Field and Greenhouse Analysis of Variation for Disease Resistance in Tobacco Somaclones

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Supported in part by funds from the North Carolina Tobacco Foundation.

Paper 11739 of the Journal Series of the North Carolina Agricultural Research Service, Raleigh 27695-7601.

We wish to thank D. Wayne Pollard for excellent technical assistance and S. T. Barnes, W. C. Clements, R. D. Coltrain, and W. W. Weeks for assistance with the field station operations and yield and leaf chemistry determinations. We also thank R. Rufty for cultivar pedigree information and S. Leath for critical review of the manuscript.

Accepted for publication 27 December 1988 (submitted for electronic processing).

ABSTRACT

Daub, M. E., and Jenns, A. E. 1989. Field and greenhouse analysis of variation for disease resistance in tobacco somaclones. Phytopathology 79:600-605.

A total of 854 somaclones of two flue-cured tobacco cultivars were generated from protoplast cultures, and their progeny analyzed in greenhouse and field tests for yield, leaf chemistry, and resistance to black shank, bacterial wilt, tobacco mosaic virus, and root knot (*Meloidogyne incognita*). Under the culture conditions established in this study, approximately 55% of the somaclones were not self-fertile. Progeny of the somaclones had normal phenotype and did not differ significantly from the parent cultivars in yield and leaf chemistry. Significant variation was found in resistance to black shank and bacterial wilt, two diseases for which the parent cultivars have low levels of resistance. The variation that was observed in response to these diseases depended on the disease as well as the genotype of the parent. Somaclones of cultivar NC2326 were similar to the parent cultivar in black shank resistance, but many had greater resistance to bacterial wilt than did NC2326. Most cultivar Coker 319 somaclones were more susceptible than the parent cultivar to black shank but had bacterial wilt resistance similar to that of Coker 319. Variation in black shank and bacterial wilt resistance was slight, and few somaclones had responses equivalent to those of the susceptible and resistant control cultivars. However, conditions used in this study reduced the amount of potential variation. No somaclones were identified with resistance to tobacco mosaic virus or *M. incognita*. We conclude that genetic variation occurred in the somaclones, that the magnitude of variation was slight, and that the variation depended on both the genotype of the parent cultivar and the trait.

Additional keywords: *Nicotiana tabacum*, *Phytophthora parasitica* var. *nicotianae*, *Pseudomonas solanacearum*, tissue culture.

In recent years there has been much interest in the use of somaclonal variation for crop improvement (14). This tissue-culture-induced variation occurs at high frequencies (7,16,30), is stable and heritable, and occurs for both monogenic and polygenic traits (3,7,14,30). Somaclonal variation has been reported to occur for traits that are useful in crop improvement such as yield, maturity, and disease resistance (6,7,14), and at least two cultivars have been released that originated from somaclones (13,19). Somaclonal variation has been reported in a large number of crop species and can be obtained through a variety of tissue culture sources including callus, protoplast, embryo, and meristem cultures (6,14). It has been shown that the frequency and type of mutations that occur in somaclones are different from those induced by chemical mutagenesis of seeds and pollen (9).

The above findings were based on early studies of somaclonal variation, which were primarily laboratory and greenhouse analyses, many utilizing small numbers of plants. Several large-scale field studies of somaclones and their progeny have been reported recently; some of these studies suggest that the amount of variation found in somaclones is less than was previously estimated (7,17,18,24). Further, most studies have dealt with agronomic traits. There are few field studies of variation in disease resistance (4,8,11,23) and none for tobacco. The purpose of this research was to determine whether somaclonal variation might be useful in generating tobacco lines with increased levels of resistance to several important tobacco pathogens. Specifically, we wanted to know if variation in disease resistance could be induced and if the variant somaclones would retain desirable yield and quality characteristics present in the parent cultivar. Here we report on greenhouse and/or field analyses of disease resistance, yield, and leaf chemistry traits of somaclones derived from two high-quality tobacco cultivars that are susceptible or have only low levels of resistance to four major diseases of tobacco.

MATERIALS AND METHODS

Somaclone production. The flue-cured tobacco cultivars NC2326 and Coker 319 were used to generate somaclones. Both cultivars have excellent agronomic characters. Both have similar, partial resistance to black shank and bacterial wilt. The 4-yr (1984–1987) average black shank disease index value compiled from tests in three southeastern states was identical for the two cultivars (disease index = 37); for bacterial wilt, 4-yr averages were 32 for NC2326 and 22 for Coker 319 (Tobacco Disease Evaluation Committee Annual Report, *unpublished*). Neither cultivar has detectable resistance to tobacco mosaic virus (TMV) or root knot nematodes.

