GENETICA POLONICA
Vol. 28 1987 No. 3

CYTOGENETICAL STUDY ON *NICOTIANA TABACUM* L. CV. NADWIŚLAŃSKI MAŁY (2*x* and 4*x*) × *NICOTIANA ALATA* LINK ET OTTO HYBRIDS[1]

APOLONIUSZ BERBEĆ[2]

Central Laboratory of Tobacco Industry, Kraków, Department of Tobacco Breeding, Cultivation and Protection, Puławy

Summary. The F_1 hybrid *Nicotiana tabacum* ($2n=48$) cv. Nadwiślański Mały × × *N. alata* ($2n=18$) was obtained as the first step in an attempt to develop tobacco resistance to tomato spotted wilt virus (TSWV). The hybrid showed a poor survival rate and complete sterility. Surviving plants were amphihaploids with 33 mitotic chromosomes. The plants formed from 0 to 10 bivalents with a modal number of 6.

The F_1 plants of *N. tabacum* cv. Nadwiślański Mały tetraploid × *N. alata* showed likewise a poor growth and high mortality. Cytologically they were sesquidiploids with 57 somatic chromosomes. From 10 to 23 univalents were found in 65.7% of the PMC's which indicated asynaptic behaviour of some of the *N. tabacum* homologues and accounted for a high percentage of aborted pollen formed by that hybrid.

The fertility of the sesquidiploids was markedly reduced and restricted solely to the *N. tabacum* × sesquidiploid and sesquidiploid × *N. alata* matings. The former gave rise to a BC_1 progeny composed mostly of Nadwiślański Mały-like plants with little plant-to-plant variation and few signs of gene transmission from *N. alata*. The latter mating resulted in near-amphihaploids with the chromosome numbers ranging from 34 to 38. Unlike regular amphihaploids, some of them were highly vigorous.

Nicotiana alata ($2n=18$), a common ornamental species of tobacco, has been consistently reported as being resistant to tomato spotted wilt virus (TSWV) an important and damaging disease of the tobacco *N. tabacum* ($2n=48$) in Poland and other countries of eastern Europe. Resistance to the virus is absent within the cultivated species and now seems confined to *N. alata* and, possible, to its two closest relatives, *N. sanderae* and *N. forgetiana* (Ivancheva-Grabovska 1984). Of the attempts to utilize *N. alata* as a source of resistance to TSWV the most noteworthy is that by Gajos (1981). The author reported on a transfer of the local lesion response to TSWV, characteristic of *N. alata*, to a breeding line of *N. tabacum*.

Although demonstrated as feasible, the interspecific gene transfer between

[1] Received for publication: March 1986.

[2] Dr. Present address: Osada Pałacowa, 24-100 Puławy — Poland.

N. alata and *N. tabacum* is by no means easy. The two species represent the products of two divergent evolutionary pathways in the genus, *N. alata* having originated through aneuploidy on the basis of a 12-chromosome-pair level ($2n=18$) and *N. tabacum* being a 48-chromosome allotetraploid (Goodspeed 1953). Furthermore, *N. tabacum* is a self-pollinating species, while *N. alata* is an obligate cross-pollinator. The crossability of the two species is rather poor and seems to be largely affected by the genotype of *N. tabacum* (Kostoff 1943). In this author's experience the hybridization products may range from no seeds at all, through abundant seeds of the maternal origin, to poorly germaniting hybrid seeds and seedlings that rarely survive beyond the transplant stage.

It was the observation made by the late Professor J. Berbeć that a fairly large percentage of vigorous hybrid plants is consistently obtained when an air-cured variety of tobacco — cv. Nadwiślański Mały — is mated to *N. alata*. On the other hand, Chaplin and Hann (1961) reported no special difficulties in hybridizing *N. tabacum* with *N. alata* when tetraploid* rather than diploid *N. tabacum* was used in the cross.

Based on those findings, *N. tabacum* cv. Nadwiślański Mały was converted into a tetraploid, and both the diploid and tetraploid were crossed with *N. alata*. It was the first step in an attempt to transfer resistance to TSWV from *N. alata* to the cultivated variety.

This paper deals with the viability, cytological status, pairing behaviour, and fertility of the resulting hybrids. The use of findings on the potential of *N. alata* as a gene donor in *N. alata-to-N. tabacum* gene transfer programs is also briefly discussed.

MATERIAL AND METHODS

Nicotiana tabacum L. cv. Nadwiślański Mały — diploid ($2n=48$) — is an air-cured variety of tobacco no longer grown commercially. It has pink-coloured flowers and is conspicuous for its very short internodes and a compact habit.

N. alata ($2n=18$) taken for this study has been maintained in the collection for a number of years. It is of the white-flowering type with no traces of violet on the corolla.

The tetraploid of *N. tabacum* cv. Nadwiślański Mały ($2n=96$) was obtained by colchicine — treatment of germinating seeds. They were immersed in a 0.2% aqueous colchicine solution for 4 hrs at 27°C, then rinsed thoroughly with distilled water and transferred to pots with a commercial soil mixture. Maturing plants were observed for the signs of increased ploidy, such as slow growth, thick and fragile leaves, irregular leaf vein pattern, and large anthers. Suspected tetraploids were verified cytologically. True tetraploids contained 96 chromosomes in their somatic cells (Fig. 1).

* Although phyllogenetically an allotetraploid *N. tabacum* has undergone considerable diploidization and at least in this context, may be regarded as a functional diploid.

The following interspecific crosses were made:

N. tabacum cv. Nadwiślański Mały ($2x$) × *N. alata*
N. tabacum cv. Nadwiślański Mały ($4x$) × *N. alata*.

No reciprocals were attempted since the failure of using female *N. alata* in crosses with male *N. tabacum* has been a common experience and, besides, there was no advantage to be gained from using the non-recurrent species as a cytoplasm parent. The resulting F_1 plants were used as both males and females in crosses with either of the parental species. Fresh pollen from newly opened flowers was used for pollinations of castrated flowers. After pollination, the pistils were protected against foreign pollen contamination by mounting on them pieces of plastic drinking straws.

Seeds were sown either when freshly collected from mature capsules or after being stored for a few months at a room temperature. They were sown in Petri dishes on several layers of blotting paper moistened with distilled water. Germinating seeds were kept in a growth chamber at 27°C with lighting provided by fluorescent tubes. After 3, 6, 10 and 14 days the seeds of each entry were observed for germination percentage.

Germinating seeds were transferred to pots with a commercial soil mixture. Seedlings were transplanted to individual pots with the same mixture or they were left undisturbed when their mortality was high and only one to four survivors were left per pot. Both the seedlings and the older plants were grown in the greenhouse.

