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CHROMOSOME PAIRING AND POLLEN FERTILITY IN THE INTERSPECIFICS F_1 HYBRIDS *NICOTIANA TABACUM* L. × *N. BENAVIDESII* GOODSPEED, *N. KNIGHTIANA* GOODSPEED×*N. TABACUM*, AND *N. RAIMONDII* MACBRIDE×*N. TABACUM*[1]

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Summary. The F_1 hybrids of *Nicotiana tabacum* ($2n=48$) with three species of the section *Paniculatae* ($2n=24$) were obtained. The F_1 hybrid *N. tabacum* cv. BP-210×*N. benavidesii* formed from 0 to 6 bivalents with a modal number of 0. The F_1 hybrids *N. knightiana* × *N. tabacum* cv. Izyda and *N. raimondii* × *N. tabacum*-F_1 cv. Zamojska 4 × cv. LB-838 each formed from 0 to 3 bivalents with a modal number of 0. The mean number of bivalents per cell in the F_1 *N. tabacum* × *N. benavidesii* was significantly higher than in the remaining hybrids indicating that, out of the three species, *N. benavidesii* had the closest affinity to *N. tabacum*. The three hybrids formed mostly aborted pollen.

The study of interspecific hybrids of *Nicotiana* was initiated by Kolreuter in the 18th century. The major contributions in this field were the monographs by Kostoff (1943) and Goodspeed (1953). For both authors the cytogenetical investigations of *Nicotiana* hybrids were primarily a tool of establishing systematic relationships within the large genus.

Nicotiana became practically used as a source of germplasm in breeding *N. tabacum* after the work of Holmes (1938) who transferred resistance to tobacco mosaic virus (TMV) from *N. glutinosa* to the variety Samsoun of *N. tabacum*. Since that time the theoretical and practical aspects of interspecific hybridization in *Nicotiana* have been closely related.

The three species — *N. benavidesii*, *N. knightiana*, and *N. raimondii* — are phyllogenetically distant from the cultivated *N. tabacum*, but they are closely related to one another as being in the same section within the genus (Goodspeed 1953). They also share the property of being immune to the necrotic strain of the potato virus Y (Sievert 1972), due to which they were chosen as potential sources of resistance to PVY in the project aimed at obtaining resistant tobacco germplasm.

The first stage of the investigations was to make the respective F_1 hybrids and

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o study their meiosis, as a probability of genetic exchange between two species is believed to be strongly dependent on the degree of chromosomal affinity between hem.

Herein are reported data on chromosomal pairing and pollen fertility in F_1 hybids resulting from crossing *N. tabacum* with *N. benavidessi*, *N. knightiana* and *N. aimondii*.

MATERIAL AND METHODS

The wild species used in the interspecific crosses were *N. benavidesii* Goodspeed, *N. knightiana* Goodspeed, and *N. raimondii* Macbride. They are classified in the ection *Paniculatae*, subgenus *Rustica* of the genus *Nicotiana* (Goodspeed 1953). Each of them has a diploid chromosome number of $2n = 24$. They are native to southern Peru, where they occur as ruderal and roadside weeds. The accessions used in his study have been maintained in the collection of this department for a number of years.

Three forms of the *N. tabacum* parent ($2n = 48$) were used: cv. BP-210, cv. Izyda, nd F_1 cv. Zamojska 4 × cv. LB-838. The following interspecific hybrids were obained:

1. *N. tabacum* cv. BP-210 × *N. benavidesii*
2. *N. knightiana* × *N. tabacum* cv. Izyda
3. *N. raimondii* × *N. tabacum*-F_1 cv. Zamojska 4 × cv. LB-838.

Interspecific pollinations were made in the greenhouse. Castrated flowers were pollinated with fresh pollen from the male parents in the cross. To prevent premature eed capsule abscission, 1% NAA in lanolin was applied to the base of the pedicels f pollinated flowers. Seeds were germinated on Petri dishes on moistened blotting aper at 27°C and light was provided by fluorescent tubes. Hybrid plants were raised ingly in pots in the greenhouse. The soil was a compost mixture.