Plants were grown in the greenhouse in a 1:1 soil:sand mixture in 15-cm pots. Protoplasts were isolated from fully expanded, young leaves from plants 2–3 mo old by the methods of Jenns et al (12) with the addition of two washing steps following flotation on sucrose. After isolation, protoplasts were incubated at a concentration of 5×10^4/ml in the medium of Nagata and Takebe (22) with 3 mg of naphthaleneacetic acid, 1 mg of 6-benzyladenine, 1 mg of 2,4-dichlorophenoxyacetic acid (2,4-D), 17 g of sucrose, and 82 g of mannitol per liter with 20 ml of sterile coconut water (Gibco Laboratories, Grand Island, NY) and 0.25 g of vitamin-free casamino acids per liter added after autoclaving (D3 medium). Protoplasts were grown in liquid medium for 6 wk with weekly additions of D3 medium without mannitol. At 6 wk, individual cell clumps were transferred to solid Murashige and Skoog (MS) medium (20) with 3 mg of indoleacetic acid (IAA) and 0.3 mg of kinetin per liter and grown for an additional 10 wk with two transfers to fresh medium after 2 and 6 wk. Callus tissue then was transferred to MS medium with 0.3 mg of IAA and 10 mg of 2-isopentenyl adenine per liter. When shoots formed, they were transferred to MS medium without hormones for rooting and finally into soil in the greenhouse. Plants (R_0 generation) were grown to flowering, the flowers were covered with bags to prevent

outcrossing, and seed was harvested at maturity. All tests were conducted with seed progeny (R_1 families) of the first-generation somaclones. Thus somaclones were evaluated based on the response of their progeny. Each R_1 family is hereafter referred to as a somaclone.

Greenhouse inoculations. Somaclones were analyzed in the greenhouse for resistance to TMV, *Meloidogyne incognita* (Kofoid and White) Chitwood, and *Phytophthora parasitica* Dast. var. *nicotianae* (Breda de Haan) Tucker. Isolate NC-40 of TMV was provided by G. V. Gooding, North Carolina State University, Raleigh. Inoculum was maintained on plants of *Nicotiana tabacum* L. 'Hicks' and was prepared for use by blending infected leaves with 0.05 M phosphate buffer, pH 7.2. The inoculum suspension was filtered, mixed with Carborundum, and atomized onto 5-wk-old seedlings (about 100 seedlings per 11-cm pot, two replications) with an airbrush sprayer. Tobacco plants expressing hypersensitive resistance to TMV die following inoculation, whereas susceptible plants survive. Plants that survived were grown for an additional 3–4 wk and scored for mosaic symptoms to screen for nonhypersensitive resistance. Each somaclone was scored as susceptible, resistant, or segregating if both susceptible and resistant seedlings were present.

A race 3 isolate of *M. incognita* was provided by K. R. Barker, North Carolina State University. Inoculum was grown by inoculating roots of 4-wk-old tomato plants with 10–12 egg masses and growing the plants for 6 wk. Inoculum was prepared by mixing soil and roots from the infected plants with 3 parts soil and 3 parts sand. Six-week-old tobacco plants were transplanted into this mixture (12 plants/21 × 15 cm plastic tray, two replications) and grown for 6 wk. Plants were rated for the presence or absence of galls. Somaclones were scored as resistant, susceptible, or segregating.

Two race 0 isolates of *P. p. nicotianae*, 1189A-3 and 455, were provided by H. D. Shew, North Carolina State University. Isolates were grown on oatmeal agar (50 g of oatmeal, 15 g of agar per liter). Tobacco seedlings were transplanted at 6 wk and inoculated 3 wk later. Inoculations were done according to the methods of Apple (1). Plants were evaluated when 100% of susceptible check plants (cultivar Hicks) had died (10–14 days after inoculation). The number of dead plants out of nine in each replication was recorded. Values given are the mean percent of dead plants in two replications.