Counts of somatic chromosomes were made in young corollas using the oxyquinoline-maltose method as described by Burns (1964). Observations of meiosis were carried out in acetocarmine-stained preparations of pollen mother cells (Collins 1979). Pollen viability was determined by staining mature pollen grains with acetocarmine. Based on the test by Sficas and Gerstel (1962) the estimated number of chromosomes capable of forming biparental associations ($\hat{n}$) and the estimated probability for a chromosome to form a bivalent ($\hat{p}$) were calculated from the observed distribution of bivalent frequencies in the F_1 *N. tabacum* cv. Nadwiślański Mały ($2x$) × *N. alata*.

RESULTS

VIABILITY AND MORPHOLOGY OF PLANTS

F_1 *N. tabacum* cv. Nadwiślański Mały ($2x$) × *N. alata*

Seeds were obtained with ease, every single pollination resulting in a well-filled seed capsule. Germination rate varied, depending on batch, from 14.7 to 75.2% (Table 1). It did not seem to be consistently affected by time of storage. Germination was extended over a long period, some seeds still germinating after as long as 14 days.

Table 1. Germination of F_1 seeds of *N. tabacum* cv. Nadwiślański Mały $(2x) \times$ *N. alata* and *N. tabacum* cv. Nadwiślański Mały $(4x) \times$ *N. alata* hybrids and their parental forms

Sowing date	Hybrids and parental forms	Germination percentage after days:			
		3	6	10	14
Fall 1984	F_1 *N. tabacum* cv. Nadwiślański Mały $(2x) \times$ *N. alata*	–	–	–	75.2
	F_1 *N. tabacum* cv. Nadwiślański Mały $(4x) \times$ *N. alata*	–	–	–	99.1
	N. tabacum cv. Nadwiślański Mały $(2x)$	–	–	–	98.0
	N. alata	–	–	–	50.0
Spring 1985 (seeds stored over winter)	F_1 *N. tabacum* cv. Nadwiślański Mały $(2x) \times$ *N. alata*	42.2	45.5	48.7	49.0
	F_1 *N. tabacum* cv. Nadwiślański Mały $(4x) \times$ *N. alata*	100.0	100.0	100.0	100.0
	N. tabacum cv. Nadwiślański Mały $(2x)$	94.7	97.3	100.0	100.0
	N. alata	95.3	97.8	100.0	100.0
	N. tabacum cv. Nadwiślański Mały $(4x)$	83.3	90.7	96.6	96.6
Fall 1985	F_1 *N tabacum* cv. Nadwiślański Mały $(2x) \times$ *N. alata**	–	12.1	–	14.7
	N. tabacum cv. Nadwiślański Mały $(2x)$**	–	100.0	–	100.0
	*N. alata***	–	100.0	–	100.0

* fresh seeds
** seeds stored over 1 year

Seedling mortality was high and usually no more than 1 - 4 seedlings out of about 30 per pot survived up to the transplant stage. The survivors grew slowly but most of them could be grown to maturity. They ranged from diminutives to fairly sizeable individuals (Fig. 2). However, none of them was found to reach the size of either of the parental species. The flowers of the F_1 showed few differences from individual to individual. They were whitish with only traces of violet on the corolla. Generally, they resembled the flowers of the alata parent both in the size and shape (Fig. 3) and in the anthocyane coloration of the anthers.

F_1 *N. tabacum* cv. Nadwiślański Mały $(4x) \times$ *N. alata*

The seeds were obtained with ease. They showed consistently good and rapid germination (Table 1). Within 3 days 100% of the seeds were found to germinate in the spring 1984 experiment, the figure being only slightly lower for the fall of 1984.

The seedlings showed good vigour and grew at approximately the same rate as the diploid tabacum parent. However, the majority of the juvenile plants started to show some leaf yellowing at about the 5th-leaf stage. After transplanting, the plants' growth became arrested and many of them died. Less than 10% of the plants resumed normal growth and reached maturity (Fig. 4) but most of them were dwarfed and showed little vigour.

The flowers of the F_1 *N. tabacum* cv. Nadwiślański Mały $(4x) \times$ *N. alata* were much like those of the F_1 involving *N. tabacum* cv. Nadwiślański Mały $(2x)$ (Fig. 3).

They were whitish and some showed patches of anthocyane blue. All had anthocyane coloured anthers but the colour was more diluted than that in the F_1 *N. tabacum* cv. Nadwiślański Maly (2*x*) × *N. alata*.

CYTOLOGY AND FERTILITY

F_1 N. tabacum cv. Nadwiślański Mały (2*x*) × *N. alata*

The hybrids possessed 33 chromosomes in their somatic cells (Fig. 5) as could be expected from crossing the 48-chromosome *N. tabacum* with the 18-chromosome *N. alata*. Thus the hybrids could be assumed to be amphihaploids with the haploid chromosome complements of both parents.

The cytological status of the hybrids was confirmed by the study of meiosis (Table 2). Seven F_1 plants analysed for chromosome pairing at Metaphase I showed a

Table 2. Chromosome pairing at Metaphase I in the PMCs of F_1 hybrid *N. tabacum* cv. Nadwiślański Mały (2*x*) × *N. alata*

Hybrid No.	Number of PMCs with bivalents[a]											Total of PMCs	Modal of bivalent
	0	1	2	3	4	5	6	7	8	9	10		
1	0	1	0	3	5	10	15	9	2	4	1	50	6
2	0	0	0	0	0	1	1	5	1	1	1	10	7
3	0	0	0	1	0	5	3	3	2	1	1	16	5
4	0	1	0	2	4	2	4	5	3	0	1	22	7
5	0	0	5	3	12	19	20	19	8	3	4	93	6
6	0	0	0	2	3	7	12	7	8	3	1	43	6
7	1	0	3	8	11	11	11	8	5	2	0	59	4–6
Total	1	2	8	19	35	55	66	56	29	14	9	293	6
% of total PMC number	0.3	0.7	2.7	6.5	11.9	18.8	22.5	19.1	9.9	4.8	3.1	111.0	

Mean no of bivalents 5.89
$\hat{n}^* = 13.71$
$\hat{p}^{**} = 0.42$

[a] Higher valencies (trivalents) classified as bivalents
* Estimated no. of chromosomes capable of forming biparental pairs
** Estimated probability to form a bivalent

fairly consistent pairing behaviour. The plants formed from 0 to 10 bivalents, the mode being 5 in one plant, 6 in three plants, 4 - 6 in one plant, and 7 in two plants. Less than three bivalents and more than nine bivalents in a PMC were found very rarely. The majority of the PMCs contained 5 to 7 bivalents, the PMCs containing 6 bivalents being the most frequent class. One to two ring bivalents were observed in diakinesis (Fig. 6A). The majority of the bivalents were rod-shaped with chiasmata ranging from distinct to vestigial (Fig. 6C, 6D).

The estimated number of chromosomes capable of forming biparental pairs was more than 9 (13.71) which, together with 10 bivalents actually observed in some of the PMCs, was indicative of a fair degree of autosyndetic pairing in the hybrid.

