

A. Berbeć

Variation among offspring of alloplasmic tobacco *Nicotiana tabacum* L. cv 'Zamojska 4' with the cytoplasm of *N. knightiana* Goodspeed

Received: 22 November 1993 / Accepted: 12 January 1994

Abstract Progeny obtained from single plants of cultivated tobacco *Nicotiana tabacum* cv 'Zamojska 4' and its isonuclear analogue with the cytoplasm of *N. knightiana* were compared for 16 agronomic traits. The alloplasmic strain showed reduced self-fertility, increased 1000-seed weight, lower plant height, lower leaf width, and reduced yield and money-value of cured leaves, most of which were fairly common effects of an alien cytoplasm. However, some of these parameters such as plant height, leaf width and length/width ratio, yield and money-value of the crop, and, less regularly, leaf number and fertility, underwent further reduction in the selfed offspring of the alloplasmic strain. Furthermore, among those offspring, especially in the S_2-and S_3-selfed generations, a significant progeny-to-progeny variation was found for the majority of traits studied, the extent of which far exceeded that encountered in the selfed offspring of the autoplasmic paternal cultivar.

Key words *Nicotiana tabacum* · Alloplasmic forms Cytoplasmic inheritance · Agronomic traits

Introduction

Cytoplasmic inheritance in higher plants, although long recognized and in many cases well documented, is still not fully understood in all its manifestations. Over the last two decades, advances in molecular genetics and the development of biotechnology, including protoplast fusion, have increased our understanding of extrachromosomal inheritance and the possibilities for manipulating genetic factors resident in the cytoplasm. None-the-less, the significance of the cytoplasm in total genetic variation continues, to some extent, to be an area of speculation rather than of systematic knowledge. Cytoplasmic male sterility and the associated effects seem to be the best explored aspects of cytoplasmic inheritance in higher plants mainly because of the economic importance of this trait. However, since it can be maintained solely by repeated backcrossing, cytoplasmic male sterility imposes some serious limitations on the study of cytoplasmic-nuclear interactions. The alloplasmic strain of *Nicotiana tabacum* L. cv 'Zamojska 4' with the cytoplasm of *N. knightiana* Goodspeed provides a genetic system, not frequent in tobacco, in which alien cytoplasm substitution is associated with partial male fertility and, consequently, the ability to produce selfed offspring (Berbeć and Doroszewska 1992). This system gives an opportunity to study the effect of cytoplasmic factors on the stability and integrity of a cultivar reproduced by selfing. In Poland, there has been an unwritten rule in tobacco breeding that domestic forms should be used as maternal components in cultivar development to secure a stable and well-adapted variety. However, that recommendation, although conscientiously followed by tobacco breeders, was based on lore rather than knowledge since no attempt was ever made to back it up by experimental evidence. The primary aim of the present study was to find out if the substitution of an alien "undomesticated" cytoplasm may in any way interfere with the true-breeding character of a cultivar.

Materials and methods

In the 1970s the flue-cured cultivar 'Zamojska' 4 was grown over large areas in Poland and employed as a standard in official variety tests. Withdrawn from cultivation several years ago, it has continued to be used as the recurrent male parent in the isonuclear collection of alloplasmic strains of tobacco involving different *Nicotiana* cytoplasms.

The alloplasmic strain of *N. tabacum* cv 'Zamojska 4' with the cytoplasm of *N. knightiana* was developed conventionally from the *N. knightiana* × *N. tabacum* hybrid (Berbeć and Doroszewska 1992). It was increased over nine generations by backcrossing to the parental cultivar 'Zamojska 4' before being used in a self-fertilization program.

