

THE INHERITANCE OF RESISTANCE TO TOBACCO
MOSAIC VIRUS IN TOBACCO INTRODUCTIONS

by

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The Inheritance of Resistance to Tobacco Mosaic Virus in Tobacco
Introductions

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ABSTRACT

Thirty-one tobacco introductions that were reported to display either a local lesion or a symptomless reaction to infection with tobacco mosaic virus (TMV) were screened for reaction to the virus (Chaplin and Gooding, 1969). Ten tobacco introductions (TI), TI 203, TI 407, TI 438, TI 450, TI 692, TI 1203, TI 1459, TI 1462, TI 1467, and TI 1500 were randomly chosen for further study to characterize their resistance to tobacco mosaic virus (TMV). Each TI line was crossed with susceptible cultivar K 326 to determine the mode of inheritance of resistance to TMV. The F₂ progeny of TIs 1459, 1462, and 1500 segregated in a 3 local lesion:1 mosaic ratio, indicating that the gene governing resistance in these three TI lines was a single, dominant trait. The F₂ progeny of TIs 203, 407, 438, 450, 692, 1203, and 1467 failed to segregate, only mosaic plants were observed. This would indicate that the gene(s) controlling resistance to TMV in these lines would not provide resistance for plant breeders to incorporate into a breeding program. Each TI line was also crossed with local lesion cultivar NC 567, which contains the N gene, in order to determine if the gene(s) governing resistance in the TI lines was allelic to the N gene in NC 567. The F₂ progeny of TIs 1459 and 1462 did not segregate. All progeny displayed the local lesion reaction to TMV

indicating that the gene governing resistance in these two lines is allelic to the N gene. The F₂ progeny of the cross between TI 1500 and NC 567 segregated in a 15 local lesion: 1 mosaic ratio, which indicates that the gene controlling resistance in TI 1500 is not allelic to the N gene. When crossed with NC 567, the F₂ progeny of TIs 407, 438 and 1467, segregated in a 3 local lesion: 1 mosaic ratio. No symptomless plants were observed. There was also segregation in the F₂ progeny of the crosses between NC 567 and TIs 203, 450, 692, and 1203. However, the segregation was in no discernible ratio. Once again the F₂ progeny of the crosses either displayed a local lesion or mosaic reaction and no symptomless progeny were observed. This would again indicate that the symptomless TI lines do not provide heritable resistance to TMV and therefore are not acceptable as an alternative source of resistance to TMV for the plant breeder. Tobacco introduction 1500 should be investigated further because a single, dominant trait that is not allelic to the N gene governs resistance to TMV in this line.

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Chapter 1

INTRODUCTION

Tobacco mosaic virus (TMV) is a single-stranded, positive-sense, rod-shaped RNA virus that is worldwide in distribution and is found in all countries where tobacco is grown. Tobacco mosaic virus reduces cured tobacco yield, quality, and average price. Because of its adverse effects on tobacco and the high dollar value of the crop, TMV is an economically important disease. Characteristic symptoms include an irregular pattern of dark green and light green leaf areas intermingled, stunted plant growth, leaf malformation, and mosaic burn.

Control of the spread of TMV in the tobacco crop includes such measures as crop rotation and sanitation practices as well as host plant resistance. The first resistance to TMV incorporated into tobacco came from the Colombian variety 'Ambalema' (Nolla and Roque, 1933). This resistance was controlled by two single recessive genes as well as some modifying genes. Once resistance was incorporated into the tobacco genome, the plants were of poor agronomic quality. Holmes (1938) used interspecific hybridization to incorporate resistance to TMV from *Nicotiana glutinosa* into the Turkish tobacco variety Samsoun. This resistance was governed by a single dominant gene and was designated the 'N' gene. The 'N' gene causes a necrotic reaction when the plant is inoculated with TMV.

Resistance derived from *Nicotiana glutinosa* was successfully incorporated into burley tobacco. However, when the 'N' gene was incorporated into flue-cured tobacco, Chaplin et al. (1961) reported reductions in cured tobacco yield and value, and Chaplin and Mann (1978)

concluded that the N factor may be inherently difficult to disassociate from the adverse yield and quality characteristics in flue-cured tobacco.

The N gene was identified molecularly and sequenced and is believed to be a cytoplasmic protein involved in a signal transduction pathway. It is hypothesized that the N gene acts as a cytoplasmic receptor to the TMV gene product and elicits a defensive hypersensitive response in the plant that results in a necrotic, or local lesion reaction. A family of related genes clustered around the N locus was identified by restriction fragment length polymorphism (RFLP) (Whitham et al., 1994).

Chaplin and Gooding (1969) screened 907 tobacco introductions (TI) for reaction to TMV. They found that there are three possible reactions to infection with TMV in the *Nicotiana* genus, a necrotic or local lesion reaction, a reaction that displays no visual symptoms, and a susceptible reaction that exhibits the characteristic mosaic response. Thirty-six TIs were identified as resistant to infection with TMV. Eleven of the lines exhibited the local lesion response and 25 lines exhibited no visual symptoms upon inoculation with the virus. Resistance to TMV derived from *N. glutinosa* is characterized by a local lesion reaction. It has not been determined if the eleven TIs that exhibit the local lesion reaction possess the same N factor for resistance as that derived from *N. glutinosa*. If the gene for resistance in these 11 TIs is found to be the same as the N gene, then it is probable that the same undesirable traits that cause reduced yield and value in flue-cured tobacco will be linked to the gene in these TI lines. If that is the case, other TIs that display no visual symptoms upon infection with TMV should be assessed as possible new sources of resistance to TMV. Tobacco introductions could provide a useful and alternative source of resistance to TMV.

Chapter 2

LITERATURE REVIEW

Tobacco Mosaic Virus

Tobacco mosaic virus (TMV) is a rigid, rod-shaped, RNA-containing virus that is worldwide in distribution, being found in all countries where tobacco is grown. Tobacco mosaic virus has a wide host range, including 199 species from 30 families. However, other *Solanaceous* hosts are the only important sources of inoculum for the tobacco crop (Shew and Lucas, 1991). The chief symptom is a characteristic “mosaic” pattern. This mosaic pattern is the result of intermingled yellow-green mottling on the foliage of the tobacco plant. Young leaves of infected plants are often malformed and may show leaf puckering, or wrinkling of the leaf tissue. Nearly mature leaves that are infected may show “mosaic burn”. Mosaic burn is characterized by large, irregular, burned or necrotic areas on the foliage that can cause extensive damage to the tobacco crop (Lucas, 1975).

Tobacco mosaic virus is sap transmissible and is one of the most infectious plant viruses. Primary sources of infection may include perennial weeds and infected crop debris in the soil. Primary infections are usually only responsible for a small proportion of infected plants in a field. Gooding (1969) reported that only about 10% of TMV infection in North Carolina is the result of primary infection by crop debris in the soil. Therefore, secondary spread of the virus accounts for most TMV infections. The virus is mainly spread by contact. Secondary infections may occur when a worker’s hands,

clothing, or equipment, previously in contact with an infected plant, comes in contact with a healthy plant. Cultivation practices such as hoeing, topping, spraying pesticides and insecticides, and other field operations can also spread the disease (Lucas, 1975).

