Resistance

Characterization of Potato Virus Y Resistance from Gametoclonal Variation in Flue-Cured Tobacco

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ABSTRACT

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A previously identified gametoclonal tobacco variant, NC 602, exhibits resistance to certain potato virus Y (PVY) strains. NC 602 was subjected to a second cycle of anther culture and colchicine treatment. The resulting doubled haploid lines were evaluated for back mutation to susceptibility and any additional mutation that might provide resistance to tobacco etch virus (TEV). The PVY resistance was stable in anther culture, and although no TEV resistance was found, three lines were identified with delayed symptom onset. NC 602 was crossed to breeding line NC 744, which contained the PVY resistance gene found in the Virgin A Mutante (VAM) (Tobacco Introduction line 1406). Maternal doubled haploid lines of this cross were screened for both resistance genes by using an array of PVY strains to distinguish genotypes. Results demonstrated that the two genes could be combined into a single genome with improved levels of resistance, thus indicating different loci for each resistance. NC 602 was also crossed to a root-knot nematode (Rk) resistant cultivar (Coker 209) in an effort to combine PVY and Rk resistance for protection against the MN strain of PVY in Rk resistant varieties. Plants were identified at the haploid level, which contained both PVY(NN) and Rk resistance, but when these plants were doubled and challenged with the PVY(MN) strain, all lines resistant to Rk showed susceptibility to the virus.

Large amounts of genetic variation have been found among tobacco plants that have undergone in vitro anther culture and chromosome doubling (4,7). This variation has been termed "gametoclonal" by Evans et al (11). Gametoclonal variation has been used by several investigators (5,12,19) to improve qualitative traits in a number of species. Witherspoon (25) reported a gametoclonal tobacco variant (NC 602) with a single gene exhibiting incomplete dominance for resistance to potato virus Y strain NN (PVY[NN]), derived by anther culture from the PVY susceptible flue-cured tobacco (*Nicotiana tabacum* L.) cultivar McNair 944 (McN 944).

PVY is an increasing problem in the tobacco belt of the southeastern United States, causing significant economic losses (18). The virus also has been known to cause losses in other economically important solanaceous crops including pepper (*Capsicum annuum* L.), potato (*Solanum tuberosum* L.), and tomato (*Lycopersicon esculentum* Mill.) (9). The virus has worldwide

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distribution and many strains. Serious crop losses have resulted from infection by native strains of the virus on every continent (13,20). The three most common strains in the southeastern United States are PVY(MM), which causes mosaic symptoms on both root-knot nematode (*Meloidogyne incognita* (Kofoid and White) Chitwood) resistant and susceptible flue-cured tobacco cultivars; PVY(MN), which causes a necrotic reaction on cultivars resistant to the root-knot nematode (Rk) but a mosaic reaction on cultivars susceptible to Rk, and PVY(NN), which produces necrotic symptoms on both Rk resistant and susceptible cultivars (14).

Tobacco etch virus (TEV), like PVY, is a member of the potyvirus group and can cause serious yield and leaf quality reductions in tobacco. Some PVY resistance mechanisms are known to provide protection against other members of the potyvirus group (15,21).

This study was undertaken to determine: 1) the stability of a gametoclonal PVY resistance source in a second cycle of anther culture, 2) whether an additional cycle of anther culture might prove useful in obtaining resistance to additional viruses of the potyvirus group, in particular, TEV, 3) whether the NC 602 PVY resistance mechanism was different from that found in the Virgin A Mutante (T.I. 1406) and if the two could be genetically combined, 4) whether this virus resistance could be combined with resistance to Rk to provide protection against both Rk and PVY(MN), and 5) the effect of this variant on the agronomic characteristics of flue-cured tobacco.

MATERIALS AND METHODS

General. All viruses were maintained in Burley 21 tobacco plants grown in insectproof cages, and inoculum was prepared from leaf lamina of infected plants. Leaf tissue was macerated with mortar and pestle in phosphate buffer (50 mM Na_2HPO_4-KH_2PO_4, pH 7.2) in the ratio of 1 g of tissue to 5 ml of buffer. Inoculum was mixed with 20 μm Carborundum powder and applied to the upper leaf surface by dipping a cotton swab into the inoculum and lightly rubbing the leaf surface. A second technique, employing an artist's airbrush, was also used to inoculate large numbers of plants. Inoculum was prepared in the manner described above, but was sprayed onto the leaves using 3.45×10^5 Pa of CO_2 to produce a water-soaked area of inoculum approximately 8–10 mm in diameter. This spot straddled the midvein on the underside of the leaf tip. Two different leaves of each plant were inoculated to reduce the possibility of missing a plant in the inoculation process.

