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GENETIC BASIS OF TOLERANCE IN MAIZE TO MAIZE STREAK VIRUS USING MOLECULAR MARKERS

Dissertation

Presented in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Graduate School of The Ohio State University.

By

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********

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To my parents, wife Gracie and son Emmanuel.
ACKNOWLEDGMENTS

I wish to express my sincere appreciation to Jr. R.C. Pratt, my adviser, Dr. M.D. McMullen, my co-adviser, and members of my advisory committee Drs. S. St. Martin and D.T. Gordon for their consistent support throughout my studies and research.

The technical assistance of Mark W. Jones, Brenda S. Schult in the laboratory, Bert Bishop and James Hacker with statistical analyses, and Dr. David Lohnes with the MAPMAKER program is highly acknowledged. I also thank Reiguang Ming with whom we shared the laboratory work load. Without the support of the Director, and the Maize Program team of Namulonge (NAARI), and Mr. W. Page of Natural Resources Institute, U.K., during my field and glasshouse work in Uganda I could not have gone far. Many thanks to Dr. V.O.A. Okoth, who was my in-country adviser in Uganda. I highly appreciate the support of Drs. D.C. Jewell, K.V. Pixley and S.R. Waddington of CIMMYT, Harare, Zimbabwe, and the CIMMYT support staff for providing the most wonderful working environment and happy stay while in Zimbabwe for my research.
Without the seed from Dr. J. Brewbaker, I could not have carried out this work. My highest regards to you. I also thank Dr. R. Louie who advised on the glasshouse evaluation method and obtained the original TZi4 accession from the National Germplasm bank in Iowa.

I could not have carried out this work without the financial and material support of the following: The Uganda Government, USAID/MFAD, The Rockefeller Foundation, and the World Bank. The support of Mr. M. Erbaugh and Dr. D. Hansen of IPA/OSU is highly appreciated. Thanks to Shelley Whitworth for assisting in putting this dissertation together.

Without the support and encouragement of my wife Gracie and our son Emmanuel I could not have reached this stage. A million thanks.
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GENERAL INTRODUCTION

Occurrence of Maize Streak

Maize streak geminivirus (MSV) disease, hereafter referred to as maize streak, was first described by Fuller (1901) as "mealie variegation" in South Africa. It was renamed "maize streak" by Storey in 1925. It is now found widely distributed in sub-Saharan Africa from South Africa northwards into East Africa as far as Egypt. It occurs through Central and West Africa into the Savanna/Sahelian countries below the Sahara. Outside continental Africa, it has been reported as occurring naturally on maize (Zea mays) from Mauritius, Reunion, Madagscar, SaoTome and Principe (Storey, 1936; Bock, 1974; Baudin, 1976; Fajemisin and Shoyinka, 1976; Guthrie, 1976; Rose, 1978; Fauquet and Thouvenel, 1980; Kim et al., 1981; Dubern, 1981; Ammar, 1983; Dadant, 1983; Rossel and Thottappilly, 1985; Dintinger et al., 1994). A virus of pearl millet (Pennisetum americanum) and wheat (Triticum aestivum) in India has been reported as MSV (Seth et al., 1972 a,b) but the exact identity of this virus and its relationship to MSV remains unconfirmed.
Host range of MSV

The host range of MSV is wide within the Poaceae (Gramineae) (Gorter, 1953; Bock, 1974; Rose 1978; Damsteegt, 1983; Rossel and Thottappilly, 1985; Pinner et al., 1988). The list includes economic crops such as maize, wheat, sorghum (Sorghum bicolor), sugarcane (Saccharum officinarum), barley (Hordeum vulgare), oats (Avena sativa), rye (Secale cereale), finger millet (Eleusine coracana), bulrush millet (Pennisetum typhoides), rice (Oryza sativa) and pearl millet. Many wild grasses are also susceptible and these may act as alternative hosts. In Nigeria, wild grasses such as Brachiaria deflexa, B. distichophylla and Axonopus compressus were found to be naturally infected with MSV (Rossel and Thottappilly, 1985). Utilizing a maize isolate of MSV from South Africa to inoculate seedlings and young grass plants, Damsteegt (1983) was able to infect certain grass species of the tribes Hordeae, Aveneae, Agrostideae, Chlorideae, Andropogoneae, Paniceae, Oryzeae and Glycerieae. Rossel and Thottappilly (1985) were, however, unable to transmit MSV isolates from Brachiaria mutica, B. deflexa, Eleusine indica, Oryza sativa, Rottboellia cochinchinensis and Panicum maximum to selected IITA (International Institute of Tropical Agriculture, Ibadan, Nigeria) maize varieties. In recent studies at IITA (Mesfin et al., 1992), 24 isolates originating from cereal crops or wild grasses were transmitted to the maize cultivar 'Golden Bantam'. But several attempts to transmit some isolates to another MSV-susceptible variety, 'Pool 16', proved unsuccessful.
Vectors of MSV

MSV is transmitted by species of the leafhopper genus *Cicadulina* China. There are 22 species of *Cicadulina* of which 18 occur in Africa (Webb, 1987). Of the 18 species, 8 have been confirmed to be vectors of MSV: *C. mbila* (Naude), *C. storeyi* (=triangula) China, *C. bipunctata* (=bipunctella) (Melichar), *C. latens* Fennah, *C. parazaeae* Ghauri, *C. arachidis* China, *C. similis* China and *C. ghaurii* Dabrowski (Storey, 1925, 1936; Fennah, 1959; Rose, 1962; Dabrowski, 1987; Okoth and Dabrowski, 1987). *C. mbila* appears to be the most important vector of MSV in East Africa (Storey, 1928) and recent studies show it is the most abundant vector across major ecological zones in Nigeria (Asanzi et al., 1994), and in Africa as a whole (Rose, 1978, 1983). Another species *C. niger*, has been shown recently to transmit MSV (Bigirwa et al., 1994) but its importance in field transmission of MSV is yet to be established. MSV is transmitted in a persistent circulative, non-propagative manner (Storey, 1933; Bock, 1974; Boulton and Markham, 1986; Nault and Ammar, 1989; Reynaud and Petershmitt, 1992). The latent period of MSV in *C. mbila* is 6-12 hours (Storey, 1925; 1928) and *C. mbila* can acquire MSV from infected plants within 15 seconds and inoculate it within 5 minutes (Storey, 1925; 1938). Different *Cicadulina* species have different capabilities of virus transmission (Okoth et al., 1988; Van Rensburg and Giliomee, 1990).
Properties of MSV

MSV is the type member of the geminivirus subgroup I (Francki et al., 1991; Mathews, 1991). It has twinned (geminate), quasi-icosahedral particles (20 x 30 nm) with a sedimentation coefficient of about 76S, 54S for the single particles (Bock et al., 1974; Howarth and Goodman, 1982; Howell, 1984). The single particles (20 x 18nm) have been observed in purified preparations in addition to geminate particles. The coat protein of MSV particles typically consists of a single species of molecular weight 28-30 kilodaltons (kd) (Bock et al., 1977; Howarth and Goodman, 1982; Clarke et al., 1989). The nucleic acid of MSV is a single-stranded DNA existing predominately as closed circular molecules of molecular weight 7.1-9.3 x 10^5 daltons (d) and about 2.7 kilobases (kb) (Harrison et al., 1977; Mullineaux et al., 1984; Harrison, 1985; Lazarowitz, 1987; 1988).

Symptoms of MSV

Maize streak symptoms on maize from onset are characterized by chlorotic, almost circular spots with a diameter of 0.5-2mm. These are mostly seen on the basal portions of the youngest leaves (Fajemisin and Shoyinka, 1976; Rose, 1978; Rossel and Thottappilly, 1985; Efron et al., 1989). Fully developed symptoms consist of prominent white chlorotic streaking developing over and along the veins on most of the leaf laminae. The parallel, chlorotic streaks may partially or completely fuse, leaving irregular green lines or islands centered between secondary and tertiary veins. Streaking develops on newly emerging leaves with
existing leaves remaining unaffected. This allows estimation of the growth stage of the plant at the time of infection (Connolly, 1987). The intensity of streaks varies greatly according to genotype and time of infection and streaks are typically of low density on leaves of resistant genotypes (Van Rensburg, 1981; Efron et al., 1989). In highly susceptible genotypes chlorotic striping may develop into uniform chlorosis of the entire lamina, which may result in progressive necrosis and eventually plant death, particularly if infection occurred at the early seedling stage. Maize plants are vulnerable from emergence to tasseling but infection at later stages, six to eight weeks old, usually has little or no effect on vigor or productivity of the plant.

Isolates of MSV

Despite the intensive research that has been carried out on MSV, there exists some contradictory information on its variability. Storey and Howland (1967) while searching for maize-infecting MSV strains in East Africa found no differences in symptom expression of those evaluated. An MSV isolate from Nigeria was found by Fajemisin et al. (1976) to react with antiserum to an MSV isolate from East Africa and this has been confirmed at IITA (Thottappilly et al., 1993). Serological variation has been reported among MSV isolates from different grass species using monoclonal antibodies and polyclonal antisera, whereas no serological variation among maize-infecting MSV isolates from different African countries was reported (Dekker et al., 1988; Pinner et al., 1988; Peterschmitt et al., 1992). Johnson (1983) reported that sources of MSV resistance from Nigeria and South Africa exhibited the
same resistance under Zimbabwean conditions but this is not universally accepted (Pham, H.N., formerly of CIMMYT, Harare, Zimbabwe, personal communication). Lazarowitz (1988) showed that based on sequence analysis of the infectious clone, a South African isolate of MSV was homologous to Kenyan and Nigerian isolates but with some significant differences from each of the published sequences.

In contrast, McClean (1947) showed that several forms, supposedly strains, of the maize-infecting MSV exist in South Africa. Bock et al (1975) reported that a variety from IITA (TZB-SR) selected as streak resistant in Nigeria and other resistant selections performed similarly well in Kenya when challenged with an East African isolate. When they were challenged with two other geographically distinct maize isolates from the Coast Province, Kenya and Western Uganda they performed differently (Bock et al., 1976). The Coast isolate was the most virulent and hence the existence of strains of maize-infecting MSV was suggested and a re-evaluation of resistant selections was called for. Ngwira (1988) using monoclonal antibodies to a Malawian maize-infecting isolate, Clarke et al (1989) using polyclonal antisera to three maize-infecting isolates (MSV-CT, MSV-PE, and MSV-SW) from widely separated locations in South Africa, and Bonga (1992) using polyclonal antisera, collected at different stages of immunization, to maize MSV isolates from Kenya, Uganda, and Zimbabwe obtained evidence for serological variation among some isolates. It has been shown further that differences occur in transmission and symptomatology among maize-infecting MSV isolates (Bock, 1980; Clarke et al., 1989; Von Wechmar and Hughes, 1992; Dintinger et al., 1994).
Economic importance of MSV

The effects of MSV on maize vary enormously, from negligible damage in some years to total devastation when epidemics occur. Under natural leafhopper infestation, the following yield reduction due to MSV have been observed in various African countries: 33% - 56% in Kenya (Guthrie, 1976), 68% in Uganda (Mukiibi, 1976), 24% - 76% in Nigeria (at IITA) (Fajemisin et al., 1986), 24% - 28% in Mauritius (Ann. 1975), 36% in Zimbabwe (Mzira, 1984), 5.6% - 94.2% in South Africa (Barrow, 1992), and up to 100% in many countries of West Africa and Zaire (Fajemisin and Shoyinka, 1976) and on fields planted to Kenyan hybrids in Uganda (personal observation). Van Rensburg and Kuhn (1977) reported that plants infected less than a week after germination produced no yield, and those infected after three or eight weeks produced about 50% yield or nearly full yield, respectively. Under controlled infestation with viruliferous leafhoppers yield reduction of 70% (Kim et al., 1981) and of 71-93% (Fajemisin et al., 1986) were reported at IITA. In Kenya, yield losses of 25 - 60% (Guthrie, 1978) under controlled infestation were reported and were shown to be directly related to the age of plant at the time of infection.

