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Floral Morphology and Some Other Characteristics of Iso-genomic Alloplasmics of *Nicotiana tabacum* L.*

by

Apoloniusz Berbeć

Institute of Soil Science and Plant Cultivation, Poland

SUMMARY

Cytoplasms of several *Nicotiana* species – *N. amplexicaulis, N. bigelovii, N. debneyi, N. eastii, N. exigua, N. glauca, N. glutinosa, N. goodspeedii, N. knightiana, N. occidentalis, N. plumbaginifolia, N. raimondii, N. suaveolens, N. undulata* – were bred into the *N. tabacum* genomic background of flue cured tobacco cv. Zamojska 4. The collection includes also a cytoplasmic male sterile (cms) analogue of cv. Zamojska 4 with mutated cytoplasm of *N. tabacum*. Some of the alloplasmics were originally obtained in this laboratory (*N. amplexicaulis, N. eastii, N. exigua, N. glauca, N. knightiana, N. raimondii*). The remaining ones were acquired from other laboratories and backcrossed into Zamojska 4. All alien cytoplasms except that of *N. knightiana* produced full male sterility in Zamojska 4. The extent of male organ modifications varied from complete absence of stamens (*N. suaveolens, N. tabacum*) to petaloid and stigmatoid structures (most common effect) to malformed stamens (*N. amplexicaulis, N. glauca*) to apparently normal stamens (*N. raimondii, N. knightiana*). The majority of the alloplasmics showed response to tentoxin that was compatible with the cytoplasm donor. The exceptions were those involving *N. exigua, N. raimondii*, (*N. raimondii* I), and the cytoplasmic mutant of *N. tabacum*. There was some variation in growth and morphology among the alloplasmic variants of Zamojska 4. Under field infestation alloplasmics with the cytoplasm of *N. plumbaginifolia* and *N. eastii* showed symptoms of blue mold whereas the remaining alloplasmics and cv. Zamojska 4 were highly tolerant of that disease. [Beitr. Tabakforsch. Int. 19 (2001) 309–14]

ZUSAMMENFASSUNG

Die zytoplasmatischen Charakteristika mehrerer *Nicotiana* Species, und zwar *N. amplexicaulis, N. bigelovii, N. debneyi, N. eastii, N. exigua, N. glauca, N. glutinosa, N. goodspeedii, N. knightiana, N. occidentalis, N. plumbaginifolia, N. raimondii, N. suaveolens, N. undulata*, wurden in das Genom des flue-cured Tabaks cv. Zamojska 4 mittels Züchtung integriert. Die Sammlung beinhaltet ebenfalls ein zytoplasmatisch bedingtes männlich steriles (cms) Analogon von cv. Zamojska 4 mit einem mutierten Cytoplasma von *N. tabacum.* Einige Alloplasmen wurden ursprünglich in diesem Labor erzeugt (*N. amplexicaulis, N. eastii, N. exigua, N. glauca, N. knightiana, N. raimondii*), die übrigen wurden von anderen Labors bezogen und in das Genom von Zamojska 4 rückgekreuzt. Alle fremden Zytoplasmen mit Ausnahme von *N. knightiana* erzeugten vollständige männliche Sterilität bei Zamojska 4. Das Ausmaß der Veränderungen bei den männlichen Organen variierte von vollständigem Fehlen des Staubgefäßes (*N. suaveolens, N. tabacum*) über petaloide und stigmatoide Strukturen (häufigste Veränderung) sowie von deformierten (*N. amplexicaulis, N. glauca*) bis zu offensichtlich normalen Staubgefäßen (*N. raimondii, N. knightiana*). Die Mehrzahl der Alloplasmen reagierte auf Tentoxin, was auch beim Zytoplasma-Donator zu beobachten war. Die Ausnahmen waren *N. exigua, N. raimondii, (N. raimondii* I) und die zytyplasmatische Mutante von *N. tabacum.* Die alloplasmatischen Varianten von Zamojska 4 variierten leicht in Größe und Morphologie. Bei Feldbefall zeigten die Alloplasmen mit dem Zytoplasma von *N. plumbaginifolia* und *N. eastii* Blauschimmelsymptome, während die übrigen Alloplasmen und cv. Zamojska 4 gegenüber dieser Krankheit sehr tolerant waren. [Beitr. Tabakforsch. Int. 19 (2001) 309–14]