Table 3. Pollen stainability in F_1 *N. tabacum* cv. Nadwiślański Mały $(2x) \times N.$ *alata*, F_1 *N. tabacum* cv. Nadwiślański Mały $(4x) \times N.$ *alata* $(2x)$ and BC_1 *N. alata* $(2x) \times (F_1$ *N. tabacum* cv. Nadwiślański Mały $(4x) \times N.$ *alata* hybrids

Hybrids	Per cent of stainable pollen
F_1 *N. tabacum* cv. Nadwiślański Mały $(2x)$ × *N. alata*	11.8
,,	1.1
,,	2.4
,,	0.5
,,	0.6
F_1 *N. tabacum* cv. Nadwiślański Mały $(4x)$ × *N. alata*	18.8
,,	21.8
,,	11.2
,,	11.3
BC_1 *N. alata* × (*N. tabacum* cv. Nadwiślański Mały $(4x)$ × *N. alata*)	0.2

The expectancy for a given chromosome to pair was relatively high (0.41). Trivalents were observed in many of the PMCs but their number did not exceed 2 per cell (Fig. 6A, 6B, 6C, 6D).

Chromatin bridges and dicentric chromosomes were rare, but they did occur in some of the PMCs (Fig. 6E). Laggards and chromosomes not included in the nuclei were frequent (Fig. 6F). In one plant observed for the phenomenon, dyads and triads accounted for 32.8% of the total number of quartets. Five F_1 plants were examined for pollen stainability (Table 3). In four of them less than 3% of stained pollen grains were found, the fourth showing 11.8% of viable pollen. However, none of the F_1 plants produced any seeds either by selfing or by backcrossing to the parental species.

F_1 *N. tabacum* cv. Nadwiślański Mały $(4x) \times N.$ *alata*

As theoretically expected, the F_1 plants obtained from mating NM tetraploid (96 chromosomes — Fig. 1) with *N. alata* contained 57 chromosomes in their somatic cells (Fig. 7A). Thus, they contained a full diploid complement of *N. tabacum* plus 9 chromosomes from *N. alata* and could be classified as sesquidiploids.

It was borne out by some of the meiotic configurations in which 24 bivalents and 9 univalents were observed (Fig. 7B).

However, the 24 II + 9 I configuration was by no means present in all the PMCs nor did it occur in the majority of the meiocytes. In 10.6% of the cells 7 or 8 univalents were observed, the remaining one or two chromosomes entering a trivalent association. In 65.7% of the PMCs more than 9 univalents were found, the number observed being up to 23 (Fig. 7C, 7D).

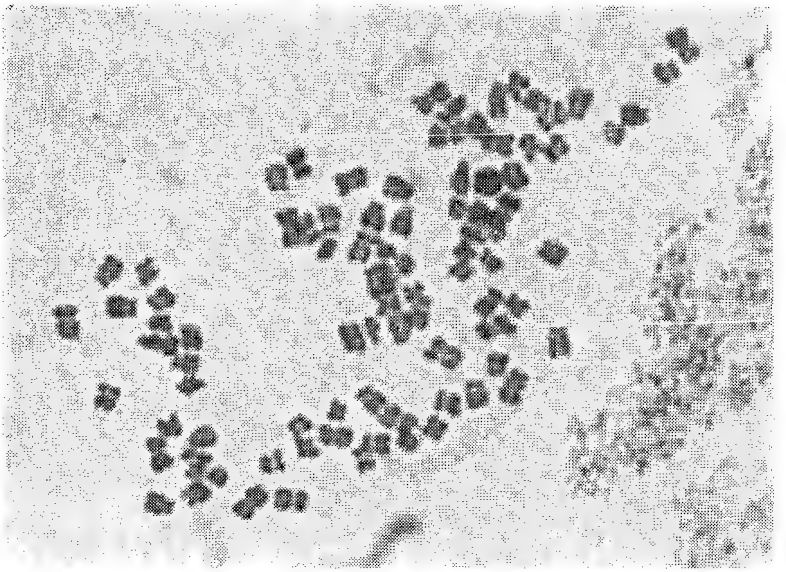

Fig. 1. *N. tabacum* cv. Nadwiślański Mały (4*x*). Mitotic metaphase: 96 chromosomes

Fig. 2. F_1 *N. tabacum* cv. Nadwiślański Mały (2*x*) × *N. alata*. Two flowering plants: a larger one (in front) and a dwarf (behind)