Control of MSV

Various cultural practices have been suggested for the control of MSV such as timely planting, having barriers of bare ground between early- and late-planted maize fields, and rotations with other crops to reduce leafhopper movement and
subsequent spread of the virus (Gorter, 1953; Rose, 1978; Rothwell, 1979). Insecticides have also been used to control the vectors (Rose, 1978; Rothwell, 1979; Mzira, 1984; Van Rensburg and Giliomee, 1991; Van Rensburg et al., 1991b; Barrow, 1992). Although insecticides can control the leafhopper vectors thus mitigating the disease, they are expensive, and may be hazardous or ineffective because they are difficult to use in low-input African farming conditions. Once a plant is infected with MSV, no further control of the disease is possible (Connolly, 1987). Therefore the development of resistant varieties seems the most appropriate, safe, and cost effective disease control (Rothwell, 1979; Soto et al., 1982; Efron et al., 1989; Brewbaker et al., 1991).

Sources of resistance to MSV

Resistance was noted as early as 1931 in South Africa (Fielding, 1933) in an open-pollinated variety (OPV) 'Peruvian Yellow'. This was confirmed by Rose (1936) who found that next to Peruvian Yellow, 'Arkell's Hickory', another OPV, was the most resistant variety. From these two varieties, intra-varietal self-pollinations were made and crossed to make a hybrid P X H which was superior to either parent (Rose, 1936). A later breeding program in South Africa produced the hybrid SA31 which combined the MSV tolerance of P X H with other maize lines adapted to South African conditions (Gorter, 1951). Extensive screening of a wide range of materials in South Africa finally identified new resistant sources in inbred Mexican
In 1954, pioneering work in the search for resistance to streak was carried out in East Africa. In Uganda some lines of Columbian and Mexican origin were identified to have combined resistance to rust (*Puccinia polysora*) and MSV (Rubaihayo, 1974). These lines were used to develop the variety 'White Star'. Through a cooperative effort between the Institut Recherche Agronomique Tropicale (IRAT) in Reunion and the Kenya Agricultural Research Institute (KARI), a new source of resistance was found and named 'La Revolution'. It is an OPV which originated from Reunion Island in the Indian Ocean (Entienne and Rat, 1973). This source of resistance has proved to be generally effective (Soto et al., 1982; Fajemisin et al., 1984; Hainzelin and Marchand, 1986; Dintinger, 1994). Streak resistance was also reported in 1976 in Tuxpeno X 'Ilonga Composite' from Tanzania (Bjarnason, 1985).

In West Africa, attempts to identify sources of resistance to streak started in Nigeria only in the early 1970's following the epidemic of 1971 (Fajemisin et al., 1976). The search was initiated by IITA in 1975 under controlled infestation with viruliferous leafhoppers (IITA 1977; Buddenhagen, 1978). IITA scientists identified sources of resistance in the population TZ-Y (Tropical Zea-Yellow) derived from a cross between Tuxpeno Planta Baja from the Centro Internacional de Mejoramiento de Maiz y Trigo (CIMMYT), Mexico, and an unknown yellow germplasm source from East Africa. Susceptibility to MSV could be eliminated from the TZ-Y population by
two generations of successive inbreeding, selection, and resynthesis (Efron et al., 1989). In one of the $S_1$ lines developed from TZ-Y (TZ-Y-32), all plants were rated as highly resistant with resistance expressed by very few and short white streaks on the leaves. The line was advanced to the $S_6$ stage by 1979 and designated as IB32 (Ibadan 32). IB32 was used to study the genetics of resistance to MSV (Kim et al., 1981; 1989).

In 1978, both white- and yellow-grain populations were established, based on TZ-Y; they were called TZSR(W) and TZSR(Y) respectively. These populations were developed by chain-crossing streak resistant plants developed from TZ-Y with a few selections of TZPB (streak susceptible white variety with high yielding potential in Nigeria and other West Africa countries) (Buddenhagen, 1978; Soto et al., 1982). These populations had good streak resistance but a rather narrow genetic base and certain limitations such as root lodging, high ear- and plant-heights. TZSR-W is the donor of streak resistance to the streak resistant parent (TZi4) (Kim et al., 1981, 1987) that was used in the present study. To broaden the genetic base, TZSR(W) and TZSR(Y), were crossed to several improved but streak-susceptible varieties/populations.

In 1982, streak resistance was also verified in uniform streak nurseries at IITA in the populations tropical late white dent (Pop. 10), tropical intermediate white flint (Pop. 11), and tropical yellow flint-dent (Pop. 12). These populations were developed in a cooperative effort between the national programs of Tanzania and Zaire and CIMMYT (Bjarnason, 1985).
Research at CIMMYT, Harare, Zimbabwe has emphasized conversion to MSV resistance and improvement of elite tropical and mid-altitude maize materials. The CIMMYT program has produced a number of populations, varieties, and inbred lines, which not only have resistance to MSV but are also adaptable to local conditions of the mid-altitude regions in Africa (Pham and Short, 1991; Pham, 1992; K.V. Pixley, CIMMYT, Harare, Zimbabwe, personal communication). Various resistance levels, as well as immunity to MSV, have been identified in these maize materials.

Recently, in South Africa white and yellow inbreds, populations and hybrids with various resistance levels to MSV have been released (Van Rensburg et al., 1991a; Barrow, 1992). Also symptomless infection indicating a high level of resistance to MSV has been detected in some inbred lines and in an experimental hybrid (Barrow, 1992).

Genetics of resistance to MSV

More efficient exploitation of available germplasm resources is facilitated if the genetic mechanisms of resistance are known (Fraser, 1986). Furthermore, decisions about the strategies of resistance gene deployment, and predictions of the likely future patterns of interaction with the evolving genetic system of the pathogen, can only be based on sound genetic knowledge.

Although the first observation by Fielding (1933) that OPV 'Peruvian Yellow' was resistant to MSV, genetic studies of its resistance were not carried out until the
1950's (Gorter, 1959). The genetics of resistance in the P X H hybrid, under natural infestation, was reported to be of a complicated nature and did not follow Mendelian segregation ratios, i.e., was quantitative (Rose, 1938; Gorter, 1959). Reexamination using controlled leafhopper infestation in the glasshouse led to the conclusion that resistance in P X H was controlled by a single gene lacking dominance, but whose level of expression was affected by the genetic background of the parents (Storey and Howland, 1967).

Soto et al (1982) screened, under controlled leafhopper infestation, several diverse populations for resistance to MSV from West Africa, East Africa, CIMMYT, and Brazil. Included among these populations were TZ-Y, Tuxpeno X 'Ilonga composite' and 'Revolution' which are MSV resistant. They found that in the few populations that were resistant, this resistance was simply inherited and easily fixed through selection.

Kim et al (1981,1982), with controlled leafhopper infestation, studied the genetics of resistance in IB32 using generation mean analysis involving three susceptible temperate inbred lines (B14, B73, Mo17). They found that additive genetic variance comprised 55.1%, nonadditive variance 26.1%, and environmental variance 19.1% implying that more than one gene was involved in MSV resistance. Using the Castle-Wright formula (Castle, 1921) it was estimated that two to three gene pairs controlled resistance. Further resistance studies in IB32, using generation mean analysis, involving four susceptible inbred lines (B14, B68, B73, Mo17) suggested that the resistance in IB32 is quantitatively inherited and involves
a relatively small number of genes (Kim et al., 1989). It was reported that the symptoms of MSV varied with the genetic background of the susceptible parent. Tang and Bjarnason (1993) evaluated the progress in selecting for resistance to MSV through full-sib family selection and backcrossing in the La Posta population. They noted a marked improvement in resistance which suggested rather simple inheritance.

Engelbrecht (1973) cited by Damsteegt (1981) identified five dominant gene pairs responsible for genetic control of the streak disease in South African germplasm. Susceptible lines had two or less dominant gene pairs and highly resistant lines contained three or more dominant gene pairs. However, Engelbrecht (1975) reported that MSV resistance was controlled by five dominant genes but it was not possible to determine whether the effects of all gene pairs were of the same magnitude. Later studies on MSV resistance, under natural infestation, using diverse maize germplasm showed there exists genetic variation for resistance against MSV. It was noted that this resistance was relatively simply inherited, with an apparently strong dominance component (Fourie and Piennar, 1983).

Fajemisin et al (1984) and later Efron et al (1989) observed a qualitative type of resistance in some maize selections developed in Tanzania. Resistant plants were virtually free of symptoms following controlled leafhopper infestation, and preliminary data suggested a single dominant gene action.

Thus, overall, it is not possible to state conclusively if multiple types of MSV resistance factors are present in maize, or, if varying experimental conditions lead
to variable interpretations. This calls for elucidation of the genetic basis of MSV resistance using careful experimentation. In this study I will use more precise methods of controlled leafhopper infestation and the recently developed molecular genetic markers that can map genes for resistance to specific chromosome regions.

There also still exists confusion regarding resistance terminology. Several terms such as resistance, tolerance, susceptible, sensitive and intolerant have been used (Gorter, 1959; Storey and Howland, 1967; Soto et al., 1982; Kim et al., 1989; Barrow, 1992; Dintinger et al., 1994) to describe the host-pathogen interaction. Quite often they have been used interchangeably. Various descriptive degrees associated with the above terms also have been used: true-breeding tolerance, highly or moderately tolerant; good-, very little good-, high level-, low level-, partial-, and intermediate-resistance; epidemiological resistance; and recently, immunity (Pham and Short, 1991). This leaves the reader confused or uncertain as to what kind of reaction the authors are referring to and how they are related. The common practice in all MSV-breeding work reported is that the researchers select plants showing mild symptoms and those showing no symptoms are avoided as they can include escapes. Also avoided are those not showing symptoms on lower leaves for they were infested late and therefore expressing less severe symptoms. Mild symptom expression especially has been inconsistently described.

The confusion in the usage of various terms describing plant responses to virus inoculation is a problem of long standing (Cooper and Jones, 1983; Jones and Cooper, 1984; Tavantis, 1984) and is not limited to researchers involved with MSV-
breeding. These authors point out that the existing confusion is caused in part by the use of resistant terminology that was established in relation to diseases caused by extracellular pathogens. Further confusion arises from the different viewpoints often taken by breeders who are largely concerned with visible changes that are subjectively deemed harmful to individual plants or populations while those of virologists are usually more concerned with the viruses themselves and with events that occur in cells or tissues. For improvements in usage of terms so as to facilitate the exchange of information unambiguously between scientists, Cooper and Jones (1983) suggested that: (1) the term 'immunity' be used as an absolute state of exemption from infection with a specified agent, a virus in this case; (2) the term 'infectible' be the antonym of immune, i.e., a plant that can be infected by a virus; (3) that the behavior of the virus should be separated from the disease response of the plant; (4) that the terms 'susceptible' and 'resistant' be used to indicate effects of an infectible individual on virus infection, multiplication, and invasion and; (5) the terms 'tolerant' and 'sensitive' be used to indicate the disease reaction of the plant to virus infection and establishment. However many plant breeders involved in MSV-resistance breeding have used the terms 'susceptible' to indicate severe expression of the virus, and 'resistant' to indicate suppression of the symptoms by the plant (Fourie and Piennar, 1983; Kim et al., 1987; Kim et al., 1989; Efron et al., 1989; Van Rensburg et al., 1991a, Barrow, 1992; Dintinger, 1994). In other cases 'susceptible' and 'tolerant' have been used for severe symptom expression and suppression of symptoms, respectively (Gorter, 1959; Soto et al., 1982; Bjarnason,
1985; Hainzelin and Marchand, 1986). In a number of others, 'tolerance' and 'resistance' have been used interchangeably (IITA, 1977). On the whole, Fraser (1986) suggests that the terminology has to be operational and evolving rather than absolute and definitive.

In this study, the terms 'susceptible' and 'tolerant' are the ones adopted. Susceptibility is used to indicate severe expression of the virus symptoms with loss of vigor, yield or eventual plant death and tolerance is used to indicate the occurrence of only mild symptoms with little or no loss in vigor or yield (Posnette, 1969; Schafer, 1971; Cooper and Jones, 1983). In this respect, it is tolerance to MSV that was found in maize and rapidly incorporated into high-yielding maize populations for use in tropical Africa (Soto et al., 1982). This is similar to the definition of Loi et al. (1986) and Edwards et al. (1988) of tolerance to barley yellow dwarf virus as the resistance to symptom formation or yield loss, rather than to virus multiplication. The definition of the term 'resistance' has been limited to inhibition of virus multiplication (Fraser, 1986; Mathews, 1991; Hull, 1994). Here virus multiplication is limited to initially infected cells or to a zone of cells around the initially infected cell. For tolerance, the virus multiplies and spreads widely throughout the plant, but produces mild or negligible disease. This toleration of the virus appears compatible with toleration of disease resulting in less damage. Buddenhagen (1981) recommended highly the breeding for tolerance to systemic pathogens, for it is easy to see and can be selected for without concern for escapes or the repeated reinfection requirement of polycyclic, non-systemic pathogens. He
suggested that for systemic diseases, there should be a separation of the phenomenon of individual plant tolerance from the tendency of a population to develop disease (disease incidence). He introduced the term 'tolremic' to refer to a cultivar if it develops few diseased plants in comparison to cultivars growing nearby under the same conditions that develop many diseased plants ('intolremic'). It is hypothesized that selecting for tolerance will also provide for cultivar tolremicity (low field spread and incidence), especially for vectored pathogens. This is what occurred for MSV, where selecting only for tolerance provided sufficient tolremicity to reduce this major disease to insignificance where resistant populations are grown (Soto et al., 1982).