Two methods were used to evaluate viral-induced stunting. One measured the stem length from the soil to the growing point in centimeters and the second used the fresh weight (in milligrams) of the plant cut off at soil level. Plants in both methods were compared with uninoculated controls.

Anther culture followed the procedures established by Nitsch and Nitsch (22). Maternally derived haploids were obtained using the *Nicotiana africana* Merxm. & Buttler method described by Burk et al (1). Chromosome doubling also used two procedures: The colchicine method developed by Burk et al (3) and the in vitro leaf midvein culturing method developed by Kasperbauer and Collins (17). Plants were initially grown in an environmentally controlled growth chamber (25 C with 16 h of fluorescent light per day). When plants were approximately 3 cm tall they were transplanted into 5-cm peat pots and transferred to the greenhouse for virus inoculation when leaves reached a length of approximately 5 cm.

Because PVY produces more severe symptoms at cooler temperatures, plants to be inoculated with PVY were taken from the growth chamber, placed in an air-conditioned greenhouse regulated to a temperature of 21 C, and illuminated under natural light conditions. TEV and Rk infections, on the other hand, exhibit more severe symptoms at higher temperatures; consequently, plants to be inoculated with these pathogens were removed from the growth chamber and placed directly in the greenhouse with an ambient temperature of approximately 30 C.

Back mutation experiment. Anthers from the doubled haploid (DH) gametoclonal variant NC 602 were cultured to determine if the PVY resistance in the variant remained stable through an additional cycle of anther culture. The resulting haploid plants were treated with colchicine and DH lines were obtained. Four plants of each DH line were inoculated with a cotton swab with PVY(NN). Uninoculated DH controls served to evaluate stunting. McN 944, NC 602, and NC 744 plants were also inoculated at the same time to serve as disease reaction controls. These controls were chosen for their parentage and their reaction to PVY(NN) (15,25). McN 944 is the parental cultivar of NC 602 and exhibits a necrotic reaction to the viral strain. NC 602 demonstrates a mosaic reaction to the strain while NC 744 shows no symptoms.

Resistance to additional viruses. Known resistance genes for PVY in Virgin A Mutante (VAM) (Tobacco Introduction line 1406) and Havana 307 have been shown to provide protection against both PVY and TEV (6,15). When a preliminary field experiment found the NC 602 resistance gene offered no protection to TEV, a controlled greenhouse experiment was conducted to determine if additional gametoclonal mutations could be found that would provide resistance to TEV as well as PVY. The same DH lines used in the back mutation experiment were inoculated with a highly virulent strain of TEV (NC isolate 155A). In addition to these DH lines, anther-derived haploids from NC 602 were also screened for TEV resistance. Those haploids that demonstrated some degree of tolerance (delayed onset of symptoms) were chromosome-doubled with the midvein culturing technique developed by Kasperbauer and Collins (17) and seeds were obtained to provide plants for a comparison test with the diploid controls, McN 944 and NC 602. Six plants of each genotype were matched by size and transferred to peat pots. Three plants were inoculated as outlined above, whereas three uninoculated plants were used for stunting estimation using the fresh weight method of comparison. Because resistance is a qualitative trait, three replications of each genotype are considered sufficient. Leaf necrosis was evaluated subjectively as mild (0–25%), moderate (25–75%), and severe (75–100%).

Combining genetic mechanisms for virus resistance. NC 602 was crossed with a second source of PVY resistance, NC 744, and the F_1 was crossed to *N. africana*. Maternally derived tobacco haploids were identified among the surviving plants of the interspecific cross as prescribed by Burk et al (1). The haploid plants were chromosome-doubled using the midvein culturing technique of Kasperbauer and Collins (17), resulting in 20 maternal doubled haploid (MDH) lines.