Communicated by K. Tsunewaki

A. Berbeć (✉)
Department of Special Crops,
Institute of Soil Science and Plant Cultivation,
Osada Palacowa,
24-100 Pulawy, Poland

Table 1 Designation of offspring derived from *Nicotiana tabacum* cv 'Zamojska 4' and its alloplasmic counterpart with the cytoplasm of *N. knightiana*

Progeny group	No. of progeny		Source of cytoplasm	Generation [a]	
	1991	1992		1991	1992
A	12	10	*N. tabacum*	–	
B	2	7	*N. knightiana*	BC_{12}	BC_{13}
C	3	3	*N. knightiana*	$BC_{11}S_1$	$BC_{12}S_1$
D	22	5	*N. knightiana*	$BC_{10}S_2$	$BC_{11}S_2$
E	5	10	*N. knightiana*	BC_9S_3	$BC_{10}S_3$
F		2	*N. knightiana*		BC_9S_4

[a] Number of selfed generations unknown (higher than 15); BC, backcrossing (fertilization with pollen of cv 'Zamojska 4'); S, selfing of the alloplasmic strain

Progeny obtained from single plants of cv 'Zamojska 4' and its alloplasmic counterpart were investigated in the present study. These progeny were divided into groups depending on their origin. The groups were assigned capital letters from A to F for easier tabular presentation of the data. The origin of the progeny in each group is explained in Table 1.

The groups comprised different, sometimes small, numbers of progeny due to technical reasons. Backcross number (BC_x) is the number of repeated backcrosses to male 'Zamojska 4' in the pedigree of a progeny group. Likewise, S_x denotes selfed generations: from 1st to 4th. The number of selfed generations for cv 'Zamojska 4' since the inception of the cultivar was difficult to establish because of its origin outside this Department. In 1990, as part of the department's germplasm collection, the cultivar had been increased from single plants for 15 generations.

The investigations reported here were carried out in the field during the years 1991–1992. Since a large number of entries were compared, relatively small one-row plots were used because of space limitations. The distances were 60 cm between rows and 40 cm between plants. There were 28 plants per plot in experiment 1991, 24 in experiment 1992 a, and 28 in 1992 b. Growth and development records were taken from 20 plants in 1991 and 15 plants in 1992. The entries were replicated three times in 1991 but only twice in 1992. Standard husbandry practices for flue-cured tobacco culture were applied.

Data were collected on the following parameters: plant height, leaf number/plant, days to flower, length/width ratio of the 8th–10th (midposition) leaves, fresh leaf weight, cured leaf weight by grades, fertile capsule number/plant, selfed seed weight/plant, and 1000-seed weight. Internode length was calculated as the plant weight/leaf number ratio. Seed weight/capsule was calculated as the seed weight per plant/capsule number per plant ratio. Leaf area was estimated as leaf length × leaf width × 0.675 according to the formula accepted for Virginia-type tobacco. The percent dry matter was calculated from the cured leaf weight/fresh leaf weight ratio; the percent light grades was the ratio of the weight of leaves in the 1st, 2nd and 3rd grades to the total weight of cured leaves; money-value/ha was the value of cured leaves harvested from a single plot, converted to 1 ha and calculated in 1991 prices.

For self-fertility studies, five plants at full flowering were picked for each entry on the same day. After removing expanded flowers and seed capsules the inflorescences were bagged. Care was taken to choose plants with similarly-developed inflourescences. After a month bags were removed and the seed heads cleared of flowers and flower buds. When mature, seed heads were harvested and the above parameters determined.

The experiments were laid out as randomized block designs. A hierarchic classification was used in the analysis of variance and the least significant differences (LSD) were calculated for each group of entries using Tukey's test at the 0.05 significance level.

