HYBRIDIZATION BETWEEN *NICOTIANA GOSSEI* DOMIN AND *N. TABACUM* L. FOR DEVELOPMENT OF ORIENTAL TOBACCO LINES RESISTANT TO TOBACCO APHIDS AND DISEASES

TOBACCO SCIENCE

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The disease- and aphid-resistant species, *N. gossei* (2n=36), was crossed with *N. tabacum* cv. Kroumovgrad 90, an oriental tobacco type. The F_1 hybrids from this cross were all sterile. Regenerates of this cross were developed by *in vitro* methods, and they were partially fertile. The F_1 hybrids, their regenerates, and seed progenies were evaluated for resistance to tobacco aphids, powdery mildew, and tobacco mosaic virus by artificial inoculation in greenhouse conditions. The inheritance of resistance to aphids, powdery mildew, and TMV was dominant in F_1 hybrids. Resistance to aphids, blue mold, and powdery mildew was also expressed in the fertile regenerates, and in the first and second seed progenies. These regenerates are of great interest for the genetics and breeding of tobacco.

Additional key words: *Nicotiana tabacum*, interspecific hybridization, host plant resistance, *in vitro* methods.

INTRODUCTION

The tobacco aphid (*Myzus nicotianae* Blackman) is an economically important pest of tobacco (*Nicotiana tabacum* L.) in Bulgaria and worldwide. In addition to reducing yield and quality of tobacco, aphids transmit a number of viral pathogens. Measures for controlling tobacco aphids include the use of chemical insecticides that may contaminate the environment. Continuous chemical control may also lead to the formation of resistant strains of *M. nicotianae*. This could lead to even greater pesticide use.

The wild species, *Nicotiana gossei* Domin (section Suaveolentes), originated from Australia. It is an annual herbaceous plant with relatively large leaves (ca. 25-cm long and 12-cm wide) that are covered with long leaf trichomes. Plants can be up to 120 cm tall, and they have relatively large white flowers. *N. gossei* is important for tobacco breeding programs because of its resistance to aphids (2, 13), black root rot (*Thielaviopsis basicola* E.[Berk. & Broome] Ferris), powdery mildew (*Erysiphe cichoracearum* DC), tobacco streak virus (1), and common strains of tobacco mosaic virus (TMV) (1).

A number of authors (3,4,5,6) have reported disease-resistant tobacco types using *in vitro* methods. The aim of the present investigation was to obtain aphid-resistant tobacco types by crossing *N. tabacum* with *N. gossei* and using *in vitro* methods.

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MATERIALS AND METHODS

Experiments were conducted between 1987 and 1992 at the Institute of Genetics, Bulgarian Academy of Science, Sofia. *N. gossei* was hybridized with a high-quality oriental tobacco cultivar, "Kroumovgrad 90," which is widely planted in Bulgaria. Kroumovgrad 90 was developed from an intervarietal hybridization between "Kroumovgrad 15" and "Trapezon," and it is resistant to TMV and blue mold (*Peronospora tabacina* D. B. Adam). It is characterized by high yields, good quality, and desirable technological and smoking properties.

Twenty-four sterile F_1 hybrids were obtained from 16 successful crosses of *N. gossei* x Kroumovgrad 90. *In vitro* methods were used to overcome the incompatibility of the sterile F_1 hybrids. Young stem segments, collected between the upper internodes of flowering hybrids, were cultivated *in vitro* on Murashige & Skoog (MS) basic medium (9). Callus was induced on MS medium supplemented with 2 mg/L naphthyl acetic acid, 0.5 mg/L kinetin, and 500 mg/L casein hydrolisate. Plants were regenerated by transferring the callus to MS medium supplemented with 0.2 mg/L indole-3-acetic acid and 3 mg/L kinetin. For further growth of the regenerates, MS medium supplemented with 2 mg/L kinetin and 0.1 mg/L gibberellic acid was used. The regenerates were rooted on MS nutrient medium with twice-reduced macronutrients, 15% sucrose, and 1 mg/L indole-3-butyric acid. The rooted plants were grown on a mixture of sterile soil and perlite under greenhouse conditions, and then they were transferred to

the field. Forty-two fertile regenerates were produced from the F_1 hybrids using these procedures. Seed progenies (20-50 per plant) were produced from the fertile regenerates.