**Genetic marker-facilitated studies**

A genetic marker is defined as a gene, whose phenotypic expression is usually easily discerned, used to identify an individual or a cell that carries it, or as a probe to mark a nucleus, chromosome or locus (King and Stansfield, 1990). To be useful as a genetic marker, the marker must meet two criteria: (1) the marker must differentiate between the parents; and (2) the marker must be accurately reproduced in the progeny (Paterson et al., 1991). Thus the discovery by Morgan (1911) of genetic linkage, i.e., that Mendelian genetic factors which lie close together on a chromosome are usually cotransmitted from parent to progeny, set forth the idea that markers could serve as a "proxy" for other nearby genes controlling traits of interest.
The use of genetic markers to study quantitatively inherited traits has been employed for about 70 years since Sax (1923) reported the association of seed coat pattern and pigmentation with seed size differences in beans (Phaseolus vulgaris). He interpreted his findings as the linkage of the single gene controlling seed color with one or more of the polygenes controlling seed size. Polygenes are defined in this context as genes which control traits whose phenotypic variation is continuous (referred to as quantitative traits) and are believed usually to have effects that are small relative to those of other sources of variation (Thompson, 1975). The term polygenes is used here interchangeably with quantitative trait loci (QTLs) ( Tanksley, 1993). Subsequent reports also indicated linkage of genes controlling quantitative variation with single gene markers. Wexelsen (1933) reported the association of a quantitative trait, length of internode of the spike, with a qualitative trait, surface texture of the awn (rough or smooth) in barley. Rasmusson (1935) demonstrated linkage of flowering time (a quantitative trait) in peas (Pisum sativum) with a simply inherited gene for flower color. Linkage of color, a quantitative trait, to corolla size, a qualitative trait, in Nicotiana spp. was reported by Smith (1937). Everson and Schaller (1955) found morphological markers which flanked a chromosomal region affecting yield in barley. In maize and barley, as discussed by Burnham (1966), translocations have been used to associate segregation for quantitative traits such as European corn borer resistance (maize) and diastatic power (barley) with individual chromosome segments. In wheat, monosomics have been used to identify association of quantitative traits with individual chromosomes (Law, 1967).
These studies employed mostly morphological and some cytological genetic markers.

The idea of using single gene markers to systematically characterize and map individual QTL controlling quantitative traits was put forth by Thoday (1961). His idea was that if the segregation of a single gene marker could be used to detect and estimate the effect of a linked QTL, and if single gene markers were distributed throughout the genome of an organism, it should be possible to map and characterize all of the QTLs affecting a character. There were major limitations to putting this theory into practice. They included the limited number of markers available, undesirable effects of many of the morphological markers on phenotype, and in the case of translocations or whole chromosome effects, the extreme size of the chromosome segments being compared (Dudley, 1993). However, these studies and ideas provided a background of theory and observation for the recent work with the development of molecular (protein and DNA) markers which have exhibited greater utility (Tanksley, 1983; Tanksley et al., 1989; Stuber, 1992). The discovery that allelic forms of enzymes (allozymes or isozymes) can be separated on electrophoretic gels and detected with histochemical stains for enzymatic activity heralded the era of molecular markers (protein, in this case) in genetics research (Hunter and Markert, 1957). The next advance in molecular markers came with the introduction of DNA-based genetic markers, the first of which was restriction fragment length polymorphism (RFLP) (Botstein et al., 1980). In the past few years a new generation based on polymerase chain reaction, random amplified
polymorphic DNAs (RAPDs) (Williams et al, 1990; Welsh and McClelland, 1990) has also been developed.

The advantage and greater utility of these molecular markers stems from their inherent properties which include the following: (1) The phenotype of most morphological markers can only be determined at the whole plant level whereas molecular markers can be assayed at whole plant, tissue, or cellular level thus allowing early screening methods to be applied. (2) Polymorphic alleles tend to be much higher in frequency for molecular markers than for morphological markers. (3) In addition, morphological mutants tend to be associated with undesirable phenotypic effects which is a problem in disease screening because of symptom expression. (4) Alleles at morphological markers interact in a dominant-recessive manner that limits the identification of heterozygous genotypes whereas (5) most molecular markers exhibit a codominant mode of inheritance that allows the genotypic identification of all individuals in a segregating population. (6) Fewer epistatic or pleiotropic effects are observed with molecular markers than with morphological markers. (7) Hence, a large number of polymorphic molecular markers can be monitored in a single cross.

Among molecular markers, it is the DNA markers that have dramatically increased the usefulness of genetic markers in plant breeding (Powell, 1992; Ragot and Hoisington, 1993). The two current techniques for detecting DNA polymorphisms are RFLPs (by chemiluminescent or radioactivity) and RAPDs (Ragot and Hoisington, 1993). RFLPs detect differences in the length of specific
DNA fragments after digestion of genomic DNA with sequence specific restriction endonucleases (Botstein et al., 1980), while RAPDs are generated by amplification of genomic DNA with short, e.g., 9-10 base pairs, oligonucleotides and thermostable DNA polymerases (Williams et al., 1990; Welsh and McClelland, 1990). Evaluation of the two molecular marker protocols by Ragot and Hoisington (1993) demonstrated that in terms of genotyping costs and time requirements, none of them had an absolute advantage over the other. However, while RAPDs are the most economical markers for relatively small-to-medium sized projects, RFLPs would be the marker of choice for extensive genotyping endeavors (Martin et al., 1991; Ragot and Hoisington, 1993). These two markers provide genetic information not only in different amounts but also in different qualities. RFLPs are codominant markers, inherited in a simple Mendelian fashion (Botstein et al., 1980) while RAPDs are usually dominant markers thus preventing the accurate detection of heterozygotes (Williams et al., 1990; Welsh and McClelland, 1990). RFLPs were the ones available in our laboratory and were employed in this study.

**RFLP - facilitated genetic studies**

Until recently, investigations of quantitative variation have relied largely upon biometrical methods to describe the characteristics of continuous phenotypic distributions. From these methods, such as generation means analysis (Mather and Jinks, 1982), the approximate number of loci affecting the trait in a particular mating design, the average gene action (e.g. dominance, recessiveness), and the
degree to which the various QTLs interact with each other and the environment in determining the phenotype could be estimated (Falconer, 1989). This approach did not pinpoint specific genomic regions involved, nor characterize the magnitude or nature of individual QTL affecting the character. In addition, they are constrained by a number of assumptions which are frequently not satisfied. The use of mapped genetic markers now provides a powerful approach for studying quantitative traits and for locating and manipulating individual genetic factors associated with the traits.

The underlying assumption of using marker loci to detect QTLs is that linkage disequilibrium exists between alleles at the marker locus and alleles at the linked QTL. Linkage disequilibrium can be defined as the non-random association of alleles at different loci in a population due to a number of factors including selection, genetic drift and physical linkage (Tanksley, 1993). In primary segregating generations (e.g., F2, F3 or backcross populations) the predominant cause of linkage disequilibrium is physical linkage of loci and thus has formed the basis for classical linkage mapping for the past century. The ability to detect a QTL with an RFLP marker is a function of the magnitude of the QTL's effect on the trait, the size of the population being studied, and the recombination frequency between the marker and the QTL (Tanksley et al., 1989).

The commonly used mapping populations include F2 populations, doubled haploids lines (DH), backcrosses, topcrosses and S1 lines (Cowen, 1988); and random inbred lines developed by single seed descent (Brim, 1966) which are
referred to in literature as recombinant inbred lines (RIL) (Burr et al., 1988; Burr and Burr, 1991).

The establishment of the RFLP-trait associations is usually determined by the crossing of parents which display sufficient phenotypic contrast for the trait of interest and subsequent scoring of the segregating populations of the progeny to ascertain the correlations between the phenotypic expression of the trait and the genotype of parental markers at different chromosomal sites. The genotypic data are obtained from RFLP analysis (as in materials and methods) of DNA samples extracted from the parents and the progeny. RFLPs, which are revealed as differences in DNA fragment lengths of the two individuals being compared, are due to base pair changes at enzyme recognition sites, to rearrangements encompassing that site or to internal deletion/insertion events within the site (Helentjaris, 1987). These changes are based in the genetic material of the individuals and are inherited in a simple Mendelian fashion. The restriction fragments can then be used as genetic markers, with the different size fragments (referred to as polymorphisms) functioning as alleles at the various RFLP loci.

There are two principal statistical approaches for determining whether a QTL is linked to a RFLP marker and both share the same basic principle. It is based on partitioning the population into different genotypic classes based on genotypes at the marker locus and then using correlative statistics to determine whether the individuals of one genotype differ significantly compared with individuals of other genotype(s) with respect to the trait being measured ( Tanksley, 1993). If the
phenotypes differ significantly, it is interpreted that a gene(s) affecting the trait is/are linked to the RFLP locus used to subdivide the population. More often than not it is not possible to determine whether the effect detected with a RFLP locus is due to one or more linked genes affecting the trait (Thompson, 1975; Tanksley, 1993) and the term locus can be loosely defined to include many gene loci (Beavis et al., 1994).

The first, and simplest approach for detecting QTL, is to analyze the data using one RFLP marker at a time, which is known as a single factor analysis of variance (SFAOV) (Soller and Brody, 1976; Edwards et al., 1987). Phenotypic means for the progeny in each marker class are compared. Significant F-values may indicate linkage of the marker and the trait of interest. Essentially this amounts to linear regression of phenotype on genotype by means of one-way analysis of variance, under the assumption of normally-distributed residual environmental variance (Lander and Botstein, 1989). Shortcomings associated with this approach if the QTL does not lie near the RFLP locus include; 1) its phenotypic effect may be underestimated due to recombination between the QTL and the marker; 2) substantially more progeny may be required to map the trait because of the confounding effects with the recombination frequencies; 3) the approach does not define the likely position of the QTL; 4) and the method cannot tell whether the markers are associated with one or more QTLs (Edwards et al., 1987; Lander and Botstein, 1989). This approach can also generate false positives; this becomes more likely as the number of markers and traits being examined increases.
The second approach suggested to overcome the limitations of SFAOV utilizes the principle of interval mapping and involves two different approaches. The first approach utilizes pairs of RFLPs in a sequential manner and estimates the phenotypic effect of the QTL and its significance in the region bracketed by the two RFLP loci in each pair (Knapp, 1989; Knapp and Bridges, 1990; Knapp et al., 1990). The statistical models arising from these genetical models are nonlinear and use maximum-likelihood methods. The second approach reported by Lander and Botstein (1989) involves interval mapping using LOD scores. A LOD score is the Log_{10} of the odds ratio, where the odds ratio is the chance that the data would have arisen from a QTL within the boundary of the two RFLP loci divided by the chance that it would have arisen given no linked loci (Lander and Botstein, 1989). That is, the LOD involves a ratio of two maximum likelihood estimates which indicate how much more probable it is for the data to have arisen by assuming the presence of a QTL, rather than by assuming its absence (Stuber, 1992). By dissecting the region between adjacent RFLP markers into small intervals, the probable position of the QTL relative to the markers can be specified. The LOD threshold (T) for the declaration of a QTL can be calculated according the formula given by Lander and Botstein (1989):
\[ T = \frac{1}{2} \left( \log_{10} e \right) (Z_{a/m})^2 \]  

Where

\( a \) = the chosen level of significance
\( m \) = the number of interval markers tested in the genome; and
\( Z \) = standardized normal variate

RFLPs have so far been used to build genetic linkage maps for a number of agriculturally important crops such as maize (Coe et al., 1990), rice (McCouch et al., 1988), potato, *Solanum tuberosum*, (Bonierbale et al., 1988; Gebhardt et al., 1989; Gerbhardt et al., 1991), barley (Graner et al., 1991; Heun et al., 1991), soybean, *Glycine max*, (Keim et al., 1990; Diers et al., 1992a) and tomato, *Lycopersicon esculentum* (Bernatzky and Tanksley, 1986). The advent of RFLP marker linkage maps in many plant species has provided the opportunity to identify QTL by their association with segregating RFLP loci, thus allowing the examination of the genetic basis of the quantitative trait.