Field evaluations. Somaclones were evaluated in the field in 1986 and 1987 for yield, leaf chemistry, and resistance to black shank and bacterial wilt (*Pseudomonas solanacearum* E. F. Sm.). Plants were grown in 20-plant, single-row plots (disease nurseries) or 22-plant, single-row plots (yield), in a completely randomized design with two to three replications per location with a plant spacing of 0.56 m within and 1.2 m between rows. Leaves were harvested four to five times during the season from the inside 20 plants of the yield plots, cured, and weighed for yield determinations. Samples of cured leaf were analyzed for percent total alkaloids and percent reducing sugars by the Tobacco Analytical Services Laboratory at North Carolina State University following the methods of Harvey et al (10). Black shank resistance evaluations were made in two disease nurseries: one at the Lower Coastal Plain Tobacco Research Station in Kinston, NC, and one at the Upper Coastal Plain Research Station in Rocky Mount, NC. These nurseries were established in 1976 and 1977, respectively, with inoculum of two race 0 isolates of *P. p. nicotianae*, 955-5 and 1189A-3. Resistance to bacterial wilt was evaluated in a nursery at the Oxford Tobacco Research Station in Oxford, NC, and in a naturally infested grower's field near Kinston, NC. At Oxford, plants were inoculated with isolate K-60 of *P. solanacearum* at transplanting by mixing inoculum with the transplant water ($A_{600nm} = 0.05$, 85 ml/plant), in addition to being planted in infested soil. Disease resistance was evaluated by taking stand counts every 2 wk starting at 2 wk after transplanting (5). With both diseases, the number of plants killed or irreversibly wilted out of 20 in each plot was recorded during each stand count. Evaluation of black shank infections also was based on blackening of the lower stem and disking of the pith in random samples. Typical symptoms of bacterial wilt started with unilateral wilting of the plant. Random samples also were taken to confirm the presence of bacteria in the vascular tissue. For both diseases, a disease index was computed according to the following formula:

$$\text{Disease index} = \frac{\sum_{y=2}^{y=n} y_n [100 - (n-2)x]}{z}$$

where n = the number of a particular stand count, $x = 100/$ the total number of stand counts minus 1, y = number of plants that have died since the previous stand count, and z = number of plants in the first stand count. High disease index values indicate high levels of disease.

All somaclones were tested for resistance to TMV, *M. incognita*, and *P. p. nicotianae* in the greenhouse. All NC2326 somaclones were tested in the field in 1986. Seed for 1987 was harvested from surviving plants (flowers were covered with bags to prevent outcrossing) in the Rocky Mount black shank nursery in 1987. Thus, not all lines were repeated in 1987. Random lines were selected from the Coker 319 somaclones. None of these somaclones performed well in the black shank test in 1986; thus different Coker 319 somaclones were planted in the field nurseries in 1987. In 1986, R_2 families harvested from a field test in 1985 also were planted for a test of variance in and among families. In all tests, two tobacco cultivars, Hicks, which is highly susceptible to all diseases tested, and Coker 86, which is resistant to TMV and *M. incognita* and has moderate to high levels of resistance to black shank and bacterial wilt, were included as controls.

RESULTS

A total of 161 somaclones of NC2326 and 693 somaclones of Coker 319 were regenerated and survived transfer into soil in the greenhouse. In early studies, cultures were maintained on medium with 2,4-D for the full 16 wk before shoot induction. Under these conditions, many plants were highly abnormal in appearance. Thus, the media sequence described in the Materials and Methods section was adopted. Under these conditions, most plants appeared phenotypically normal. Some phenotypic abnormalities were noted, such as altered leaf shape, altered petioles, reduced internode distance, leaf variegation, and altered flower color and shape. Variation was induced by our methods as evidenced by the fact that only 46 and 45%, respectively, of the NC2326 and Coker 319 R_0 somaclones were self-fertile. Because we wanted to analyze only variation that is transmitted by seed, we discarded plants that were not self-fertile.

Correlations were obtained among the responses of the somaclones to black shank in the three different tests. In both years, results of the tests in the two field locations were correlated ($r = 0.493$, $P = 0.0001$ [1986], $r = 0.631$, $P = 0.0001$ [1987]). Results of the greenhouse black shank tests also were correlated with results at Rocky Mount ($r = 0.336$, $P = 0.0003$ [1986], $r = 0.290$, $P = 0.0369$ [1987]) and Kinston ($r = 0.243$, $P = 0.0092$ [1986], $r = 0.409$, $P = 0.0026$ [1987]). The same was not true for the two bacterial wilt tests at Kinston and Oxford. Correlation coefficients were 0.131 ($P = 0.1580$) for 1986 and 0.022 ($P = 0.8651$) for 1987. Based on the response of the susceptible cultivar Hicks, disease was more severe at Oxford in 1987 than in 1986 (Fig. 1). At Kinston, the opposite was true; more severe disease occurred in 1986 than in 1987 (Fig. 2). With black shank more disease occurred in both locations in 1987 than in 1986 (Figs. 3 and 4; black shank disease index value for Hicks at Kinston in 1986 = 60).