Another source of new infection is air-dried tobacco. Workers who smoke cigarettes, or who use chewing tobacco or snuff containing air-cured tobacco, may introduce the virus to the plants. This is especially common when the worker is performing plant bed operations. Leaf symptoms do not usually have time to develop before transplanting, so mosaic is rarely seen in the plant beds. However, if TMV is present in the plant beds, then it could easily be spread at transplanting time when the infected plants come in contact with workers and equipment.

Effects of TMV on Tobacco

Tobacco mosaic virus is the most widespread virus in tobacco. Because of the high value of the crop, it is an economically important disease. TMV has been reported to reduce cured tobacco yield, value, average price, and quality index. Inoculation of Maryland tobacco 4 wk after transplanting reduced cured tobacco yield by 25.8%, value by 28.2%, average price by 3.4%, and quality index values by 8.5% (Diallo and Mulchi, 1981). During the period from 1992 to 1996, the North Carolina Extension Service estimated that TMV was responsible for annual losses in the value of flue-cured tobacco, from a low of 0.18% (\$1,986,645) in 1995, to a high of 0.57% (\$6,287,598) in 1992 (Melton et al., 1996). Tobacco mosaic virus was estimated to cause a 1.8% reduction in crop value for tobacco in Virginia in 1993, an estimated loss of \$266,508 (C.S. Johnson, personal communication).

Researchers have attempted to characterize the effects of TMV on agronomic properties of tobacco. Several researchers have reported a relationship between the time of infection with respect to plant growth and development, and the severity of the infection. McMurtrey (1929), Valleau and Johnson (1927), Wolf and Moss (1933), and Diallo and Mulchi (1981) have all reported that time of infection is of major importance with regard to the effect of TMV on tobacco yield and value, with greater losses occurring when plants are infected early in the growth of the plant, around transplanting. Plants that are infected later in their development, around time of topping, may yield comparably to plants that have not been infected. Wolf and Moss (1933) reported a 31.4% reduction in yield and a 54.6% reduction in value when flue-cured tobacco was infected at transplanting time compared to a 17.2% reduction in yield and a 23.8% reduction in value when infection took place at topping.

A more recent study by Johnson et al. (1983) on the effects of TMV on flue-cured tobacco in North Carolina found that the percent incidence of infection was a more important factor in predicting yield and value loss than was time of infection. They found that the effects of inoculation date were not always significant. Most of TMV infection was the result of secondary spread, which occurred primarily through contact with infected and healthy plants by equipment and laborers. Tobacco plants grow rapidly between 4 and 8 wk after transplanting, which is also when many cultural practices such as lay by and application of insecticides and herbicides are performed. Therefore, this is an ideal time for TMV to spread. Johnson and Main (1983) concluded that TMV incidence was, therefore, more important than date of inoculation in predicting flue-cured loss due to TMV infection. They developed a crop loss assessment model in which a single assessment of TMV

incidence made 8 to 10 wk after transplanting was an appropriate method for TMV loss assessment. These assessments will be useful in determining which disease management strategies are appropriate.

Control of TMV

Preventative and Eradicative Measures

Attempts to control the spread of TMV have included both preventative and eradicated measures in cultural practices as well as host plant resistance. According to Lucas (1975), “The most efficient way to control mosaic is to keep the crop TMV-free”. Some preventative measures include keeping hands, clothing, and equipment free of TMV through rigorous sanitation; rotating crops to prevent infection from perennial weeds and other crop debris; plant bed sanitation, which would help to reduce the primary infections; and using cultivation practices that do not call for much handling of the plants, for instance, using herbicides as weed killers in the field instead of weeding by hand, in order to reduce secondary spread of the disease. This would reduce the incidence of infection by TMV by limiting contact between an infected plant and a healthy plant through equipment and laborers, thus reducing secondary spread via worker contact.

Hare and Lucas (1959) found that milk acted as an antiviral agent. When applied 24 h before transplanting, milk could be used to control the mechanical spread of TMV. Two fungicides, dodine and glyodin, have also been found to reduce the amount of damage caused by TMV when applied in the field, by acting as foliar protectants for controlling contact transmission of

the virus (Chow and Rodgers, 1973). However, neither of these fungicides are used practically in controlling transmission of the virus.

Host resistance

Modified cultural practices are only one way to limit the spread and reduce the damage caused by TMV. Another way to limit the damage caused by TMV is to incorporate resistance to the pathogen into the host. Host resistance is an effective means of controlling most pathogens, in many cases host resistance eliminates or limits dependence on pesticides for disease control. This makes host resistance both economical and environmentally sound.

Clayton (1953) found that when tobacco production stabilized and intensified in certain regions of the country, disease problems in the crop began to become acute. He found that preventative and eradication measures to control disease were inadequate when used alone. These measures are most effective when used in combination with heritable forms of resistance. When the proper preventative measures have been observed and when the incidence of TMV exceeds certain threshold levels, resistant cultivars could be recommended as the best tactic for managing the disease (Johnson and Main, 1983). Apple et al. (1963) found that host resistance derived from *N. glutinosa* reduced losses in yield and value to TMV more than did milk treatment of seedlings. Milk treatment of seedlings increased yield of inoculated tobacco plots and also gave partial protection against other undesirable effects of inoculation, however, this treatment still showed reductions in yield and value compared to uninoculated plots. Host resistance of inoculated plants gave 500 more pounds per acre and increased the value of

each acre by \$380 compared to inoculated milk treated plots. In 1983, Ford and Tolin compared a recently isolated strain of TMV with a “common” strain of the virus isolated in 1927. They found that the virus had a high degree of stability in nature. This implies that once resistance is incorporated into a host, it will not be lost due to adaptation of the pathogen to overcome the host resistance. Thus, the combined advantages of limiting the damage caused by a pathogen and the stability of the inherited resistance, make host plant resistance a very economical option for growers.

Resistance to TMV

Ambalema resistance

Nicotiana tabacum is believed to have originated from a chance cross between *N. sylvestris* and *N. tomentosiformis* in a region that includes northwest Argentina and adjacent Bolivia (Burk and Heggestad, 1966). There are 64 other species in the genus that have exploitable characteristics, providing a broad base of germplasm for plant breeders to work with. Nolla and Roque (1933) first used intraspecific hybrids (crosses made between two different cultivars of the same species) to introduce resistance to TMV into *N. tabacum*. They used the Colombian tobacco variety ‘Ambalema’ as the source of resistance. Ambalema displayed a symptomless response to inoculation with TMV, neither the typical mottling response was seen nor necrotic spots. Re infectivity assays of inoculated Ambalema plants revealed that virus was present throughout the plant. Ambalema resistance was found to be controlled by two recessive Mendelian factors. Later, Clayton et al. (1938) reported that there were also modifying genes involved which reduced

TMV resistance. Because Ambalema resistance is governed by two recessive factors, along with modifying genes, it is difficult to obtain a plant that has significant TMV resistance. Once identified, these plants usually had very poor plant type. Much backcrossing to the recurrent *N. tabacum* parent was needed in order to obtain a plant with both resistance to TMV and the desirable tobacco characteristics. Ambalema crossed with burley tobacco plants produced plants that were both high yielding and resistant to TMV. However, the quality was poor and the plants suffered from severe wilting on hot days from which they sometimes failed to recover (Valleau, 1952). Even after repeated backcrossing to the parent phenotype, Ambalema with incorporated TMV resistance failed to recover good quality, and breeders began to look for other sources of TMV resistance.