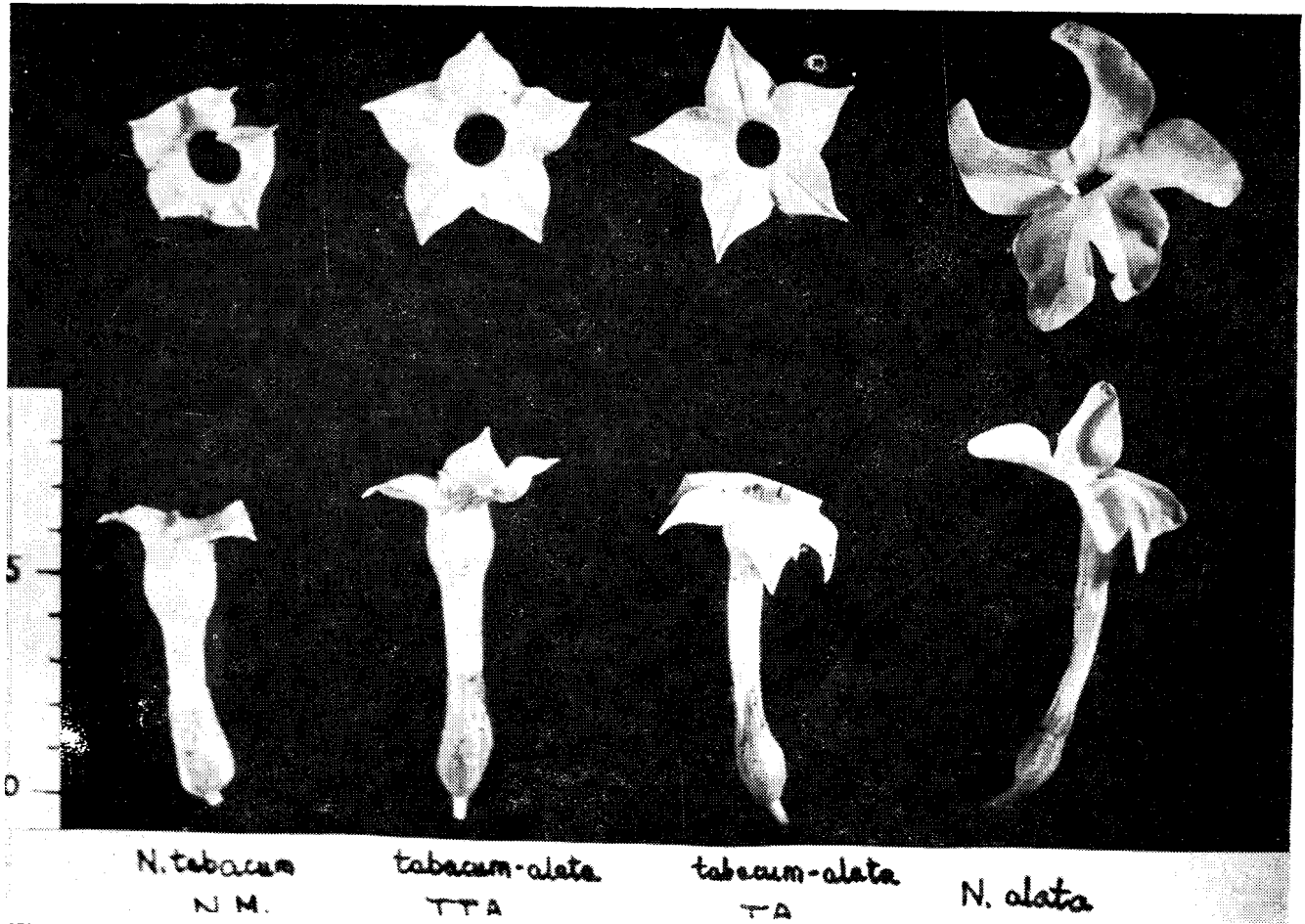

Fig. 3. Flowers (from left to right): *N. tabacum* cv. Nadwiślański Mały (2*x*) F_1 *N. tabacum* cv. Nadwiślański Mały (4*x*) × *N. alata* F_1 *N. tabacum* cv. Nadwiślański Mały (4*x*) × *N. alata*

Fig. 4. F_1 *N. tabacum* cv. Nadwiślański Mały $(4x) \times N.$ *alata*. Flowering plants

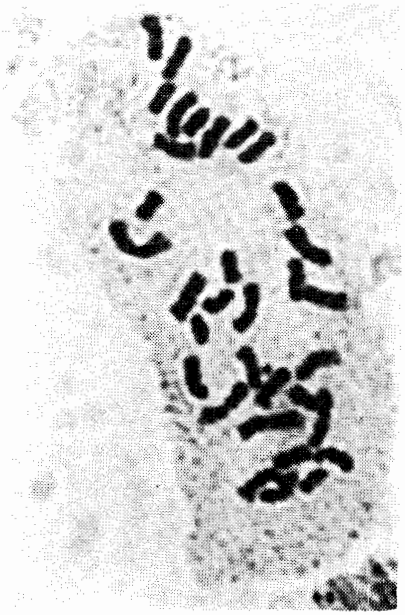

Fig. 5. F_1 *N. tabacum* cv. Nadwiślański Mały $(2x) \times N.$ *alata*. Mitotic metaphase: 33 chromosomes

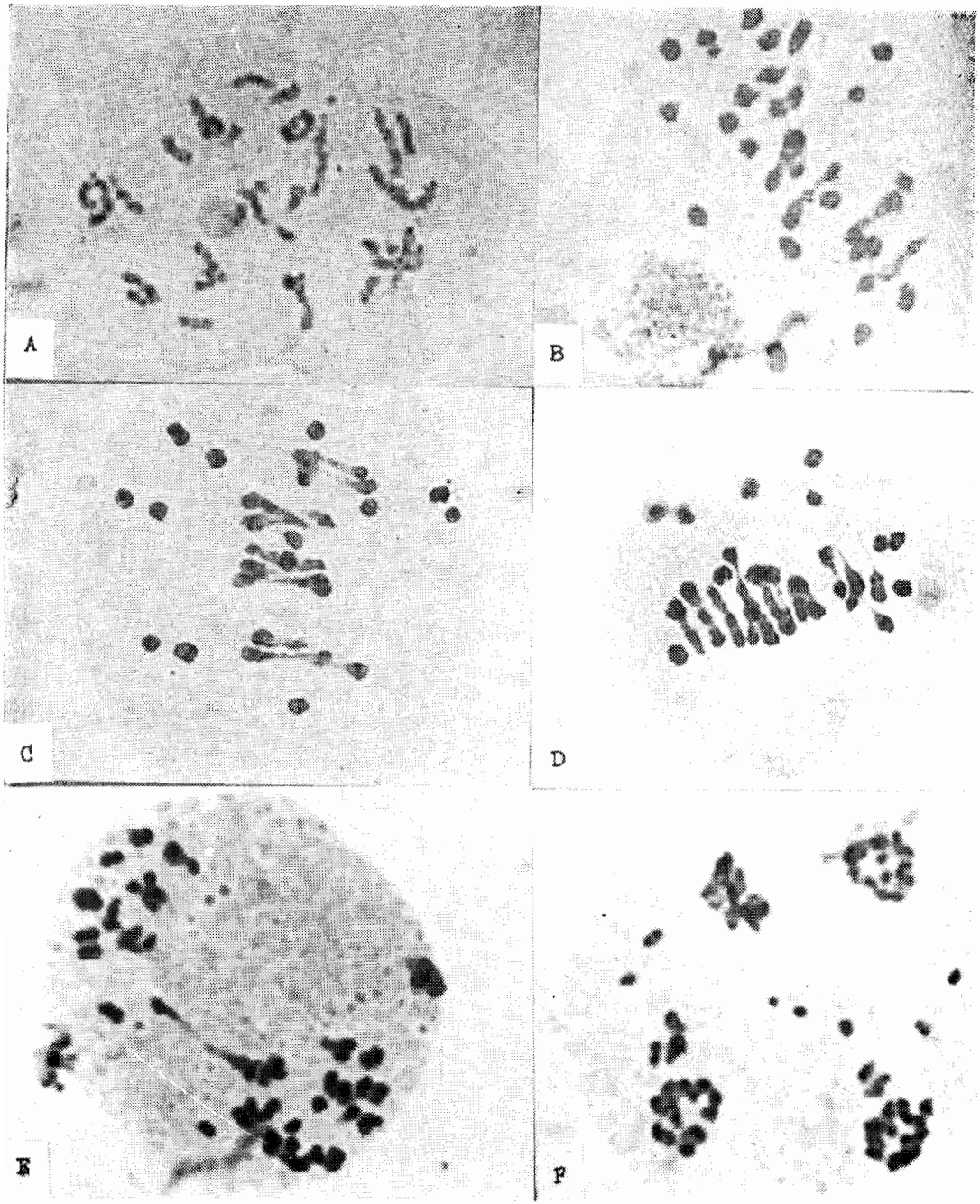

Fig. 6. *F_1 N. tabacum* cv. Nadwiślański Maly $(2x) \times$ *N. alata*. Meiosis. A) Diakinesis: 2 trivalents, 2 ring-shaped bivalents, 4 one-chiasma bivalents; B) Metaphase I: 1 ring-shaped bivalent 4 rod-shaped bivalents, and 23 univalents; C) Metaphase I: 2 trivalents, 6 bivalents, random chromosome associations and univalents; E) Telophase I: a dicentric chromosome; F) Telophase II: chromosomes not included in the nuclei

