Allopolyploidy and Aneuploidy in the Genus *Nicotiana*

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Interspecific hybridization between the species *Nicotiana umbratica* and *Nicotiana glutinosa* followed by colchiploidy resulted in allopolyploid ($2n=70$) aneuploids ($2n=66, 68$) and hyperploid ($2n=72$) of the above hybrid. These polyploids are stable in morphological and cytological features. However abnormalities such as apical bud suppression and heteromorphic pollen were observed from C_3 generation irrespective of its chromosomal complement. The above stable polyploids supports Goodspeed's postulate (1954).

Material and methods

Colchicine treatment was successful in the F_1 hybrid of *N. umbratica* $\times$ *N. glutinosa*. Cytological screening was made as per Swaminathan *et al.* (1954).

Observations

The colchicine treated plants revealed the characteristic symptoms of the drug such as stunted growth, thickened leaves, delayed flowering, dark pink flowers with good pollen fertility and capsule setting. General morphological characters were recorded from 40 plants in each generation (C_2 to C_5) and tabulated in Table 1. The progenies in general were uniform for morphological characters (Figs. 1 and 2) and more vigorous in growth. Pollen fertility and capsule setting were good. In the C_3 generation a few abnormalities such as apical bud suppression and heteromorphic pollen were recorded (Subhashini 1974).

Cytological screening of the colchiploid plants revealed a complement of $2n=67, 68, 70$. Detail studies were made in all the progenies of the colchiploids and here only the progeny of $2n=67$ is given. At diakinesis (Fig. 3) the configuration was $33^{II}+1^I$. There was a tendency for early separation of the rod bivalents at diakinesis itself. Post metaphase I irregularities of the aneuploid 67 are tabulated below.

Chromosome number	Configuration at diakinesis	Configuration at metaphase I	Anaphase I	Anaphase II	Pollen fertility
$2n=67$	$33^{II}+1^I$	$32^{II}+3^I$	Delayed and unequal distribution and laggards	Irregular with laggards	90%

In spite of the above irregularities the pollen fertility was as high as 90%. Germination of the pollen grains on the stigmatic surface as well as on the artificial

Table 1. Morphological description of *N. umbratica*, *N. glutinosa* and the colchipsoid

Parent or hybrid character	<i>N. umbratica</i>	<i>N. glutinosa</i>	Colchiploids	C ₃ generation
Chromosome number 2n= General appearance	46	24	67-70 Mostly intermediate in some respects resembled <i>N. glutinosa</i>	As the C ₁ plants
Plant height at flowering	about 10.5 cm	about 32.0 cm	about 38.0 cm	about 30.0 cm
Stem	Main axis suppressed profuse branches from the axils. Color of the branches light green	Main axis thick round, elongated color dark green with stiff axillary branches	As in <i>N. glutinosa</i> with violet tinge on the main axis	As the C ₁
Leaf color texture margin	light green herbaceous undulate	dark green coriaceous entire and leaf tip twisted towards main axis	light green coriaceous undulate and leaf tip as in <i>N. glutinosa</i>	light green coriaceous As the C ₁
Number of days for flowering	90	65	68	83
Flower color	greenish white	yellowish light red	greenish light pink	As the C ₁
tube	tube throat constricted on both sides	bell shaped throat	bloated throat	
length	about 5.20 cm	2.9 cm	Range 3.3 to 4.3cm	As the C ₁
spread	about 2.20 cm	1.9 cm	Range 2.0 to 2.9cm	
stamens	subdidynamous and epipetalous 98%	subdidynamous and free 98%	half epipetalous 80-90% and subdidynamous	95% heteromorphic
PF				
Gynoecium	normal	normal	normal	Heterostyle.
Number of seeds/capsule	More than 300	More than 1000	About 115	115±10

Figs. 1-2. 1, *Nicotiana umbratica-glutinosa* (2n=69). 2, *Nicotiana umbratica-glutinosa* (2n=68).

media was good.