Normality tests (26,28) indicated that, with few exceptions, variation of somaclones for black shank and bacterial wilt resistance, yield, and leaf chemistry was normally distributed. In several tests, the mean of all the somaclone lines was significantly different from the mean of the parental cultivar. In both 1986 and 1987, the NC2326 somaclones as a group performed significantly better in the Oxford bacterial wilt test than did NC2326 (Fig. 1); significant differences were not seen at Kinston, although in both years the mean disease index of the somaclones was lower than that of NC2326 (Fig. 2). In the field black shank tests, the somaclones

often performed significantly worse than did the parental cultivar. This was true for the Coker 319 somaclones at Rocky Mount in both 1986 and 1987 (Fig. 3) and for both sets of somaclones in the Kinston black shank nursery in 1987 (Fig. 4). Somaclone means did not differ significantly from parent means in the other field tests. Means also did not differ in the greenhouse black shank tests (Fig. 5). Lack of significant differences between the mean of all the somaclones and the parental mean does not necessarily indicate that no significant somaclonal variation for those traits exists. Significant somaclonal variation was detected for both cultivars and both diseases in at least one year by an F-test of the somaclone term in a combined analysis of variance across locations ($P \leqslant 0.05$). Somaclone and parental means did not differ significantly for yield and leaf chemistry characteristics (Table 1).

There were definite differences between parent cultivars in the responses of their somaclones. Depending on the disease, Coker 319 somaclones performed the same as the Coker 319 parent or worse, whereas NC2326 somaclones performed the same or better than NC2326. The mean black shank disease index of the Coker 319 somaclones was significantly worse than the mean of the parent at Rocky Mount in 1986 and 1987 and at Kinston in 1987 (Figs. 3 and 4B). Differences at Kinston in 1986 were not significant, but low disease levels at Kinston in 1986 obscured differences in resistance. By contrast, in only one case was the mean disease index of the NC2326 somaclones significantly higher than that of NC2326 (Kinston, 1987, see Fig. 4A). In the other three trials, there was no difference between the mean of the NC2326 somaclones and NC2326 (disease index values for Rocky Mount 1986 and 1987 and Kinston 1986, respectively, were 34 and 28, 49 and 47, and 9 and 5 for the somaclones and NC2326, respectively). The tendency for NC2326 somaclones to perform better than Coker 319 somaclones also was evident with the bacterial wilt data.

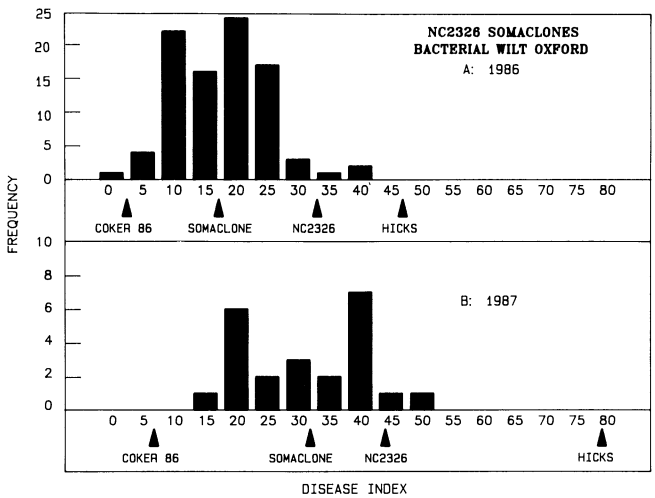

Fig. 1. Response of NC2326 somaclones to bacterial wilt at Oxford, NC, in **A,** 1986 and **B,** 1987. Arrows indicate mean disease index values for the parent cultivar NC2326, the control cultivars Coker 86 and Hicks, and the mean of all the somaclones. High disease index values indicate high levels of disease. Frequency is the number of somaclones with mean disease index values indicated ± 2.5. Differences between the mean of NC2326 and the mean of all somaclone lines were significant for both 1986 ($P = 0.0005$) and 1987 ($P = 0.0004$).

