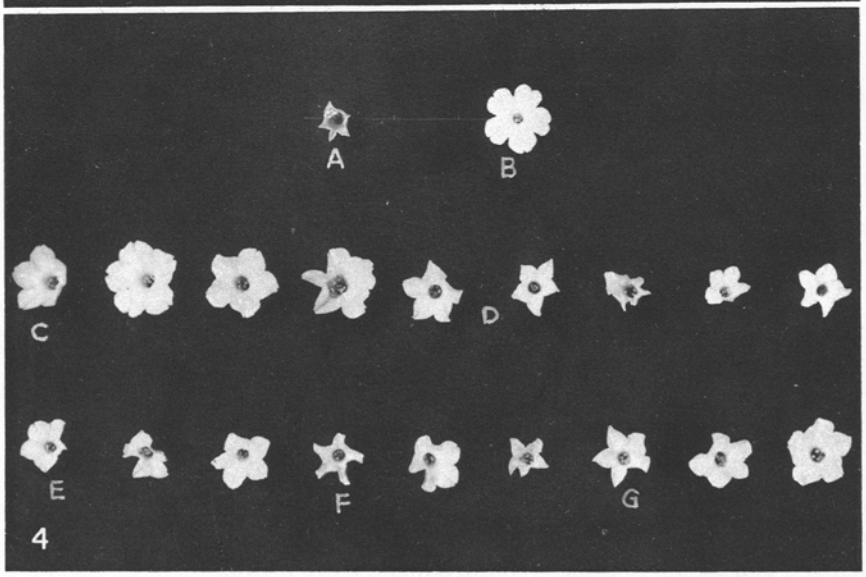


Fig. 1. *A N. glutinosa, B N. megalosiphon, C* F_1 hybrid

Fig. 2. Only plantlet by in vitro hybridization in *N. glutinosa* × *N. megalosiphon*

Fig. 3. Leaves of *A N. glutinosa, B N. megalosiphon* and F_1 hybrid, *C* 2n = 32, *D* 2n = 31, *E* 2n = 30, *F* 2n = 29, *G* 2n = 28

Fig. 4. Limb spread in flowers. *A N. glutinosa, B N. megalosiphon, C* 2n = 32, *D* 2n = 31, *E* 2n = 30, *F* 2n = 29, *G* 2n = 28

Table 1. Phenotypic characters of *N. glutinosa, N. megalosiphon* and the F_1 hybrid

	N. glutinosa	*N. megalosiphon*	P_8	P_{14}	P_9	P_{10}	P_4	P_{13}	P_{21}
Chromosome no. (2n)	24	40	28	28	30	30	31	31	32
Plant height (cm) at FB (cms)	68	80	13	19	19	16	19	12	18
No. of leaves/plant	18	15	9	15	12	10	13	13	13
Leaf shape	Cordate	Lanceolate elliptic	Oblong	Cordate ovate	Ovate oblong	Cordate	Ovate	Ovate oblong	Oblong
Petiole	Clean petiole	Winged petiole	petiolate	Winged petiole	Winged petiole	petiolate	petiolate	petiolate	Winged petiole
Leaf size l	12.5	10.0	9.6	7.3	8.3	7.6	9.6	8.2	8.6
(cms) b	8.0	6.2	6.2	5.6	5.3	5.0	6.6	6.2	6.5
Leaf margin	Entire	Wavy	Entire	undulate	Entire	undulate	undulate	undulate	undulate
Leaf tip	Acute	Acute	Obtuse	Obtuse	Acute	Acute	Obtuse	Obtuse	Acute
Flower colour	Reddish pink	White	Light pink	Pink	Very light pink	Very light pink	Pink	Almost colour-less	Pink
Corolla size									
Tube length (cm)	2.9	8.5	6.9	5.8	5.8	5.1	5.8	5.8	6.6
Limb spread	1.9	2.5	3.0	3.0	2.8	2.8	2.4	2.4	2.8
PF	98%	98%	1%	Nil	1%	1%	Nil	Nil	Nil

F_1 hybrids

About 50 healthy plants were raised. The phenotypic characters of the parents and F_1 are presented in Table 1. Lack of uniformity was observed even when the plants were young. There was variation in morphological characters (Table 1, Figs. 3 and 4). The hybrid plants resembled the *N. glutinosa* parent at the time of flowering while the flowers resembled *N. megalosiphon* in shape and length. Stamens were normal and heterostyly was observed. There was no capsule setting upon selfing.