**RFLP facilitated host-resistance genetic studies**

RFLP markers have been used to study the genetics of host-plant resistance in plants for a number of fungal, bacterial and viral pathogens, nematodes and insect pests. These include *Fusarium oxysporum*, *Stemphylium* and *Pseudomonas syringae* in tomato, (Sarfatti et al., 1989; Behare et al., 1991; Martin et al., 1991), powdery mildew in barley (Hinze et al., 1991), race 2 of *Plasmodiophora brassicae*
in Brassica (Landry et al., 1992), leaf blast (*Magnaporthe grisea* Barr) resistance in rice (Yu et al., 1991), *Phytophthora sojae* (Kauf. and Gende) resistance in soybean (Diers et al., 1992b), resistance to white-backed plant hopper of rice (McLouch et al., 1991), the *Mi* resistance gene to the nematode (*Melodogyne incognita* Kofoid and White) in tomato (Messeguer et al., 1991), *Globodera rostochiensis* (Woll) in potato (Barone et al., 1990) and trichome-mediated insect resistance in potato (Bornierbale et al., 1994).

The use of RFLPs to study the genetics of resistance to viruses has been demonstrated in a few cases using different strategies. Young et al. (1988) used two pairs of nearly isogenic lines (NILS) (Vendor and Vendor-*Tm-2a*, and Craigella and Craigella-*Tm-2a*) to identify RFLP markers tightly linked to the *Tm-2a* gene which confers resistance to tobacco mosaic tobamovirus (TMV) in tomato. To identify genomic clones located near the *Tm-2a* gene, up to ten tomato genomic clones especially of single copy sequences, were simultaneously co-labeled and probed on *Tm-2a*-survey filters to speed up the screening process. *Tm-2a* was mapped to chromosome 9 near RFLP markers TG101 and TG79. RFLP analysis, of primary trisomics which possess an additional copy of one chromosome, confirmed earlier studies of the *Tm-2a* gene which had shown that this gene is located on the long arm of chromosome 9 very close to the centromere (Schroeder et al., 1967).

McMullen and Louie (1989) mapped a major gene, designated as *Mdm1*, for resistance to maize dwarf mosaic potyvirus strain A (MDMV-A) in maize. They
used backcrosses derived from hybrids between resistant inbred, Pa405, and two susceptible inbreds (K55 and yM14) backcrossed to the respective susceptible inbred parents. Linkage of Mdm1 to the chromosome 6 gene for yellow endosperm (Y1) reported by Scott (1986) was confirmed by RFLP analysis. The gene order on chromosome 6 with RFLP markers was determined, by multiple three point linkage analysis, to be umc85, mdm1, bnl6.29, y1, umc59 and umc21. Results from these studies showed that the degree of resistance conferred by Mdm1 is dependent on the genotype of the susceptible parent.

Louie et al (1991) reported the genetic basis of resistance to individual MDMV strains A, B, D, E, and F in inbred Pa405 using three approaches. These were: 1) field studies to identify genes for resistance by translocation markers which had been incorporated into inbred M14 (a susceptible); 2) greenhouse studies to determine the linkage of resistance to a morphological marker of yellow endosperm gene (Y1) in inbred M14; and 3) RFLP analysis using RFLP-marker loci umc85, bnl6.29, umc59 and umc21 identified by McMullen and Louie (1989). Results from classical linkage analysis of the three approaches using backcrosses of the type (M14 translocation X Pa405) X M14 all indicated a gene or genes on either the short arm or the proximal region of the long arm (proximal to Y1) of chromosome 6 controlling resistance to all five strains of MDMV.

The genetic basis of resistance to wheat streak mosaic potyvirus, strain Wooster (WSMV-W), in maize was studied by McMullen and Louie (1991) in polymitotic (po) B73 lines, (po/po or po/+) B73, and in (Pa405 X Oh28) F2 and F1
backcross to Oh28. It was found that the gene for resistance to WSMV, designated as $Wsm1$, was linked to the genetic locus $po$ which had been located on the short arm of chromosome 6 (Coe et al., 1988). Three point linkage analysis of RFLP markers located $Wsm1$ on either the short arm of chromosome 6 or the long arm proximal to the RFLP marker locus umc59. This region includes a major gene for resistance to MDMV, $Mdm1$, reported earlier (McMullen and Louie, 1989). Further RFLP analysis of (Pa405 X Oh28) F$_2$ and backcross plants suggested the presence of multiple genes for resistance to WSMV in Pa405 including $Wsm1$. Two other genes have since been located on chromosomes 3 and 10 (McMullen, M.D., personal communication).

Two segregating F$_1$ populations, resulting from crossing diploid lines, were used to map two major genes in potato controlling extreme resistance to potato X potexvirus (PVX) strain PVX-BS from tobacco variety Xanthi (Ritter et al., 1991). Multipoint linkage analysis of RFLP markers of the F$_1$ segregating populations mapped resistance genes $Rx1$ to the distal end of chromosome X11 and $Rx2$ at an intermediate position on linkage group V. Two approaches were used to map resistance genes in parental lines that were heterozygous for the resistant allele. One approach which involved mapping of $Rx1$, in a cross of P18 (resistant) X P40 (susceptible), was the use of probes chosen according to their map position. The other approach, from which $Rx2$ was mapped, used the F$_1$ of a cross P34 (resistant) X P16 (susceptible), based on the assumption of possible linkage between the resistance gene and specific RFLP alleles introduced from a wild potato species.
The mapping of the two genes is in agreement with Cockerham (1970) who suggested that there are two independent genes for extreme resistance to PVX.

RFLP analysis of the progeny of F1 anther-derived, doubled haploid (DH) barley lines identified a recessive gene, ym4, that confers immunity to barley yellow mosaic (BaYMV) and barley mild mosaic (BaMMV) viruses (Graner and Bauer, 1993). In the cross between cultivars `Igri' (susceptible) and `Franka' (resistant) ym4 was mapped to the long arm of barley chromosome 3H where it is closely flanked by RFLP marker loci MWG838 and MWG10. The mapping of ym4 to the long arm of chromosome 3H by linkage analysis using MAPMAKER program (Lander et al., 1987) confirmed earlier results obtained by trisomic and telotrisomic analysis (Kaiser and Friedt, 1989, 1992).

Nodari et al (1993) mapped the I gene which conditions resistance to bean common mosaic potyvirus (BCMV) in common bean, Phaseolus vulgaris. Linkage analysis was performed with MAPMAKER program (Lander et al., 1987) and LINKAGE-1 (Suiter et al., 1983). RFLP analysis of F2 plants derived from a cross between BAT93 (resistant) and Jalo EEP 558 (susceptible) and subsequent phenotypic analysis of F3 families facilitated the mapping of the I gene to Linkage group D2. The I gene conditions dominant resistance to the non-necrosis-inducing strains of BCMV and hypersensitivity to the necrosis-inducing strains (Driffhout et al., 1978).

Timmerman et al (1993) used RFLP, RAPD, and isozyme markers to map a recessive gene, sbm-1, conferring resistance to pea seed-borne mosaic potyvirus
pathotype P-1 (PSbMV-P-1) in pea, *Pisum sativum*. RFLP marker GS185 was closely linked to *sbm-1* which was mapped on chromosome 6 by linkage analysis using MAPMAKER program version 2.0 (Lander et al., 1987), and it was advocated for use in marker-assisted selection (MAS) for resistance to PSbMV. RFLP analysis was carried out on F\textsubscript{2} plants or pooled F\textsubscript{3} plants of each family to reconstitute the F\textsubscript{2} genotype. Virus resistance responses were scored on F\textsubscript{3} and F\textsubscript{4} progeny of two crosses (88V1.11 (resistant) X 425 susceptible) and 88V1.11 x Almota (susceptible).

RFLP analysis of F\textsubscript{2} plants and subsequent phenotypic classification of the F\textsubscript{3} progeny grown in the field, and F\textsubscript{4} progeny grown in the greenhouse, confirmed that resistance to pea common mosaic virus in pea is controlled by a single gene, *mo*, (Dirlewanger et al., 1994). This gene previously had been reported to be on chromosome 2 (Marx and Provvidenti, 1979) and this was confirmed. It was mapped near RFLP marker Locus p252 in the resistant variety 'Erygel' using MAPMAKER (Lander et al., 1987) and one-way ANOVA using the SAS GLM procedure.

Using backcross populations and NILs, Zamir et al. (1994) mapped a major, incompletely dominant, tolerance gene, *Ty-1*, to tomato yellow-leaf curl geminivirus (TYLCV). The tolerance factor mapped to chromosome 6 in wild tomato (*Lycopersicon chilense*). Linkage analysis was carried out using MAPMAKER. Two other minor loci whose effects were detected only in certain experiments were mapped to chromosomes 3 and 7.
In many of the RFLP-assisted virus resistance genetic studies reported above, RFLP analysis was carried out to confirm and map what earlier studies had suggested to be the number and chromosomal locations of genes involved. This study was carried to determine the genetic basis of resistance to MSV with RFLPs in a current, resistant genotype, which has not been characterized.

The objectives of this study, therefore, are:

1. To characterize MSV resistance loci in a segregating maize population by:
   a. identifying the number of QTLs linked to MSV resistance,
   b. identifying the approximate location of these QTLs on the chromosome,
   c. identifying marker-loci that could be used in indirect selection for MSV tolerance.

2. To estimate the magnitude of resistance contributed by each of the QTLs involved.

3. To evaluate expression of these QTLs in different environments.
CHAPTER I

GENETIC ANALYSIS OF MAIZE STREAK VIRUS TOLERANCE IN MAIZE
USING RESTRICTION FRAGMENT LENGTH POLYMORPHISMS

Introduction

"It is pleasing that modern molecular methods of genetic analysis are bringing clarity to areas where previous attempts to determine the genotype by assessment of the phenotype have suggested more complex solutions". Fraser, 1992.

Maize streak, incited by maize streak geminivirus, is an important economic disease occurring in most of the African countries. Yield losses in maize due to maize streak range from a trace to virtually 100% when epidemics occur on susceptible OPVs and hybrids (Guthrie, 1976, 1978; Fajemisin and Shoyinka, 1976; Kim et al., 1981; Mzira, 1984; Fajemisin et al., 1986; Barrow, 1992).

The potential threat of MSV to maize production is not limited to the African continent. Damsteegt (1980) found that apart from three African selections (two South African lines, Vaal Harts Komposiet and J2705, and Revolution) which were tolerant to MSV, all other maize germplasm evaluated from commercial corn seed companies, State experiment stations and CIMMYT, Mexico, were susceptible.
Disease severity caused by MSV, in comparison with maize dwarf mosaic virus (MDMV) and maize chlorotic dwarf virus (MCDV), the most widely distributed and important maize viruses in USA (Gordon et al., 1981), showed that MSV alone caused as much reduction in plant height and total dry weight as any virus combination (Damsteegt, 1980). MSV dominated the host symptomatology although MDMV and MCDV were also present in the younger leaves.

Scientists at IITA in collaboration with scientists from National maize programs in Africa have developed over 100 different improved open-pollinated and hybrid varieties of maize with tolerance to MSV (Soto et al., 1982; Efron et al., 1989; Kim et al., 1989). Research at CIMMYT, Harare, Zimbabwe, has also resulted in a number of MSV-tolerant germplasm releases which have been distributed for evaluation by the National programs (Pham, 1992; K.V. Pixley, CIMMYT, Harare, Zimbabwe, personal communication). Several MSV tolerant inbreds, hybrids, and populations have recently been released for cultivation in South Africa (Van Rensburg et al., 1991a; Barrow, 1992).