Cytological examinations included counts of mitotic chromosomes, observaions of pairing at Metaphase I in the pollen mother cells, and determinations of polen viability. Smear preparations of mitotic chromosomes were made from young orollas. They were pretreated with an oxyquinoline-maltose solution (0.44% oxyuinoline solution) to aid shortening and spreading of chromosomes, fixed with :3:1 ethanol-chloroform-acetic acid Carnoy's fluid, hydrolysed for 6 min in a mixure of three parts of the above fixative and 2 parts of 10% HCl at 60°C, and stained ith acetocarmine (Burns 1964). Anthers for PMC preparations were fixed in arnoy's fluid and stained with acetocarmine (Collins 1979). Pollen viability was stimated as percentage of mature pollen grains stainable in acetocarmine. Photoicrographs were taken using Zeiss's Ergaval microscope with a standard microhotographic gear.

Duncan's test was used to estimate the significance of differences in the mean bialent numbers per cell between the hybrids (Oktaba 1966).

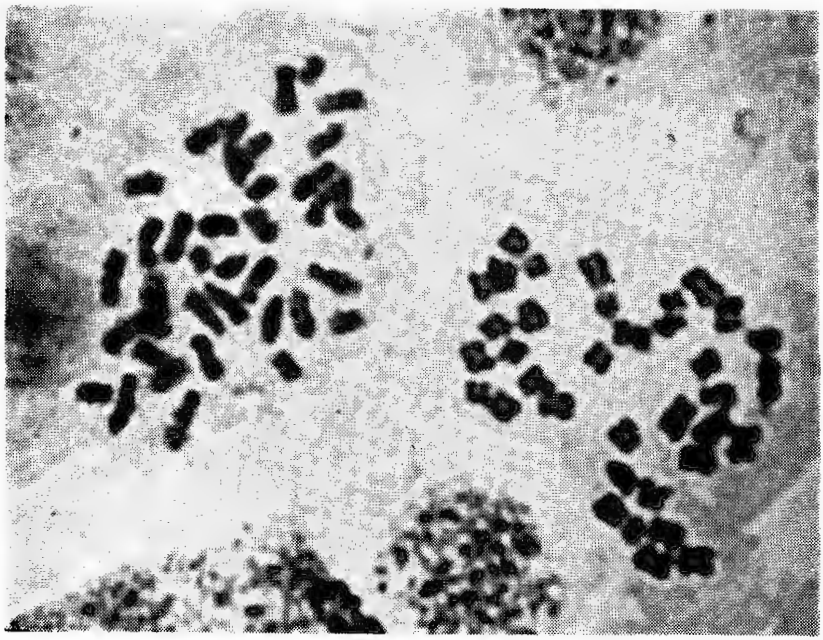

Fig. 1. Mitotic metaphase in the F_1 *N. tabacum* cv. BP-210 × *N. benavidesii*. 36 chromosomes