We tried to identify the four expected resistance combinations of MDH lines by using different strains of PVY under the assumption of additive gene effects. PVY strains VAM-B (NC isolate 182), NN (NC isolate 78), and SA (NC isolate 187) were used to separate the four genotypes. NC 744, NC 602, and McN 944 were used as controls. The predicted reactions of these controls to the strains used are presented in Table 1. Each of the eight replications of the completely randomized experiment consisted of four plants each of the MDH lines and the three control cultivars. One plant of each genotype was inoculated with one strain of the virus, and the fourth was used as the uninoculated control. Inoculum was applied with a cotton swab.

Combining the resistances to PVY and Rk. The gametoclonal variant NC 602 was crossed with flue-cured cultivar Coker 209 (C209), which is resistant to the Rk. The F_1 was crossed to *N. africana* to obtain a series of maternal haploids representing the complete gametic array. Maternal haploids were recovered, grown in a growth chamber until 12–15 cm high, and then transplanted into 15-cm clay pots in the greenhouse. When the plants began to flower, they were repotted into 23-cm clay pots and topped to induce growth from the leaf axils. Four sets of axillary shoots were removed from each plant and rooted in deep-dish glass culture dishes; a rooting compound was used to stimulate root initiation. The dishes were covered and placed in a growth chamber until roots were visible in the bottom of the dish. The rooted shoots were then transplanted into 15-cm clay pots in the greenhouse. At the same time all haploid plants were midvein-cultured to double the chromosome complement.

One set of the transplanted shoots was inoculated with 20,000 eggs of *M. incognita* race 3 per plant and one was inoculated with PVY(NN). Each inoculated set had an additional uninoculated set for use as a control. The plants inoculated with PVY were evaluated 3 wk after inoculation for viral symptoms. The Rk-inoculated plants were evaluated 6 wk after inoculation for nematode infection. Plants were scored for weight of root mass, percentage of the root mass with infected nodules, and numbers of eggs present. All genotypes that showed resistance to both pathogens and one from each of the other possible resistance categories (resistant to PVY only, resistant ot Rk only, and resistant to neither pathogen) were continued in midvein culture. These 'other' category plants served as controls in the final diploid test. Chromosome-doubled plants from midvein cultures of the desired genotypes were selfed to obtain seeds. Six plants of each remaining genotype were inoculated with PVY(NN), PVY(MN), and 20,000 eggs of race 3 of *M. incognita* to determine responses.

Effects of the virus-resistant gene on agronomic traits. Because a gene had been added or altered to the genome to achieve this resistance, and the biochemical aspects of the resistance mechanism were unknown, efforts were made to determine if any effects on agronomic characteristics might result from the use of this variant in a breeding program. NC 602 was crossed to the parental cultivar, McN 944, 250 F_2 plants were screened for PVY(NN) resistance, and resistant plants were selfed for two generations. Fifty-eight resulting resistant lines were randomly selected and field tested for agronomic values. The 58 lines were randomly assigned to two sets, which also included NC 602 and McN 944 as control cultivars. The test was planted in a randomized complete block at Kinston, NC (28501), in 1987 with each set replicated four times. Plots consisted of 22-plant rows with 56 cm between plants and 120 cm between rows. End plants were used as competitive border plants and were not included in the data. Data were recorded for plant height (cm), days from transplanting to flowering, numbers of leaves per plant, cured leaf yield (kg/ha), cured leaf quality as measured by the grade index method (24), and percent total alkaloids and reducing sugars (16). The analysis of variance assumed a mixed model with fixed treatments and was analyzed as an experiment with sets in the manner of Comstock and Robinson (8). Treatment mean squares were partitioned into contrasts between the checks, the midparent and the resistant lines, and among the resistant lines.

RESULTS

Back mutation experiment. Anther culture of NC 602 produced 1,128 haploid plants, 192 of which were chromosome doubled to produce DH lines. When inoculated with PVY, all lines exhibited mild to moderate mosaic symptoms as found in NC 602. Variation in stunting was observed not only across genotypes, but also among plants within genotypes. The stem height method was used to measure stunting in two replications of inoculated plants. Viral infection resulted in an 18.5% reduction in height averaged over all DH genotypes. The fresh plant weight method was used to measure stunting in the remaining replications and demonstrated on average overall reduction in weight of 17.2%.