Tolerance to MSV was recognized in the early thirties in South Africa (Fielding, 1933) but owing to difficulties inherent in genetic studies under natural field infestation, researchers could not arrive at any hypothesis for the manner in which tolerance was inherited (Rose, 1938; Gorter, 1959). Storey and Howland (1967) were the first to report inheritance studies resulting from controlled infestation with viruliferous leafhoppers, *Cicadulina mbila*. Genetic studies and evaluations of MSV-tolerant germplasm were later greatly enhanced with the

So far studies on genetics of tolerance to MSV have attempted to fit the progeny of tolerant x susceptible crosses into symptom classes predicted by various models of gene number and dominance effects. Tolerance in P X H was reported to be quantitative (Rose 1938; Gorter, 1959) but Storey and Howland (1967) found that it was conditioned by a single gene lacking dominance. MSV tolerance in the tolerant maize germplasm in South Africa was controlled by five dominant genes (Engelbrecht, 1975). Later, Fourie and Piennar (1983) reported that MSV tolerance in the South African materials was relatively simply inherited with an apparently strong dominance component. MSV tolerance among diverse populations including TZ-Y, from which IB32 was developed, was considered to be simply inherited (Soto et al., 1982) but Kim et al (1989) reported that tolerance in IB32 was quantitatively inherited. It is possible that many genetic systems exist. However, there are factors which complicate interpretations of results obtained and these include different leafhopper infestation methods (natural vs controlled), different MSV isolates, different genotypes, different disease rating scales to measure host reaction, or different statistical analyses and the various environment x genotype interaction. These may have contributed to the different conclusions arrived at in a number of cases where same/similar genotypes were used and creates doubt whether different genetic systems for MSV tolerance exist.
To determine the mode of gene action for tolerance in IB32 to MSV, Kim et al. (1989) used unadapted USA Corn Belt inbred lines, as the susceptible parents. They suggested that these inbred lines could have biased the results obtained. This would be expected if streak symptoms could be modified depending on the genetic background of the susceptible parent.

In this study, recombinant inbred lines (RILs) of tropical origin were used. Apart from being adapted, each genotype is represented by one RIL in which segregation is fixed, rather than by an individual and this allows a better estimate of the genetic component of variance (Burr et al., 1988; Burr and Burr, 1991) underlying tolerance to MSV (Brewbaker, 1992). We investigated the genetics of tolerance to MSV in RILs using controlled infestation, both in the glasshouse and field, at two different locations. Genotypic analysis and linkage to the phenotypic scores was carried out using molecular markers (RFLPs) and linkage analysis programs such as MAPMAKER (Lander and Botstein, 1989).
MATERIALS AND METHODS

Plant material

The two parents (Hi34 and TZi4) of the RILs were selected because they had divergent origins and exhibited contrasting MSV responses. Hi34 is a tropical inbred line, a yellow flint derived from Antigua 2D rated as 5.0 (susceptible) on MSV scale (Brewbaker et al., 1991). TZi4 is a streak resistant tropical white flint inbred line developed at IITA, Nigeria, and rated as 1.0 on MSV scale (Kim et al., 1987). It was derived from Guanacaste 7729 X TZSR. The donor for streak tolerance in TZi4 was TZSR which comprises tolerance from TZ-Y, and it is the latter from which inbred IB32 was derived (Kim et al., 1989). The two parents also differ in morphological and agronomic traits such as total number of leaves, flowering date, number of husk leaves, plant and ear heights, kernel rows and kernel weight. The cross (Hi34 X TZi4) was made in Hawaii by Dr. Brewbaker in 1986. F1s plants were advanced to F2s in 1987. In 1990 single seed descent without selection was used to advance each F2 plant in the population (R.Ming, Dr. Brewbaker's student, personal communication). The procedure involved planting each selfed ear to a 5-10 plant row from which two plants were chosen at random for self-pollination (Brewbaker, 1992). One ear was taken to produce the next progeny. The 116 RILs seeds received were at the S6 generation of inbreeding.
RFLP Analysis - at OARDC, Wooster, Ohio

**Plant DNA preparation**

Twenty five kernels of each of 116 RILs, the two parents (Hi34 and TZi4) and the F1 were planted in heated raised soil beds in the greenhouse. At three weeks after planting (about V3 stage, Ritchie et al., 1989) each row was harvested (seedlings cut one inch above the soil level) placed into a shoot bag, stapled and quickly stored in an icebox containing liquid nitrogen or dry ice and stored at -70°C. At a later date the samples were lyophilized for 3-5 days depending on their moisture content. The lyophilized samples were ground to a fine powder with a Wiley mill (40 mesh) and stored in Model 2059 14 mL Falcon snap top tubes (Becton Dickinson & Co., NJ) and kept in a cold room (4°C).

**DNA extraction**

Genomic DNA was extracted based on the method described by Saghai-Marof et al (1984). Three-tenths gram of lyophilized ground tissue was used per 8 ml CTAB (Hexadecyltri-Methylammonium Bromide) extraction buffer (100 mM Tris pH 7.5, 0.7 M NaCL, 1% CTAB, 10 mM NaEDTA and 1% β-mercaptoethanol). The mixture was incubated at 65°C for 30-60 minutes with occasional mixing to denature proteins. 4.5 ml of 24:1 chloroform : octanol (v/v) was added and the solution was mixed by inversion to form an emulsion. This emulsion was centrifuged for 10 minutes at 1800 g in a table top centrifuge (Beckman Model TJ-6 centrifuge) at room temperature. The supernatant was removed and 6 ml of cold isopropanol
added and mixed gently by tube inversions to precipitate DNA. The precipitated DNA was lifted out with a glass hook, transferred to 1.5 mL Eppendorf tubes containing 1 mL of wash solution (76% ethanol and 0.2 M NaOAC) for 20 minutes, and then dipped in a rinse solution (76% ethanol and 10 mM NH₄OAc) for 5 seconds. DNA of each sample was dissolved in 400 mL TE (10 mM Tris, pH 8, and 1 mM EDTA) overnight at 4°C by rocking. The solution was centrifuged for 10-15 minutes at 16000 g in a table top Eppendorf centrifuge model 5415C and the supernatant transferred to a clean 1.5 mL Eppendorf tube. DNA concentration was then determined using a TKO 100 Dedicated Mini Fluorometer (Hoefer Scientific Instruments, San Francisco, CA.) and the samples were stored in a -20°C freezer. The DNA was diluted to a concentration of 0.3 μg/μl in TE of which 30 μl (about 10 μg) was taken for digestion.

**DNA digestion**

A survey was first carried out to identify which enzyme/probe combinations generated informative polymorphisms between the parents. Genomic DNA samples (10 μg) from the two parents and F₁ was digested with 20 units of each of the five restriction enzymes used (i.e., 2 U/μg DNA) in 4 μl 1X manufacturer's buffer and 0.01 μg RNase A (Sigma). The five enzymes used were EcoRI, BamHI, HindIII, Dral and EcoRV (New England BioLabs, Beverly, MA). Restriction digestion was carried out for 6-10 hours at 37°C in an oven.
**Gel electrophoresis**

The digested genomic DNA (approximately 10 µg of DNA/lane) mixed with 1X TBE Loading buffer (0.089 M Tris-borate, 0.089 M boric acid, 0.002 M EDTA, 25% glycerol, and one drop of bromphenol blue) was loaded into a 0.8% (w/v) agarose gel (10 cm x 14 cm) in 1X RBE buffer (40 mM Tris-Acetate, pH 7.3, 2 mM Na₂EDTA, 0.02 mM NaOAc, 32 mM glacial acetic acid) as described by Simcox and McMullen (1993). Electrophoresis was carried out for 10-12 hours at 20-25 volts, on double - stacked gels each containing 32 wells.

**Southern transfer**

After electrophoresis the gel was stained with ethidium bromide (250 µg/l) to permit visualization of the DNA and photographed. The procedure used for Southern transfer was the 'modified dry blot' described by Simcox and McMullen (1993) which is an adaptation of the 'dry blot' procedure of Kempter et al (1991). The gel was prepared for DNA transfer in 2X gel volumes of solution for each of the following steps: 1) Depurination in 0.25 M HCL for 2 x 10 minutes and to aid in the transfer of large segments; 2) Denaturation in 0.4 M NaOH for 2 x 15 minutes; 3) Neutralization in 0.25 M Tris-Acetate, 0-1 M NaCL, pH 8.0 for 2 x 15 minutes and; 4) Incubation in the 0.025 M Tris-Acetate, 0.1 M NaCL, pH 8.0 transfer solution for 2 x 15 minutes. During these steps the gel was slowly agitated using a rotary platform and between each step, except the last, the gel was rinsed twice in deionized distilled water (ddH₂O).
The DNA was transferred from the gel to a Gene Screen Plus hybridization membrane (DuPont, Boston, MA) which had been cut to the same size as that of the gel and labeled accordingly. First, a Whatman 3M chromatography paper, cut to the same gel size and soaked in the transfer solution, was placed on plastic wrap laid on a table. The gel was laid on this paper and the membrane laid on top. Care was taken when laying the membrane, which was pre-wetted in ddH₂O and equilibrated in the transfer solution, on the gel to gently roll out air bubbles using a pipet. A second 3M paper soaked in the transfer solution was laid on top of the membrane and the air bubbles rolled out. Four more dry 3M papers were stacked on top followed by a 2.5 cm stack of paper towels slightly larger than the gel. Added on top of this stack was a flat 1kg weight. The transfer process was left overnight after which the stack was disassembled and the membrane rinsed in 2X SSC (0.3 M NaCl and 0.03 M Na citrate) to remove residual agarose. The membrane was air dried for a minimum of two hours at room temperature. The types of membranes consisting of DNA samples of the two parents and F₁ digested with the five enzymes are referred to as 'Survey blots' or 'Parental blots'.

*Southern hybridization*

The same procedure of hybridization and autoradiography as reported by Simcox and McMullen (1993) was used. The membranes (1-8) were pre-hybridized in the 'prehybe solution' (5X SSC, 5X Denharts, 0.01 M Tris-HCL, pH 8.0, 0.002 M Na₂EDTA, 0.5% SDS, and 1 ml of 10 mg/ml boiled salmon sperm) in a 65°C water
bath for 4-8 hours. The membranes were sealed in a Dazey Micro-Seal pouch 8"x12" (Model 6003, Dazey Corporation, KS) with a ClampCo sealer (Fischer Scientific, Pittsburgh, PA) with about 30 ml of the solution. The pre-hybridization solution was replaced with hybridization solution (same as the 'prehybe solution' except 10% dextran sulphate was added).

A set of 126 maize RFLP markers spaced approximately 20 centiMorgan (cM) throughout the ten maize chromosomes were selected based on the maize genetic linkage map (Coe, 1993) to screen the parental blots for polymorphisms. Markers were obtained from the University of Missouri, Columbia, MO, (umc), Brookhaven National Laboratory, Long Island, NY, (bnl), and Pioneer Hi-Bred International Johnston, IA (php). Gel-purified inserts of these genomic RFLP clones were labeled by random priming (Feinberg and Vogelstein, 1983). Approximately 25 ng of insert was boiled for 5 minutes, quenched on ice and 11.5 μl LS mix (0.446 M TM, 0.225 M random primer hexamers, and 0.223 M Hepes, pH 6.0), plus 1.0 μl of 10 mg/ml bovine serum albumin (BSA) were added. The isolated insert of each genomic clone was radiolabeled with 20 μCi ³²P-dCTP (Amersham, Chicago, IL, and New England Nucleotide, DuPont) and 2.5 units of Klenow fragment (New England BioLabs) was added. The solution was mixed gently by stirring and pipetting and was incubated for two hours at room temperature. This radiolabeled probe was separated from unincorporated ³²P-dCTP on Pharmacia Nick-Column prepacked with Sephadex G-50 (Pharmacia LKB, Uppsala, Sweden). The purified radiolabeled probe was boiled for 5 minutes and added to the hybridization pouch.
Air bubbles were removed with a slender rod, the pouch was sealed and incubated 24 to 48 hours in a 65°C water bath.

To remove excess probe the membranes were washed five times for 8-10 minutes each in a low salt stringency solution (2X SSC and 0.1% SDS) at 65°C (400 ml/wash) and another five times in a high salt stringency solution (0.1X SSC and 0.1% SDS) at 65°C. The membranes were wrapped in Saran wrap and exposed to Kodak XAR-5 film (Eastman Kodak, Rochester, NY) at -70°C using two intensifying screens for 24 to 48 hours depending on the intensity of the signal. Autoradiographs were obtained by developing films in a Kodak X-OMAT M20 Processor.

The membranes were stripped in 0.4 M NaOH for 30 minutes and neutralized in 0.4 M Tris-HCL, 0.1X SSC, and 0.1% SDS for 30 minutes. The membranes were either returned to the pre-hybridization solution for further hybridization with other probes or wrapped in saran wrap to prevent them from drying and kept in the refrigerator for reuse (up to 8 times).