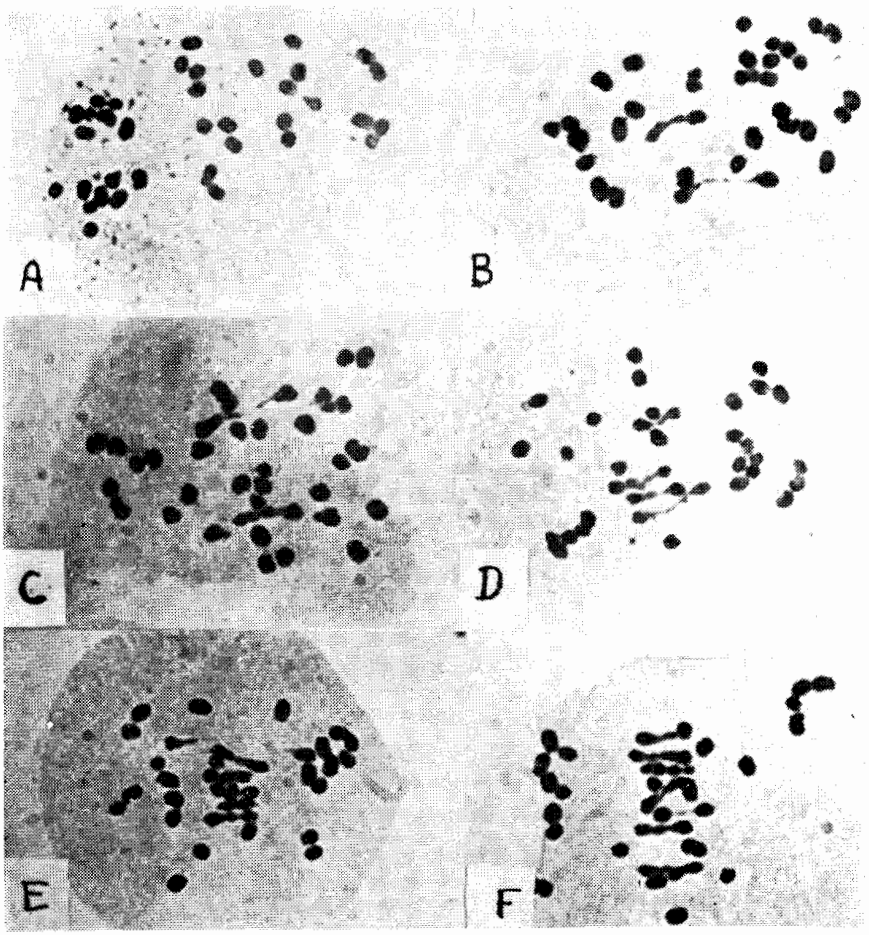

Fig. 2. Metaphase I in the F_1 *N. tabacum* cv. BP-210 × *N. benavidesii*: A 36 univalents; B and C 32 univalents, 1 rod-shaped bivalent with a well-formed chiasma and 1 bivalent with a vestigial chiasma; D 28 univalents and 4 bivalents including one heteromorphic (second from top); E 24 univalents, 1 bivalent showing precoccious disjunction or a pseudo-bivalent (first from top). and 5 regular rod-shaped bivalents; F 21 univalents 1 random univalent association with a pseudochiasma (third from bottom) 5 regular bivalents, and 1 trivalent (first from bottom)