Nicotiana glutinosa resistance

Shortly after resistance to TMV was incorporated into tobacco from Ambalema, Holmes (1938) incorporated a different source of resistance into tobacco. He used interspecific hybridization, (a cross made between two species) to introduce resistance from *N. glutinosa* into *N. tabacum*. He demonstrated that this resistance is conveyed by a single dominant genetic factor that he designated as 'N'. Gerstel (1943) showed that Holmes had transferred an entire *N. glutinosa* chromosome into the tobacco genome. The *tabacum* chromosome was retrieved through repeated backcrosses to a recurrent *N. tabacum* parent. Through crossing over, the N gene was incorporated into the *N. tabacum* chromosome. The N gene provides complete resistance to TMV in both burley and flue-cured cultivars of tobacco by causing a local lesion, or necrotic reaction (Holmes, 1938). Legg et al. (1979) assessed isogenic lines of burley tobacco for the influence of the

N gene and linked genes on agronomic, chemical, and morphological characteristics. The N gene did not reduce cured tobacco yield, value, or other agronomic characteristics. Today, most burley cultivars possess resistance to TMV derived from the N gene in *N. glutinosa*.

Flue-cured tobacco cultivars with resistance to TMV derived from *N. glutinosa* have not been as successful because the N gene does negatively affect agronomic characteristics such as cured tobacco yield and value. Chaplin et al. (1961) studied isogenic lines of flue-cured tobacco to determine the influence of the N gene and/or linked genes on yield, value, and other agronomically important characteristics. The flue-cured tobacco lines containing the N gene were found to have reduced yield and value even in the absence of the virus. In a later study, Chaplin et al. (1966) compared homozygous resistant cultivars (NN) with parental mosaic-susceptible cultivars (nn) and F₁ hybrids (Nn). Once again, the homozygous resistant cultivars (NN) showed reduced yield and value compared to the recurrent parent line (nn). The F₁ hybrids were found to yield somewhere between the homozygous resistant cultivars (NN) and the recurrent parent cultivars (nn), suggesting that the deleterious effects of the N gene were not as great when the gene was present in a single dosage as when the gene was present in duplicate. Chaplin and Mann (1978) concluded that the N gene may be inherently difficult to disassociate from the adverse yield and quality characteristics in flue-cured tobacco.

The N gene

The N gene was the first plant disease resistance gene to be isolated and sequenced. Whitham et al. (1994) isolated the N gene by using a

technique known as insertional mutagenesis. A maize transposon, denoted as Ac, was inserted into the tobacco genome of tobacco plants containing the N gene. These plants were normally TMV resistant. A positive selection scheme was employed to identify the plants where the insertional event occurred in the N gene. If the Ac element were inserted in the N gene, then the function of the gene would be disrupted and the plant would no longer be TMV resistant. Once TMV susceptible mutants were identified, researchers were able to use the Ac fragment as a genetic tag and sequence the flanking genomic DNA of the tobacco plant. The flanking genomic DNA sequences were then used to screen a library and identify genomic DNA clones containing the N sequence. Complementation tests using a TMV susceptible plant and a genomic DNA fragment thought to contain the N gene restored resistance to TMV and proved that the N gene had been cloned.

The structure and sequence of the N gene product were analyzed (Whitham et al., 1994). It was determined that the N gene product had a molecular mass of 131.4 kDa. The amino acid sequence of the N protein was compared with the GenBank database and three domains that have functional significance were identified. The first is called a nucleotide-binding site (NBS), which was identified by the presence of three structural motifs that are commonly found in proteins that are known to bind ATP and/or GTP such as kinases and ATPases. The second domain is a leucine rich region (LRR). The LRR of the N gene contains four imperfect tandem repeats of a 26 amino acid sequence. This region mediates protein-protein interactions and is found in other proteins such as yeast adenylyl cyclase and chaoptin. The last domain is an amino-terminal domain that has homology to the cytoplasmic regions of the *Drosophila* Toll protein and the human interleukin-1R protein. The presence of these three domains suggests that the N protein is a cytoplasmic

protein involved in a signal transduction pathway. The N gene may act as a receptor for the gene product of TMV, that when triggered elicits a hypersensitive defense response.

Whitham et al. (1994) also identified a cluster of related repetitive sequences at the N locus using restriction fragment length polymorphisms (RFLPs). This cluster of repeated sequences is actually a family of genes that are all similar to the N gene. Using RFLPs, they demonstrated that the related repetitive sequences at the N locus in *N. glutinosa* were transferred to the *N. tabacum* chromosome when the two species were crossed. These repeating regions are physically linked to each other and are clustered near the N locus. It is possible that these repeating sequences could be other resistance genes. However, their function has never been investigated.

The gene products of TMV

Tobacco mosaic virus (TMV) is a single-stranded, rod-shaped, positive-sense RNA virus. The virus measures about 300 X 18 nm. The virus was the first virus to be crystallized and has been the subject of extensive study. The genome of TMV has five open reading frames that each encode for a protein product. The 126K and 183K proteins are RNA-dependent polymerases, the 17K protein is a coat protein, the 54K protein has not yet been identified, and the 30K protein is the movement protein of TMV (Goelet et al., 1982). Next to the coat protein, the most studied protein product of TMV is the movement protein, denoted TMV MP. A virus must be able to replicate and move from cell-to-cell in the plant in order to establish an

infection. The movement protein of TMV is responsible for cell-to-cell movement in the host plant.

Citovsky et al. (1992) demonstrated that TMV MP cooperatively binds single-stranded nucleic acids in a nonspecific manner. These complexes are long, unfolded and very thin in diameter (1.5 to 2.0 nm). Heinlein et al. (1995) determined that TMV MP aligns with microtubule components of the cytoskeleton and exploits this structure as a trafficking system to reach the plasmodesmata. Plasmodesmata are cytoplasmic bridges, or membrane-lined microchannels that function as transport pathways between cells. Plasmodesmal channels typically limit molecular traffic to small molecules and metabolites with a molecular mass of about 1 kD. This translates to a size exclusion limit (SEL) of 0.75 nm. However, Wolf et al. (1989) found that transgenic tobacco plants expressing TMV MP possessed plasmodesmata that permitted passage of particles up to 10 kD in size (2.4 to 3.1 nm). Therefore, the TMV MP interacts with the plasmodesmata and increases the normal SEL of 0.75 nm to 2.4-3.1 nm. This allows for the transport of the MP-nucleic acid complex through the plasmodesmata and into the next cell.