Results

The performance of the investigated progenies is listed in Tables 2 to 5. Group means for plant height, leaf number, leaf width, leaf area, yields, and the money-value of cured leaves, were consistently higher in progenies derived from cv 'Zamojska 4' (group A) than in their isonuclear alloplasmic counterparts (groups B–E or F). Leaf length/width ratio was consistently larger in the alloplasmics than in the counterpart autoplasmics. The self-fertility of the progenies studied was also dependent on the source of cytoplasm. The alien cytoplasm of *N. knightiana* drastically reduced the number of seed capsules set within a specified period and, to an even larger extent, the seed weight per plant and the number of seeds per capsule, but significantly increased the weight of 1 000 seeds. The response of the other traits to the source of cytoplasm was less regular. The autoplasmic cultivar had longer leaves than its alloplasmic counterpart but only in experiment 1992 a. Internode length, days to flower, dry-matter percentage in cured leaves and percentage of superior (light) grades, were little affected by the alien cytoplasm. However, among the alloplasmic groups there were differences for a number of traits. Plant height, leaf width, yield of cured leaves, money-value of the crop and, less consistently, leaf number and self-fertility parameters, tended to be lower in alloplasmics derived from selfing compared to their counterparts maintained by backcrossing to the autoplasmic cultivar. Crop-quality indicators (dry-matter percentage and light grades) also showed some group-to-group differences within the alloplasmic strain but those differences did not follow any regular pattern.

The range between the highest and the lowest progeny mean within a group (see Tables 2 to 5) was compared with the appropriate LSD value and the outcome was used as a measure of genetic diversity within the groups (Table 6). As expected of a true-breeding variety, in all but two cases the progeny-to-progeny differences within the autoplasmic group A were insignificant for the 16 parameters studied. In all alloplasmic groups, except the very small group F, the number of maximum within-group differences above the significance level was higher. It was four for the backcross-derived group B, five for the selfed group C (S_1 generation), and as much as 24 and 32 for groups D and E (generations S_2 and S_3), respectively. In terms of percentage, the incidence of the maximum within-group difference higher than the LSD calculated over the years and experiments was from 3.6% for group F to 72.3% for group E.

Discussion

The depressive effect of alien cytoplasm on the agronomic performance of alloplasmic substitution races is a fairly common phenomenon known from many crop species and is a frequent problem in developing cms cultivars and hy-

Table 2 Performance of single-plant progenies derived from *N. tabacum* cv 'Zamojska 4' (group A) and its alloplasmic counterpart with the cytoplasm of *N. knightiana* (groups B–F): growth characteristics

Group	Plant height (cm)			Leaf number			Internode length (cm)			Days to flower		
	Mean	Range		Mean	Range		Mean	Range		Mean	Range	
		Max	Min		Max	Min		Max	Min		Max	Min
(a) Experiment 1991												
A	178.3	186.5	170.8	29.2	30.7	28.1	6.12	6.58	5.84	101.1	111.0	91.7
B	166.8	168.3	165.2	25.7	26.0	25.5	6.48	6.49	6.47	98.8	99.0	98.7
C	159.0	163.7	152.9	27.2	27.9	26.2	6.26	6.26	5.60	104.2	111.7	97.7
D	148.2	170.0	118.1	26.3	29.4	23.8	6.58	6.58	4.28	104.4	116.7	92.3
E	154.1	172.2	139.6	25.3	27.8	23.2	7.07	7.07	5.03	99.3	114.0	87.3
LSD	10.8			0.8			0.46			5.1		
(b) Experiment 1992 a												
A	194.9	198.2	191.7	31.0	32.2	30.3	6.32	6.52	6.13	87.9	90.9	85.4
B	176.9	181.8	173.0	29.3	29.7	28.4	6.07	6.23	5.83	85.6	88.7	82.7
C	169.9	170.6	168.8	28.9	29.4	28.6	5.91	5.96	5.86	88.7	89.2	87.9
D	171.1	176.8	160.7	28.3	29.4	25.8	6.07	6.23	5.84	85.0	88.4	81.8
E	168.2	179.1	155.3	27.9	30.5	26.0	6.07	6.85	5.60	87.3	93.4	82.0
F	157.0	169.0	155.1	27.5	27.8	27.1	5.71	5.73	5.72	88.7	84.9	82.5
LSD	9.2			1.4			0.41			4.3		
(c) Experiment 1992 b												
A	113.7	116.5	108.0	32.5	33.9	31.1	3.51	3.68	3.32	93.4	96.8	89.2
B	97.0	106.9	86.4	28.5	29.9	26.7	3.44	3.84	3.06	92.2	96.0	86.4
C	91.4	95.5	89.0	28.6	28.8	28.3	3.20	3.30	3.14	98.8	101.0	95.7
D	92.0	94.4	86.9	27.9	30.7	25.3	3.33	3.71	3.06	91.5	96.4	85.5
E	91.5	99.5	76.1	27.1	29.5	24.7	3.41	3.94	2.88	91.1	97.0	85.5
F	89.2	91.2	87.2	26.7	27.3	26.1	3.39	3.52	3.26	88.4	91.0	85.7
LSD	9.2			1.9			0.31			5.0		