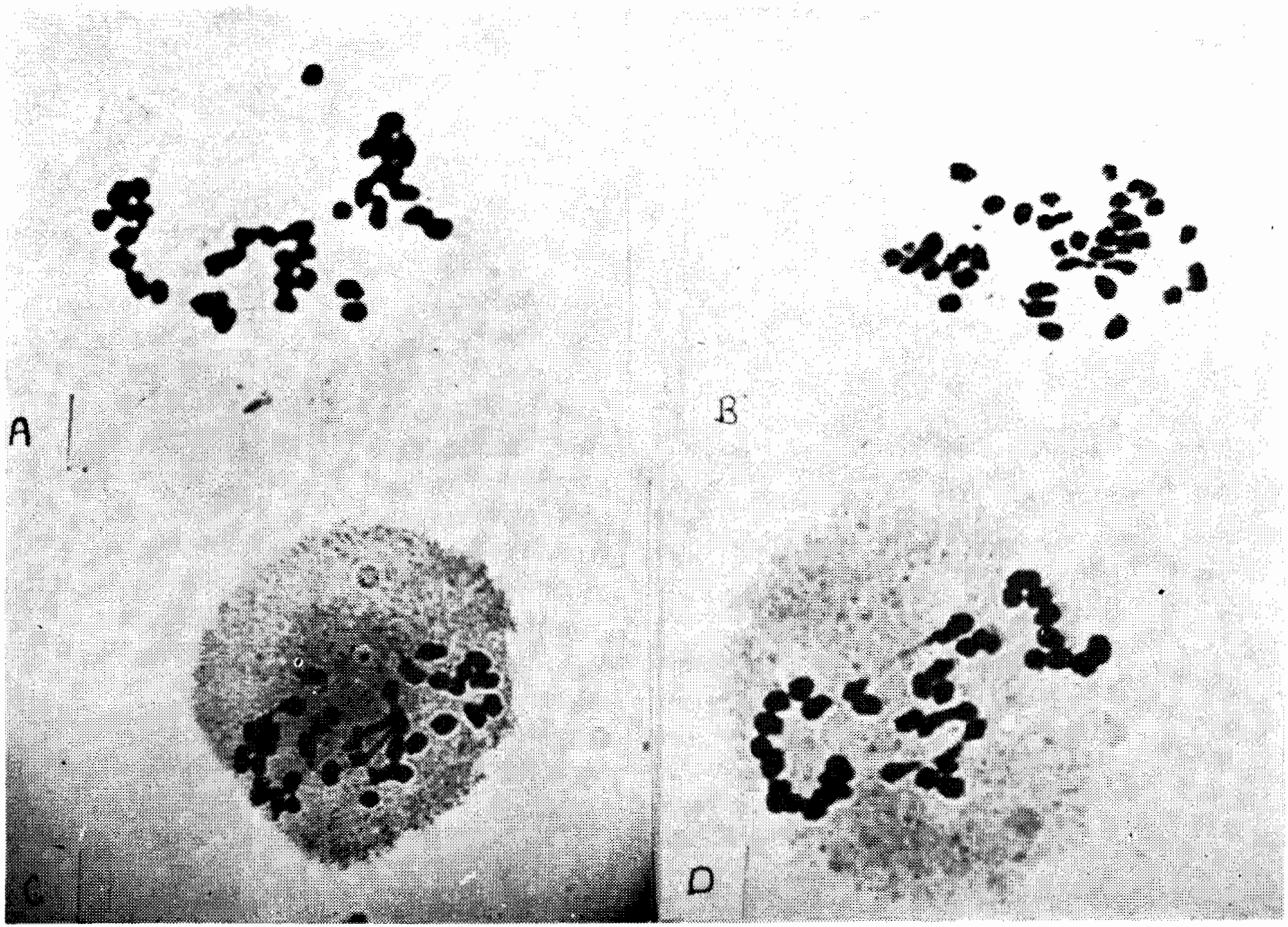

Fig. 3. Metaphase I in the F_1 *N. knightiana* × *N. tabacum* cv. Izyda: A 36 univalents; B 34 univalents and 1 bivalent; C 32 univalents and 2 bivalents including ane heteromorphic; D 30 univalents, 2 bivalents precocciously disjoined, and 1 regular bivalent

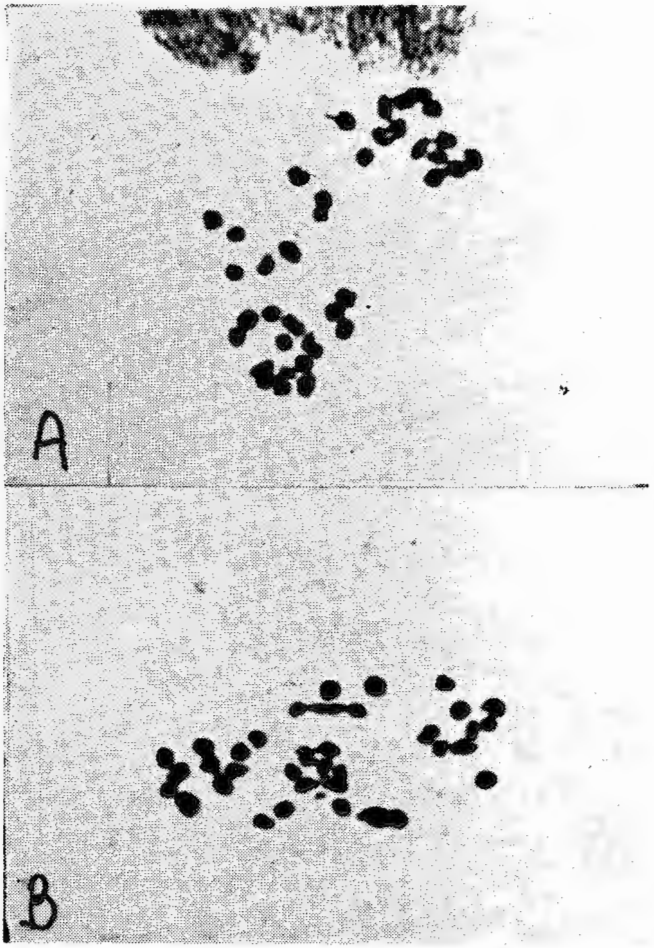

Fig. 4. Metaphase I in the F_1 *N. raimondii* × *N. tabacum* F_1 cv. Zamojska 4× cv. LB-838: A 36 univalents; B 34 univalents and 1 bivalent

