INTERSPECIFIC HYBRIDIZATION AND BREEDING FOR PEST RESISTANCE IN TOBACCO[1]

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Breeding for pest resistance in tobacco has received great emphasis because of potential detrimental effects of pesticide residue on human health. Failure in finding resistance to plant pests among *N. tabacum* varieties has led to extensive screening of the wild species in the genus *Nicotiana*, among which *N. gossei* was found to be toxic to aphids and caterpillars. Using this species as a donor source, a breeding program is in progress to incorporate resistance factors into commercial flue-cured tobacco varieties. Initially an autotetraploid *N. tabacum* variety Delcrest was used as a recipient parent. The F_1, S_1, BC_1, S_2 and BC_2 progenies were screened for resistance. Results showed that it may be possible to obtain plants resistant to aphids (*Myzus persicae*), tobacco caterpillars (*Spodoptera litura*) or both species.

INTRODUCTION

The concept of incorporating heritable factors for pest resistance in crop plants is not new and enormous work has been done in this field (3,13,19). In tobacco (*Nicotiana tabacum* L.) the extensive damage caused by such insect pests as aphids, caterpillars and whiteflies, plus the destructive role of aphids and whiteflies in transmitting virus diseases, needs no emphasis. But compared to the progress achieved in evolving disease resistant varieties, breeding for insect resistance has received little attention. Chemical control, though highly effective, may lead to indiscriminate use of pesticides resulting in excess residues in/on cured leaves. Industry's refusal to accept the crop with harmful residues, which can affect human health, has created an awareness among tobacco breeders for a need to produce resistant varieties.

The lack of an adequate donor source of resistance within strictly *N. tabacum* germplasm was one of the major obstacles for the slow pace of insect resistance breeding program in tobacco. However, a survey of the wild species of the genus *Nicotiana* has shown that a good number of them are resistant to one or more insects (6,7,8,9,16,20,21). Among them *N. gossei* (n = 18) was found most promising, having resistance to green peach aphid (*Myzus persicae* Sulzer), budworm (*Heliothis virescens* F.) and hornworm (*Manduca sexta* Johannson). Laboratory testing of this species by Central Tobacco Research Institute, Rajahmundry, revealed that it was resistant to tobacco caterpillar (*Spodoptera litura* F.) and green peach aphid (12). Earlier attempts to produce direct hybrids between flue-cured *N. tabacum* and *N. gossei* resulted in failure due to seedling lethality (2,5). However, success has been reported recently when a cigar-wrapper variety, Florida-17, was used as a maternal parent (4). To overcome lethality at the diploid level, we used an autotetraploid form of *N. tabacum* as the female parent.

During 1973-76, the sesquidiploid (F_1), selfed generations (S_1 and S_2) and backcross progenies (BC_1 and BC_2) were screened to isolate the plants that were resistant to green peach aphid and tobacco caterpillar. Results are reported herein.

MATERIALS AND METHODS

Interspecific hybridization was successful when an autotetraploid form of the variety Delcrest was pollinated by *N.*

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Table 1. Showing the frequencies of various chromosomal groups and the type of resistance to aphids, caterpillars or both.

Chromosomal Types	F_1				S_1				BC_1				S_2				BC_2			
	S	AR	CR	ACR	S	AR	CR	ACR	S	AR	CR	ACR	S	AR	CR	ACR	S	AR	CR	ACR
1. $24_{II} + 2_I$	57	11	–	12	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
2. 24 normal bivalents	–	–	–	–	1487	–	–	–	1339	–	–	–	1572	–	–	–	2584	–	–	–
3. 24_{II} (23_{II} + 1 heteromorphic bivalent)	–	–	–	–	–	14	–	–	–	37	–	–	–	–	–	–	–	7	–	–
4. 24_{II} (23_{II} + 1 heteromorphic bivalent + a fragment)	–	–	–	–	–	–	–	3	–	–	–	3	–	19	–	1	–	–	–	1
5. 24_{II} (23_{II} + 1 heteromorphic bivalent) + 1_I	–	–	–	–	–	7	–	–	–	16	–	–	–	–	–	–	–	5	–	–
6. 24_{II} (23_{II} + 1 heteromorphic bivalent) + 1_I + a fragment	–	–	–	–	–	–	–	1	–	–	–	3	–	12	–	2	–	–	–	3
7. 24 normal bivalents + 1_I + a fragment	–	–	–	–	–	–	–	–	–	–	–	–	–	–	3	–	–	2	–	
8. 24 normal bivalents + a fragment	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1	–	–	2	–	