From these parental autoradiographs it was determined which enzyme/probe combinations gave the clearest fragment length polymorphism and would be best for segregation analysis (e.g., Figure 1a). Thus DNA from the RILs was digested and transferred in a similar manner to produce "RIL blots" (e.g., Figure 1b). Each set of 116 RILs DNA was digested and probed with the respective enzyme and probe identified. RIL autoradiographs were scored three times to verify the results obtained. In the scoring, arbitrarily a value of 1 or A was assigned to all alleles from
the susceptible parent, Hi34, a value of 2 or B to the alleles from the resistant parent, TZi4, and a value of 12 or H to the heterozygous class. Occasionally outliers, bands not identified with either parent, were found and designated as 3, or if found present with either parent's band were designated as 13, or 23 accordingly. These designations, numeric or alphabetic, were assigned depending on the program that was to be used for linkage analysis.
Figure 1. Autoradiographs from parental and RIL blots.

(a) Parental blot showing informative polymorphism (with \textit{BamH}	extsubscript{I}, lanes 4-6) among parents and \textit{F}	extsubscript{1}. (RIP = recombinant inbred parental blot, \textit{E} = \textit{EcoRI}, lanes 1-3, \textit{B} = \textit{BamHI}, lanes 4-6, \textit{H} = \textit{HindIII}, lanes 7-9 \textit{D} = \textit{DraI}, lanes 10-13, \textit{V} = \textit{EcoRV}, lanes 14-16).

(b) RIL blot showing parents (lanes 1-2) and RILs (lanes 3-31) segregating on a double stacked blot.
MSV Screening

Because of quarantine regulations field inoculation and evaluation of MSV could not be performed in the USA. Inoculation and evaluation are possible only in Africa where the disease is endemic. MSV infection and rating were carried out at two different locations in Africa: Uganda at the Namulonge Agricultural and Animal Production Research Institute (NAARI), and Zimbabwe at the CIMMYT, Mt. Pleasant Research Station, Harare.

Experiment 1 - NAARI, Uganda

Seeds of 10 parental sublines each of Hi34 and Tzi4, F1 and 123 RILs totalling to 144 entries were received from Mr. H.G. Moon, University of Hawaii, courtesy of Dr. Brewbaker. This set included entries which had not been typed in the laboratory at OARDC, Wooster.

NAARI is located 27 km North-East of Kampala, latitude 0° 31'N, Longitude 32° E and at an altitude of about 1150 meters above sea level (m.a.s.l.). In collaboration with the Natural Resources Institute (NRI), U.K., and NAARI, a research project on epidemiology and vector ecology of MSV, under the National Maize Research Program, was initiated at NAARI in 1993.

C. mbila colonies were increased on pearl millet in rearing cages. Insufficient leafhoppers were raised to infect the field-planted experiment so a method similar to that of Louie and Anderson (1993) was employed to screen the above entries for their response to MSV infection in the glasshouse (Figure 2a). 'Cone-Tainers'
measuring 16.4 by 2.5 cm (Stuewe and Sons, Corvallis, OR) were filled with sterilized soil fertilized at 1g NPK (15%N), 0.33g Urea (46%N) and 0.80g SSP per 1kg soil as recommended by the Soil Section at NAARI. These cylindrical tubes, referred to as pine cells, were held upright by a plastic rack measuring 30 cm square by 17 cm high. Each rack was of 100-tube capacity. The racks, with the tubes containing soil, were placed on glasshouse bench with rectangular troughs. Troughs were filled with water up to one quarter way up the tubes. The soil was wet by capillary adsorption for 24 hours, to the top of the tubes. This was to ensure that the soil was packed properly and water could rise by capillary action to the top layer where the seeds were to be planted. The rack and tubes containing wetted soil were placed in a plastic basin (32 cm square by 16 cm high) with water 2-3 cm deep. The top edges of the basin and the edges of the rack were covered with a continuous elastic cloth to prevent the leafhoppers from drowning.

Seeds were planted one seed per tube and 5 tubes per entry in a row, randomized in a 12 x 12 lattice design. Three susceptible and four resistant local checks were included, replacing some of the parental sublines. Since each rack could hold 100 plants, i.e., only 20 entries, that meant some entries of one block that couldn't fit on one rack were planted on another. After infestation these racks were placed next to each other on the glasshouse bench. The second replicate was planted one week after the first. Flats and trays similar to size A of Hummer International, St. Louis, MO, were used. The flats measured 5.6 cm square by 6.8 cm high with bottom drain holes. There were 30 flats per tray (46.4 cm x 38.9 cm).
Two to three seeds, depending on the amount of seed available, were planted in these flats on wetted soil of the same composition as that used in the tubes.

After planting the racks were placed in a net-cloth covered cage (45 cm square by 60 cm high) with a glass door. This is comparable to the deacon-organdy-covered cage of Louie and Anderson (1993). The flats and trays were placed in cages and frequently hand-watered. The infestation procedure and data recording were similar in both cases.

Two weeks after planting, at about the V3 stage (Ritchie et al., 1989), the plants were infested with viruliferous leafhoppers. *C. mbila* populations were placed in an acquisition feeding cage for 2 days on plants collected at the station showing very severe streak symptoms. The screening was thus carried out with a viruliferous local natural inoculum. The leafhoppers were collected in small plastic vials. Immediately before infestation they were treated with a small volume of carbon-dioxide to anaesthetize and immobilize them for placement into the leaf whorl. Carbon-dioxide was released from a portable anesthetizing and dispensing unit consisting of an inner tube (approx. 32 cm diameter) with an attached rubber hose equipped with a valve (Leuschner et al., 1980). Leafhoppers (3-4) were shaken out through a small hole in the lid of the vial into the leaf whorl of each individual plant. Shortly after being released, the leafhoppers revived and began to feed. After 3 days, following occasional disturbing of the leafhoppers so that they would move around the plants, they were removed with a modified hand operated vacuum cleaner and placed in the acquisition feeding cages. Streak symptoms
started to appear on the third day after infestation and were prominent on the fourth day. One week after the appearance of the symptoms the two replicates were placed on the screenhouse bench with troughs containing water. Virus symptoms were noted at weekly intervals at first but later at two weeks with mid-week observation to check if any entry showed more symptoms following the previous recording. Recording was discontinued after the fourth score when there were no further changes in symptom expression. Individual plant scores per entry were recorded and used to compute the mean score for that entry for each replicate.

Symptom rating in this study was based on the chlorotic/streaked areas on the leaf surface which has been the standard procedure used in MSV screening as reported by Kuhn (1979), Soto et al (1982), Kim et al (1989) and Barrow (1992). Stunting of the plant was considered with care since these materials were inbreds and could be stunted by the virtue of the genotype itself and not the disease. However, in all cases stunting due to infection was evident. The rating was based on a 1-5 scale (Figure 2b) as follows: 1 = very few streaks and widely dispersed spots on leaves. 2 = light streaking and multiple spots on older leaves, gradually decreasing on young leaves. 3 = Moderate streaking on old and young leaves. 4 = severe streaking on all leaves (about 60% of leaf area), causing general yellow appearance of the plants. 5 = severe streaking on all leaves, very little green leaf tissue visible (80-100% chlorosis), plant growth severely stunted or plant dead. A semi-quantitative scale was used whereby if the response was between two scores,
a middle class was recorded. Thus effectively it was a 9 class scale with increments of 0.5 between scores, i.e., 1.0, 1.5, 2.0.....up to 5.
Figure 2. Bioassay method used in the glasshouse and the MSV rating Scale.

(a) "Cone-Tainers" used for the bioassay in the glasshouse.

(b) MSV scale used in this study showing symptom severity levels.
Experiment 2 - CIMMYT, Mt. Pleasant, Harare, Zimbabwe

CIMMYT, Harare, is located on the University of Zimbabwe Farm, about 13 km from Harare, latitude 17° 41'S, longitude 31° E and at an altitude of 1500 m.a.s.l. The soils belong to the Fersiallitic group (Kaolinitic order): moderately deep to deep reddish brown granular clays formed on mafic rocks. The summer season, November to May, is the main growing season with mean temperatures ranging from minimum of 16°C to maximum of 30°C and mean rainfall of about 800 mm.

Two sets of entries, 10 parental sublines of each of Hi34 and TZi4, F₁ and 123 RILs, similar to that planted in Uganda were obtained from Hawaii. Each set contained seeds for two replications. One set was to be planted at Mt. Pleasant, Harare and the other at Mzarabani, a lowland (400 m.a.s.l.) dry area. It was not possible to plant at the latter location so both sets were planted at the same location, two and half weeks apart.

The standard management practices for maize production used on the station are those based on large scale commercial farms in the sub-humid Natural Region II of Zimbabwe. Land was prepared by disc-plowing (2 passes) and one disc harrowing. Basal fertilizer at 400 kg ha⁻¹ Compound Z (8:14:7, N: P₂O₅:K₂O; and 0.8% Zinc) was broadcast and incorporated into the soil. An additional 160 kg ha⁻¹ of Compound Z was applied in the planting hole at planting. Approximately 85 kg ha⁻¹ N, as ammonium nitrate, was topdressed in a 2-way split. 42 kg ha⁻¹ N at 4-5 weeks after crop emergence and another 42 kg ha⁻¹ N at anthesis. Weed control by hand hoeing was carried out three times.
Masses of *C. mbila* leafhoppers were reared in virus free, large greenhouse cages and allowed to acquire virus on stocks of infected MSV susceptible maize plants for 2 days. They were then collected into vials by suction using a modified vacuum cleaner. The same procedure of infestation as described by Leuschner et al (1980) was used.

The entries were planted in 12 x 12 lattice design with two replications, on December 20, 1993. 10 hills per entry were planted to two seeds per hole at 25 cm within row and 75 cm between rows. When streak symptoms appeared plants were thinned to one plant per hill, leaving a population of about 54000 plants per hectare. Compensation among hills within a row was done to ensure that each row had the same number of plants. 3-5 leafhoppers were dropped into the plant whorl at the V-3 stage (Ritchie et al., 1989), about 3 weeks after planting. Streaked spots were noticed on the fifth day after infestation and some streaking was evident on some plants one week after infestation. Scoring of streak was first done at two weeks after infestation and three more recordings were taken fortnightly.

Individual plant ratings based on symptom severity/streaking, were recorded and scores for all plants per row were used to complete the mean score for each entry per replicate per each rating time. The same rating scale of 1 to 5, as in Experiment 1, was used. However, every week, in between two consecutive recordings, scores based on per row and not on individual plants were taken and compared with the last recording to find out if there were any changes.
Experiment 3 - CIMMYT, Mt. Pleasant, Harare, Zimbabwe

Experiment 2 was attacked by mice and the plant stand was rather poor in some entries. It was not possible to plant in Mzarabani so the second set of seeds was planted on January 6, 1994 at the same Harare site, but in a more protected area. Seeds were planted at 50 cm within row, 10 m long, by 75 cm between rows spacing. Three or in some cases more than three seeds were planted and thinned to two plants per hill after infestation and streak symptoms appeared, leaving a plant stand of about 54000 plants per hectare. The design was also a 12x12 lattice with two replications and management, leafhopper infestation, and MSV rating procedures were the same as in Experiment 2 except that there was a second leafhopper infestation. Field observation six days after the first infestation showed inconsistency in disease development over the two replications. This was attributed to many leafhoppers having drowned, for it rained immediately after infestation, before they revived from the carbon-dioxide administered.

MSV data analysis

Out of 123 RILs received for MSV screening, only 87 RILs had been genotyped. These 87 RILs, the two parents, and F₁, were analyzed as a randomized complete block design with two replicates using SAS Proc GLM statistical package (SAS Institute, 1989). Data from each scoring date was analyzed separately for each experiment. The mean value based on each of the four dates was then calculated and analyzed.
To test the hypothesis of no differences among the scoring dates within each experiment, data from each assessment date were analyzed as a split plot in time with genotypes as mainplots and scoring dates as subplots. Across locations analysis of each scoring date was carried out to determine if treatment response of the genotypes was the same at the three locations at all rating times. Area under the disease progress curve (AUDPC) estimates were computed based on RIL scores for the four dates. The following formula modified from that of Ceballos et al (1991) and Campbell and Madden (1990) was used:

\[ \text{AUDPC} = \sum_{i=1}^{n} \left( \frac{x_{i+1} + x_i}{2} \right) (t_{i+1} - t_i) / T \]  

AUDPC values of the three experiments were analyzed to find out if the amount of disease was the same in the two locations. To determine how the different scoring dates of each experiment relate to each other, Pearson correlation coefficients between consecutive dates were calculated using SAS Proc CORR (SAS Institute, 1989).