RESULTS

The three hybrids — F_1 *N. tabacum* cv. BP-210 × *N. benavidesii*, *N. knightiana* × *N. tabacum* cv. Izyda, and *N. raimondii* × *N. tabacum* F_1 cv. Zamojska 4 × cv. LB-838 — each had 36 chromosomes in their somatic cells (Fig. 1). These numbers indicated that the hybrids were amphihaploids i.e. that they combined haploid chromosome complements of their respective parents.

The amphihaploid nature of the hybrids was confirmed by meiotic configurations found at Metaphase I. They were from fully asynaptic with 36 univalents distributed at random in a PMC (Fig. 2A, 3A, 4A) to partly synaptic with 1 to 6 bivalents at the equatorial plane of the cell (Fig. 2B through 2F, 3B through 3D ynd 4B). There were no large differences in both the bivalent number and bivalent morphology shown by the three F_1 hybrids. As seen in Table 1, the hybrid *N. tabacum* cv. BP-210 × *N. benavidesii* showed a somewhat higher pairing range (from 0 to 6) than the remaining two hybrids (from 0 to 3). Likewise, the mean bivalent number per cell was significantly higher in the F_1 *N. tabacum* cv. BP-210 × *N. benavidesii* (0.96) than in the F_1 *N. knightiana* × *N. tabacum* cv. Izyda (0.20) or in the F_1 *N. raimondii* × *N. tabacum* F_1 cv. Zamojska 4 × cv. LB-838 (0.48). However, the average modal number of bivalents was equal to 0 in all three hybrids.

Within the three hybrids there occurred a certain plant-to-plant variation in the degree of chromosome pairing, especially in the F_1 *N. tabacum* cv. BP-210 × *N. benavidesii*. It seems, that the major source of that variation was the sampling error resulting from a limited number of the observed PMCs.

The bivalents observed in the three hybrids were rod-shaped with one terminal chiasma (Fig. 2, 3 and 4). The chiasma varied from very distinct to vestigal (Fig. 2 and 3) and some of the apparent bivalents could actually be univalents that stuck together at random. Bivalents observed were mostly roughly homomorphic, though some heteromorphic bivalents were also found (Fig. 2F, 3C). Trivalents were very rare and were observed only in the F_1 *N. tabacum* cv. BP-210 × *N. benavidesii* (Fig. 2F).

The plants of the three F_1 hybrids were self- and cross-sterile. On average, stainable pollen grains accounted for 0.5, 3.0, and 0.6% of the total number of pollen grains in the F_1 of *N. tabacum* cv. BP-210 × *N. benavidesii*, *N. knightiana* × *N. tabacum* cv. Izyda, and *N. raimondii* × *N. tabacum* F_1 cv. Zamojska 4 × cv. LB-838, respectively (Table 2). For comparison, those values were over 90% in two cultivated varietes of *N. tabacum* — BP-210 and Zamojska 4.

DISCUSSION

The interspecific F_1 hybrids described in this report can be classified, according to the criterion by Goodspeed (1953), under the "minimum pairing" category as showing the modal number of bivalents equal to 0. Those data are not in complete agreement with the observations made by Goodspeed himself (Goodspeed 1953).

Table 1. Chromosome pairing at Metaphase I in the interspecific F_1 hybrids *N. tabacum* cv. BP-210 × *N. benavidesii*, *N. knightiana* × *N. tabacum* cv. Izyda, and *N. raimondii* × *N. tabacum*-F_1 cv. Zamojska 4 × cv. LB-838