gossei. The F_1 seeds were sown in petri dishes and upon germination were planted in pots. In November, a single row of six to eight week old seedlings were transplanted in an endemic "Lanka" area (Islands within the river Godavari). A local cheroot variety DR-1, which is highly susceptible to aphids and caterpillars, was planted on both sides of the F_1 plants. Two to three irrigations were given to the crop which along with the cold weather prevailing during the crop growth period (November to January), created favorable conditions for severe infestation by the insects. Under these conditions a rigorous plant to plant screening was done. The classification for resistance was based on the method developed by Chesnokov (10) suitably modified to our field conditions.

The selected plants were then selfed and backcrossed to commercial flue-cured tobacco varieties. Seedlings from the selfed (S_1 and S_2) and backcross (BC_1 and BC_2) populations were grown in nursery beds and transplanted to the field. From each selfed plant and backcross, 100-150 plants were grown, along with the susceptible control variety DR-1 on both sides of the line. The screening methodology adopted for the F_1 plants was used in isolating resistant plants in these generations. Flower buds were fixed in Carnoys' fluid and stained in aceto-carmine for cytological analysis. Pollen fertility was estimated by staining the cells in 1% aceto-orcein.

RESULTS

(a) Morphological characters: The sesquidiploid plants resembled *N. tabacum* for most of the morphological characters and very little interplant variation was observed within the population. The plants were slightly shorter than normal, had long elliptic leaves, medium internodes and typical *N. tabacum* like flowers except for slightly longer and slender corolla tube.

Pollen fertility ranged between 75% and 90% and good capsules containing viable seeds were produced upon selfing and backcrossing. In the S_2 and BC_2 generations, the plants attained more uniformity with almost all the morphological features of *N. tabacum*.

(b) Screening and selection of resistant plants:. In the F_1 generation, all the selected resistant plants possessed resistance to aphids as well as caterpillars. In the S_1, BC_1, S_2 and BC_2 generations, where a large population was grown in each category, segregation was observed for resistance. Plants having resistance to aphids, caterpillars or both insects were isolated separately. For this, the following procedure was adopted.

(i) Isolation of aphid resistant (AR) plants.

Immediately after commencement of flowering, when the aphid infestation is maximum, plants were screened and classified by visual assessment into four groups depending upon the intensity of infestation.

(a) highly resistant	0-50 aphids per plant
(b) resistant	51-200 aphids per plant
(c) moderately resistant	201-500 aphids per plant
(d) highly susceptible	more than 500 aphids per plant

Plants in category (a) were designated as AR and selected for further work. Plants in category (b) were kept in reserve.

(ii) Isolation of caterpillar resistant (CR) plants

The mortality of larvae soon after hatching and the extent of damage caused by the surviving larvae, were used as criteria for resistance. Uusually egg masses are found on the lower side of the 3rd, 4th or 5th leaves. Highly susceptible plants can be easily identified by the extent of insect damage. To isolate resistant plants, the lower surface of the leaf was checked for freshly hatched egg masses and the condition of the first instar larvae. Only those plants showing hatched egg masses along with the dead first instar larvae were considered resistant and accordingly classified into

(a) highly resistant (designated as CR)

Where most of the larvae died within a couple of days after hatching causing negligible damage to less than 5% of the total area. These few which survived, had retarded growth and died prematurely.

(b) moderately resistant

5-10% damage to the leaf with less than ten apparently healthy larvae.

(c) susceptible (S)

A loss of more than 10% of the leaf area with a large population of healthy larvae.

Plants from category 'a' were retained for further work.

(iii) Isolation of aphid and caterpillar resistant (ACR) plants.

Plants found resistant to aphids were checked for caterpillar resistance. Similarly caterpillar resistant plants were checked for aphid resistance. Plants resistant to both insect pests were designated as ACR and selected for further studies.