Responses to TMV in *Nicotiana* genus

Several responses to infection by TMV have been observed in the *Nicotiana* genus. The first is the local lesion, or necrotic reaction, around the area of infection. This induced cell death helps to contain the virus and limits or prevents its spread through the rest of the plant. The second response is the mottling, mosaic pattern seen in susceptible plants. A third reaction to TMV infection is described by Chaplin and Gooding (1969) as symptomless, because these plants display no visible signs of infection. The mode of

inheritance for the genes that control these different responses, and their allelic relationships to each other, have not been determined in tobacco introductions.

Chaplin and Gooding (1969) screened 907 tobacco introductions for resistance to TMV. Eleven of these introductions were found to exhibit the local lesion response to infection with TMV. They also found 25 lines that exhibited no visual symptoms to the virus. Reinfectivity assays on the symptomless plants revealed that virus was present in each of the inoculated parent TIs (Chaplin and Gooding, 1969). Resistance to TMV derived from *N. glutinosa* is also characterized by the local lesion reaction. It has not been determined whether the eleven tobacco introductions characterized by the local lesion reaction convey the same N gene for resistance as that derived from *N. glutinosa*. If the gene for resistance in these tobacco introductions is found to be the same as the gene for resistance derived from *N. glutinosa*, then it is probable that the gene in the TIs also has the linked traits that adversely affect cured tobacco yield and value in flue-cured tobacco. If this is the case, other tobacco introductions that show no visual symptoms upon infection with the virus should be assessed as possible new sources of resistance to TMV. Host resistance to TMV would enhance cultural practices aimed at limiting or preventing the spread of TMV, which would be a very economical and effective way of controlling the disease. Tobacco introductions could provide a useful and alternative source of resistance to TMV.



Figure 1. Susceptible (upper) and resistant (lower) tobacco leaf inoculated with tobacco mosaic virus displaying mosaic and local lesion symptoms, respectively.

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Chapter 3

Inheritance of Resistance to Tobacco Mosaic Virus in Tobacco Introductions 1459, 1462, and 1500

Abstract

Greenhouse studies were performed to characterize the resistance of three tobacco introductions (TI), TI 1459, TI 1462, and TI 1500 to tobacco mosaic virus (TMV). Each TI line was crossed with TMV susceptible cultivar K 326 to determine the mode of inheritance of resistance to TMV. Each TI line was also crossed with TMV resistant cultivar NC 567 to determine if the gene conferring resistance to TMV in the TI lines was the same as or allelic to the N gene found in NC 567. All F₂ populations of the crosses between K 326 and the TI lines segregated in a 3:1 ratio with local lesion being dominant to mosaic. The absence of mosaic segregants in F₂ populations of the crosses between NC 567 and TI 1459 and TI 1462, indicate that the single dominant gene conferring TMV resistance in TI 1459 and TI 1462 is allelic to the gene in NC 567. The F₂ populations of the cross TI 1500 X NC 567 segregated in a 15:1 ratio with local lesion being dominant to mosaic, indicating that the single dominant gene in TI 1500 is not the same as or allelic to the N gene in NC 567.

Introduction

Tobacco mosaic virus (TMV) is a single-stranded, positive-sense, rod-shaped, RNA virus that is found in all countries where tobacco is grown. It adversely affects yield, quality, and average price in flue-cured tobacco.

Characteristic symptoms include the typical mosaic pattern of dark green and light green leaf areas, stunted plant growth, leaf malformation, and mosaic burn.

The first resistance to TMV incorporated into tobacco came from Ambalema. Ambalema resistance was controlled by two single recessive genes as well as some modifying genes. Plants with this incorporated resistance were of poor economic quality (Nolla and Roque, 1933). Consequently, an alternative source of resistance to TMV that would not adversely affect agronomic characteristics was needed. Resistance to TMV from *N. glutinosa* was incorporated into tobacco in 1938 (Holmes, 1938). *Nicotiana glutinosa* resistance was governed by a single, dominant gene, denoted as the N gene. The N gene causes a necrotic reaction when the plant is infected with TMV. While the N gene was successfully incorporated into burley tobacco, flue-cured tobacco with the N gene was adversely affected (Legg et al., 1979). Reductions in cured tobacco yield and value were reported (Chaplin et al., 1961). A study by Chaplin and Mann (1978) concluded that the N factor may be inherently difficult to disassociate from the adverse yield and quality characteristics in flue-cured tobacco. A flue-cured hybrid with TMV resistance has recently been released which is acceptable for yield and quality (Reed et al., 1997).

The diverse *Nicotiana* genus was searched for an alternative source of resistance to TMV. Chaplin and Gooding (1969) screened 907 TI lines for reaction to TMV, three possible reactions were identified. The first was a necrotic or local lesion reaction. The second was a susceptible reaction that exhibits the characteristic mosaic response, and finally a reaction that displays no visual symptoms to infection was identified. Chaplin and Gooding (1969) called this response the symptomless response. Reinfecitivity assays

performed on these lines revealed that virus was indeed present throughout the plant.

Eleven of the TI lines exhibited the local lesion response upon infection with the virus. Resistance to TMV derived from *N. glutinosa* is also characterized by the local lesion response. It is not known if the gene for resistance in these 11 TI lines is the same N gene found in *N. glutinosa*. If the local lesion TI lines are different they could provide a useful and alternative source of resistance to TMV.

The objectives of these experiments were to determine the inheritance of TMV resistance to these TI lines and to determine if the gene conferring TMV resistance in these TI lines was the same as or allelic to, the N gene in NC 567.

Materials and Method

Greenhouse experiments were conducted at the Southern Piedmont Agricultural Research and Extension Center in Blackstone, Virginia, in 1996 and 1997. Seed of eight tobacco introductions obtained from Dr. Verne Sisson, were sown in 7.62 cm clay pots filled with vermiculite. The tobacco introductions were selected based on previous research by Chaplin and Gooding (1969) and expressed the local lesion reaction upon infection with TMV. Seventy-five three-week-old seedlings of each tobacco introduction were transplanted into 10.16 cm peat pots filled with a 2:1 sand:vermiculite mixture. Four weeks after transplanting, when plants were at the four to six leaf stage, one leaf of each seedling was inoculated with 100 µg/mL of TMV in a potassium phosphate buffer (0.01M) with 1% Celite at a pH of 7.0. The purified TMV was obtained from Dr. Sue A. Tolin of Virginia Tech. A cotton swab was used to apply the inoculum to the top side of the leaf. The

inoculated leaf was then rinsed with water to wash away any viral inhibitors on the leaf and to standardize the process. The plants were scored for reaction to the virus at 7 and 14 d after inoculation. The results of these evaluations were used to verify Chaplin and Gooding's (1969) original classification of the eight tobacco introductions. K 326 was used as a susceptible check and NC 567 was used as a necrotic local lesion check as it possesses the *N. glutinosa* resistance gene.