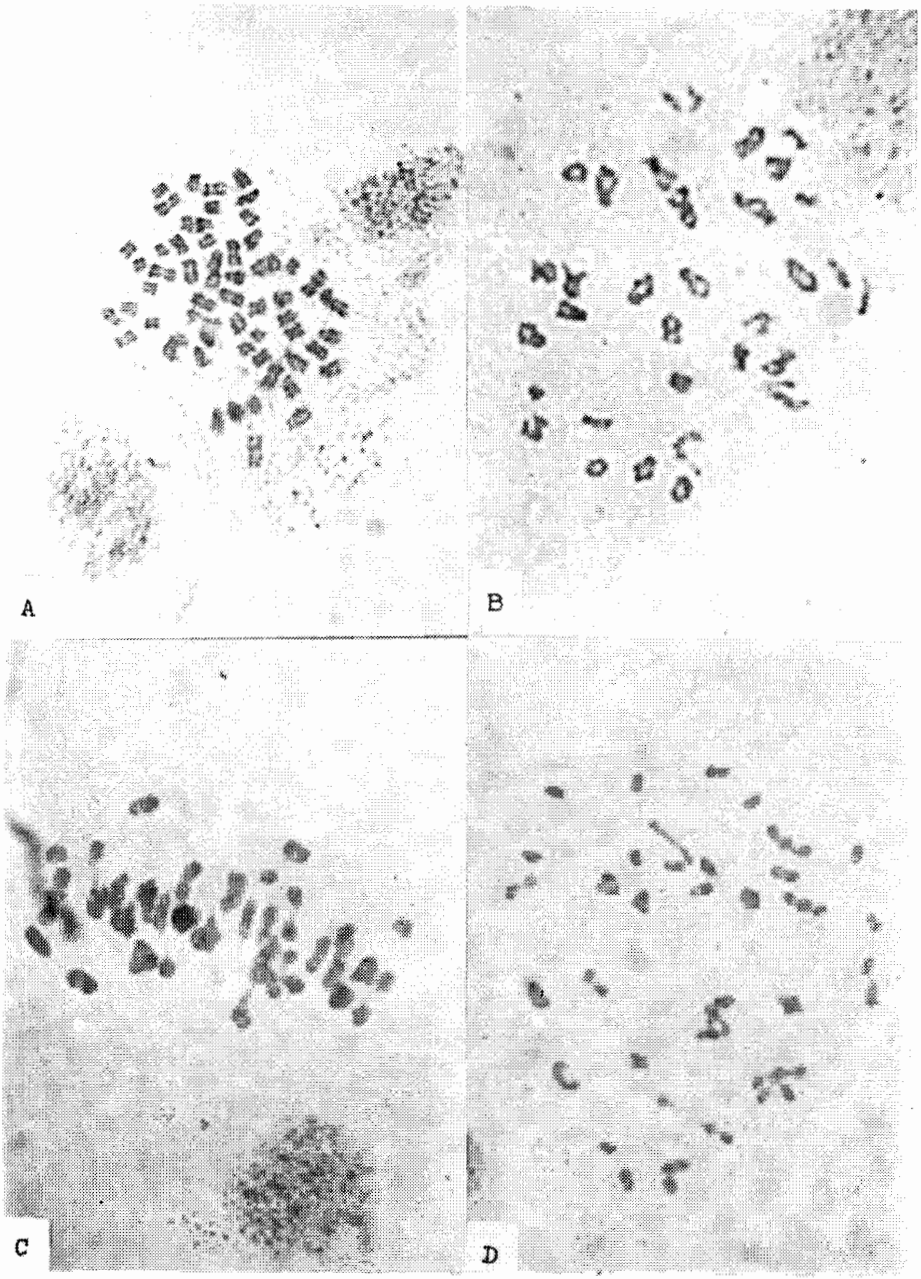

Fig. 7. F_1 *N. tabacum* cv. Nadwiślański Mały $(5x) \times N.$ *alata*. Mitosis and meiosis. A) Mitotic mataphase: 57 chromosomes; B) Diakinesis: 24 bivalents and 9 univalents; C) Metaphase I: 22 bivalents (including 5 with a very weak chiasma) and 13 univalents; D) Metaphase I: 17 bivalents and 3 univalents