TABLE 1. Predicted disease reactions of tobacco genotypic categories to three strains of potato virus Y

Entry	Genotype[a]	PVY strain		
		VAM-B	NN	SA
NC 602[b]	PPVV	Mosaic	Mosaic	Mild necrosis
NC 744[c]	ppvv	Death	No symptoms	No symptoms
McN 944[b]	ppVV	Death	Death	Mild necrosis
Additive[d]	PPvv	Mosaic	No symptoms	No symptoms

[a] Genotype refers to Figure 1.
[b] From Witherspoon (25).
[c] From Gooding (13).
[d] Assumption of additive gene effects.

Resistance to additional viruses. Twenty-two of the 192 anther-derived DH lines with milder or delayed symptoms to TEV compared with the control were selected for further analysis. Of the 1,008 anther-derived haploid plants of NC 602 screened directly for TEV resistance, six demonstrated varying degrees of resistance. Upon chromosome doubling of these six plants, three DH lines produced plants both with and without visual virus symptoms (leaf mosaic). In these three lines, seeds from plants both with and without mosaic symptoms were collected and further tested with the controls. No difference was observed for disease development between the two symptom types within these three lines.

The 22 DH lines obtained from colchicine treatment and screened as diploids and the nine DH lines obtained from midvein culture after initial screening as haploids were compared to NC 602 and McN 944 for viral symptoms and time of symptom onset. Of these 31 DH lines, all exhibited the typical symptoms of stunting and necrosis associated with TEV infection in flue-cured tobacco (21). Whereas most of the genotypes exhibited some necrosis 7 days after inoculation and became severely necrotic after 11 days, three genotypes exhibited a 1-wk delay in symptom onset and did not become severely necrotic until 18 days after inoculation. When we used fresh plant weight as a measure, mean stunting was estimated as 60%. The three lines showing delayed symptoms also exhibited reduced stunting (37%).

Combining genetic mechanisms for PVY resistance. Observed reactions of the control genotypes, NC 744, McN 944, and NC 602, corresponded to those predicted in Table 1, except that the SA strain produced only mosaic symptoms on McN 944 and NC 602. A fourth distinct reaction category was detected that corresponded to the predicted reaction of the combined resistance under the assumption of additive gene action. Thus, the screening techniques developed in this test provided adequate separation of symptoms to distinguish between four different combinations of resistance (Table 2) and provided evidence that the two resistance mechanisms in VAM and NC 602 result from genes at different loci. A chi-square test indicated that the distribution of the four classes was not significantly different from that which was expected if the two resistance mechanisms were controlled by single genes at independent loci (Fig. 1).

Combining resistance to PVY and Rk. Each of the selected DH lines with resistance to Rk gave a necrotic reaction when

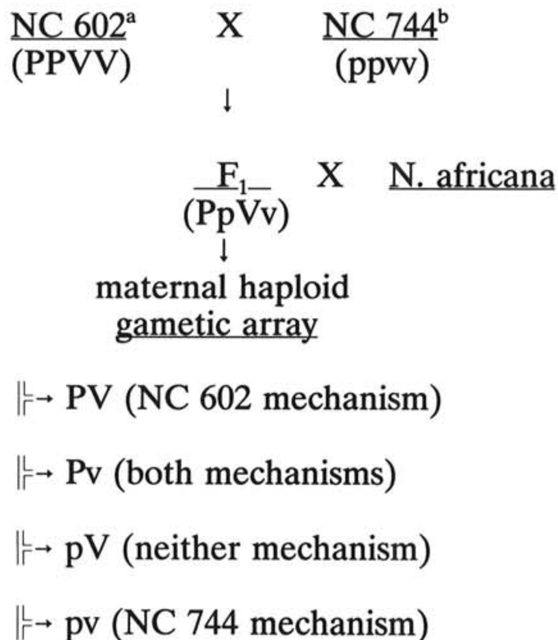
TABLE 2. Observed reactions of tobacco genotypes to three strains of potato virus Y (PVY)