Table 3 Performance of single-plant progenies derived from *N. tabacum* cv 'Zamojska 4' (group A) and its alloplasmic counterpart with the cytoplasm of *N. knightiana* (groups B–F): middle leaf measurements

Group	Length (cm)			Width (cm)			Length/width ratio			Area (cm^2)		
	Mean	Range		Mean	Range		Mean	Range		Mean	Range	
		Max	Min		Max	Min		Max	Min		Max	Min
(a) Experiment 1991												
A	40.2	43.5	37.5	23.1	25.0	21.0	1.76	1.82	1.69	641	727	545
B	40.9	41.5	40.3	21.9	22.1	21.8	1.92	1.92	1.92	604	614	594
C	38.9	40.1	37.6	19.4	20.4	18.6	2.01	1.98	1.98	514	555	477
D	38.8	42.7	33.5	19.6	22.6	17.0	2.00	1.78	1.78	524	624	412
E	38.6	41.1	36.6	20.0	21.3	19.2	1.95	1.86	1.86	530	601	499
LSD	1.7			1.3			0.06			54		
(b) Experiment 1992 a												
A	44.3	45.8	41.9	25.7	26.6	23.9	1.74	1.80	1.70	748	819	678
B	39.1	42.1	37.3	20.0	21.2	18.9	1.97	2.01	1.93	502	606	480
C	38.6	39.7	37.9	19.6	20.2	19.2	1.98	1.99	1.98	490	546	499
D	38.6	40.1	37.5	20.3	21.2	19.3	1.92	2.03	1.84	529	579	511
E	40.7	45.8	32.4	20.9	24.8	16.7	1.98	2.13	1.82	518	761	410
F	37.7	38.3	37.2	20.0	20.5	18.6	1.95	2.02	1.87	449	539	473
LSD	2.5			1.8			0.10			78		
(c) Experiment 1992 b												
A	33.5	34.7	32.2	19.3	19.9	18.9	1.74	1.78	1.68	447	471	428
B	32.2	33.6	31.2	17.0	17.8	16.0	1.91	1.96	1.88	376	407	348
C	31.7	33.1	30.8	16.0	17.5	15.2	1.99	2.08	1.91	354	402	328
D	31.3	32.2	30.2	16.2	17.3	15.4	1.94	2.02	1.86	352	387	319
E	33.2	34.9	30.6	16.7	20.0	15.1	1.99	2.17	1.68	387	459	318
F	31.6	32.2	31.1	15.9	16.4	15.4	2.00	2.02	1.98	348	361	336
LSD	1.6			1.4			0.08			47		

Table 4 Performance of single-plant progenies derived from *N. tabacum* cv 'Zamojska 4' (group A) and its alloplasmic counterpart with the cytoplasm of *N. knightiana* (groups B–F): yield parameters