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RESUME

Les cytoplasmes de plusieurs espèces de *Nicotiana : N. amplexicaulis, N. bigelovii, N. debneyi, N. eastii, N. exigua, N. glauca, N. glutinosa, N. goodspeedii, N. knightiana, N. occidentalis, N. plumbaginifolia, N. raimondii, N. suaveolens et N. undulata*, ont été introduits par sélection dans le génome du tabac flue-cured cv. Zamojska 4. La collection comporte également un analogue mâle-stérile cytoplasmique (cms) du cv. Zamojska 4 avec un cytoplasme muté de *N. tabacum*. Certaines des lignées alloplasmiques ont été obtenues dans ce laboratoire (*N. amplexicaulis, N. eastii, N. exigua, N. glauca, N. knightiana et N. raimondii*), les autres ont été acquises d'autres laboratoires et retrocroisés avec Zamojska 4. Tous les cytoplasmes étrangers, sauf celui de *N. knightiana*, ont produit une stérilité mâle complète chez Zamojska 4. L'importance des modifications de l'organe mâle allait de l'absence complète d'étamines (*N. suaveolens, N. tabacum*) à des structures pétaloïdes et stigmatoïdes (le plus courant), à des étamines déformées (*N. amplexicaulis, N. glauca*), et à des étamines apparemment normales (*N. raimondii, N. knightiana*). La majorité des lignées alloplasmiques a présenté une réponse à la tentoxine qui était compatible avec le donneur de cytoplasme, exceptés *N. exigua, N. raimondii, (N. raimondii* I), et le mutant cytoplasmique de *N. tabacum*. On constate des différences de croissance et de morphologie parmi les variants alloplasmiques de Zamojska 4. En cas d'infestation par le mildiou (*P. tabacina*) des plantes en plein champ, les lignées alloplasmiques ayant le cytoplasme de *N. plumbaginifolia* et de *N. eastii* ont présenté des symptômes de la maladie tandis que les autres lignées alloplasmiques et le cv. Zamojska 4 se montrent très tolérants. [Beitr. Tabakforsch. Int. 19 (2001) 309–14]

INTRODUCTION

An interaction between nuclear genes of one species with cytoplasmic genes of another often results in disturbed development of male organs and the supression of the male gametophyte, most commonly referred to as cytoplasmic male sterility (cms). In *Nicotiana*, many alloplasmic combinations of this kind have been reported and studied (8,9). Indeed, nearly all known substitutions of an alien cytoplasm in the genome of cultivated *Nicotiana tabacum* have produced cms. Although the end result of alloplasmic substitution is the same i.e. inability to produce viable pollen, its expression may vary considerably depending on interacting nuclear and cytoplasmic genes. More often than not, substantial alterations in flower morphology are involved and the growth and development of the whole alloplasmic plant is often also affected, though usually to a lesser extent.

In the early seventies, a collection of alloplasmic substitutions of *Nicotiana tabacum* bred into one local variety cv. Zamojska 4 was started at the Institute of Soil Science & Plant Cultivation (IUNG), Puławy, and was gradually built up involving alloplasmics originally obtained in this laboratory and accessions from other research units. Since it was based on a single *N. tabacum* genotype the collection offered a unique opportunity to compare the effect of cytoplasms of several *Nicotiana* species in one genotypic *milieu*. This paper provides the description of the alloplasmics thus obtained with the main focus on the most characteristic feature, the morphology of the flower.

Table 1.
Alloplasmics of *N. tabacum* cv. Zamojska 4 held at the IUNG, Puławy, Poland

No.	Alien cytoplasm substitution	Origin	Back-crosses[a]
1	*N. amplexicaulis*	IUNG Puławy, Poland	12
2	*N. bigelovii*	T RB, Harare, Zimbabwe	5
3	*N. debneyi*	IUNG Puławy, Poland	15
4	*N. eastii,*	IUNG Puławy, Poland	15
5	*N. exigua*	IUNG Puławy, Poland	15
6	*N. glauca*	IUNG Puławy, Poland	6
7	*N. glutinosa*	WITIM Krasnodar, Russia	12
8	*N. goodspeedii*	T RB, Harare, Zimbabwe	5
9	*N. knightiana*	IUNG Puławy, Poland	15
10	*N. megalosiphon*	WITIM Krasnodar, Russia	12
11	*N. occidentalis*	WITIM Krasnodar, Russia	12
12	*N. plumbaginifolia*	WITIM Krasnodar, Russia	12
13	*N. raimondii* I	IUNG Puławy, Poland	13
14	*N. raimondii* II	IUNG Puławy, Poland	6
15	*N. suaveolens*	T RB, Harare, Zimbabwe	5
16	*N. tabacum*	IUNG Puławy, Poland	15
17	*N. undulata*	WITIM Krasnodar, Russia	12

[a]No. of backcrosses to cv. Zamojska 4 (for the 1997 seed lot).