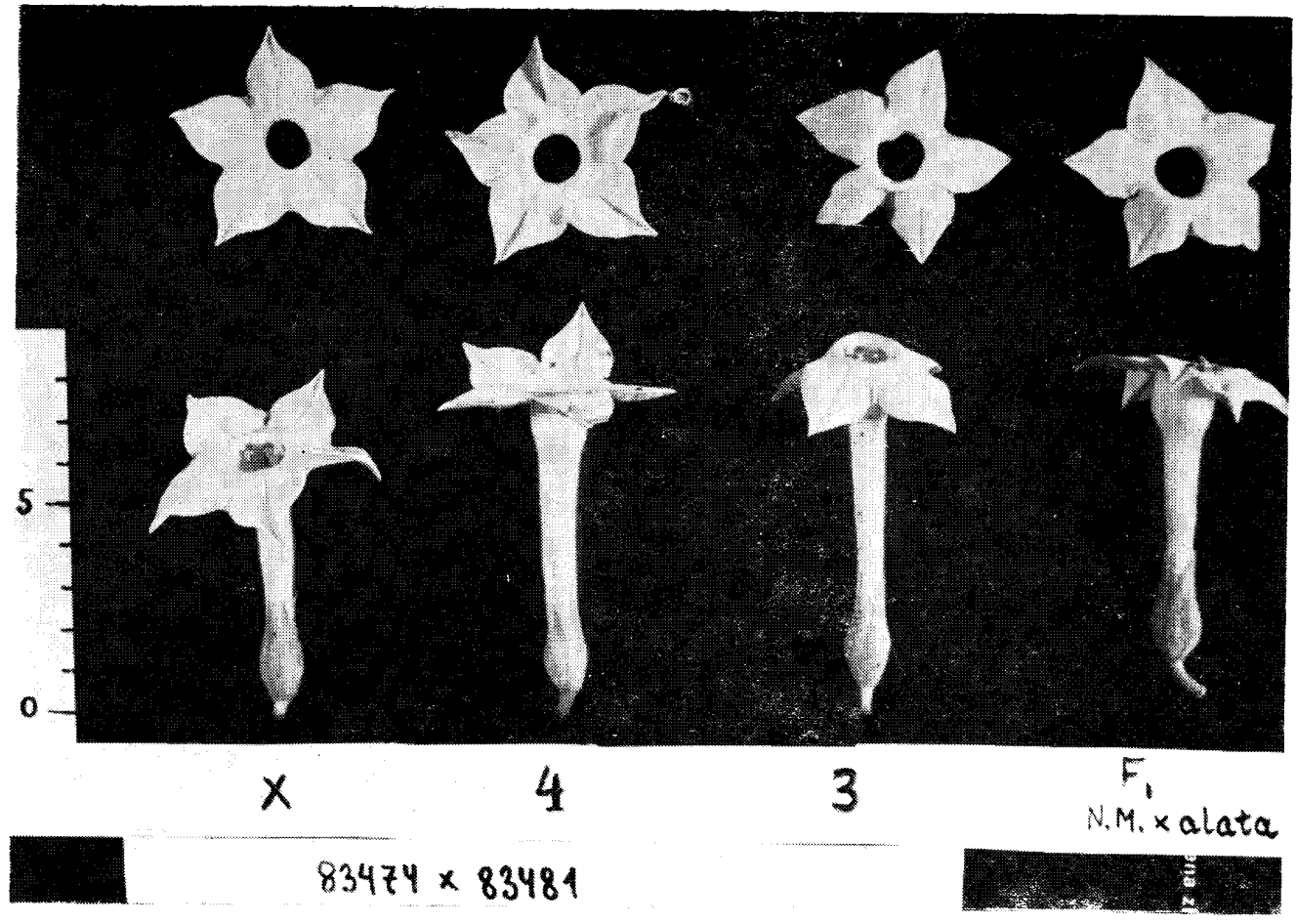

Fig. 8. Flowers (from left to right): 3 segregants BC_1 (F_1 *N. tabacum* cv. Nadwiślański Mały $(5x) \times$ *N. alata* F_1 *N. tabacum* cv. Nadwiślański Mały $(2x) \times$ *N. alata*

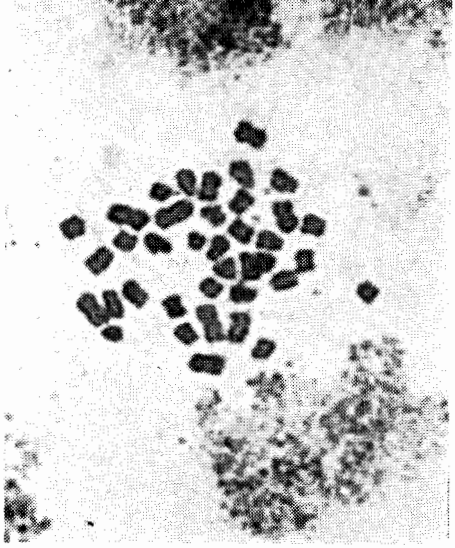

Fig. 9. BC_1 (F_1 *N. tabacum* cv. Nadwiślański Mały $(4x) \times N$. *alata* $\times$ *N. alata*. Mitotic metaphase: 38 chromosomes

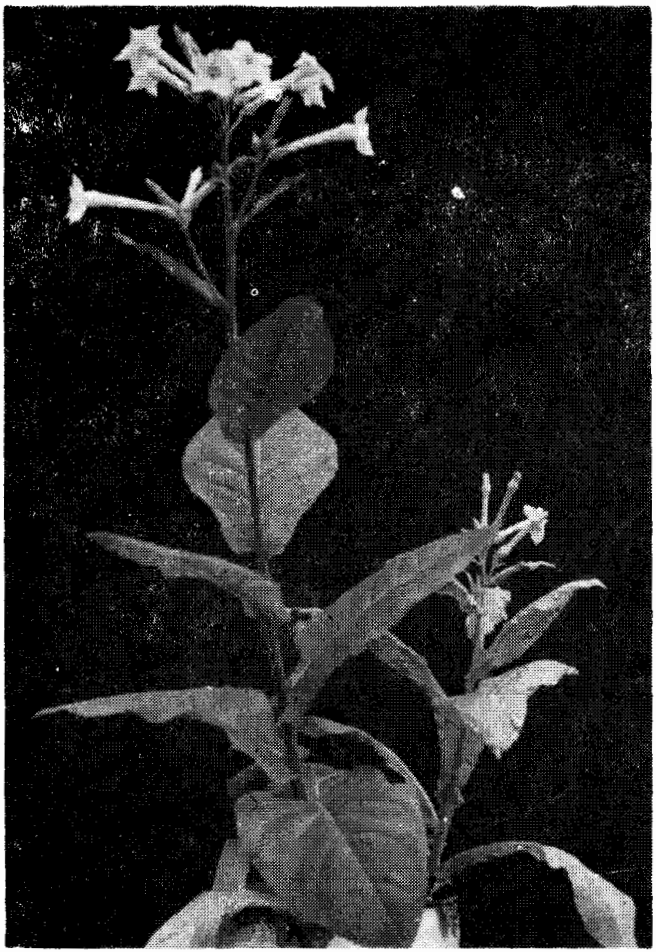

Fig. 10. BC_1 (F_1 *N. tabacum* cv. Nadwiślański Mały $(4x)$% *N. alata* $\times$ *N. alata*. Flowering plants

Sesquidiploids NM tetraploid × *N. alata* produced from 9.7 to 21.8% of viable pollen (Table 3). The plants were completely self-sterile and also did not set seeds when crossed as females to NM diploid. They showed partial fertility when used as pollen parents in crosses with NM di and when fertilized with pollen of *N. alata*.

BC_1F_1 PROGENIES FROM BACKCROSSING THE SESQUIDIPLOIDS TO *N. TABACUM* CV. NADWIŚLAŃSKI MAŁY (4*x*) AND *N. ALATA*

Although a more detailed analysis of the BC_1 and subsequent generations is intended to be the subject of the next paper, some general information is provided below as relevant to the fertility of the sesquidiploids *N. tabacum* cv. Nadwiślański Mały (4*x*) × *N. alata*.

Matings of female *N. tabacum* cv. Nadwiślański Mały (2*x*) plants with male sesquidiploids resulted in a poor seed set. Approximately half of the fertilized flowers dropped, and the other half formed shrunken capsules, each containing from 1 to no more than 50 seeds.

There was a considerable variation in germination percentage of the BC_1 seeds thus obtained (Table 4) depending on progeny. It ranged from 60.0% to 3.5% and the effect of the time of storage did not seem to be consistent.

Of 36 plants of that generation which were grown to maturity, 29 closely resembled the NM diploid parent and were fully self-fertile. The remaining 7 plants departed morphologically from the type of the recurrent parent and some of them showed reduced fertility. In none of the plants of that generation the presence of violet anthers was detected.