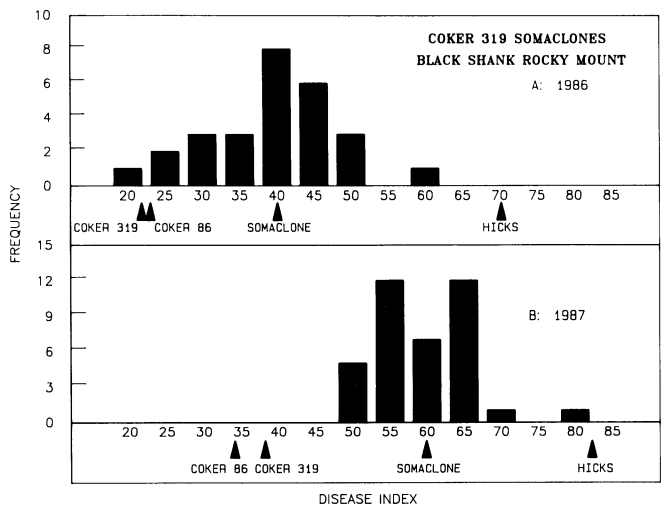

Fig. 3. Response of Coker 319 somaclones to black shank at Rocky Mount, NC, in **A,** 1986 and **B,** 1987. Arrows indicate mean disease index values for the parent cultivar Coker 319, the control cultivars Coker 86 and Hicks, and the mean of all the somaclones. Frequency is the number of somaclones with mean disease index values indicated ± 2.5. Differences between the mean of NC2326 and the mean of all somaclone lines were significant for both 1986 ($P = 0.0018$) and 1987 ($P = 0.0015$).

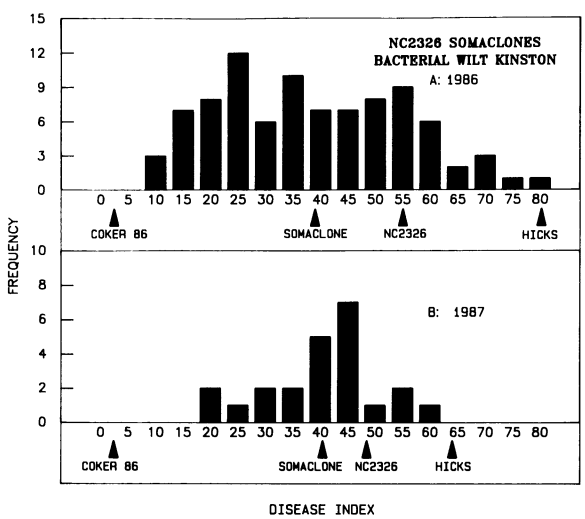

Fig. 2. Response of NC2326 somaclones to bacterial wilt at Kinston, NC, in **A,** 1986 and **B,** 1987. Arrows indicate mean disease index values for the parent cultivar NC2326, the control cultivars Coker 86 and Hicks, and the mean of all the somaclones. Frequency is the number of somaclones with mean disease index values indicated ± 2.5. Differences between the mean of NC2326 and the mean of all somaclone lines were not significant in either year.

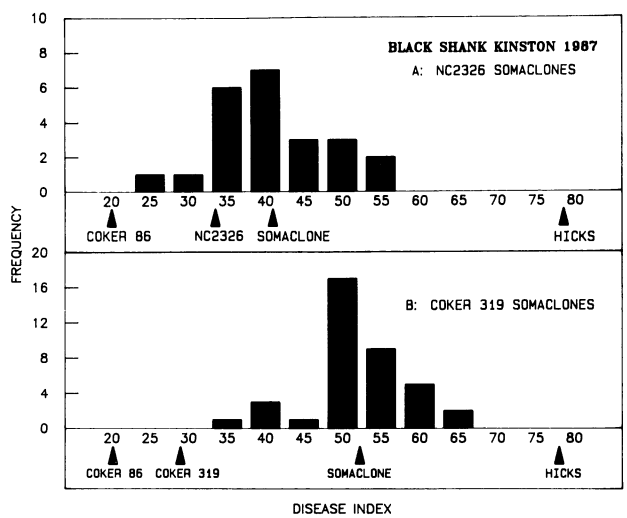

Fig. 4. Response of **A,** NC2326 and **B,** Coker 319 somaclones to black shank at Kinston, NC, in 1987. Arrows indicate mean disease index values for the parent cultivars NC2326 and Coker 319 and the control cultivars Coker 86 and Hicks, and the mean of each set of somaclones. Frequency is the number of somaclones with mean disease index values indicated ± 2.5. Differences between means were significant for NC2326 and the NC2326 somaclones ($P = 0.017$) and for Coker 319 and the Coker 319 somaclones ($P = 0.0001$).

The mean disease index for NC2326 somaclones was always lower than that of NC2326 (Figs. 1 and 2). With the Coker 319 somaclones, in only one test (Kinston 1986) was the somaclone disease index mean less than that of the parent (disease index values of 48 and 63, respectively, for the somaclones and Coker 319) and this difference was not significant. Differences between the NC2326 and Coker 319 somaclones also are quite evident in the greenhouse black shank data (Fig. 5). The NC2326 somaclones are fairly evenly distributed on either side of the parent, whereas the Coker 319 somaclones are shifted toward susceptibility.