MATERIALS AND METHODS

Materials

A local flue-cured variety of tobacco cv. Zamojska 4 was used as the recurrent male *N. tabacum* parent in setting up and maintaining the collection of tobacco alloplasmics. The sources of alien cytoplasms and their place of origin are listed in Table 1. Of the 17 cytoplasm sources 9 listed as originating from the IUNG Puławy were originally bred into the genomic background of *N. tabacum* in this laboratory. Those acquired from foreign laboratories were obtained as ready-made alien cytoplasm substitutions originally involving *N. tabacum* genomes other than cv. Zamojska 4.

Mode of origin of the alloplasmics

The alloplasmics of cv. Zamojska 4 were obtained and have been maintained by a series of successive backcrosses to the paternal variety. The seed lots used in the 1997 experiment represented varying numbers of backcrosses to Zamojska 4, from 5 to 15 in 1997 (Table 1). The seeds from a consecutive backcross were used in the 1998

Table 2.
Reaction to tentoxin by the seedlings of the alloplasmics of *N. tabacum* cv. Zamojska 4

No.	Source of alien cytoplasm	Response to tentoxin	Agreement with cytopl. source spec.
1	*N. amplexicaulis*	Sensitive	Yes
2	*N. bigelovii*	Sensitive	Yes
3	*N. debneyi*	Insensitive	Yes
4	*N. eastii*	Sensitive	Yes
5	*N. exigua*	Insensitive	No
6	*N. glauca*	Sensitive	Yes
7	*N. glutinosa*	Sensitive	Yes
8	*N. goodspeedii*	Sensitive	Yes
9	*N. knightiana*	Insensitive	Yes
10	*N. megalosiphon*	Sensitive	Yes
11	*N. occidentalis*	Sensitive	Yes
12	*N. plumbaginifolia*	Sensitive	Yes
13	*N. raimondii* I	Insensitive	No
14	*N. raimondii* II	Sensitive	Yes
15	*N. suaveolens*	Sensitive	Yes
16	*N. tabacum*	Sensitive	No
17	*N. undulata*	Insensitive	Yes

experiment. There was no observable phenotypic variation within the different alloplasmic lines.

Of the alloplasmics obtained in this laboratory, all except *eastii* were obtained through conventional interspecific hybridization (3,4,6). The original *N. tabacum* parents were other than Zamojska 4. The origin of *eastii* departed from the conventional scheme. A male sterile but female fertile *N. tabacum*-like plant was found in the F_1 generation of the cross *N. eastii* × *N. tabacum*. Although the origin of the plant was never fully explained it may have been the product of the androgenetic development of an *N. tabacum* sperm cell in the embryo sac of *N. eastii* (5). The *N. tabacum*-type cytoplasmic male sterility was discovered as a spontaneous male sterile mutation in the field of normal self-fertile tobacco (1). No details concerning the origin of the foreign accessions are known.

Two types of cms *raimondii* were obtained in the course of backcrossing the original *raimondii-tabacum* hybrid to paternal Zamojska 4. They are referred to as *raimondii* I and *raimondii* II. The former was described previously (2), the latter is of more recent provenance and is reported for the first time here.

The alloplasmics were grown in the field on the Agricultural Experiment Station in Puławy under standard fluecured tobacco management.

The germinating seeds of the alloplasmics were tested for their response to a 0.002% aqueous solution of tentoxin, a cyclic tetrapeptide which interferes with chloroplast development in sensitive species and the sensitivity to which is inherited maternally (7).

Description of the alloplasmics

Although the response to tentoxin of the majority of alloplasmics was in agreement with that of the cytoplasm donor species there were notable exceptions (Table 2). They included cv. Zamojska cms *exigua* and cms *raimondii* I which, unlike their respective cytoplasm donors *N. exigua* and *N. raimondii* were tentoxin insensitive, and cms *tabacum* which was tentoxin sensitive, unlike normal *N. tabacum*.