Group	Yield ($t \cdot ha^{-1}$)			Dry matter (%)			Money value 1 000 000 $zl \cdot ha^{-1}$)			Light grades (%)		
	Mean	Range		Mean	Range		Mean	Range		Mean	Range	
		Max	Min		Max	Min		Max	Min		Max	Min
(a) Experiment 1991												
A	1.90	2.31	1.57	15.5	16.4	14.6	20.0	23.2	15.5	20.8	35.3	13.9
B	1.86	1.87	1.81	16.2	16.4	16.0	19.3	19.5	19.2	11.8	11.8	11.7
C	1.61	1.71	1.54	17.0	17.1	16.9	17.6	18.4	16.7	26.0	31.4	19.7
D	1.40	1.94	0.75	16.4	18.7	12.7	14.3	18.8	8.6	19.0	31.2	13.3
E	1.16	1.25	0.95	17.3	20.0	14.6	21.1	13.2	8.7	12.2	22.0	6.2
LSD	0.10			S*			2.3			S*		
(b) Experiment 1992 a												
A	2.76	2.83	2.62	15.8	16.4	15.4	32.1	34.3	30.6	27.6	35.5	21.4
B	2.30	2.50	2.16	14.9	15.5	13.9	26.4	29.1	24.5	24.6	35.0	17.5
C	2.08	2.22	1.84	14.8	15.2	14.6	23.8	25.7	20.3	25.7	28.8	20.6
D	2.13	2.38	1.96	15.2	16.3	14.4	25.5	28.2	22.9	33.4	55.2	21.8
E	2.06	2.62	1.53	15.3	16.7	12.9	24.2	31.4	17.0	29.3	47.9	17.0
F	2.02	2.11	1.93	14.7	14.7	14.7	22.4	22.8	22.0	24.1	33.5	14.7
LSD	0.25			S*			3.4			S*		
(c) Experiment 1992 b												
A	1.38	1.45	1.28	20.0	20.4	19.4	24.2	27.7	22.5	23.8	27.5	17.0
B	1.19	1.28	1.11	19.8	20.6	19.3	20.9	21.8	17.5	21.7	28.8	13.3
C	1.23	1.36	1.03	20.1	20.2	19.9	20.7	22.5	18.1	25.2	25.8	24.4
D	1.03	1.16	0.96	19.4	21.0	18.3	19.6	21.5	17.6	16.6	23.5	13.3
E	1.00	1.18	0.90	18.8	20.9	17.2	18.3	24.4	16.1	26.6	38.8	16.0
F	1.01	1.04	1.00	20.2	20.8	19.6	18.7	20.3	17.2	28.0	28.7	27.3
LSD	0.10			NS*			2.7			S*		

* S (significant) vs NS (non-significant) rather than numeric LSD values are given for percentage-expressed traits because the analysis of variance was performed on Bliss angular degrees

Table 5 Performance of single-plant progenies derived from *N. tabacum* cv 'Zamojska 4' (group A) and its alloplasmic counterpart with the cytoplasm of *N. knightiana* (groups B–F): self-fertility indicators

Group	Capsule number/plant			Seed weight/plant			Seed number/capsule (1 000)			1 000-seed weight (g)		
	Mean	Range		Mean	Range		Mean	Range		Mean	Range	
		Max	Min		Max	Min		Max	Min		Max	Min
(a) Experiment 1991												
A	127	144	100	15.57	18.14	13.50	2.04	2.41	1.63	0.059	0.061	0.058
B	47	54	39	0.53	0.76	0.46	0.16	0.22	0.12	0.072	0.072	0.071
C	29	61	16	0.18	0.45	0.10	0.08	0.08	0.08	0.074	0.074	0.071
D	32	100	7	0.19	0.62	0.04	0.10	0.16	0.02	0.075	0.075	0.066
E	40	77	14	0.28	0.41	0.12	0.11	0.16	0.07	0.076	0.076	0.072
LSD	11			0.09			0.03			0.003		
(b) Experiment 1992 a												
A	164	181	129	19.92	21.70	16.90	1.86	2.05	1.63	0.065	0.067	0.063
B	62	71	47	0.68	0.87	0.61	0.16	0.18	0.13	0.070	0.072	0.066
C	52	58	41	0.56	0.69	0.44	0.16	0.19	0.11	0.070	0.071	0.068
D	64	87	50	0.54	0.88	0.30	0.12	0.17	0.07	0.072	0.076	0.068
E	46	72	20	0.42	0.64	0.15	0.14	0.32	0.08	0.070	0.076	0.057
F	37	46	28	0.36	0.42	0.30	0.14	0.15	0.13	0.069	0.070	0.067
LSD	9			0.14			0.04			0.003		