The meiotic chromosome number of 26 F_1 plants was studied. While the expected chromosome number of the hybrid was 2n = 32, 18 plants showed a range in chromosomal complement of 2n = 28 to 31 (Fig. 7). The remainder of the plants had a full chromosome complement. One plant showed 2n = 32 (Fig. 6) and a chimeral branch from this plant had 2n = 29. Such chromosomal chimeras were also observed in aneuploid plants. At diakinesis, an association of four chromosomes to form chains was noticed in few plants. In almost all cells only one rod bivalent was observed at diakinesis, the remainder were univalents (Fig. 6). There was no clear-cut metaphase plate formation but there was meta-anaphase distribution, i.e. some of the univalents showed anaphase 1 movement while the other univalents were at M1. Meiotic irregularities such as unequal segregation at A_1, i.e. 12–19 (Fig. 7), 12 to one side and the rest forming two unequal groups (Fig. 8) were manifested. At A_{11}, micronucleus and polyad formation were also observed.

Colchiploids

Six plants were restored to fertility by colchicine treatment (0.5%/8 h/3 days). These plants grew vigourously (Fig. 5) with phenotypic differences among themselves. There was a range in pollen fertility from 55 to 90% and capsule setting was satisfactory. Selfed capsules were collected.

Meiotic studies from the six plants showed aneuploids as well as amphiploids (2n = 56; 2n = 64 (30″ + 4′)). Metaphase plate formation was observed, but the univalents separated early. Irregularities at A_1 and A_{11} resulted in aneuploid gametes.

Discussion

The sexually incompatible cross between the species *N. glutinosa* × *N. megalosiphon* is successful by in vitro

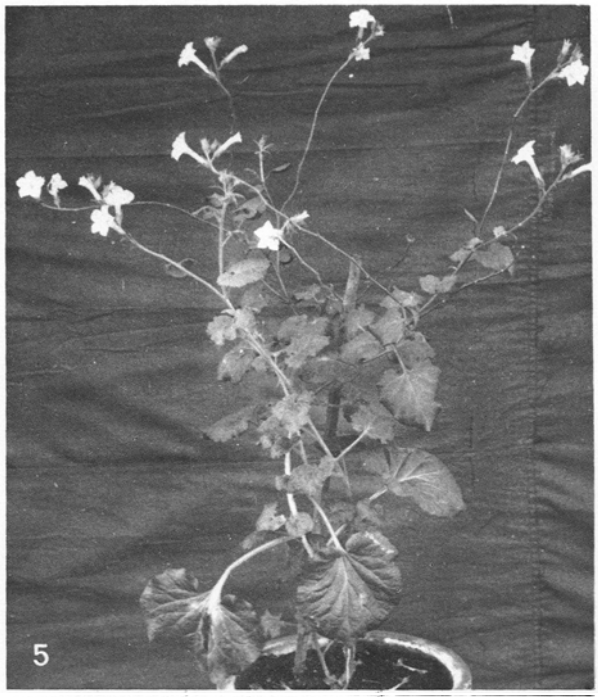
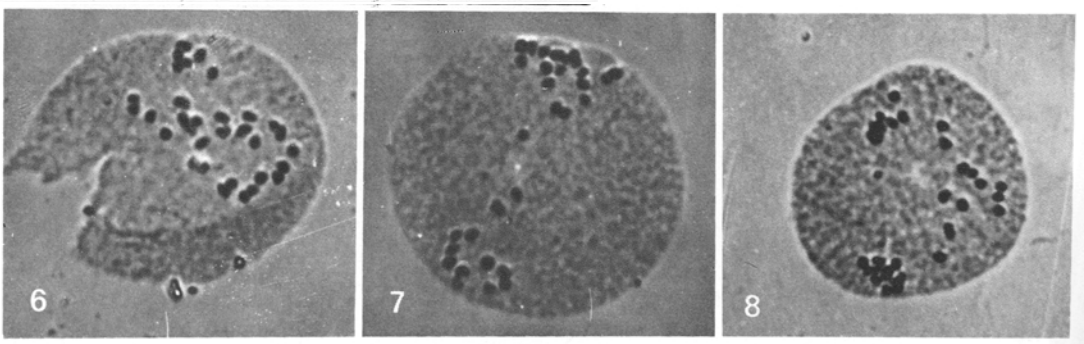