**Linkage analysis**

To identify the linkage groups, pairwise comparison and grouping of markers were performed with MAPMAKER/EXP. Version 3.0 (Lincoln et al., 1992a), which uses multipoint linkage analysis. Segregation data for all polymorphic RFLP markers were analyzed and putative linkage groups were identified using LOD 3.0.
Some markers that were not identified with any linkage group were assigned according to the published maize genetic linkage map (Coe, 1993). Some markers that mapped to different positions than those published were also anchored accordingly. Four markers could not be assigned to any linkage group.

Genotypic analysis.

To identify genomic regions associated with MSV tolerance, three approaches were used. The first was single factor analysis of variance (SFAOV) (Soller and Brody, 1976; Edwards et al., 1987) using SAS Proc GLM (SAS Institute, 1989). This analysis considers each RFLP marker as a treatment and compares each marker with each MSV score. The second one was interval mapping using pairs of RFLPs that are adjacent to each other in a linkage group (Knapp, 1989; Knapp and Bridges, 1990; Knapp et al., 1990) using SAS Proc GLM. The third was interval mapping by MAPMAKER/QTL program which searches for the effects of QTL between sets of linked markers using LOD scores (Lander and Botstein, 1989). Genotypes were, however, not established for every one of the 87 RILs, which were genotyped at each of the 71 loci. This is because some bands could not be read easily from the autoradiographs. Also during the analysis heterozygotes and outlier loci were recorded as missing data, and were not analyzed; this being a requirement of the analytical approaches.
RESULTS AND DISCUSSION

Genotypes reaction to MSV.

The resistant parent, previously reported as a 1.0 on the severity assessment scale (Kim et al., 1987) had a mean score across locations of 3.25 in these experiments. This difference in scores could be due to interaction with testing procedures or environments, different MSV isolates, disease assessment factors (different scales - I could have rated more severely), and/or heterogeneity among different TZi4 accessions.

Analysis of variance of the data from both the glasshouse and field experiments showed highly significant differences among the RILs (Table 1). In the two Harare experiments transgressive segregation (Simmonds, 1979) for MSV tolerance was observed whereby some RILs had more tolerance than the MSV-tolerant parent, TZi4 (Table 1). The performance of TZi4 across locations is similar implying that although not highly tolerant, expression of tolerance is stable across the three environments. The F1's reaction to MSV infestation was intermediate or similar to the tolerant parent which implies that a degree of dominance is associated with expression of tolerance to MSV in TZi4. MSV severity means for the susceptible check and parent, and susceptible RILs were 5.0 indicating that high infection levels were achieved (Figure3). Susceptible RILs and Hi34 (susceptible parent) died by 3-4 weeks after infestation in the field experiments.
Table 1. Mean MSV disease severity values (scale 1-5) of the parents, F₁, and RILs in three experiments conducted in two locations.

<table>
<thead>
<tr>
<th></th>
<th>Namulonge</th>
<th>Harare expt.1</th>
<th>Harare expt.2</th>
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<tr>
<td>Dates</td>
<td>1  2  3  4</td>
<td>1  2  3  4</td>
<td>1  2  3  4</td>
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<tr>
<td>Hi34</td>
<td>3.5a‡ 4.0a 4.5a 4.5a</td>
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<tr>
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<tr>
<td>LSD₀.₀₅</td>
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<td>0.94 0.52 0.56 0.46</td>
<td>0.76 0.6 0.62 0.54</td>
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</table>

‡ Means followed by the same letter within columns do not differ at the 0.05 probability level (LSD).
Figure 3. MSV symptoms in the glasshouse in Namulonge (Top), and in the field, Harare expt.2 (Bottom).
Figure 3
Analysis of MSV scores for the four dates across the three locations showed that there was no location effect (Table 2). This observation is inconsistent with marked phenotypic differences in disease severity being the result of MSV isolates at the two locations; however, there was a location by genotype interaction.

Table 2. Mean squares from analysis of MSV scores for disease assessment dates across three locations.

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<td>Location (L)</td>
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<td>6.25&lt;sup&gt;NS&lt;/sup&gt;</td>
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<td>0.41&lt;sup&gt;***&lt;/sup&gt;</td>
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<td>0.29&lt;sup&gt;***&lt;/sup&gt;</td>
<td>0.27&lt;sup&gt;***&lt;/sup&gt;</td>
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<sup>NS</sup> not significant. <sup>***</sup> Significant at 0.001 probability level.

To further test the possibility that differences in the responses of genotypes at different locations could be due to changes in magnitude of disease expression, rather than in genotypic rank, analysis of variance across any two locations, per individual date, was carried out (Table 3).
Table 3. Pairwise comparison of location by genotype mean squares from analysis of MSV scores for the four dates across locations.

<table>
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<th>Dates</th>
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<td>NAM vs HRE2</td>
<td>0.44***</td>
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<tr>
<td>HRE1 vs HRE2</td>
<td>0.20NS</td>
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</table>

NAM Namulonge. HRE1 Harare expt.1. HRE2 Harare expt.2.
NS not significant. **, *** Significant at 0.01 and 0.001 probability levels respectively.

The above table shows that the response of genotypes at Namulonge were different from those in Harare experiments (Figure 4). In Figure 4, the fourth rating data of the two Harare experiments have been averaged together since they were similar to each other. The different response in the two locations is most likely due to the different environmental conditions encountered in the glasshouse bioassay than in the field. The temperature conditions obviously differed in the glasshouse versus field experiments. The temperature in the glasshouse ranged from 20°C in the mornings to above 50°C in most of the afternoons while the temperature in the field experiments was between 15°C at night to 30°C during daytime. In general, phenotypic performance depends on both genetic and non-genetic influences on plant development.
Figure 4. Bar graph showing distribution of RILs and the range where the parents and F₁ are found.

NAM4 Namulonge fourth rating. T tolerant families. S susceptible families. AvH₁H₂ average of the two Harare experiments' fourth rating.
Thus the relative rankings of genotypes may well differ across environments in a manner more complex (Allard and Bradshaw, 1964) than that attributed to the effect of disease itself. Environmental variables such as light, temperature, moisture, and nutrition have pronounced effects on the growth and development of plants and likewise influence disease symptoms (van Loon, 1987).

Temperature as a key factor among environmental factors influencing streak disease development was pointed out by Storey and Howland (1967), Van Rensburg (1979), Rasaiah (1981), Damsteegt (1984) and Van Rensburg et al (1991c). Storey and Howland (1967) suggested a partial inactivation of streak at higher temperatures (39°C) as compared to lower temperatures (28°C). Van Rensburg (1979) attributed the inability of maize streak to attain epidemic levels in some areas partly to temperature limitations. Rasaiah (1981) reported that MSV symptoms appeared between 4 and 7 days after MSV infestation depending on glasshouse temperature with higher temperatures favoring more rapid symptom development. Damsteegt (1984), using fluctuating temperature between 0°C and 27°C, found the time from infestation with MSV to symptom development in maize to be inversely related to increase in temperature. Similarly, Van Rensburg et al (1991c) reported that MSV incubation in maize was inversely related to post-infestation temperature which ranged from 15°C to 36°C.

Response of genotypes to MSV infestation at different rating dates was evaluated by analyzing the experiments as a split-plot in time. The mainplot treatment was "genotypes" and "dates" was the subplot treatment (Table 4).
Table 4. Response of genotypes to MSV infestation across time and locations.

<table>
<thead>
<tr>
<th>Source</th>
<th>d.f.</th>
<th>Namulonge</th>
<th>Harare expt.1</th>
<th>Harare expt.2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotypes (G)</td>
<td>89</td>
<td>2.65***</td>
<td>6.01***</td>
<td>6.30***</td>
</tr>
<tr>
<td>Dates (D)</td>
<td>3</td>
<td>101.79***</td>
<td>5.61***</td>
<td>16.52***</td>
</tr>
<tr>
<td>G x D</td>
<td>267</td>
<td>0.27NS</td>
<td>0.09NS</td>
<td>0.14***</td>
</tr>
</tbody>
</table>

NS not significant. *** Significant at 0.001 probability level.

Disease responses across the four dates were significantly different with higher scores observed in the later dates as the disease progressed. With the exception of Harare experiment 2, there were no genotype by date interactions. This means that genotypes responded similarly to MSV infestation across rating times and experimental locations. Those genotypes that were tolerant or susceptible were tolerant and susceptible, respectively, when rated early or late in all environments. This was reflected in the pairwise comparisons of any two subsequent MSV ratings in each experiment. They were highly positively correlated in most cases (Table 5).

The genotype x date interaction observed in Harare experiment 2 can be explained by a low *Cicadulina* leafhopper population left after the first field infestation. It is possible that many leafhoppers drowned before they revived properly from carbon-dioxide treatment because it rained immediately after the first infestation. In this experiment, few lines consistently showed streak symptoms six days after infestation and a second leafhopper infestation was carried out.
Table 5. Pearson correlation coefficient of consecutive MSV ratings in each experiment.

<table>
<thead>
<tr>
<th>Dates</th>
<th>Namulonge</th>
<th>Harare expt.1</th>
<th>Harare expt.2</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 vs 2</td>
<td>0.49</td>
<td>0.89</td>
<td>0.86</td>
</tr>
<tr>
<td>2 vs 3</td>
<td>0.72</td>
<td>0.95</td>
<td>0.93</td>
</tr>
<tr>
<td>3 vs 4</td>
<td>0.91</td>
<td>0.98</td>
<td>0.97</td>
</tr>
</tbody>
</table>

Mean separation of MSV scores averaged over genotypes per each date (Table 6) shows that the first and second dates were significantly different from each other and both were different from the third and fourth dates. After the third date there was no further disease progress. The third date was about one week prior to anthesis. In practice, selection and evaluation of MSV tolerance has been carried out prior to flowering or about two weeks after flowering (Kuhn, 1979; Soto et al., 1982; Kim et al., 1989; Barrow, 1992). This period corresponds to the time when there was no further disease development in the field studies. At the fourth rating, MSV scores averaged over genotypes show that many tolerant RILs were rated between 3.0-4.0 (Figure 4).
Table 6. Mean separation of average MSV scores of the parents, F1, and RILs across the four dates.

<table>
<thead>
<tr>
<th>Dates</th>
<th>Namulonge</th>
<th>Harare expt.1</th>
<th>Harare expt.2</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.53c‡</td>
<td>3.79c</td>
<td>3.38c</td>
</tr>
<tr>
<td>2</td>
<td>3.81b</td>
<td>4.01b</td>
<td>3.63b</td>
</tr>
<tr>
<td>3</td>
<td>4.13a</td>
<td>4.19a</td>
<td>3.99a</td>
</tr>
<tr>
<td>4</td>
<td>4.14a</td>
<td>4.15a</td>
<td>4.03a</td>
</tr>
</tbody>
</table>

‡ Means followed by the same letter within columns do not differ at the 0.05 probability level (LSD).

AUDPCs can be a descriptor of disease severity and can identify RILs with slower development of symptoms that were not easily detected by the other analyses of variance. Since there was also a genotype by date interaction in Harare expt. 2, AUDPCs were calculated and analyzed (Table 7). Similar results to those from analyses of disease ratings were obtained with the susceptible parents showing higher disease severity and the occurrence of transgressive segregation, in the two Harare experiments, for higher MSV tolerance. AUDPC, in this case, was similar to the mean value of the disease rating averaged across all the four ratings per experiment. Analysis of AUDPCs across locations showed a significant location by genotype interaction. Across analysis of any two locations showed that this interaction was due to Namulonge location which was different from the two Harare locations, that were similar to one another.
Table 7. AUDPC values of parents, F₁, and RILs of MSV scores of the four dates for each experiment.