Table 6 Results of analysis of variance in groups of single-plant progenies derived from *N. tabacum* cv 'Zamojska 4' and its alloplasmic counterpart with the cytoplasm of *N. knightiana*: significance of the maximum difference of means (range) within a group[a]

Source of variation	A			B			C			D			E			F		
	1991	1992 a	1992 b	1991	1992 a	1992 b	1991	1992 a	1992 b	1991	1992 a	1992 b	1991	1992 a	1992 b	1991	1992 a	1992 b
1. Plant height	NS	NS	NS	NS	NS	NS	NS	NS	NS	*	NS	NS	*	NS	*		NS	NS
2. Leaf number	NS	NS	NS	NS	NS	NS	NS	NS	NS	*	*	*	*	*	*		NS	NS
3. Internode length	NS	NS	NS	NS	NS	*	NS	NS	NS	*	NS	*	*	*	*		NS	NS
4. Days to flower	*	NS	NS	NS	NS	NS	*	NS	NS	*	NS	*	*	*	NS		NS	NS
5. Leaf length	NS	NS	NS	NS	NS	NS	NS	NS	NS	*	NS	NS	NS	*	*		NS	NS
6. Leaf width	NS	NS	NS	NS	NS	NS	NS	NS	NS	*	NS	NS	NS	*	*		NS	NS
7. Length/width ratio	NS	NS	NS	NS	NS	NS	NS	*	NS	*	*	NS	NS	*	*		NS	NS
8. Leaf area	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	*	*		NS	NS
9. Cured leaf yield	NS	NS	NS	NS	NS	NS	NS	NS	*	*	NS	NS	NS	*	*		NS	NS
10. Percent dry matter	NS	NS	NS	NS	NS	NS	NS	NS	NS	*	*	NS	*	*	NS		NS	NS
11. Money value per ha	NS	NS	NS	NS	NS	NS	NS	NS	NS	*	NS	NS	NS	*	*		NS	NS
12. Percent light grades	*	NS	NS	NS	NS	NS	NS	NS	NS	NS	*	NS	*	*	NS		NS	NS
13. Capsule number/plant	NS	NS		NS	*		*	NS		*	*		*	*			*	
14. Seed weight/plant	NS	NS		*	NS		*	NS		*	*		*	*			NS	
15. Seed number/capsule	NS	NS		*	NS		NS	NS		*	NS		NS	*			NS	
16. 1000-seed weight	NS	NS		NS	NS		NS	NS		*	*		*	NS			NS	
% of significant differences	4.5			9.1			11.4			54.5			72.3			3.6		

[a], * and NS: significant and non-significant at the 0.05 level, respectively

brids. Thus, the negative influence of *N. knightiana* cytoplasm on several agronomic traits observed in this study is in line with what has been repeatedly observed for other alien cytoplasm sources (Gerstel 1980).