Meiotic analyses of the pollen mother cells in 36 regenerates were studied by fixing flowers at the early button stage. The observations were carried out after fixation in Clarke's mixture (1:3 [v:v] glacial acetic acid:96% ethanol) and staining with 4% acetocarmine after treatment with 2% ferrous ammonium sulphate. The determination of pollen fertility was carried out on preparations stained with acetocarmine:glycerine (1:1 [v:v]); 244-900 pollen grains were examined from each regenerate.

The F_1 hybrids, fertile regenerates, their seed progenies (20-50 plants per regenerate), and the susceptible control plants ("Harmanliiska basma 163") were evaluated for their resistance to tobacco aphids, TMV, and powdery mildew in greenhouse experiments. The sterile F_1 hybrids were kept in the greenhouse. During the experimental period some plants died; therefore, the number of hybrids used in subsequent experiments declined. The seed progenies were also evaluated for blue mold in the greenhouse and the field.

Evaluations of resistance to tobacco aphids were carried out in the greenhouse using Thurston's methods (13). Twenty adult aphids were placed on each plant. Two weeks after inoculation, the number of aphids was recorded each day for 10 days. Plants were subjectively rated in the button stage as follows: 0 = plants without aphids; 1 = plants very slightly affected; 2 = plants slightly affected; 3 = plants moderately affected; and 4 = plants strongly affected. In the F_1 hybrids, only resistant (rating = 0) and susceptible (rating = 4) plants were obtained.

Inoculations of TMV were carried out artificially with both the tobacco and tomato strains (11). Resistance to TMV was recorded in the plantlet stage using Ternovsky's (12) scale: 0 = plants have a necrotic reaction and mosaics do not appear (when artificially inoculated); 1 = sensitive, necrotic reaction does not appear and mosaics develop; 2 = strongly sensitive, inhibition of growth and break-down of the plant. Resistance to powdery mildew was recorded in the plantlet stage using Ternovsky's (12) scale: 0 = resistant; 1 = slightly affected; 2 = strongly affected.

Seed-progeny plants were tested for blue mold resistance in the button and flowering stage in the field under natural conditions and in the greenhouse under artificial conditions. Plant inoculations in the greenhouse were carried out with spore suspensions. Between 50 and 150 plants from each variant were infected. The response to infection was recorded using the scale of Hill & Mandryk (8) where: 0 = immunity; 1 = high resistance; 2 = moderate resistance; 3 = moderate susceptibility; 4 = susceptibility. For better accuracy in the evaluation of plant resistance to blue mold, we used the modifications of this scale described by Palakarcheva & Stoyanova (10), where intermediate grades of 0.5, 1.5, 2.5, and 3.5 were included. Harmanliiska basma 163 plants were used as a susceptible control.

During these investigations, resistance to powdery mildew was recorded first. This was followed by screening for TMV and aphid resistance. The experimental data were statistically processed according to Gentchev et al. (7).

RESULTS AND DISCUSSION

Table 1 presents plant heights, leaf numbers, and the sizes of the leaves of the F_1 hybrids, their regenerates, and the *N. tabacum* parent (Kroumovgrad 90). Hybrids were 48-164 cm tall (avg. 111.5 cm) and the upper portion of each stalk was branched. There were 18-28 leaves per plant (avg. 24.5). Leaf lengths and widths for the lower, middle, and upper stalk positions of the F_1 hybrids averaged 30 x 17 cm, 29 x 16 cm, and 22 x 11 cm, respectively. Flowers of the branched inflorescences from the F_1 hybrids were large and white. All F_1 hybrids were sterile.