There was a wide spectrum of variation at the C_2 level from the aneuploid $2n=67$ (Pedigree Chart). The different chromosomal types encountered were 62, 65, 68, 69 and 70 of which the progenies of 62, 65 and 70 are given in detail.

General trend of the different chromosomal complements in the progenies

The amphiploid 70 had a disturbed cytological behaviour in C_2 generation resulting in types 72, 70 and 68 in C_3 which however bred true in C_4 and C_5 . One of the amphiploids which had a chromosomal complement of $2n=70$ showed good pollen fertility but there was no capsule setting (Pedigree chart). The aneuploid 65 exhibited irregular meiosis and segregated to 66 and 65 of which the 66 bred true. The aneuploid 62 showed irregular meiosis at C_3 level and in C_4 only the type 68 was isolated which bred true.

Cytology

$2n=70$. A total number of 22 plants screened cytologically had a complement of $2n=70$ (Fig. 4) as expected for the amphiploid *N. umbratica glutinosa*. Meiosis was irregular at diakinesis and M_1 due to the presence of univalents. The univalents showed random movement towards the poles even at M_1 stage without congregating on to the equatorial plate. Some of the irregularities observed at A_1 are given below.

Meiotic irregularities in the chromosomal type $2n=70$

Total number of cells analysed	A_1 regular distribution		A_1 irregular distribution				Laggards	
	35-35	% of cells	39-31	%	37-33	%	Cells	%
48	32	67	10	21	2	4	4	8

The above irregularities resulted in unequal distribution of chromosomes (Fig. 5) in the daughter cells. Since the A_{11} was normal the spore quartets that resulted always were suspected to have hyperploid and deficient microspores. In spite of these irregularities the pollen fertility was normal, indicating that the sporads with any complement ranging from 31 to 39 were functional. One of the amphiploid of C_2 showed regular meiosis having sporads with a complement of 35 and good pollen fertility, but there was no capsule setting. Cytological screening was made from the progeny of the amphiploid which exhibited meiotic irregularities and good fertility. It is quite interesting to note the progeny showed deficient allopolyploid $2n=68$ (Fig. 6, 2 plants), the amphiploid $2n=70$ (7 plants) and hyperploid with a complement of $2n=72$ (1 plant). At diakinesis and M_1 perfect bivalent formation (34^{II} , 35^{II} and 36^{II}) was recorded in the above types. At A_1 and A_{11} the separation was normal. The parent plant gave functional sporads with a complement ranging from 31 to 39 chromosomes. Random fusion of the gametes with 37-31, 35-35, and 39-33 might have resulted in plants with a complement of 68, 70 and 72. However the other types expected from random fusion of gametes were not encountered. The above three types bred true in C_3 generation.

$2n=65$. Two plants showed a chromosomal complement of $2n=65$ both of which differed in its meiotic behaviour. One plant showed complete pairing with one univalent $32^{II}+1^I$ while the other had $30^{II}+5^I$ (Fig. 7). The chromosomal

Figs. 3-7. 3, colchiploid at diakinesis $33^{II}+1^I$. 4, diakinesis showing 35^{II} . 5, unequal segregation at A_1 39-31 in the amphiploid $2n=70$. 6, diakinesis 34^{II} . 7, diakinesis $30^{II}+5^I$.

segregation at A_1 was 33-32 in most of the cells resulting in sporads with a complement of 33 to 32. Progeny studies in C_3 were made from one of the selfed plants. In the C_3 generation, two types were encountered *i.e.* $2n=66$ and 65. The aneuploid 66 bred true in C_4 and C_5 . The type 65 showed segregation in C_4 and C_5 of

which only the aneuploid 66 and 65 were identified.

$2n=62$. Only one sample in C_2 had a chromosomal complement of $2n=62$. The configuration at M_1 was $27^{II}+8^I$. The irregularities noted at A_1 were delayed separation, the unusual movement of the bivalents without separation to the poles resulting in unequal segregation of chromosomes and laggards at A_1 and A_{11} . In spite of these irregularities the pollen fertility was good. In the C_3 progeny only the aneuploid 68 was isolated and the parental complement was not encountered. The aneuploid $2n=68$ exhibited regular meiosis. This type bred true in C_4 and C_5 .