As mentioned above, there were differences in the response of the somaclones to bacterial wilt and black shank. In general, disease index values of both sets of somaclones were equal to or lower than those of the parents for bacterial wilt, and equal to or higher than those of the parent for black shank. With the NC2326 somaclones, mean bacterial wilt disease index values were never higher for the somaclones than for NC2326 (Figs. 1 and 2); for black shank they were never lower (Fig. 4A). With Coker 319 somaclones, there were no significant differences in bacterial wilt disease index values between the somaclones and Coker 319, whereas in three of the four black shank trials, the somaclones performed significantly worse than did Coker 319 (Figs. 3 and 4B). These differences are clear from the percentage of the somaclones that lie outside the 95% confidence limits of the parent cultivars (Table 2). Very few of the NC2326 somaclones, for example, had

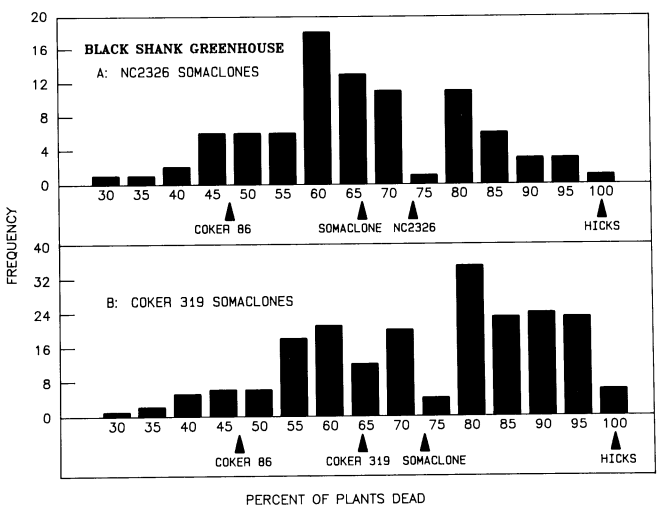

Fig. 5. Response of **A,** NC2326 and **B,** Coker 319 somaclones to black shank in the greenhouse. Arrows indicate mean values for percent of plants dead for the parent cultivars NC2326 and Coker 319, the control cultivars Coker 86 and Hicks, and the mean of each set of somaclones. Frequency is the number of somaclones with values for percent of plants dead indicated ± 2.5. Differences between the mean of the parent cultivars and the somaclones were not significant for either cultivar.

disease index values for black shank that were significantly lower (more resistant) than NC2326, but many were more susceptible. The reverse was true for bacterial wilt. None of the Coker 319 somaclones were more resistant to black shank than Coker 319, whereas there were lines both more resistant and lines more susceptible to bacterial wilt than the parent.

These data (Table 2) also were useful measures of variation in the somaclone population. In all tests a large proportion of the somaclones was outside the 95% confidence interval of the parent mean (either more susceptible or more resistant), demonstrating that significant levels of variation had been induced in the somaclones. The magnitude of the variation was slight, however, as evidenced by comparisons with the resistant and susceptible control cultivars (Coker 86 and Hicks) that were included in these studies. In most tests, few somaclones had disease index values within the 95% confidence intervals of the controls (that is, few were as resistant or as susceptible as Coker 86 and Hicks) (Table 3). Somaclone means fell within the 95% confidence intervals of the controls most commonly when disease levels were low (black shank tests at both Kinston and Rocky Mount in 1986); in these tests the differences in disease index values between Coker 86 and the parent cultivars NC2326 and Coker 319 were slight. Somaclone means were more commonly within the 95% confidence intervals of Coker 86 than within those of Hicks. Further, in only one case (NC2326 somaclones in the 1986 Rocky Mount black shank test) were any of the somaclones significantly more resistant than Coker 86, and in no case were any more susceptible than Hicks (Table 3). The fact that highly resistant and highly susceptible lines were not generated by our methods is apparent from Figures 1–4. With few exceptions, disease index values of the somaclones lie between those of Coker 86 and Hicks.