The mating of female sesquidiploids to male *N. alata* yielded capsules at a rate

Table 4. Germination of BC_1 hybrid seeds of *N. tabacum* cv. Nadwiślański Mały (2*x*) × (F_1 *N. tabacum* cv. Nadwiślański Mały (4*x*) × *N. alata*) and (F_1 *N. tabacum* cv. Nadwiślański Mały (4*x*) × *N. alata*) × *N. alata*

Progeny no.	Hybrids	Sowing date	Per cent germinated seeds (after 14 days)
1	BC_1 *N. tabacum* cv. Nadwiślański Mały (2*x*) × (F_1 *N. tabacum* cv. Nadwiślański Mały (4*x*) × *N. alata*)	Fall 1984 (fresh seeds)	16.4
2	,,		26.9
1	BC_1 (F_1 *N. tabacum* cv. Nadwiślański Mały (4*x*) × *N. alata*) × *N. alata*		3.5
2	,,		38.4
3	,,		2.4
1	BC_1 *N. tabacum* cv. Nadwiślański Mały (2*x*) × (F_1 *N. tabacum* cv. Nadwiślański Mały (4*x*) × *N. alata*)	Spring 1985 (seeds stored over winter	48.3
2	,,		16.9
3	,,		31.9
1	BC_1 *N. tabacum* cv. Nadwiślański Mały (2*x*) × (F_1 *N. tabacum* cv. Nadwiślański Mały (4*x*) × *N. alata*)	Fall 1985 (fresh seeds)	60.0

of approximately 1 capsule/10 pollinated flowers. The capsules were poorly filled. The seed germination rate was from 38.4 to 2.4% (Table 4). That generation was composed of plants very similar in flower morphology to the F_1 *N. tabacum* cv. Nadwiślański Mały ($2x$) × *N. alata* (Fig. 8). The segregants had from 34 to 38 chromosomes (Fig. 9), formed blue anthers and were completely sterile. Both the chromosome number and the morphology indicated that the plants must have originated through the fusion of gametes of *N. alata* with those of the sesquidiploids, the latter containing, in addition to the haploid complement of *N. tabacum*, some univalents from *N. alata*. Some of the plants thus obtained were remarkable for vegetative vigour (Fig. 10), far exceeding that of regular amphihaploids.

DISCUSSION

The range of chromosome pairing in the F_1 *N. tabacum* cv. Nadwiślański Mały ($2x$) × *N. alata* was wider than that given by Kostoff (1943) the latter investigator reporting from 4 to 9 bivalents. Malecka (1977) investigated a very close hybrid combination i. e. *N. sanderae* × *N. tabacum* and found 6 bivalents as most frequently occurring which agrees well with our data. Since autosyndetic associations must have accounted for some of the bivalents formed by the hybrid, the actual number of biparental pairs might have been a little lower than the bivalent numbers observed. As it is, the chances of genetic recombination in the tabacum-alata amphidiploid seem to be good as both the regular number of bivalents and the presence of chromatin bridges and dicentric chromosomes may be regarded as cytological consequences of crossover events taking place in the hybrid's meiosis. However, the *N. tabacum* cv. Nadwiślański Mały ($2x$) × *N. alata* amphihaploids were completely self- and cross-sterile and, due to reduced vegetative vigour, not readily amenable to chromosome-doubling treatments.

The sesquidiploids *N. tabacum* cv. Nadwiślański Mały ($4x$) × *N. alata* obtained directly from the tetra-di mating were found to have some residual fertility unlike the completely sterile sesquidiploids obtained in the same manner by Chaplin and Mann (1961). However, Chaplin's sesquidiploids involved a different tetraploid tabacum parent. The fertility of our sesquidiploids though much improved when compared with Chaplin's was, as it is, very poor for that class of hybrids and restricted to the ♀ sesquidiploid × ♂ *N. alata* and ♀ *N. tabacum* × ♂ sesquidiploid matings.

It is a common knowledge that in crosses between parents of which one is self-compatible and the other is self-incompatible, the mating ♂ self-compatible × ♂ self-incompatible is often successful while the reciprocal fails to yield seeds. It should be noted, however, that Ivancheva-Gabrovska and Manolov (1982) reported some self-fertility in the F_1 tabacum-alata hybrids involving both diploid and tetraploid *N. tabacum*. In the case of F_1 *N. tabacum* cv. Nadwiślański Mały ($4x$) × *N. alata* the incompatibility seems to extend to the sesquidiploid generation as well. Since extra chromosomes are usually better transmitted through the egg

than through the pollen (Chaplin, Mann 1961), the chance of a gene transfer via univalent transmission may be negatively affected.

The irregular pairing behaviour of some of the tabacum homologues seems to account, at least in part, for the poor fertility of the sesquidiploids *N. tabacum* cv. Nadwiślański Mały ($4x$) × *N. alata*. However, other factors such as lethals may be involved. The involvement of lethal genes might explain the low germination rate of BC_1 *N. tabacum* cv. Nadwiślański Mały ($2x$) × (F_1 *N. tabacum* cv. Nadwiślański Mały ($4x$) × *N. alata*). *N. alata*, as an outcrossing species, is likely to maintain a substantial load of lethal recessives for which *N. tabacum* may lack appropriate dominants. The notion seems to be supported by a surprisingly high vigour of some of the BC_1 (F_1 *N. tabacum* cv. Nadwiślański Mały ($4x$) × *N. alata*) × *N. alata* segregants which, in all probability, had one or more *N. alata* chromosomes in disomic condition.

The BC_1 *N. tabacum* cv. Nadwiślański Mały ($2x$) × (*N. tabacum* cv. Nadwiślański Mały ($4x$) × *N. alata*) was the so-called "breakdown generation", in which the haploid genome of *N. alata* was broken down into individual, independently segregating chromosomes. The preliminary observations of that generation seem to indicate that it was largely composed of *N. tabacum* cv. Nadwiślański Mały ($2x$) -like plants with little plant-to-plant variation and little, if any, segregation for dominants from *N. alata*. The relative uniformity of the BC_1 generation was suggestive of a very low rate of interspecific transfer from the alien species.

In the tetra-di combination a certain reduction of genetic exchange rate seems to be inherent. Patel and Gerstel (1961) demonstrated that sesquidiploids showed high degree of preferential pairing and, consequently, little trivalent formation. In conformity with those data, few trivalents were formed by the sesquidiploid *N. tabacum* cv. Nadwiślański Mały ($4x$) × *N. alata*. In the latter hybrid the situation seems to be further aggravated by the fact that the sesquidiploids could only be used as pollen parents in backcrosses with *N. tabacum*, thereby reducing the chance of a gene transfer based on transmission of extra univalents. Thus, to make up for restricted gene exchange between *N. alata* and *N. tabacum* in gene transfer programmes utilizing the tetra-di combination, very large BC_1 populations should be obtained and screened for a desired trait.