Entry	PVY strain			Genotype[a]
	VAM-B	NN	SA	
NC 602	Mosaic	Mosaic	Mosaic	PPVV
NC 744	Death	No symptoms	No symptoms	ppvv
McN 944	Death	Death	Mosaic	ppVV
GH86 1881-1	Death	Death	Mosaic	ppVV
GH86 1881-2	Death	Death	Mosaic	ppVV
GH86 1881-3	Mosaic	No symptoms	No symptoms	PPvv
GH86 1881-5	Death	Death	Mosaic	ppVV
GH86 1881-6	Death	No symptoms	No symptoms	ppvv
GH86 1881-8	Mosaic	Mosaic	Mosaic	PPVV
GH86 1881-9	Mosaic	No symptoms	No symptoms	PPvv
GH86 1881-10	Death	Death	Mosaic	ppVV
GH86 1881-11	Mosaic	No symptoms	No symptoms	PPvv
GH86 1881-13	Death	Death	Mosaic	ppVV
GH86 1881-14	Mosaic	No symptoms	No symptoms	PPvv
GH86 1881-15	Mosaic	Mosaic	Mosaic	PPVV
GH86 1881-17	Mosaic	Mosaic	Mosaic	PPVV
GH86 1881-18	Death	No symptoms	No symptoms	ppvv
GH86 1881-20	Death	Death	Mosaic	ppVV
GH86 1881-22	Death	No symptoms	No symptoms	ppvv
GH86 1881-23	Death	Death	Mosaic	ppVV
GH86 1881-24	Mosaic	No symptoms	No symptoms	PPvv
GH86 1881-25	Mosaic	No symptoms	No symptoms	PPvv
GH86 1881-28	Death	No symptoms	No symptoms	ppvv

Actual $\chi^2 = 2.0$; Tabular $\chi^2_{(3\ df;\ P=0.05)} = 7.81$

[a] Genotype refers to Figure 1.

inoculated with the PVY strain MN, regardless of the presence of the NC 602 gene for PVY resistance. As in the previous experiment, haploid production of a complete gametic array was demonstrated with 6/23 plants resistant to both PVY and Rk, 8/23 resistant to neither, 4/23 resistant to PVY only, and 5/23 resistant to root-knot only. Because each resistance is controlled by a single dominant gene (10,21), these numbers give a nonsignificant chi-square value of 1.52, indicating that the data fit the expected ratio.



[a] Additive resistance gene of NC 602 denoted by "P"

[b] Recessive resistance gene of NC 744 denoted by "v"

Fig 1. Procedure for combining the genes for resistance to potato virus Y from two tobacco breeding lines into a single genotype.

Effects of the PVY resistance gene on agronomic traits. Significant differences for yield and days from transplanting to flowering were observed among the genotypes tested. Contrasts also revealed significant differences between the check cultivars (McN 944 and NC 602) for these two traits. In contrasting the midparent value with the mean of the resistant lines, differences were found in plant height and days from transplanting to flowering. Among line variation within the population was significant for reducing sugars and yield (Table 3). A comparison of the means of the check cultivars and the resistant lines indicated that the population of resistant lines resembled McN 944 for plant height and NC 602 for days from transplanting to flowering (Table 4). Although the mean value for yield among the resistant lines was equivalent to the midparent value of the two checks, considerable genetic variation exists within the population and 10% of the lines exhibit yield differences from McN 944 of less than 3%.

DISCUSSION

When stunting in these experiments was estimated, environmental variation and random error within genotypes resulted in some lines exhibiting apparent increased growth in the inoculated plants when compared with controls. Although we tried to match plants by size within genotypes before inoculation, the plants grew at different rates in response to the microenvironment. Crowding and shading resulted, which further increased differential growth. Because of the number of controls used in these experiments, a clear superiority of the fresh weight method in estimating stunting was not established, because results were similar for both fresh weight and plant height techniques. However, because stunting is not limited solely to stem length but includes the entire plant structure, the use of plant fresh weight is expected to produce a more accurate estimate of stunting than plant height alone. Additional testing is needed.

The 192 genotypes evaluated were all DH and are representative of the expected number of homozygotes to be found in a population of 36,864 conventionally bred F_2 plants; therefore, the population is considered adequate to evaluate back mutation tendencies. Based on this work, it appears that the gametoclonal variant NC 602 does not readily revert to a susceptible reaction

TABLE 3. Partitioning of treatment mean squares for quantitative traits for determination of the effect of tobacco line NC 602 potato virus resistance on agronomic characteristics

		Mean squares						
Source	df	Plant height	Leaf number	Days to flower	Grade index[a]	Total alkaloids	Reducing sugars	Yield
Treatments (sets)	60	35.65	2.11	4.71*[b]	12.48	0.092	3.01	110,153.99**
Between parents (sets)	2	78.63	2.00	13.63*	8.13	0.205	0.14	572,985.63**
Between midparent and lines (sets)	2	123.56**	2.87	11.56*	21.30	0.010	0.53	21,574.88
Among lines (sets)	56	30.98	2.09	4.15	12.32	0.091	3.20*	96,787.82**
Error	180	25.99	1.09	3.27	11.73	0.078	2.17	59,110.09

[a] Grade index is a measure of leaf quality; higher numbers indicate superior quality.
[b] Single asterisk indicates significance at the 0.05 level; double asterisk indicates significance at 0.01 level.