Three tobacco introductions that displayed the necrotic local lesion reaction upon infection with TMV were randomly chosen for further study and characterization. These tobacco introductions were TI 1459, TI 1462, and TI 1500. Tobacco introduction 1459 had been donated to the National Germplasm Bank by Germany and is a burley-type introduction. Tobacco introduction 1462 was also donated by Germany but is a flue-cured type tobacco. Tobacco introduction 1500 was collected in the Former Soviet Union and is a flue-cured type.

In order to study the mode of inheritance of resistance to TMV for the tobacco introductions, crosses were made between the TMV susceptible tobacco cultivar K 326 and each of the tobacco introductions. Crosses were also made between each of the tobacco introductions and the resistant cultivar NC 567, to determine if the gene conferring resistance in each of the TI lines was the same as, or allelic to, the gene in the TMV resistant cultivar NC 567. Tobacco mosaic virus resistance in NC 567 is believed to have come from *N. glutinosa* via breeding line MRS-3 which has resistance derived from *N. glutinosa* (Chaplin et al., 1969). Seventy-five F₁ seedlings were inoculated and evaluated as previously described. Two reactions were expected, a necrotic local lesion reaction, or a susceptible reaction showing the characteristic mottling response to the virus. Two F₁ seedlings from each

cross were selected to self-pollinate and were advanced to the F_2 generation. One hundred fifty F_2 seedlings from each advanced F_1 were grown, inoculated, and scored in the usual manner. Data from all segregating F_2 progeny were subjected to chi-square analysis.

Several plants exhibiting each phenotype (local lesion, mosaic symptoms) from each F_2 family were selected for self-pollination and advanced to the $F_{2:3}$ generation. Again, 100 $F_{2:3}$ seedlings from each advanced F_2 plant were grown, inoculated, and scored in the usual manner. Data from all segregating F_2 and $F_{2:3}$ progeny were subjected to chi-square analysis for both a 3:1 and 15:1 ratio and assigned to the class for which the chi-square value was lowest.

Three results were predicted for the resistant X resistant crosses. If both parents carried the same or allelic genes, then all of the F_2 and $F_{2:3}$ progeny rows would be resistant. If the parents carried different independent genes, the progeny rows would fall into one of four phenotypic classes. The four classes would be all resistant (R), 15R:1S (susceptible), 3R:1S, and all susceptible.

Results and Discussion

Eight tobacco introductions were inoculated with TMV, evaluated for reaction to the virus, and compared with the results of Chaplin and Gooding's (1969) original study. Tobacco introductions 1459, 1462, 1473, 1500, and 1504 all displayed a local lesion reaction in response to infection with TMV (Table 1). These results agreed with those found by Chaplin and Gooding (1969). Tobacco introductions 1409, 1463, and 1501 all displayed a mosaic reaction upon infection with TMV (Table 1). These results differed from those of Chaplin and Gooding (1969), who reported that these three tobacco

introductions displayed a local lesion reaction upon infection with the virus. The differing result may be due to the use of a different strain of TMV for inoculation or the use of seed from a different lot. Tobacco introductions 1459, 1462, and 1500 were randomly chosen from among the TI lines displaying a local lesion reaction to TMV for further study and characterization.

TI 1459 and TI 1462

Tobacco introductions 1459 and 1462 both displayed a local lesion reaction to TMV. There was no segregation in reaction to inoculation with TMV in either of the parent populations screened, which indicates that both were homogeneous populations. These results also agreed with those found by Chaplin and Gooding (1969). Neither the K 326 X TI 1459 F_1 nor the K 326 X TI 1462 F_1 progeny segregated in reaction to infection with TMV. All F_1 seedlings displayed a local lesion reaction (Table 2).

The F_2 progeny of both the K 326 X TI 1459 cross and the K 326 X TI 1462 cross segregated in reaction to infection with TMV (Table 3). The results were subjected to chi-square analysis for a 3 local lesion:1 mosaic and a 15 local lesion:1 mosaic ratio. The chi-square values indicate that the F_2 progeny of both crosses clearly fit a 3 local lesion:1 mosaic ratio. This indicates that the gene conferring resistance to TMV in TI 1459 and in TI 1462 is a single dominant gene.

Plants of each F_2 phenotype (local lesion and mosaic) were selected for advancement to the next generation. The $F_{2:3}$ progeny families were screened and evaluated. Three classes of segregation were expected in the $F_{2:3}$ progeny families. Plants from self-pollinated F_2 plants displaying a mosaic reaction, would produce $F_{2:3}$ progeny families that were all susceptible to the

virus (all mosaic). Because F_2 plants displaying a local lesion phenotype could either be homozygous or heterozygous for the local lesion genotype, two classes of progeny families were possible when the seeds from the self-pollinated F_2 plant were sown and evaluated. If the F_2 parent plant had been homozygous for the local lesion genotype, then the $F_{2:3}$ progeny row would be all resistant (all local lesion phenotypes) and no segregation would be observed. If the F_2 parent had been heterozygous for the local lesion genotype then a 3 resistant: 1 susceptible ratio (3 local lesion: 1 mosaic) would be present in the $F_{2:3}$ progeny row. All three classes of segregation in reaction to infection with TMV were observed in the $F_{2:3}$ progeny families of both the K 326 X TI 1459 and K 326 X TI 1462 crosses (Table 4). The $F_{2:3}$ data supports the F_2 data indicating that a single dominant gene controls resistance to TMV in both TI 1459 and in TI 1462.

The F_1 progeny of the crosses between TI 1459 and TI 1462 X NC 567 did not segregate, all plants displayed the local lesion reaction upon infection with TMV (Table 2). The F_2 progeny of these crosses did not segregate either, all plants displayed a local lesion phenotype (Table 3). This indicates that the gene controlling resistance to TMV in TI 1459 and TI 1462 is the same as or allelic to the N gene in NC 567. $F_{2:3}$ progeny families of both crosses were seeded, inoculated, and evaluated. No segregation was observed in any of the progeny families, all plants displayed the local lesion reaction to the virus (Table 4). This is further evidence that the gene controlling resistance to TMV in TI 1459 and in TI 1462 is the same as or allelic to the N gene in NC 567.

TI 1500

Chaplin and Gooding (1969) classified TI 1500 as a local lesion tobacco introduction. Inoculation and evaluation of TI 1500 verified these results, all plants displayed local lesions after infection with the virus. The F_1 progeny of the cross K 326 X TI 1500 also displayed all local lesions after infection with TMV; there was no segregation (Table 2). The local lesions observed in TI 1500 seemed to be of the same size and number as the local lesions observed in TI 1459, 1462, and NC 567.

The F_2 progeny of the cross K 326 X TI 1500 segregated (Table 3). These results were subjected to chi-square analysis for both a 3 local lesion:1 mosaic and a 15 local lesion:1 mosaic ratio. The data clearly fit a 3 local lesion:1 mosaic ratio. This suggests that the gene controlling resistance to TMV in TI 1500 is a single, dominant gene. The $F_{2:3}$ progeny families of K 326 X TI 1500 fell into the same three classes of segregation seen previously in the other two TI lines (Table 4). The $F_{2:3}$ data supports the F_2 data, indicating that a single, dominant gene is responsible for reaction to infection with TMV in TI 1500.