The vigorous amphihaploid-like plants with supernumerary chromosomes obtained from backcrossing the sesquidiploids to *N. alata* may offer some hope in circumventing the disadvantages of the tetra-di combination. They show enough vigour to be a good material for further breeding work including colchicine-treatment. Thus they may hold promise as a source of improved genetic recombination at the amphihaploid level.

REFERENCES

1. Burns J. A. (1964). A technique for making preparations of mitotic chromosomes from Nicotiana flowers. Tob. Sci., 8: 1 - 2.
2. Chaplin J. F., Mann T. J. (1961). Interspecific hybridization, gene transfer, and chromosomal substitution in *Nicotiana*. North Carolina Agric. Exp. Sta. Tech. Bul., 145.

3. Collins G. B. (1979). Cytogenetic techniques (in): *Nicotiana*. Procedures for experimental use. USDA Tech. Bul., 1586: 17 - 22.
4. Gajos Z. (1981). Przeniesienie odporności na wirus brązowej plamistości pomidora (Tomato spotted wilt virus) z *Nicotiana alata* Link et Otto do tytoniu szlachetnego przez skrzyżowanie obu gatunków. Biul. CLPT 1 - 2: 3 - 24.
5. Goodspeed T. H. (1953). The genus *Nicotiana*. Chronica Botanica. Waltham. Mass.
6. Ivancheva-Gabrovska T. (1984). Studies on tomato spotted wilt virus of tobacco in Bulgaria. Doctoral thesis. (Summary). Plovdiv.
7. Ivancheva-Gabrovska T., Manolov A. (1982). Investigations of the resistance of interspecific hybrids *Nicotiana tabacum* × *N. alata* and *N. tabacum* × *N. sanderae* to tomato spotted wilt virus. Rep. 2nd National Symposium of Plant Immunity, 2: 65 - 78.
8. Kostoff D. (1943). Cytogenetics of the genus *Nicotiana*. State Printing House. Sofia.
9. Malecka J. (1977). Cytoembryological studies in *Nicotiana* hybrids. Acta Biol. Cracov. Ser. Bot., 20: 89 - 99.
10. Patel K. A., Gerstel D. U. (1961). Additional information on the mechanism of chromosome substitution in *Nicotiana*. Tob. Sci., 5: 19 - 20.
11. Sficas A. G., Gerstel D. U. (1962). Statistical analysis of chromosome pairing in interspecific hybrids. Genetics, 47: 1171 - 1185.

BADANIA CYTOGENETYCZNE MIESZAŃCÓW MIĘDZYGATUNKOWYCH OTRZYMANYCH ZE SKRZYŻOWANIA DI- I TETRAPLOIDALNEJ FORMY *NICOTIANA TABACUM* L. CV. NADWIŚLAŃSKI MAŁY Z *NICOTIANA ALATA* LINK ET OTTO

Streszczenie

Uzyskanie mieszańca *N. tabacum* var. Nadwiślański Mały ($2n=48$) × *N. alata* ($2n=18$) było pierwszym etapem badań zmierzających do wyhodowania tytoniu odpornego na *Lycopersicon* virus 3 (TSWV). Rośliny mieszańcowe wykazywały słabą żywotność i były całkowicie bezpłodne. Osobniki, które osiągnęły stadium kwitnienia były amfihaploidami o liczbie chromosomów — 33. Rośliny F_1 tworzyły w mejozie od 0 do 10 biwalentów (średnia modalna — 6).

Z formy diploidalnej *N. tabacum* var. Nadwiślański Mały wyprowadzono formę tetraploidalną ($2n=96$) i skrzyżowano ją także z *N. alata*. Rośliny F_1 pochodzące z tego krzyżowania wykazywały również słabą żywotność i niski stopień przeżywalności. Cytologicznie były seskwidiploidami o liczbie chromosomów — 57. W 65,7% komórek macierzystych pyłku stwierdzono występowanie od 10 do 23 uniwalentów, co wskazywało na brak koniugacji niektórych homologicznych chromosomów *N. tabacum* i powodowało niską płodność ziarn pyłku tej formy.

Płodność roślin seskwidiploidalnych była niska i ograniczona do kombinacji *N. tabacum* × seskwidiploid i seskwidiploid × *N. alata*. W pokoleniu BC_1 pierwszej kombinacji krzyżowania obserwowano rośliny o morfologii zbliżonej do odmiany Nadwiślański Mały, wykazujące małą zmienność osobniczą i niewiele cech wskazujących na transmisję genów z gatunku *N. alata*. W wyniku drugiej kombinacji krzyżowania otrzymano rośliny zbliżone cytologicznie do amfihaploidów, o liczbie chromosomów od 34 do 38. Niektóre z tych roślin wykazywały wysoki stopień żywotności.

ЦИТОГЕНЕТИЧЕСКИЕ ИССЛЕДОВАНИЯ МЕЖВИДОВЫХ ГИБРИДОВ, ПОЛУЧЕННЫХ ОТ СКРЕЩИВАНИЯ ДИПЛОИДНОЙ И ТЕТРАПЛОИДНОЙ ФОРМ *NICOTIANA TABACUM* L. VAR. NADWIŚLAŃSKI MAŁY С ВИДОМ *N. ALATA* LINK ET OTTO

Резюме

Гибрид F_1 *N. tabacum* var. Nadwiślański Mały ($2n=48$) × *N. alata* является первым этапом исследований попытки выведения табака устойчивого к *Lycopersicom* virus 3 (TSWV). Гибрид проявлял слабую выживаемость и был полностью стерильным. Растения, которые достигли стадию

цветения, были амфигаплоидами с числом хромосом 33. Растения F_1 производили в мейозе от 0 до 10 бивалентов при средней модальной, составляющей 6.

Растения F_1, происходящие от скрещивания *N. tabacum* var. Nadwiślański Mały тетраплоид ($2n=96$) с *N. alata* также росли слабо и имели низкую степень выживаемости. Цитологически были они сесквидиплоидами с числом хромосом 57. У 65,7% материнских клеток пыльцы обнаружено появление от 10 до 23 унивалентов, что указывало на отсутствие коньюгации некоторых гомологических хромосом *N. tabacum* и одновременно объясняло низкую плодородность пыльцы этой формы.

Плодородность сесквидиплоидов была значительно снижена и ограничена до комбинаций скрещивания: *N. tabacum* сесквидиплоид и сесквидиплоид × *N. alata*. Первая из этих комбинаций дала поколение BC_1, состоящее в большинстве из растений подобных сорту Nadwiślański Mały, обнаруживающих небольшую изменчивость между индивидуальными растениями и немного признаков, указывающих на трансмиссию генов из *N. alata*. В другой комбинации скрещивания получено растения цитологически близкие амфигаплоидам с числом хромосом от 34 до 38. В отличие от регулярных амфигаплоидов, некоторые из этих растений имели высокий уровень выживаемости.