By this procedure plants having resistance to aphids (AR), caterpillars (CR) and both (ACR) were selected in each generation.

(c) Cytology: Cytological analysis of the PMCs of F_1 plants showed gross elimination of *N. gossei* chromosomes. All the plants analyzed showed a configuration of 24_{II} presumably from *N. tabacum* and two univalents from *N.* gossei at metaphase I.

Breakage and reunion involving one *N. tabacum* chromosome and an *N. gossei* univalent resulting in a heteromorphic bivalent and a fragment, with or without the other univalent chromosome was observed in the S_1 resistant plants. Such a segmental interchange resulted in bridges and laggard at anaphase I. In the BC_1, S_2 and BC_2 there was segregation into various chromosomal categories.

(i) 24 normal bivalents
(ii) 24_{II} (23_{II} + 1 heteromorphic bivalent)
(iii) 24_{II} (23_{II} + 1 heteromorphic bivalent) + a fragment
(iv) 24_{II} (23_{II} + 1 heteromorphic bivalent) + 1_I
(v) 24_{II} (23_{II} + 1 heteromorphic bivalent) + 1_I + a fragment
(vi) 24 normal bivalents + 1_I + a fragment
(vii) 24 normal bivalents + a fragment

The frequencies of the chromosomal groups and the type of resistance to the insects are given in **Table 1.** The analysis showed that there is no definite trend in the frequency of various chromosomal types in the selfed and backcrossed generations. Similarly the resistance to one or both the insects could not be correlated to a particular chromosomal group. But, since most of the resistant lines are being obtained from the plants having chromosome configuration 23_{II} + 1 heteromorphic II, 23_{II} + 1 heteromorphic II + I_I, 23_{II} + 1 heteromorphic II + 1 + a fragment, we presume that true breeding lines could be obtained from them in future.

DISCUSSION

Painter (15) has given a comprehensive review of accomplishments in breeding for insect pest resistance in field crops where intervarietal transfer of resistance traits is possible. In the absence of such donor varieties in tobacco, plant breeders have resorted to interspecific hybridization to produce resistant tobacco types. Since direct hybridization between *N. tabacum* and *N gossei* usually results in seedling lethality, we chose the sesquipoidy technique in our work.

Although chromosomal elimination is common in sesquiploids, it is interesting to note that all but two *N. gossei* chromosomes were eliminated in the F_1 plants. The segregation of the F_1 plants into insect resistant and susceptible types show that only a few plants possess the *N. gossei* chromosomes that carry resistance factors. Since a full genome of *N. tabacum* was present, the presence of two *N. gossei* chromosomes in susceptible or resistant plants did not greatly affect any morphological characters of the F_1 plants.

The chromosome loss in F_1 hybrids is one of the instabilities observed in interspecific hybrids of the genus *Nicotiana* (18). Factors responsible are found in some *Nicotiana* species which possess some alatoid components in their chromosome makeup presumably transferred from their parents (14). When these species are used in crosses, the hybrids show instability. It is presumed that *N. gossei* and two other species namely *N. excelsior* (n = 19) and *N. megalosiphon* (n = 20) had a common origin as aneuploid derivatives from the hybrid *N. fragrans* (n = 24) x *N. suaveolens* (n = 16) (11). It was reported earlier that *N. megalosiphon* possessed instability factors (17). The gross elimination of all but two *N. gossei* chromosomes in (4n *N. tabacum*) x *N. gossei* hybrid shows that *N. gossei* also contains such instability factors.

Cytological analysis of S_1 and BC_1 plants showed occurrence of segregation into various chromosomal types. This may be due to a segmental interchange between an *N. tabacum* chromosome and one of the two *N. gossei* univalents. The segregation of plants into AR, CR and ACR types in the S_2 and BC_2 generations may be attributed to the irregular meitotic distribution of the heteromorphic bivalent, fragment and the univalent.

The biochemical cause for resistance to the green peach aphid in some *Nicotiana* species have been attributed to the toxic exudates secreted by the aerial parts of the plant (22). Later it was found that the aphids were killed by contact with the secretions from trichomes and resistance to *Myzus persicae* results from this mortality (1). Presumably the same mechanism may be responsible for the death of the first instar larvae of *Spodoptera litura.*

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