The F_1 progeny of the TI 1500 X NC 567 cross did not segregate, all plants displayed the local lesion response (Table 2). The F_2 progeny of this cross did segregate (Table 3). These results were subjected to chi-square analysis for both a 3:1 and 15:1 ratio and the values show that the data clearly fits a 15 local lesion:1 mosaic ratio. This ratio indicates that there are two single, dominant, independent genes controlling resistance to TMV in this cross. One of the genes controlling resistance to TMV in this cross is the N gene in NC 567. The other gene controlling resistance to TMV is in TI 1500. The 15:1 ratio indicates that the two genes segregate independently of each

other, therefore, the gene in TI 1500 is not the same as, or allelic to the N gene in NC 567.

Because a 15:1 ratio was seen in the F_2 generation, progeny families in the $F_{2:3}$ generation were expected to fall into one of four classes. Seeds from self-pollinated F_2 plants displaying a mosaic reaction, would produce $F_{2:3}$ progeny families that were all susceptible (all mosaic) to the virus. Plants from self-pollinated F_2 plants displaying a local lesion reaction, would have one of five genotypes. The first genotype would be homozygous dominant for both genes, the second genotype would be homozygous dominant for one of the genes and heterozygous for the other. The third genotype would be homozygous dominant for one trait and homozygous recessive for the other. The fourth genotype would be homozygous recessive for one trait and heterozygous for the other. The fifth and final genotype would be heterozygous for both traits. Seeds from F_2 plants that were homozygous dominant for both traits would produce $F_{2:3}$ progeny families that were all resistant (all local lesion) to the virus. Likewise, seeds from F_2 plants that were homozygous dominant for at least one of the genes would also produce $F_{2:3}$ families that were all resistant to the virus. Seeds from F_2 plants that were heterozygous for both genes would produce $F_{2:3}$ families that segregated in a 15 local lesion : 1 mosaic ratio. Seeds from F_2 plants that were homozygous recessive for one gene and heterozygous for the other would produce $F_{2:3}$ progeny families that segregated in a 3 local lesion : 1 mosaic ratio. All four classes were seen in the $F_{2:3}$ progeny of the cross TI 1500 X NC 567 (Table 4). The $F_{2:3}$ data supports the F_2 data, indicating that the gene responsible for reaction to TMV in TI 1500 is a single, dominant gene that is not the same as or allelic to the gene in NC 567, namely the N gene.

Conclusions

In all three of the tobacco introductions studied, TI 1459, TI 1462, and TI 1500, the mode of inheritance of resistance to TMV is that of a single, dominant gene. Because no segregation is seen among F_1 , F_2 , or $F_{2:3}$ progeny from the crosses TI 1459 X NC 567 and TI 1462 X NC 567, the gene controlling resistance to TMV in these two TI lines is the same as, or allelic to the N gene found in cultivar NC 567. F_2 progeny from the cross TI 1500 X NC 567 segregate in a 15 local lesion:1 mosaic ratio. This means that another gene besides the N gene in NC 567 was controlling resistance to TMV in this cross. Therefore, the gene in TI 1500 is not the same as or allelic to the N gene.

The N gene has adverse traits linked to it that result in poor agronomic quality in flue-cured cultivars. Tobacco introductions 1459 and 1462 contain a single, dominant gene for resistance to TMV that is allelic to the N gene. It is possible that these TIs may have the same adverse traits linked to the gene controlling resistance. Further studies should be done to determine if the gene controlling resistance to TMV in TI 1459 and TI 1462 has linked characteristics that adversely affect agronomic qualities in flue-cured tobacco.

Tobacco introduction 1500 possesses a single, dominant gene that controls resistance to TMV that is not allelic to the N gene. Because it is not allelic to the N gene, it may not have the same adverse traits linked to it. A single, dominant gene is an easy trait for plant breeders to incorporate into a new breeding program. It has also been classified as a flue-cured type introduction, which may make it easier to incorporate into a flue-cured background. When used in concert with cultural practices that are proven to control TMV spread, host resistance is an effective and economical way to control TMV. A source of resistance to TMV that does not have adverse

traits linked to it would be very promising for breeders. Tobacco introduction 1500 may be that new source of resistance.

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Table 1. Reaction of eight tobacco introductions and susceptible and local lesion checks (K 326 and NC 567) to infection with tobacco mosaic virus.

Parent Cultivar	Number of plants		
	Local Lesion	Mosaic	Symptomless
K 326	0	75	0
NC 567	75	0	0
TI 1409	0	75	0
TI 1459	75	0	0
TI 1462	75	0	0
TI 1463	0	75	0
TI 1473	75	0	0
TI 1500	75	0	0
TI 1501	0	75	0
TI 1504	75	0	0

Table 2. Reaction of F₁ progeny of crosses between tobacco introductions and susceptible cultivar K 326 or local lesion cultivar NC 567 inoculated with tobacco mosaic virus.

Cross	Number of F₁ plants		
	Local Lesion	Mosaic	Symptomless
K 326 X TI 1459	75	0	0
K 326 X TI 1462	75	0	0
K 326 X TI 1500	75	0	0
TI 1459 X NC 567	75	0	0
TI 1462 X NC 567	75	0	0
TI 1500 X NC 567	75	0	0

Table 3. Reaction of F₂ progenies of crosses between tobacco introductions and susceptible cultivar K 326 or local lesion cultivar NC 567 inoculated with tobacco mosaic virus.

Cross	<u>No. of plants</u>			<u>Chi-square</u>			
	LL*	M	S	3:1	P-value	15:1	P-value
K 326 X TI 1459 F ₂	116	41	0	0.104	0.70-0.80	105.733	<0.01
K 326 X TI 1462 F ₂	114	29	0	1.699	0.20-0.30	48.037	<0.01
K 326 X TI 1500 F ₂	113	32	0	0.664	0.30-0.50	61.926	<0.01
TI 1459 X NC 567 F ₂	150	0	0				
TI 1462 X NC 567 F ₂	150	0	0				
TI 1500 X NC 567 F ₂	160	13	0	28.311	<0.01	0.473	0.30-0.50

*LL=Local Lesion, M=Mosaic, S=Symptomless

Table 4. Reaction of F_{2:3} progeny families to infection with tobacco mosaic virus.