Thirty-six regenerates from the F_1 hybrids were 50-120 cm tall (avg. 84.7 cm) with 24-32 leaves per plant (avg. 29.0) (**Table 1**). The regenerates were straight and unbranched, and their leaves were similar in size and shape to Kroumovgrad 90. Inflorescence lengths and widths were 10.3 and 5.3 cm, respectively. Flowers were a pale rosy hue, and corollas were about 4 cm long and 2 cm wide.

All F_1 hybrid plants tested during 1988-1990 (n = 57) were resistant to powdery mildew and to tobacco aphids. Most of the regenerates obtained by *in vitro* methods also were highly resistant to aphids, powdery mildew, and two strains of TMV. Even when there was a heavy aphid infestation on the control tobacco (166 aphids/leaf), most of the regenerates were not affected. Thirty-four of 36 regenerates were resistant to both the tomato

Table 1. Morphological characterization of F_1 hybrids and fertile regenerates produced from *Nicotiana gossei* x *N. tabacum* (Kroumovgrad 90).

Plant type	Plant height[a]	Number of Leaves[a]	14th leaf			20th leaf			25th leaf			Inflorescence	
			Length[a]	Width[a]	L/W	Length[a]	Width[a]	L/W	Length[a]	Width[a]	L/W	Length	Width
	--- cm ---		--- cm ---	--- cm ---		--- cm ---	--- cm ---		--- cm ---	--- cm ---		--- cm ---	--- cm ---
Kroumovgrad 90[b]	158.0±5.9	26.0±0.5	28.0±1.7	16.0±1.0	1.75	24.0±1.2	12.0±0.8	2.00	18.0±1.3	9.0±0.9	2.00	23.0	15.0
N. gossei x *N. tabacum* F_1 hybrids[c]	111.5±9.0	24.5±0.8	29.0±1.7	16.0±1.1	1.90	21.5±6.0	11.0±3.2	1.95	---	---	---	---	---
N. gossei x *N. tabacum* regenerates[d]	84.7±6.6	29.0±0.6	18.0±0.6	10.3±0.5	1.75	18.1±1.4	9.1±0.3	2.00	9.2±1.1	5.7±0.7	1.69	10.3	5.3

[a]± standard error of the mean.
[b]Average of 10 plants.
[c]Average of 12 F_1 hybrids.
[d]Average of 36 regenerates.

and tobacco strains of TMV (**Table 2**).

The numbers of aphids on the sterile F_1 hybrids and on the regenerates over a 10-day period are presented in **Table 3**. All aphids on the F_1 hybrids died after 2-4 days. There was only a small number of aphids (5-155 aphids/plant) on most of the regenerates. Three of the regenerates responded like F_1 hybrids, and all aphids on these plants died within 2-3 days after infestation.

All of the F_1 hybrids had 100% nonviable pollen (826 pollen grains examined), and all of these plants were sterile. On the other hand, Harmanliiska basma 163 had 97.4% fertile pollen (201 pollen grains examined). Pollen fertility of the *N. gossei* x *N. tabacum* regenerates averaged 71.9%, but this was quite variable (range 34.8-93.8% viable pollen, 244-524 pollen grains examined). Regenerates from *N. tabacum* averaged 93.0% pollen viability (900 pollen grains examined).

The *N. gossei* x *N. tabacum* F_1 hybrids were all sterile because of abnormal meiosis. There were also some disturbances in the meiotic process in the first-seed progeny of the regenerates, but the percentage of abnormalities varied. The cytological investigation (**Table 4**) showed partial fertility of the regenerates.

Table 2. Resistance to tobacco aphids, powdery mildew, and tobacco mosaic virus of regenerates from the hybrid *N. gossei* x *N. tabacum* (Kroumovgrad 90), 1991.