TABLE 4. Means of quantitative traits measured in the agronomic evaluation of the NC 602 resistance mechanism

Genotype	Plant height (cm)	Leaf number	Days to flower	Grade index[a]	Total alkaloids (%)	Reducing sugars (%)	Yield (kg/ha)
McNair 944	97	19	61	34	3.75	15.5	2,738
NC 602	91	19	59	34	4.06	15.3	2,214
Mean of 58 resistant lines	98	19	59	35	3.90	15.6	2,459
Extreme high	106	21	61	41	4.19	17.4	2,762
Extreme low	91	17	56	32	3.47	13.6	2,082
LSD 0.05[b]	4	1	1	2	0.20	1.1	173

[a] Grade index is a measure of leaf quality; higher numbers indicate superior quality.
[b] LSD to determine differences between the maternal doubled haploid lines and check cultivars.

when placed in anther culture. Because the resistant allele shows no instability in culture, it may be used to develop resistance in cultivars using either conventional or tissue culture breeding programs.

No genotypes expressing immunity to TEV were identified in the evaluation of the DH lines. Although all genotypes derived from anther culture of NC 602 were susceptible to TEV, three DH lines were rated as partially tolerant because of the delayed onset of symptoms and reduced stunting. The fact that no TEV resistance was found supports the belief that this PVY resistance is not related to previously known resistances and has been obtained as a direct result of anther culture. The ability to combine the two PVY resistances found in NC 602 and NC 744 demonstrates that different genes are involved and that they exhibit additive gene effects. The genetic combination of these resistances establishes a broader base than currently exists from which to develop resistant cultivars and provides the breeder with an additional tool to combat PVY.

Rufty et al (23) proposed that the gene responsible for Rk resistance has a pleiotropic effect and imparts susceptibility to the necrotic action of PVY(MN). Although genotypes were identified with both the NC 602 resistance to PVY and the C209 resistance to Rk, the ability of PVY(MN) strain to cause necrotic reactions on all Rk resistant lines regardless of the presence of the NC 602 resistance mechanism in the genome provides further evidence that virus resistance and nematode resistance genes are not epistatic. Burk et al (2) reached a similar conclusion with the PVY resistance found in NC 744 (VAM). Thus, two different PVY resistances, when combined with Rk resistance, give identical necrotic responses to the PVY(MN) strain and provide further indication that Rk resistance may have a pleiotropic effect on different viral resistance mechanisms.

Whereas mean yield for the resistant lines was substantially less than the original cultivar, McN 944, one of the lines exceeded the yield of McN 944. The data show that NC 602 sustained an average yield reduction of nearly 20%. Because a single backcross recovered one-half of the lost yield and considerable genetic variation for yield remains in the resistant population, this reduction is not considered related to the virus resistance and it should be possible to select lines with virus resistance and yielding ability equal to McN 944. Therefore, the presence of the NC 602 resistance mechanism in the genome appears to have little adverse effect on agronomic performance of flue-cured tobacco cultivars.