Cross	<u>Number of F_{2:3} progeny families</u>			
	All Resistant	3:1	15:1	All susceptible
K 326 X TI 1459 F _{2:3}	1	3	0	7
K 326 X TI 1462 F _{2:3}	3	4	0	6
K 326 X TI 1500 F _{2:3}	3	1	0	5
NC 567 X TI 1459 F _{2:3}	5	0	0	0
NC 567 X TI 1462 F _{2:3}	4	0	0	0
NC 567 X TI 1500 F _{2:3}	6	4	1	6

Chapter 4

Inheritance of Resistance to Tobacco Mosaic Virus in Tobacco Introductions 203, 407, 438, 450, 692, 1203, and 1467

Abstract

Seven tobacco introductions (TI), TI 203, TI 407, TI 438, TI 450, TI 692, TI 1203, and TI 1467 were studied to characterize their resistance to tobacco mosaic virus (TMV) in the greenhouse. Each TI line was crossed with TMV susceptible cultivar K 326 to determine the mode of inheritance of resistance to TMV. Each TI line was also crossed with TMV resistant cultivar NC 567 in order to determine the relationship between the symptomless gene(s) and the N gene which results in local lesions when inoculated with TMV. All F₂ populations of the crosses between K 326 and the TI lines showed no segregation, all plants displayed mosaic symptoms. The crosses between TI 407, TI 438, and TI 1467 X NC 567 segregated in a 3 local lesion:1 mosaic ratio, no symptomless plants were observed. The crosses between TI 203, TI 450, TI 692, and TI 1203 also showed segregation although they did not fit a particular ratio. These results indicate that the gene(s) controlling resistance to TMV in these seven symptomless TI lines is not an acceptable new source of resistance to TMV for plant breeders because it does not appear to be a heritable trait. However, further virological study of the symptomless TI lines may reveal interesting and pertinent information about the interaction between virus and genotype.

Introduction

Tobacco mosaic virus is a single-stranded, rod-shaped, RNA virus that is worldwide in distribution. It is found in all countries where tobacco is grown. The virus is named for the characteristic mosaic pattern of intermingled dark green and light green areas in infected leaves. Leaf malformation, stunted plant growth, and “mosaic burn”, a necrosis of infected tissues, are also typical symptoms of mosaic infection (Lucas, 1975). It is an economically important disease of tobacco because it reduces agronomic traits and characteristics such as cured tobacco yield, quality, and average price.

The virus is sap transmissible and one of the most infectious plant viruses. It is easily spread through contact. Practices such as crop rotation and sanitation of equipment and laborers are important in controlling the diseases spread in the field. These preventative and eradication measures can be made even more effective in combating the spread of the virus when used in conjunction with plant host resistance.

Nolla and Roque (1933) first incorporated resistance to TMV into tobacco using the variety Ambalema. Resistance to TMV in Ambalema was controlled by two single recessive genes as well as some modifying genes. This made incorporating resistance into tobacco difficult for breeders. Plants with this incorporated resistance were of poor economic and agronomic quality. Holmes next incorporated resistance to TMV into tobacco from *N. glutinosa* (Holmes, 1938). Resistance in *N. glutinosa* was governed by a single, dominant gene, called the N gene. When a plant containing the N gene is infected with TMV, the result is a necrotic or local lesion reaction. The N gene was successfully incorporated into burley tobacco. However, yield and

value of flue-cured tobacco with the N gene was adversely affected (Chaplin et al., 1961). Chaplin and Mann (1978) later concluded that the N factor may be inherently difficult to disassociate from the adverse yield and quality characteristics in flue-cured tobacco.

Chaplin and Gooding (1969) screened the diverse *Nicotiana* genus for alternative sources of resistance to TMV. They screened 907 TI lines and identified three possible reactions to infection with TMV. The first was the necrotic reaction, the second the typical mosaic reaction, and the third response was called a symptomless response where no visual symptoms to infection were identified.

Twenty-five of the TI lines screened exhibited the symptomless response upon infection with the virus. The mode of inheritance of the resistance in these 25 TI lines is not known. Also, the relationship between the gene(s) controlling resistance to TMV in the symptomless lines and the N gene derived from *N. glutinosa* is not known. It is possible that the symptomless TI lines are a novel source of resistance to TMV that could be incorporated into flue-cured tobacco with no adverse agronomic affect.

Materials and Method

Greenhouse experiments were conducted at the Southern Piedmont Agricultural Research and Extension Center in Blackstone, Virginia, in 1996 and 1997. Seed of twenty-three tobacco introductions obtained from Dr. Verne Sisson of N.C. State, were sown in 7.62 cm clay pots filled with vermiculite. The tobacco introductions were selected based on previous research by Chaplin and Gooding (1969) and expressed a reaction that displayed no visual symptoms upon inoculation with TMV. Reinfecitivity assays performed by Chaplin and Gooding on the symptomless TI lines

revealed that some virus was recovered from all plants. Fifteen three-week-old seedlings of each introduction were transplanted into 10.16 cm peat pots filled with a 2:1 sand:vermiculite mixture. Four weeks after transplanting when the plants were at the four to six leaf stage, one leaf of each seedling was inoculated with 100 µg/mL of TMV in a potassium phosphate buffer (0.01) with 1% Celite at a pH of 7.0. The purified TMV was obtained from Dr. Sue A. Tolin of Virginia Tech. A cotton swab was used to apply the inoculum to the top side of the leaf. The inoculated leaves were rinsed with tap water to wash off any viral inhibitors present on the leaf surface and to standardize the procedure. The plants were scored for reaction to the virus at 7 and 14 d after inoculation. The results of these evaluations were used to verify Chaplin and Gooding's (1969) original classification of the tobacco introductions. K 326 was used as a susceptible check and NC 567 was used as a necrotic local lesion check as it possesses the N gene.

Seven tobacco introductions displaying the symptomless response to inoculation with TMV were randomly chosen for further study and characterization. These seven tobacco introductions were TI 203, TI 407, TI 438, TI 450, TI 692, TI 1203, and TI 1467. Tobacco introduction 203 was donated by the District of Columbia and is classified as a primitive type introduction. Tobacco introductions 407, 438, and 450 are all cigar filler type introductions, however, TI 407 was collected in Colombia, TI 438 was donated by the District of Columbia, and TI 450 was collected in the Chekiang Province of China. Tobacco introduction 692 is a flue-cured type introduction collected in Ecuador, while TI 1203 and TI 1467 are both cigar filler type introductions, collected in Guatemala and Colombia respectively.

In order to study the mode of inheritance of resistance to TMV for the tobacco introductions, crosses were made between the TMV susceptible

tobacco cultivar K 326 and each of the tobacco introductions. Crosses were also made between each of the tobacco introductions and the resistant cultivar NC 567, to determine the relationship between the gene(s) controlling resistance in the symptomless TIs and the N gene. Seventy-five F_1 seedlings from each cross were grown, inoculated, and evaluated. Three reactions were predicted, a local lesion reaction, a reaction that displayed no visual symptoms, and a susceptible reaction, which showed the characteristic mottling response to the virus. Two F_1 seedlings from each cross were selected to self-pollinate and be advanced to the F_2 generation. One hundred F_2 seedlings from each advanced F_1 were grown, inoculated, and scored in the usual manner. A chi-square test of homogeneity was performed to determine if the data from each of the F_2 families could be combined. Data from segregating F_2 progeny was subjected to chi-square analysis.

Reinfectivity assays to test for presence of the virus were performed on the symptomless parent TI lines. One gram of tissue from the inoculated leaf of each parent TI line was ground in a mortar and pestle in potassium phosphate buffer (0.01M) pH7.0 with 1% Celite. A cotton swab was then used to apply the inoculum to the top side of one leaf on a NC 567 seedling., the inoculated leaf was then rinsed with water. The NC 567 seedling was at the four to six leaf stage. Approximately one week after inoculation, the NC 567 plants were scored for reaction to the virus.