Finally, we used variance among and within R_2 families to get a measure of genotypic versus environmental variation. Twenty R_1 lines were planted in the Rocky Mount black shank nursery in 1985. Seed was harvested from individual surviving plants. Seed progeny from these individual plants (R_2 families) was analyzed in

TABLE 1. Mean yields, percent reducing sugars, and percent total alkaloids of parent cultivars and somaclone populations[a]

Year	Population	Yield (kg/ha)	Percent sugar	Percent alkaloids
1986	NC2326 parent	2,745	20.8	2.40
	NC2326 somaclones	2,743	20.9	2.39
	Coker 319 parent	2,905	21.4	1.94
	Coker 319 somaclones	2,913	20.3	1.91
1987	NC2326 parent	2,333	23.6	1.98
	NC2326 somaclones	2,062	22.7	1.97
	Coker 319 parent	3,344	22.5	1.88
	Coker 319 somaclones	3,154	23.1	1.85

[a] Analysis of variance showed that somaclones did not differ significantly ($P \leq 0.05$) from their respective parents for yield, percent sugar, and percent alkaloids in either year.

TABLE 2. Range of variation in somaclones for black shank and bacterial wilt resistance as compared with the parent cultivars NC2326 and Coker 319

			Percent of somaclones outside 95% confidence limits of parent			
			More resistant[a]		More susceptible[b]	
Parent	Disease	Location	1986	1987	1986	1987
NC2326[c]	Black shank	Rocky Mount	6	9	38	26
		Kinston	0	0	38	48
	Bacterial wilt	Kinston	52	30	3	0
		Oxford	82	39	1	0
Coker 319[c]	Black shank	Rocky Mount	0	0	78	95
		Kinston	0	0	19	100
	Bacterial wilt	Kinston	48	8	0	37
		Oxford	4	0	15	34

[a] Lower disease index.
[b] Higher disease index.
[c] Different sets of lines were analyzed in 1986 and 1987, and therefore comparisons cannot be made between the 2 yr.

the 1986 field studies. Progenies from a total of 43 plants from six R_1 families were tested. For all disease tests except the Kinston black shank test, variance among families was significantly greater ($P \leq 0.05$) than variance within families. These findings support our hypothesis that variation seen in these tests is due to genotype differences and not to environmental effects. The lack of significance in the Kinston black shank test is, again, probably due to the low levels of disease in that test. Variation among R_2 families for yield, percent sugars, and total alkaloids was not significantly greater than environmental (within family) variation.

All of the NC2326 and Coker 319 somaclones were tested for TMV and *M. incognita* resistance in the greenhouse. No lines were identified with resistance to these diseases.

DISCUSSION

The results of this study demonstrate that genetic variation was present in somaclones derived from two tobacco cultivars. Variance among R_2 families for several tests was significantly greater than variance within families, indicating that the differences seen among the somaclones are real and are not just a measure of environmental variation. Correlations were obtained between tests for black shank resistance in the greenhouse and in the two field locations. Significant differences were seen in many tests between the mean response of the somaclones and the parents. Further, disease index values for a large proportion of the somaclones were outside the 95% confidence intervals of the parent mean. All these results support the fact that significant genetic variation was induced by our methods.

A significant correlation was not seen between the two bacterial wilt tests. This lack of correlation was probably due to several differences between the two tests. The test at Oxford was conducted in an established disease nursery; plants also were inoculated at transplanting. At Kinston, lines were planted in a grower's field with a history of problems with bacterial wilt. Plants displayed typical bacterial wilt symptoms and the presence of bacteria in the vascular tissue was confirmed in random samples. However, we cannot rule out the possible occurrence of other diseases in that field that could have altered the disease response.

Our results demonstrate that variation induced in somaclones is affected by the genotype of the parent cultivar. Coker 319 and NC2326 respond in a very similar manner to the diseases investigated in this study, and there are similarities in their pedigrees (21; R. Rufty, *personal communication*). NC2326 is derived from a cross between Hicks and line 9102, backcrossed three times to Hicks. Line 9102 contains genes from *Nicotiana plumbaginifolia* and several flue-cured lines, including line 400. Coker 319 is derived from a cross between Hicks and Coker 139. Coker 139 contains genes from several flue-cured lines, again including line 400. Somaclones derived from these cultivars, however, showed very different patterns of response. Depending on the test, NC2326 somaclones generally performed as well as or better than NC2326, whereas Coker 319 somaclones performed as well as or worse than Coker 319. These results support those of other investigations that demonstrated that the amount of variability seen in somaclones is dependent on the genetic background of the parent plant (15,27,29).