LITERATURE CITED

1. Burk, L. G., Gerstel, D. U., and Wernsman, E. A. 1979. Maternal haploids of *Nicotiana tabacum* L. from seed. Science 206:585.
2. Burk, L. G., Gooding, G. V., Jr., and Chaplin, J. F. 1979. Reaction to necrotic strain of potato virus Y by tobacco lines with combinations of resistance and susceptibility to root-knot and potato virus Y. Tob. Sci. 23:45-46.
3. Burk, L. G., Gwynn, G. R., and Chaplin, J. F. 1972. Diploidized haploids from aseptically cultured anthers of *Nicotiana tabacum*: A colchicine method applicable to plant breeding. J. Hered. 63:355-360.
4. Burk, L. G., and Matzinger, D. F. 1976. Variation among anther derived doubled haploids from an inbred line of tobacco. J. Hered. 67:381-384.
5. Chaleff, R. S., and Keil, R. L. 1981. Genetic and physiological variability among cultured cells and regenerated plants of *Nicotiona tabacum*. Mol. Gen. Genet. 181:254-258.
6. Chaplin, J. F., Burk, L. G., Gooding, G. V., Jr., and Powell, N. T. 1980. Registration of NC 744 tobacco germplasm. Crop Sci. 20:677.
7. Collins, G. B., Legg, P. D., and Litton, C. C. 1972. The use of anther-derived haploids in *Nicotiana* II. Comparison of doubled haploid lines with lines obtained by commercial breeding methods. Tob. Sci. 18:40-42.
8. Comstock, R. E., and Robinson, H. F. 1952. Estimation of average dominance of genes. Pages 494-535 in: Heterosis. John W. Gowen, ed. Iowa State College Press, Ames.
9. de Bokx, J. A., and Huttinga, H. H. 1981. Potato virus Y. No. 242 in: Descriptions of Plant Viruses. Commonw. Mycol Inst., Assoc. Appl. Biol., Kew, Surrey, England.
10. Drolsom, P. N., Moore, E. L., and Graham, T. W. 1958. Inheritance of resistance to root-knot nematodes in tobacco. Phytopathology 48:686-689.
11. Evans, D. A., Sharp, W. R., and Medina-Filho, H. P. 1984. Somaclonal and gametoclonal variation. Am. J. Bot. 71:759-774.
12. Gengenbach, B. G., Green, C. E., and Donovan, C. M. 1977. Inheritance of selected pathotoxin resistance in maize plants regenerated from cell culture. Proc. Natl. Acad. Sci. USA 74:5113-5117.
13. Gooding, G. V., Jr. 1985. Relationship between strains of potato virus Y and breeding for resistance, cross protection and interference. Tob. Sci. 29:99-104.
14. Gooding, G. V., Jr., and Tolin, S. A. 1973. Strains of potato virus Y affecting flue cured tobacco in the southeastern United States. Plant Dis. Rep. 57:200-204.
15. Gooding, G. V., Jr., Wernsman, E. A., and Rufty, R. C. 1985. Reaction of *Nicotiona tabacum* L. cultivar Havana 307 to potato virus Y, tobacco vein mottling virus, tobacco etch virus and *Peronospora tabacina*. Tob. Sci. 29:32-35.
16. Harvey, W. R., Stahr, H. M., and Smith, W. C. 1969. Automated determination of reducing sugars and nicotine alkaloids on the same extract of tobacco leaf. Tob. Sci. 13:13-15.
17. Kasperbauer, M. J., and Collins, G. H. 1972. Reconstitution of diploids from leaf tissue of anther-derived haploids in tobacco. Crop Sci. 12:98-101.
18. Lapp, N. A., and Gooding, G. V., Jr. 1976. Occurrence and sources of inoculum of potato virus Y in tobacco in North Carolina. Plant Dis. Rep. 60:1014-1016.
19. Larkin, P. J., and Scowcroft, W. R. 1983. Somaclonal variation and eyespot toxin tolerance in sugarcane. Plant Cell Tissue Organ Cult. 2:111-121.
20. Lattorre, B. A., Andrade, O., Penaloza, E., and Escaffi, O. 1982. A severe outbreak of potato virus Y in Chilean tobacco. Plant Dis. 66:893-895.
21. Lucas, G. B. 1975. Pages 457-469 in: Diseases of Tobacco. 3rd ed. Biological Consulting Associates, Raleigh, NC.
22. Nitsch, J. P., and Nitsch, C. 1969. Haploid plants from pollen grains. Science 163:85-87.
23. Rufty, R. C., Powell, N. T., and Gooding, G. V., Jr. 1983. Relationship between resistance to *M. incognita* and a necrotic response to infection by a strain of PVY in tobacco. Phytopathology 73:1418-1423.
24. Wernsman, E. A., and Price, E. L. 1975. North Carolina grade index for flue-cured tobacco. Tob. Sci. 19:119.
25. Witherspoon, W. D., Jr. 1987. Utilization of the haploid sporophyte as the selection unit in tobacco breeding. Ph.D. dissertation. North Carolina State University, Raleigh. Diss. Abstr. 87-12560.