Plant type	Number of plants examined	Number of Plants Rated				
		0	1	2	3	4
		Aphids[a]				
N. gossei x *N. tabacum* regenerates	36	21	10	3	1	1
Harmanliiska basma 163	40	0	0	0	0	40
		Powdery mildew[b]				
N. gossei x *N. tabacum* regenerates	36	36	0	0	-	-
Harmanliiska basma 163	40	0	0	40	-	-
		Tobacco mosaic virus[c]				
N. gossei x *N. tabacum* regenerates	36	34	0	2	-	-
Harmanliiska basma 163	40	0	0	40	-	-

[a]Rating scale two weeks after inoculation with aphids: 0 = none; 1 = very light; 2 = light; 3 = moderate; 4 = heavy.
[b]Rating scale: 0 = resistant; 1 = slightly affected; 2 = strongly affected.
[c]Tested against both the tomato and tobacco strains of TMV. Rating scale: 0= plants react with a necrotic reaction and mosaics do not appear; 1 = sensitive, necrotic reaction does not appear and the mosaics develop; 2 = strongly sensitive, inhibition of the growth and breaking down of the plant

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Table 3. Number of tobacco aphids on *N. tabacum* and on sterile *N. gossei* x *N. tabacum* F_1 hybrids and on regenerates for a period of 10 days.

Plant type	Number of plants examined	Average Number of aphids/plant on day				
		1	3	5	7	10
Harmanliiska basma 163	40	20.0	38.0	140.0	201.0	258.0
N. gossei x *N. tabacum* F_1 hybrids	11	20.0	0.0	0.0	0.0	0.0
N. gossei x *N. tabacum* regenerates	23	20.0	15.6	24.6	40.3	59.2

Some of the regenerates produced viable seed, which gave rise to the fertile progenies R1 and R2. Five of 11 second-seed progeny from the *N. gossei* x *N. tabacum* regenerates were resistant to tobacco aphids. The other six second-seed progeny and Kroumovgrad 90 were susceptible to tobacco aphids. All 11 of the second-seed progeny were resistant to blue mold (APT-3 race), as was Kroumovgrad 90. The *N. gossei* parent was not resistant to the APT-3 race of *P. tabicina*, which is spread widely in Bulgaria.

Interspecific hybridization between *N. gossei* and *N. tabacum* resulted in F_1 hybrids that were resistant to tobacco aphids, TMV, powdery mildew, and blue mold, but were sterile. In the F_1 hybrids, resistance to these pests was dominantly inherited. Partially sterile regenerates were produced by *in vitro* techniques, which gave rise to fertile R1 and R2 progenies. The progenies possessed 48 chromosomes (2n). Their resistance to aphids, TMV, and powdery mildew was based on translocations, occurring during the *in vitro* cultivation process. Similar results were reported for other species and other diseases (4,5,6). These lines may be of importance to breeding programs aimed at producing tobacco cultivars resistant to aphids and diseases. The results of this study are of importance to tobacco immunogenetics and immunobreeding.

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Table 4. Meiotic abnormalities in pollen mother cells (PMC) of regenerates from *N. gossei* x *N. tabacum* F_1 hybrids.

Plant type	Metaphase I		Metaphase II		Ana-telophase I		Ana-telophase II		Tetrades	
	PMC studied	% Deviations	PMC studied	% Deviations	PMC studied	% Deviations	PMC studied	% Deviations	PMC studied	% Deviations
N. gossei x *N. tabacum* regenerates										
N3	80	7.1	79	8.9	62	6.3	130	7.3	361	9.1
N4	78	7.7	132	6.8	85	5.9	179	6.1	121	7.4
N23	134	94.0	96	93.8	150	94.0	89	92.1	---	---
Average	---	36.2	---	36.5	---	35.4	---	35.2	---	8.3
Harmanliiska basma 163	258	3.1	178	3.4	236	4.7	171	3.6	396	2.8

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