Plant no.	Hybrid	Number of PMCs with bivalents*							Mean bivalent no. per cell	Modal bivalent no.	Total no. of PMCs
		0	1	2	3	4	5	6			
1	*N. tabacum* cv. BP-210 × *N. benavidesii*	65	39	25	7	2	—	—	0.86	0	138
2	,,	24	13	9	4	1	1	—	1.00	0	52
3	,,	29	27	19	12	4	—	1	1.34	0	92
4	,,	48	15	3	3	—	—	—	0.43	0	69
5	,,	3	6	1	5	4	4	—	2.56	1	23
6	,,	34	21	4	1	—	1	—	0.60	0	61
	Mean	34.0	20.2	10.2	5.3	1.8	1.0	0.2	0.96a	0	72.5
1	*N. knightiana* × *N. tabacum* cv. Izyda	84	10	—	—	—	—	—	0.11	0	94
2	,,	69	9	—	1	—	—	—	0.15	0	78
3	,,	66	12	4	1	—	—	—	0.28	0	83
4	,,	80	21	3	—	—	—	—	0.26	0	104
	Mean	74.5	13.0	1.8	0.5	—	—	—	0.20b	0	89.8
1	*N. raimondii* × *N. tabacum*-F_1 cv Z.4 × cv. LB-838	31	9	6	1	—	—	—	0.51	0	47
2	,,	45	14	7	2	—	—	—	0.50	0	68
3	,,	40	11	8	1	—	—	—	0.50	0	60
4	,,	25	8	2	—	—	—	—	0.34	0	35
	Mean	32.5	10.5	5.8	1.0	—	—	—	0.48b	0	52.5

* Higher valencies (trivalents) classified as bivalents

Table 2. Pollen viability in the interspecific F_1 hybrids: *N. tabacum* cv. BP-210 × *N. benavidesii*, *N. knightiana* × *N. tabacum* cv. Izyda, and *N. raimondii* × *N. tabacum* F_1 cv. Zamojska 4 × cv. LB-838 and in two cultivars of *N. tabacum*

Plant no.	Hybrid	Total no. of pollen grains	No. of stainable grains	% of stainable grains
1	*N. tabacum* cv. BP-210 × *N. benavidesii*	567	3	0.5
2	,,	504	1	0.2
3	,,	650	2	0.3
4	,,	732	7	1.0
	Mean	613.2	3.2	0.5
1	*N. knightiana* × *N. tabacum* cv. Izyda	388	5	1.3
2	,,	1196	43	3.6
	Mean	792	24.0	3.0
1	*N. raimondii* × *N. tabacum*-F_1	550	4	0.7
2	cv. Z. 4 × cv. LB-838	446	1	0.2
3	,,	654	6	0.3
4	,,	522	3	0.6
	Mean	543.0	3.5	0.6
	N. tabacum cv. BP-210	345	321	93.0
	N. tabacum cv. Zamojska 4	450	443	98.5

The latter investigator included the hybrids *N. tabacum* × *N. benavidesii* and *N. raimondii* × *N. tabacum* in the "low variable pairing" group, based on the range of 0 - 6 bivalents with a mode of 3 in the former hybrid and the range of 0 - 7 with a mode of 2 in the latter one. Kostoff (1943) reported an even higher pairing range for his F_1 *N. raimondii* × *N. tabacum* (from 3 to 6). A pairing range wider than that observed in this study was also reported for the F_1 *N. knightiana* × *N. tabacum* (Takenaka 1962).

There may be numerous causes of that discrepancy such as different parental genotypes or effect of ambient temperature. However, the most plausible explanation seems to be that related to the observation error. In low-pairing interspecific hybrids such as those dealt with in this study there frequently occur random univalent associations (Goodspeed 1953) and the distinction between these associations and true bivalents is often blurred. In this study all doubtful cases were tended to be treated as univalents joined by chance rather than paired bivalents. Such a policy might affect the actual numbers observed and it may account for the consistently lower chromosome pairing values as compared to those obtained by other authors.

Both our data and those reported earlier, regardless of the absolute figures, indicate a somewhat higher chromosome homology in the F_1 *N. tabacum* × *N. benavidesii* than in either *N. knightiana* × *N. tabacum* or *N. raimondii* × *N. tabacum*. It is consistent with the systematic position of *N. benavidesii* in the section *Paniculatae*. Unlike the "core species", *N. knightiana* and *N. raimondii*, *N. benavidesii* is an off-center species. Through *N. glutinosa*, to which it bears considerable resemblance, it is related to the section *Tomentosae* from which comes one of the postulated progenitors of *N. tabacum*.