Details of the flower morphology of the alloplasmics are given in Table 3. They include modifications and abnormalities of stamens, pistils and corollas. The external appearance of stamens including anthers was apparently unaffected by two plasmatypes: *N. knightiana* and *N. raimondii* II. In both cases apparently normal pollen was produced but it was incapable of germination and fertilization, either wholly (*N. raimondii* II) or partially (*N. knightiana*). In *raimondii* I normal stamen morphology was also retained but stamens were visibly smaller and produced no pollen.

All the other alloplasmics exhibited varying degrees of stamen modifications accompanied by full male sterility (cms). Reduced or vestigial stamens displayed by cms *glauca* and cms *tabacum* plasmatypes were characterized by shrivelled anthers on reduced filaments of varying length: from almost normally developed all five stamens to one or two on very short filaments to none at all. Petaloid or petal-like structures also represented different forms: from small, petal-like outgrowths on malformed anthers to fully expanded *quasi* petals. Likewise, stigmatoid or pistil-like structures also varied from very small outgrowths which sometimes occurred on the anthers of cms *knightiana* or petaloids of cms *eastii, plumbaginifolia* or *glutinosa* to structures that resembled fully grown pistils and were hardly to be distinguished from the regular pistil (cms *debneyi*, cms *suaveolens*). The most frequent modification of the female organ, the pistil, was the occurrence of more than two locules of the stigma. Multiple stigmas were often accompanied by fasciation of the pistil and its considerable shortening.

Modifications of corolla, if any, included drastic shortening of the tube accompanied by reduction of the lobes resulting in a protruding pistil (cms *eastii*, *glutinosa*, *plumbaginifolia*). Inflation of a shortened corolla tube was another modification (cms *goodspeedii*, cms *suaveolens*).

Whereas in some alloplasmics floral modifications were very stable within a growing season and across different growing seasons (cms *amplexicaulis*, *raimondii* I and II, *knightiana*, eastii, *glutinosa*, *plumbaginifolia*) in some others they were quite variable even during the same season. Pistils, e.g., could be anything from normal to highly malformed on the same plant and at the same time (cms *debneyi*). A very curious phenomenon observed early in the season of 1998 were large pistil-like structures (stigmatoids) in the *tabacum* and *glauca* alloplasmics. Such modifications had never been recorded before in those lines. The stigmatoids disappeared from subsequently expanding flowers to be replaced by vestigial stamens, usually observed in those alloplasmics.

The extent of floral and other modifications observed in the alloplasmics of *N. tabacum* cv. Zamojska 4 is sum-

Table 3.
Flower morphology of the alloplasmics of *N. tabacum* cv. Zamojska 4 held at the Institute of Soil Science and Plant Cultivation in Puławy (Stamens: N = normal-looking, m = shrivelled anthers on filaments of normal length, mm = malformed anthers on shortened filaments, v = vestigial stamens at the bottom of the flower, 0 = no stamens, p = small petaloids, P = sizeable petaloids, PP = very sizeable and profuse petaloids, s = minute stigmatoids, S = stigmatoids, SS = well-developed pistil-like stigmatoids; Pistil: N = bi-loculed of normal length, ml = multi-loculed, s = shortened, f = fasciated; Corolla: N = unchanged, s = shortened, ss = very shortened, f = inflated corolla tube)

Alloplasmic	Flower morphology – 1997			Flower morphology – 1998 early flowering			Flower morphology – 1998 late flowering[a]		
	Stamens	Pistil	Corolla	Stamens	Pistil	Corolla	Stamens	Pistil	Corolla
Subgenus *rustica*									
N. glauca	0 or v	N	N	SS	N	N	mm	N	N
N. knightiana	N[b]	N	N	N[b]	N	N			
N. raimondii I	N[c]	N	N	N[c]	N	N			
N. raimondii II	N[d]	N	N	N[d]	N	N			
Subgenus *tabacum*									
N. glutinosa	PP and s	N	ss	PP and s	N	ss			
N. tabacum	0, v	N	N	SS	N	N	mm	N	N
Subgenus *petunioides*									
N. amplexicaulis	m	N	N	m	N	N	Na	N	N
N. bigelovii	—[e]	—[e]	—[e]	S	N, ml	N			
N. debneyi	S	s, f, ml	s	SS, S	f, ml, N	s			
N. eastii	PP and s	N	ss	PP and s	N	ss			
N. exigua	S	N	s	S	s,ml, N	N			
N. goodspeedii	S, p	ml, s	s	S, P	s, ml, f	s, f			
N. megalosiphon	—[e]	—[e]	—[e]	0	ml	s	0 or s	ml	s
N. occidentalis	P, S	ml, s, f N	ss	S, P, 0	N, s, ml, f	ss	PP	N, s, ml, f	
N. plumbaginifolia	PP and s	N	very s	PP and s	N	ss			
N. suaveolens	0, p	N	f	S, SS	ml, f	f			
N. undulata	—[e]	—[e]	—[e]	S	N, ml	N	S	s, ml	N