Results and Discussion

Twenty-three tobacco introductions were inoculated with TMV, evaluated for reaction to the virus, and compared with the results of Chaplin and Gooding's (1969) original study. Tobacco introductions 25, 203, 384, 407, 410, 411, 412, 431, 436, 437, 438, 439, 448, 448A, 449, 450, 465, 468,

470, 471, 692, 1203, and 1467 displayed no visual response to the virus which agreed with Chaplin and Gooding's original findings (Table 1). There was no segregation in any of the parent populations, indicating that these populations were all homogeneous in regards to reaction to TMV. Susceptible cultivar K 326 and local lesion cultivar NC 567 were used as checks and exhibited mosaic symptoms and local lesions respectively.

Seven tobacco introductions were randomly chosen for further study and characterization. These seven tobacco introductions were TI 203, TI 407, TI 438, TI 450, TI 692, TI 1203, and TI 1467. Reinfectivity assays were performed on each inoculated symptomless parent TI line to determine if virus was present in the inoculated leaf. Inoculum was prepared from each symptomless parent TI line and applied to the necrotic local lesion cultivar NC 567. All NC 567 plants displayed local lesions within 4 to 7 days of inoculation. Lesions were of the same size, shape, and number for all symptomless parent TI lines. The reinfectivity assays show that TMV was indeed present in the tissue of the inoculated leaves of the symptomless parent TIs. Crosses were made between these seven TI lines and susceptible cultivar K 326. The F_1 progeny of these crosses were inoculated and evaluated (Table 2). There was no segregation in any of the F_1 progeny populations, all plants exhibited a mosaic reaction upon infection with TMV. A second set of crosses were made between the seven TI lines and local lesion cultivar NC 567 (Table 2). All of the F_1 progeny from these crosses exhibited a local lesion reaction upon infection with the virus. Once again there was no segregation in any of the F_1 populations.

None of the F_2 progeny of the crosses between the susceptible check K 326 and the symptomless TI lines segregated (Table 3). All of the progeny from these seven crosses exhibited mosaic symptoms. Because there was no

segregation for reaction to TMV in these lines whatsoever, it appears that the gene(s) conditioning the symptomless phenotype of the parents are not simply inherited traits and thus difficult for plant breeders to try to incorporate into a breeding program.

Segregation was apparent in the F_2 progeny of the TI line X NC 567 crosses (Table 3). However, no symptomless plants were observed in any of the crosses. The F_2 progeny all displayed either a local lesion or mosaic reaction to infection with the virus. Tobacco introductions 407, 438, and 1467 segregated in a 3 local lesion:1 mosaic ratio. Tobacco introductions 203, 450, 692, and 1203 did not segregate in any obvious ratio. Due to limited time and space the $F_{2:3}$ generation of each set of crosses was not evaluated and should be in the future.

The absence of any symptomless plants in the F_2 generation demonstrates that these seven tobacco introductions are not good candidates for a source of resistance to TMV other than the N gene, because they have failed to demonstrate heritable resistance. Very little is known regarding the gene(s) controlling the symptomless phenotype. Reinfectivity assays on the symptomless parent plants suggest that the virus is able to establish infection in the plant (Chaplin and Gooding, 1969). It is possible that the virus titre in the plant does not exceed a certain threshold level which is required for the appearance of symptoms. Further studies should be designed to test for virus concentration in symptomless TI lines compared to TMV susceptible varieties. The symptomless plants may have a lower virus concentration in the plant than susceptible plants do. While this may be a form of resistance, it still allows for inoculum build up in the field, which is undesirable. The symptomless plants have also failed to demonstrate heritability of the

symptomless phenotype, therefore, these seven TI lines are not useful for tobacco breeders in a resistance breeding program.

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Table 1. Reaction of twenty-three tobacco introductions and susceptible and local lesion checks (K 326 and NC 567) to infection with tobacco mosaic virus.

Parent Cultivar	Number of plants		
	Local Lesion	Mosaic	Symptomless
K 326	0	75	0
NC 567	75	0	0
TI 25	0	0	75
TI 203	0	0	75
TI 384	0	0	75
TI 407	0	0	75
TI 410	0	0	75
TI 411	0	0	75
TI 412	0	0	75
TI 431	0	0	75
TI 436	0	0	75
TI 437	0	0	75
TI 438	0	0	75
TI 439	0	0	75
TI 448	0	0	75
TI 448A	0	0	75
TI 449	0	0	75
TI 450	0	0	75
TI 465	0	0	75
TI 468	0	0	75
TI 470	0	0	75
TI 471	0	0	75
TI 692	0	0	75
TI 1203	0	0	75
TI 1467	0	0	75

Table 2. Reaction of F₁ progeny of crosses between tobacco introductions and susceptible K 326 or local lesion NC 567 to inoculation with TMV.

Cross	Number of plants		
	Local Lesion	Mosaic	Symptomless
K 326 X TI 203	0	75	0
K 326 X TI 384	0	75	0
K 326 X TI 407	0	75	0
K 326 X TI 438	0	75	0
K 326 X TI 450	0	75	0
K 326 X TI 692	0	75	0
K 326 X TI 1203	0	75	0
K 326 X TI 1467	0	75	0
TI 203 X NC 567	75	0	0
TI 384 X NC 567	75	0	0
TI 407 X NC 567	75	0	0
TI 438 X NC 567	75	0	0
TI 450 X NC 567	75	0	0
TI 692 X NC 567	75	0	0
TI 1203 X NC 567	75	0	0
TI 1467 X NC 567	75	0	0

Table 3. Reaction of F₂ progenies to tobacco mosaic virus from the crosses K 326 (susceptible) X TI lines and TI lines X NC 567 (local lesion).

Cross	<u>No. of plants</u>			<u>Chi-square</u>	
	LL*	M	S	3:1	P-value
K 326 X TI 203 F ₂	0	190	0		
K 326 X TI 407 F ₂	0	179	0		
K 326 X TI 438 F ₂	0	183	0		
K 326 X TI 450 F ₂	0	181	0		
K 326 X TI 692 F ₂	0	190	0		
K 326 X TI 1203 F ₂	0	185	0		
K 326 X TI 1467 F ₂	0	184	0		
TI 203 X NC 567 F ₂	156	31	0	7.075	<0.01
TI 407 X NC 567 F ₂	153	41	0	1.547	0.20-0.30
TI 438 X NC 567 F ₂	148	36	0	2.899	0.05-0.20
TI 450 X NC 567 F ₂	162	29	0	9.817	<0.01
TI 692 X NC 567 F ₂	162	32	0	7.484	<0.01
TI 1203 X NC 567 F ₂	162	28	0	10.673	<0.01
TI 1467 X NC 567 F ₂	124	50	0	1.295	0.20-0.30

*LL=Local Lesion, M=Mosaic, S=Symptomless

VITA

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