The irregular and random chromosome distribution to the gametes, an obvious consequence of the uneven numbers of parental chromosomes and of the lack of homology between them, was the major cause of sterility in the three hybrids.

It can be assumed with a fair degree of certainty that the few pollen grains formed by the hybrids were derived from restitution nuclei. Kostoff (1943) hypothesized that crossing-over in inverted chromosome fragments during a hybrid meiosis may generate acentric chromosome fragments and their loss may lead to the formation of genetically deficient and unviable restitution gametes. Thus the lowest pollen viability encountered in the F_1 *N. tabacum* cv. BP-210 × *N. benavidesii* may be explained in terms of Kostoff's hypothesis as related to a relatively higher chromosome homology in that hybrid.

The data on chromosome pairing in the three hybrids suggest that, of the three wild species under consideration, *N. benavidesii* has the best potential for gene transfer to *N. tabacum*. Such an assumption is based on a well-established notion that chromosome homology or partial homology may be critical for a segmental substitution to occur (Clausen, Cameron 1957) and it can also facilitate the substitution of an entire chromosome (Gerstel 1946).

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KONIUGACJA CHROMOSOMÓW I ŻYWOTNOŚĆ ZIARN PYŁKU TRZECH MIĘDZYGATUNKOWYCH MIESZAŃCÓW F_1: *N. TABACUM* × *N. BENAVIDESII*, *N. KNIGHTIANA* × *N. TABACUM* I *N. RAIMONDII* × *N. TABACUM*

Streszczenie

W wyniku krzyżowania tytoniu uprawnego *N. tabacum* ($2n=48$) z trzema gatunkami sekcji ***Paniculatae*** ($2n=24$) otrzymano mieszańce F_1. Mieszaniec F_1 *N. tabacum* cv. BP-210 × *N. benavidesii* tworzył od 0 do 6 biwalentów, przy średniej modalnej równej 0. Mieszańce F_1 *N. knightiana* × *N. tabacum* cv. Izyda i *N. raimondii* × *N. tabacum*-F_1 cv. Zamojska 4 × cv.

LB-838 tworzyły od 0 do 3 biwalentów, przy czym średnia modalna wynosiła również 0. Średnia arytmetyczna liczby biwalentów przypadających na 1 komórkę macierzystą pyłku była u mieszańca F_1 *N. tabacum* cv. BP-210 × *N. benavidesii* istotnie wyższa niż w przypadku pozostałych dwóch mieszańców, co wskazuje na bliższy stopień pokrewieństwa *N. benavidesii* z *N. tabacum*. Badane trzy mieszańce wytwarzały prawie całkowicie nieżywotny pyłek.

КОНЬЮГАЦИЯ ХРОМОСОМ И ПЛОДОВИТОСТЬ ПЫЛЬЦЫ У МЕЖВИДОВЫХ ГИБРИДОВ F_1 ОТ СКРЕЩИВАНИЯ *NICOTIANA TABACUM L.* × *N. BENAVIDESII* GOODSPEED, *N. KNIGHTIANA* GOODSPEED × *N. TABACUM* И *N. RAIMONDII* MACBRIDE × *N. TABACUM*

Резюме

Получены гибриды F_1 от скрещивания *Nicotiana tabacum* ($2n=48$) с тремя видами секции *Paniculatae* ($2n=24$). Гибрид F_1 от скрещивания *N. tabacum* cv. BP-210 × *N. benavidesii* имел от 0 до 6 бивалентов с модальным числом 0. Гибриды F_1 от скрещивания *N. knightiana* × *N. tabacum* cv. Izyda, *N. raimondii* × *N. tabacum* и cv. Zamojska 4 × cv. LB-838 имели от 0 до 3 бивалентов каждый с модальным числом 0. Среднее число бивалентов на клетку у гибрида F_1 от скрещивания *N. tabacum* × *N. benavidesii* было значительно выше, чем у остальных гибридов, что указывало на то, что из трёх видов *N. benavidesii* имело наиблисшее родство с *N. tabacum*. Эти три гибрида производили в основном стерильную пыльцу.