[a]No data means that flower morphology did not differ from that early in the season.
[b]Anthers with stainable, partly fertile pollen.
[c]Anthers void of pollen.
[d]Anthers with stainable, sterile pollen.
[e]Data unavailable for 1997.

Table 4.
Alterations of floral parts and of plant habit of the alloplasmics of *N. tabacum* cv. Zamojska 4 as induced by different alien cytoplasm sources

Stamens					Pistil				
Normal	Vestigial or none	Petaloid structures	Petaloids and stigmatoids	Stigmatoids	Normal	Variable (normal or malformed)	Malformed	Modified corolla	Modified plant habit
knightiana *raimondii* I *raimondii* II	*amplexicaulis* *glauca* *tabacum* *megalosiphon* *suaveolens*	*eastii* *glutinosa* *plumbaginifolia*	*occidentalis* *goodspeedii*	*exigua* *debneyi* *bigelovii* *suaveolens* *undulata*	*amplexicaulis* *eastii* *glauca* *glutinosa* *knightiana* *raimondii* I *raimondii* II *plumbaginifolia* *tabacum*	*bigelovii* *debneyi* *exigua* *suaveolens* *undulata*	*goodspeedii* *occidentalis*	*eastii* *glutinosa* *plumbaginifolia* *goodspeedi* *occidentalis* *suaveolens*	*debneyi* *eastii* *exigua* *glutinosa* *goodspeedii*[a] *megalosiphon*[a] *plumbaginifolia* *undulata*

[a]Substantially departs from the habit of cv. Zamojska 4.

marized in Table 4. There does not seem to be any particular relationship between the character of cytoplasm-induced floral alterations and taxonomical relationships. Nor does the extent of floral abnormalities correlate with modifications to the plant habit. In this report the term "plant habit" denotes mostly height of the plant and number of leaves and "modified plant habit" implies the reduction of both.

Another interesting phenomenon that could be linked to the influence of alien cytoplasm was the increased susceptibility to blue mold of cms *eastii* and cms *plumbaginifolia* observed during the massive outbreak of the disease in 1997. On the other hand, cv. Zamojska 4 and the remaining alloplasmic analogues of that variety showed high tolerance to that disease.

There were some doubts and questions in connection with the alloplasmic lines of cv. Zamojska 4 which could not be resolved by this study. They include very close similarities of such alloplasmics as *glauca* and *tabacum* on the one hand, and *eastii*, *glutinosa* and *plumbaginifolia* on the other, some of the results of the tentoxin test and the obtaining of two different cms types from one source.

CONCLUSIONS

i. All of 17 alloplasmics of *N. tabacum* cv. Zamojska 4, except that with the cytoplasm of *N. knightiana* showed full male sterility.
ii. Among the alloplasmics the extent of stamen modifications varied from apparently normal stamens with fully developed anthers to different degrees of staminal malformations: shrivelled anthers on shortened filaments, vestigial or absent stamens, petaloidal and stigmatoidal structures. The latter were the most frequently observed staminal abnormalities.
iii. The morphology of the female organ, pistil, was also affected in many of the alloplasmics including those with the cytoplasm of *N. suaveolens* and *N. undulata* – the most widely used sources of commercially usable cms in tobacco.
iv. Other traits such as plant habit and degree of tolerance of blue mold were also influenced by alien cytoplasm in some alloplasmic analogues of cv. Zamojska 4.

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Address for correspondence

Institute of Soil Science and Plant Cultivation
Department of Special Crops
Osada Palacowa
Poland - 24-100 Pulawy