Our results also demonstrate that the amount and type of variation seen is dependent on the trait being measured. With black shank, most somaclones either were equal to or more susceptible than the parent cultivar, whereas with bacterial wilt, somaclones mostly were equal to or more resistant than the parent cultivar. Also, significant variation was seen in response to these two diseases, but not in yield and leaf chemistry and not in response to infection by TMV or *M. incognita*. Similar results were obtained by Evans et al (8) who isolated potato somaclones with elevated levels of scab resistance but none with increased resistance to cyst nematodes. Our studies with TMV and *M. incognita* were limited to greenhouse inoculation experiments and used methods that would only have detected major changes in disease resistance. Thus, our inability to detect changes may reflect the level of sensitivity of our inoculation procedures. Another factor to be considered, however, is that the parent cultivars lack resistance to these pathogens, whereas they have partial resistance to black shank and bacterial wilt. The inability to see variation in traits not expressed by the parent used to generate the somaclones is not uncommon. In two independent studies with sugarcane, 70,000 and 30,000 somaclones from susceptible sugarcane cultivars were screened for resistance to rust and smut, respectively, and no increased resistance was found (P. S. Carlson, J. Schnell, *personal communications*). Gavazzi and co-workers (9) were unable to identify increases in resistance to Verticillium wilt, TMV, or *M. incognita* in somaclones derived from two susceptible tomato cultivars. By contrast, Barden and co-workers (2) identified 6 out of 370 somaclones from the TMV-susceptible tomato line GCRI-26 with increased levels of resistance to TMV; Shahin and Spivey (25) identified Fusarium wilt-resistant somaclones derived from the susceptible tomato cultivar UC-82; and Witherspoon and co-workers isolated a variant from anther culture of a tobacco cultivar susceptible to potato virus Y (McNair 944) that was resistant to several strains of potato virus Y (W. D. Witherspoon, E. A. Wernsman, G. V. Gooding, and R. C. Rufty, *unpublished*). Many of these results are difficult to evaluate. "Resistant" and "susceptible" are subjective terms and the degree of variation observed also may depend on the sensitivity of the assays used to measure disease. However, it appears that the likelihood of obtaining a desirable variant may be critically dependent on the choice of the plant used to generate somaclones, and in terms of disease resistance, it is probably wise to start with a parent with the highest level of disease resistance available in a desirable agronomic background.

Another reason for starting with plants with good levels of disease resistance is that only small amounts of variation usually are seen in somaclone populations. In our study, few lines gave a

TABLE 3. Range of variation in NC2326 and Coker 319 somaclones for black shank and bacterial wilt resistance as compared with the resistant and susceptible cultivars Coker 86 and Hicks

Parent	Disease	Location	Percent of somaclones within or beyond 95% confidence limits of: Coker 86 1986	Coker 86 1987	Hicks 1986	Hicks 1987
NC2326	Black shank[a]	Rocky Mount	40[b]	4	0	0
		Kinston	48	4	0	0
	Bacterial wilt	Kinston	10	0	6	13
		Oxford	17	30	1	0
Coker 319	Black shank[a]	Rocky Mount	22	0	0	3
		Kinston	63	0	0	0
	Bacterial wilt	Kinston	4	0	11	26
		Oxford	15	8	0	8

[a] Disease levels at Rocky Mount and Kinston in 1986 were very low, leading to the high proportion of somaclones with means within the 95% confidence interval of Coker 86.

[b] One somaclone mean was below the 95% confidence limit of Coker 86.

response equivalent to the response of susceptible control Hicks. Although more lines had a disease response similiar to Coker 86, this happened primarily under conditions of low disease when there was little difference between Coker 86 and the somaclone parents. The methods we chose to use in this study reduced the potential amount of variation. We manipulated the culturing conditions so that the majority of regenerants were phenotypically normal. We regenerated somaclones without any selection pressure. Also, we discarded any regenerants that were not self-fertile, thus eliminating some known variants. In our opinion, somaclonal variation will only be useful in crop improvement if it is possible to get variation in particular traits without severely altering the basic yield and quality characteristics of the parent. Given all these constraints, it is significant that we were successful in obtaining a population of agronomically normal lines that did show variation in disease resistance. The disadvantage of our approach is that the variation is small.

Several NC2326 somaclones were identified that had higher levels of disease resistance than NC2326 in the two years of this study, but these lines are not as resistant as other currently available cultivars. We recently have generated somaclones from two cultivars with high levels of resistance to bacterial wilt, one that also has moderate resistance to *Meloidogyne arenaria* (Neal) Chitwood. In both cases, cultivars currently available to growers do not provide adequate disease control without the use of other control measures. In preliminary greenhouse and field studies, we have identified several somaclones that show higher resistance than the parents. This approach of generating somaclones from parents with moderate to high resistance should provide material useful for germ plasm improvement in tobacco.

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