However, possible effects that the alien cytoplasm might have on the integrity of a cultivated variety increased by selfing has not been the subject of any extensive study. The interest of tobacco breeders in cytoplasmic systems has been limited almost exclusively to obtaining and transferring male sterility, and cytoplasmic male-sterile stocks, by their very nature, are maintained by backcrossing. In species other than tobacco, there are several reports that indicate the existence of links between the chromosomal and the organelle-based systems of heredity. At the molecular level, homologous DNA sequences were found, e.g., in the mitochondria and the nuclei of maize (Kemble et al. 1983) and the chloroplasts and nuclei of spinach (Scott and Timmis 1984), an interesting clue that there may be events involving an exchange of genetic information between the chromosomes and the organelles. Another route through which the cytoplasm may influence the makeup of the nuclear genome is in the process of meiosis. The cytoplasm was found to modify the rate of crossovers (Luginin et al. 1987) and to cause possible chromosomal structural changes since anaphase chromatin bridges were observed in some alloplasmic substitutions (Zenkteler 1962; Berbeć 1993). Transmission rates of certain recessive genes in wheat (Davydenko 1989) and peanuts (Ashri 1976) were also modified by the cytoplasm as too were the dominance effects of certain chromosome-borne traits (Molchan and Lezzhova 1981). Yet another effect of cytoplasm was on the frequency of mutations caused by irradiation. In Davydenko's (1989) opinion, the cytoplasm may significantly modify the frequency and expression of nuclear alleles through influencing mutagenic processes, recombination, and gamete selection. In tobacco, one of the few documented cases of the link between the cytoplasmic and nuclear genetic systems is the induction of cytoplasmic male sterility in two interspecific hybrids of tobacco involving the wild species *N. glutinosa* and *N. plumbaginifolia* as male parents (Burk 1960). In these two hybrids the induction of cms must have occurred in early backcross generations, possibly in a fashion similar to the induction of cytoplasmically-inherited male sterility by a nuclear gene in maize (Rhoades 1950). A notable piece of direct evidence that alien cytoplasm may increase genetic variability in a cultivated species was provided by Tsunewaki et al. (1985) who studied F_1 and F_2 generations of an intervarietal wheat hybrid with the cytoplasm of *Aegilops kotschyi*.

Cytoplasmic inheritance per se is considered to be highly conservative (Rode et al. 1985) and cytoplasmic traits are assumed to be subject to little variation. Hence, the increased variation in the offspring of a semi-male-sterile alloplasmic strain of tobacco with the cytoplasm of *N. knightiana* may be more plausibly explained by the effect of the alien cytoplasm on the nuclear makeup of the cultivar and especially on the frequency of mutations. Those small mutations may have gradually accumulated resulting in the fairly substantial variability observed in advanced selfed generations.

A certain analogy can be drawn between the phenomena reported in this study and the variability observed among the offspring of dihaploids obtained from anther culture in tobacco (Schnell and Wernsman 1986). The variability of dihaploids is most probably caused by the mutagenicity of androgenesis in vitro (Reed et al. 1991). Both in the present study and in the investigations of the dihaploids, the end result was the apparent "disintegration" of the initial genotype into lines that differed from one another for a number of traits. Another common feature of the variation among the offspring of dihaploids and the offspring of the alloplasmic strain was the generally depressive character of the observed variation. For some traits investigated in this study (plant height, leaf yield, crop value, yield of selfed seeds) that depressive effect tended to increase with selfing, a phenomenon in a way similar to those associated with the inbreeding of allogamous forms.

Of other possible explanations of the observed variability, the effect of residual nuclear germplasm from *N. knightiana* can be safely discarded. *N. knightiana* shows minimal chromosomal homology with *N. tabacum* (Berbeć 1987); hence, any alien germplasm introgressed through rare recombination events is sure to have been eliminated over nine repeated backcrosses to the male cultivated parent.

However, the strain used in this study was atypical in that its ability to produce functional germinating pollen was strongly impaired (Berbeć 1994). Consequently, another viable explanation of the existing variation among its offspring can be based on the practically equal opportunity of all sperm cells to reach the ovary and fertilize the egg cells because of the drastically-reduced competition of male gametophytes. Thus, spontaneous minor mutations that had a negative selective value at the gametophyte level, and hence normally eliminated, might have had a chance to be transferred to offspring. On the other hand, by token of the very same process, any nuclear mutations caused by the presence of alien cytoplasm could have had a substantially-better chance of being transmitted to and expressed in the offspring.