Fig. 5. Fertile hybrid of *N. glutinosa – megalosiphon*

Fig. 6. Diakinesis in F_1 *N. glutinosa* × *N. megalosiphon* $1^{II}+30^{I}$

Fig. 7. Unequal segregation (2n = 31) at A_1 12–19

Fig. 8. Abnormal A_1 12 chromosomes to one side (*N. glutinosa?*) and the rest (*N. megalosiphon?*) to poles



culture of fertilized ovules. Each capsule of *N. glutinosa* contains more than 1,000 seeds. When 22 developing capsules were inoculated, only one seed showed germination. Plants regenerated from this hybrid exhibited variation (Table 1). There was random elimination of chromosomes from the full complement of 2n = 32. While plants with the same chromosome number were not identical, plants with varying chromosomal complements also varied from each other. There was random elimination of chromosomes from the full complement. Such behaviour was also observed in the reciprocal hybrid obtained (Satyanarayana and Subhashini 1964) by conventional methods. The colchiploids also differed in ploidy level. Chromosomes may be eliminated either before or after colchicine treatment – both give fertile polyploids.

The elimination of chromosomes in the F_1 population is of much interest. Is it due to somaclonal variation – as all these plants are regenerated from callus – or does it result from the functional disharmony between chromosome sets of two unrelated species, resulting in the elimination of chromosomes. The only F_1 plant may be composed of cells with different genotypes occurring in a random mosaic fashion, since the two species involved in the cross are of polyploid origin. Genetic mosaicism can be tolerated because of the genetic buffering between the multiple chromosome sets.

Instances of chromosomal instability in the species hybrids of *Nicotiana* are many. These include interplant and intraplant variability in chromosome number and variation in morphology and habit (Satyanarayana and Subhashini 1964; Subhashini 1976). In all of the above, one of the parent species was a member of the section suaveolentes, which has its origin from alatae. The members of section suaveolentes carry in their genetic makeup the basic instability factors derived from the alatoid members. As a consequence, hybrids arising

from species with members of section suaveolentes as one of the parents usually result in instability as evidenced in the present work and also in the reciprocal cross.

Acknowledgements. The authors wish to thank Dr. N. C. Gopalachari, Director, for his constant encouragement, Sri K. Appa Rao for his helpful suggestions and Sri N. Veerraju for his help with the photography.

References

Bajaj YPS, Kumar P, Singh MM, Labana KS (1982) Interspecific hybridization in the genus *Arachis* through embryo culture. Euphytica 31:365–370

Gill MS, Bajaj YPS (1984) Interspecific hybridization in the genus *Gossypium* through embryo culture. Euphytica 33: 305–311

Gosal SS, Bajaj YPS (1983) Interspecific hybridization between *Vigna mungo* and *Vigna radiata* through embryo culture. Euphytica 32:129–137

Mahapatra D, Bajaj YPS (1984) In vitro hybridization in an incompatible cross – *Brassica juncea* and *Brassica hirta.* Curr Sci 53:489–490

Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol Plant 15:473–497

Nitsch JP (1969) Experimental androgenesis in *Nicotiana.* Phytomorphology 19:389–404

Satyanarayana KV, Subhashini L (1964) Interspecific hybridization and aneuploidy in the genus *Nicotiana.* Indian J Genet 24:264–271

Subhashini U (1976) Allopolyploidy and aneuploidy in the genus *Nicotiana.* Cytologia 41:645–651

Swaminathan MS, Magoon ML, Mehra KL (1954) A simple propionocarmine PMC smear method for plants with small chromosomes. Indian J Genet 14:87–88