To sumup, the progeny studies from the aneuploid 67 of the C_2 generation gave two stable aneuploids, one stable hyperploid besides the amphiploid with good fertility.

Discussion

Interspecific hybridization followed by colchipoideity resulted in unstable polyploids. Instances of cytogenetic instability in the species hybrids of *Nicotiana* are many. These include variation in morphology and habit during plant development (Kehr and Smith 1952), interplant and intraplant variability in chromosome number (K. V. Krishnamurthy and K. V. Satyanarayana 1962, K. V. Satyanarayana and L. Subhashini 1964). In all the above instances one of the parent species was either a member of the section *suaveolentes* or *genuinae* which have their origin from *alatae*. *N. umbratica* which was used in different crosses, is an aneuploid member of the section *suaveolentes* which originated from a triple complex involving the section *alatae*, *noctiflorae* and *acuminatae*. The members of the section *suaveolentes* carry in their genetic makeup the basic instability factors derived from the *alatoide* members. As a consequence of this, hybrids arising from species where *suaveolentes* members are one of the parents, usually result in instability as evidenced from the works reported above.

The partial chromosomal doubling observed in this hybrid is common in the genus *Nicotiana*. Smith (1959) reported aneuploids of *Nicotiana* by colchipoideity. He was of the opinion that loss of chromosomes can take place either with or without doubling of the whole complement. Colchicine treatment in certain plants at least has been almost as constant a method for eliminating chromosomes as for doubling them.

The aneuploids in these studies showed univalents at diakinesis and M_1 as a result of precocious separation of rod bivalents. Swaminathan and Howards (1953) came across a similar case in *Solanum* hybrid which was due to separation of rod bivalents and it was attributed to segmental and genetic differences. Such a mechanism may be operating in the present material resulting in the formation of univalents, which showed random segregation giving rise to gametes with different complements ($n\pm 1$). Such a case was also reported in the F_1 *Nicotiana* hybrids of *N. megalosiphon* ($n=20$) $\times$ *N. glutinosa* ($n=12$) by Satyanarayana and Subhashini (1964) with a complement ranging from $2n=31$, 32 and 34.

The amphiploid *N. umbratica-glutinosa* had a chromosome number of $2n=70$ but this similarity in possessing the same chromosome number need not necessarily

mean their genetic makeup also would be identical (C_2). As a matter of fact each one of these exhibited dissimilar pattern in meiotic behaviour producing aneuploid and hyperploid gametes. The aneuploid gametes in the genus *Nicotiana* are functional as the genus had polyploid origin and the chromosome loss of one species is being compensated by the other. Thus due to the functioning of aneuploid and hyperploid gametes in the C_2 generation, a number of aneuploids and hyperploid ($2n=72$) were isolated. It would appear from the progeny studies of this hybrid that the aneuploid gametes had a better chance for survival rather than hyperploid gametes.

One of the amphiploids ($2n=70$) showed regular meiosis, resulting in good pollen fertility, but there was no capsule setting (Pedigree chart). A similar case was reported in *Nicotiana* hybrids (Apparao and Krishnamurthy 1963, Satyanarayana and Subhashini 1964). Clausen, Keck and Heisey (1945) were of the opinion that not every sterile hybrid is converted by polyploidy into a vigorous fertile form, probably a great majority of them are eliminated as misfit. The proper gene combination, therefore should go hand in hand with hybridization and chromosome doubling if full advantage is to be taken of allopolyploidy.

The present studies which are showing a chromosomal complement of $2n=66, 68, 70$ and 72 are stable and fertile. These stable polyploids supports Goodspeed's (1954) postulate wherein he has suggested that the future of the genus will include polyploid level above the 24 paired one.

Summary

By colchiploidy a number of unstable polyploids were obtained. The progeny of the unstable aneuploid $2n=67$ gave a number of stable polyploids $2n=66, 68, 70, 72$ in the later generations. These stable polyploids which are above the 24 paired level is a positive proof of what Goodspeed (1954) has postulated.

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