Whether the phenomenon observed in this study can be actually related to changes in the nuclear genotype brought on by the cytoplasm, and especially whether such an explanation is valid beyond this particular plasmon/genome combination, are moot questions at this stage. However, although by no means direct or conclusive, it seems to be the first piece of evidence in support of the rationality of the long-standing tradition of choosing domestic varieties as maternal parents in tobacco breeding projects.

References

Ashri A (1976) Plasmon divergence in peanuts (*Arachis hypogea*). A third plasmon and locus affecting growth habit. Theor Appl Genet 48:17–21

Berbeć A (1987) Chromosome pairing and pollen fertility in the interspecific F_1 hybrids *Nicotiana tabacum* L.×*N. benavidesii* Goodspeed, *N. knightiana* Goodspeed×*N. tabacum* and *N. raimondii* Macbride×*N. tabacum*. Genet Polon 28:263–269

Berbeć A (1994) Microsporogenesis in two alloplasmic isonuclear analogues of tobacco cv 'Zamojska 4' with the cytoplasm of *N. knightiana* Goodspeed and *N. raimondii* Macbride. Genet Polon 35:33–41

Berbeć A, Doroszewska T (1992) Alloplasmic forms of cultivated tobacco with the substituted cytoplasms of *Nicotiana amplexicaulis*, *N. knightiana* and *N. raimondii* (in Polish). Pamiętnik Puławski 100:142–150

Burk LG (1960) Male-sterile anomalies in interspecific tobacco hybrids. J Hered 51:27–31

Davydenko OG (1989) The role of cytoplasmic variation in plant evolution and breeding (in Russian). Cytol Genet 23:72–78

Gerstel DU (1980) Cytoplasmic male sterility in *Nicotiana* (a review). North Carolina ARS Tech Bull 283

Kemble RJ, Mans RJ, Gabay-Laughnan S, Laughnan JR (1983) Sequences homologous to episomal mitochondrial DNA in the maize nuclear genome. Nature 304:744–747

Luginin HB, Davydenko OG, Wekshin CP (1987) Effect of cytoplasm on the frequency of sister chromatid exchanges in wheat (in Russian). Citol Genet 21:330–333

Molchan IM, Lezzhova TB (1981) Genetics of the awned ear and the development of a winter wheat cultivar (in Russian). Genetika 17:1842–1849

Reed SM, Wernsman EA, Burns JA (1991) Aberrant cytological behavior in tobacco androgenetic doubled haploid × parental cultivar hybrids. Crop Sci 31:97–101

Rhoades MM (1950) Gene-induced mutation of a cytoplasmic factor producing male sterility in maize. Proc Natl Acad Sci USA:634–635

Rode A, Hartmann C, Dron M, Picard E, Quetier F (1985) Organelle genome stability in anther-derived haploids of wheat (*Triticum aestivum* L. cv Moisson). Theor Appl Genet 71:320–324

Schnell RJ, Wernsman EA (1986) Androgenic somaclonal variation in tobacco and estimation of its value as a source of novel genetic variability. Crop Sci 26:84–88

Scott NS, Timmis JN (1984) Homologies between nuclear and plastid DNA in spinach. Theor Appl Genet 67:279–288

Tsunewaki K, Spetsov P, Yonezawa K (1985) Increasing genetic variability in common wheat by utilization of alien cytoplasms – cytoplasmic effects on the performance and interplant variability of the F_1 and F_2 generations of the cross *Triticum aestivum* cv Norin 26×cv Norin 61. Jpn J Breed 35:398–412

Zenkteler M (1962) Microsporogenesis and tapetal development in normal and male-sterile carrots. Am J Bot 49: 341–348