<table>
<thead>
<tr>
<th>Source</th>
<th>Location</th>
<th>Namulonje</th>
<th>Harare expt.1</th>
<th>Harare expt.2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hi34</td>
<td></td>
<td>4.31a‡</td>
<td>4.96a</td>
<td>4.55a</td>
</tr>
<tr>
<td>TZi4</td>
<td></td>
<td>3.20b</td>
<td>3.50b</td>
<td>3.22b</td>
</tr>
<tr>
<td>F₁</td>
<td></td>
<td>3.40ab</td>
<td>3.50b</td>
<td>3.41b</td>
</tr>
<tr>
<td>RILs range</td>
<td></td>
<td>1.50 - 4.87</td>
<td>1.58 - 5.0</td>
<td>1.88 - 4.83</td>
</tr>
<tr>
<td>LSD₀.₀₅</td>
<td></td>
<td>1.05</td>
<td>1.16</td>
<td>0.49</td>
</tr>
</tbody>
</table>

‡ Means followed by the same letter within columns do not differ at the 0.05 probability level (LSD).
Mapping of MSV tolerance

Out of the 126 RFLP markers that were selected to screen the parents only 71 (56%) were polymorphic (Table 8).

Table 8. Chromosomal location of 71 RFLP markers used in this study.

<table>
<thead>
<tr>
<th>Chromosome</th>
<th>Loci*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>bnl5.62, bnl12.06, npi262, umc167, umc67, php20855, umc128 bnl8.29, umc84, bnl6.32</td>
</tr>
<tr>
<td>2</td>
<td>npi239, umc34, umc131, umc5, umc4, umc49, umc198b, umc36</td>
</tr>
<tr>
<td>3</td>
<td>umc32, umc121, umc92, umc102, bnl1.297, umc16, umc63, umc96</td>
</tr>
<tr>
<td>4</td>
<td>umc31, umc15, umc52, php20608, bnl8.23</td>
</tr>
<tr>
<td>5</td>
<td>bnl6.25, umc147, umc27, umc43, bnl5.71, umc108, umc68, php10017</td>
</tr>
<tr>
<td>6</td>
<td>umc85, umc65, umc173b, umc62</td>
</tr>
<tr>
<td>7</td>
<td>csu13, php20581, umc136, umc110, bnl14.07, bnl16.06, umc35</td>
</tr>
<tr>
<td>8</td>
<td>bnl9.11, umc173a, umc189, umc30, umc7</td>
</tr>
<tr>
<td>9</td>
<td>umc109, umc105, umc81, umc190, umc140, csu59, bnl5.09</td>
</tr>
<tr>
<td>10</td>
<td>bnl3.04, npi285b, umc130, umc163, umc44</td>
</tr>
<tr>
<td>Unassigned</td>
<td>umc2, npi285a, umc198a, umc193</td>
</tr>
</tbody>
</table>

* Loci listed in relative chromosomal order (Coe, 1993).

A few loci did not map to the current maize linkage map and were assigned to positions according to Coe (1993) maize map. Others whose map positions could not be located were not assigned to any chromosome. Unassigned loci were included in the phenotype-genotype association analysis using SFAOV but not the other two interval-mapping methods.
Chi-square analysis was used to test single locus goodness of fit to the expected 1:1 Mendelian ratio at 0.05 probability level. Significant deviations from the expected 1:1 segregation were observed for 16 markers (22%). Of these 16 RFLP markers, one marker (bnl12.06) was later found to be significantly associated with the genomic region for MSV tolerance. Abberant segregation ratios as a result of unintentional selection favoring either parent have been reported before in two recombinant inbred families of maize (Burr et al., 1988) and in many other mapping studies in maize such as Edwards et al (1987), Beavis and Grant (1991), Reiter et al (1991), Gardener et al (1993), Bubeck et al (1993), and Goldman et al (1994). These distorted ratios can be a result of either inadvertant or natural selection taking place during sporogenesis, gametogenesis, fertilization, seed development, seed germination and/or plant growth (Grant, 1975).

**MSV traits**

RFLP marker-trait association analysis was carried out on the following maize streak scores, considered in this study as MSV traits;

1) streak scores recorded on each of four dates in each experiment;

2) the average of the four scores for each experiment; and

3) AUDPC values of streak scores for each experiment.
Results from SFAOV

Results from SFAOV show a significant association of MSV tolerance with 6 RFLP markers in the Namulonge experiment and 5 markers in the two Harare experiments (Table 9). These loci are found on chromosome 1 (Coe, 1993) (Figure 5). The probability level taken to consider a locus or loci significantly associated with MSV tolerance, with SFAOV, was 0.05 (Soller ad Brody, 1976). This relaxed level was taken in consideration as the first run to maximize the identification of chromosomal regions that may be associated with MSV. By using a less stringent probability level, chances of committing a Type 2 error, falsely rejecting an association, are reduced (Dudley, 1993), although this is at the expense of committing a Type 1 error (false positives accepted). The proportion of phenotypic variation explained by individual marker loci ranged from 6% (i.e. $R^2 \times 100$) to 76%. The largest significant $R^2$ values were generally obtained at the fourth rating time (date 4). High $R^2$ values may reflect QTLs having a big effect or tight linkage with the marker locus. Small $R^2$ values may reflect either QTLs having a small effect or a larger effect but less linkage with the marker locus (Edwards et al., 1987). RFLP marker npi262 exhibited the largest $R^2$ (25% at date 1 in Namulonge to 76% at date 4 in Harare expt.2) values in the three experiments, followed by umc167. RFLP marker bnl8.29 contributed the least phenotypic variation and none in Harare expt.2. With all the six loci, tolerance was associated with the allele from the tolerant parent (TZi4).
Figure 5. Loci mapped to Chromosome 1 in RILs of Hi34 x TZi4.
R² values from SFAOV method in these experiments are very high compared to those reported e.g. 51% accounted for by two loci for powdery mildew (Erysiphe polygoni) resistance in mungbean (Vigna radiata) at 65 days after planting (Young et al., 1993). At 85 days after planting three RFLP markers accounted for 58% of the total variation in powdery mildew response. Bubeck et al (1993) reported loci contributing the highest variation associated with gray leaf spot of 13%, 14%, and 20% in three environments. Two RFLP markers were significantly associated with soybean cyst nematode resistance in soybean accounting for 51.7% of the total phenotypic variation (Concibido et al., 1994). Dirlewanger et al (1994) reported variation of 67% in resistance to Ascochyta blight disease in pea (Pisum sativum) explained by two loci. Wang et al (1994) reported 16 markers, which defined 5 chromosomal regions, significantly associated with complete resistance to rice blast disease. The marker strongly associated with rice blast accounted for 25.4%.

The probability levels in Table 9 are much lower than the 0.0001 level reported but the analysis program setting does not go beyond this level. Significant marker-MSV tolerance association was observed from the first through the last rating. It thus seems that MSV tolerance in maize is expressed early in the seedling stage.
Table 9. Results from single factor analysis of variance showing loci significantly associated with MSV tolerance, on Chromosome 1, across dates in three experiments.

<table>
<thead>
<tr>
<th>Locus²</th>
<th>Trait</th>
<th>Namulonge Prob.</th>
<th>Namulonge R²</th>
<th>Harare expt.1 Prob.</th>
<th>Harare expt.1 R²</th>
<th>Harare expt.2 Prob.</th>
<th>Harare expt.2 R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>bnl12.06</td>
<td>date1</td>
<td>0.030</td>
<td>0.08</td>
<td>0.0001</td>
<td>0.29</td>
<td>0.0001</td>
<td>0.23</td>
</tr>
<tr>
<td></td>
<td>date2</td>
<td>0.002</td>
<td>0.16</td>
<td>0.0001</td>
<td>0.32</td>
<td>0.0001</td>
<td>0.31</td>
</tr>
<tr>
<td></td>
<td>date3</td>
<td>0.020</td>
<td>0.09</td>
<td>0.0001</td>
<td>0.29</td>
<td>0.0001</td>
<td>0.36</td>
</tr>
<tr>
<td></td>
<td>date4</td>
<td>0.010</td>
<td>0.11</td>
<td>0.0001</td>
<td>0.40</td>
<td>0.0001</td>
<td>0.37</td>
</tr>
<tr>
<td></td>
<td>average</td>
<td>0.009</td>
<td>0.11</td>
<td>0.0001</td>
<td>0.32</td>
<td>0.0001</td>
<td>0.34</td>
</tr>
<tr>
<td></td>
<td>AUDPC</td>
<td>0.010</td>
<td>0.11</td>
<td>0.0001</td>
<td>0.30</td>
<td>0.0001</td>
<td>0.34</td>
</tr>
<tr>
<td>npi262</td>
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<td>0.0001</td>
<td>0.56</td>
<td>0.0001</td>
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</tr>
<tr>
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<td>0.75</td>
</tr>
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<td>0.44</td>
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<td>0.0001</td>
<td>0.46</td>
<td>0.0001</td>
<td>0.57</td>
</tr>
<tr>
<td>Locus</td>
<td>Trait</td>
<td>Namulonge</td>
<td></td>
<td>Harare exp.1</td>
<td></td>
<td>Harare exp.2</td>
<td></td>
</tr>
<tr>
<td>---------</td>
<td>-------</td>
<td>-----------</td>
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<td>--------------</td>
<td>-----</td>
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<td>-----</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Prob.</td>
<td>R²</td>
<td>Prob.</td>
<td>R²</td>
<td>Prob.</td>
<td>R²</td>
</tr>
<tr>
<td>umc67</td>
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<td>0.0001</td>
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</tr>
<tr>
<td></td>
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<td>0.0001</td>
<td>0.23</td>
<td>0.0001</td>
<td>0.32</td>
<td>0.0001</td>
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<tr>
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<td>0.32</td>
<td>0.0001</td>
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<tr>
<td>php20855</td>
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<td>0.030</td>
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<td>0.009</td>
<td>0.09</td>
<td>0.010</td>
<td>0.08</td>
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<tr>
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<td>0.003</td>
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</tr>
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</tr>
<tr>
<td></td>
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<td>0.001</td>
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<td>0.002</td>
<td>0.12</td>
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<td>0.010</td>
<td>0.09</td>
<td>0.002</td>
<td>0.12</td>
</tr>
<tr>
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<td>-</td>
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<td>&quot;</td>
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<td>&quot;</td>
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<td>-</td>
<td>&quot;</td>
<td>&quot;</td>
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<td>0.05</td>
</tr>
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<td>&quot;</td>
<td>&quot;</td>
<td>0.020</td>
<td>0.06</td>
</tr>
<tr>
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<td>AUDPC</td>
<td>0.006</td>
<td>0.09</td>
<td>&quot;</td>
<td>&quot;</td>
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a Loci listed in relative order on chromosome 1 (Coe, 1993). 
Prob. significant probability level. AUDPC area under disease progress curve. 
NS not significant. R² phenotypic variation associated with respective loci.
Results from Knapp and Bridges interval mapping

Results similar to those obtained with SFAOV were obtained with Knapp and Bridges (1990) SAS program written for QTL analysis of RIL populations (Table 10). Unlike the results of the SFAOV, a more stringent probability level of 0.01 was considered necessary for a significant trait-marker association to avoid any false positives (Lander and Botstein, 1989). The interval between markers bnl12.06-npi262 and npi262-umc167 was so significantly associated with MSV tolerance in the two Harare experiments that the probability level exceeded a power of 10^-17 and the program default setting reported a value of zero. RFLP marker bnl5.62 was not significantly associated with MSV tolerance following SFAOV but was observed to be associated following interval mapping. However, the interval bnl5.62-bnl12.06 was not significant in the Namulonge experiment. Another marker interval, php20855-umc128, was associated only with MSV tolerance in the Namulonge experiment. This interval is, however, barely significant at 0.01 probability level and it is not considered to contribute much to the variation in MSV tolerance. Thus four intervals, bnl12.06-npi262, npi262-umc167, umc167-umc67 and umc67-php20855 were common in all experiments.
Table 10. Results from Knapp and Bridges interval mapping showing probability levels of loci on chromosome 1 associated with MSV tolerance across dates in the three experiments.

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<td>umc167 - umc67</td>
<td>umc67 - php20855</td>
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* loci in intervals listed in relative order on chromosome 1 (Coe, 1993). NS not significant.
† significant probability level values. AUDPC area under disease progress curve.
Results from analysis with MAPMAKER program

Linkage groups were established with MAPMAKER/EXP 3.0 (Lincoln et al., 1992a) which utilizes RI algorithm to analyze RILs. However MAPMAKER/QTL, which together with MAPMAKER/EXP comprise the linkage analysis package called MAPMAKER, is not presently designated to analyze RILs. To identify QTLs that are associated with MSV tolerance using MAPMAKER/QTL version 1.1 Beta Release 3.0b (Lincoln et al., 1992b), RILs were analyzed as an 'F2 intercross' (Reiter et al., 1992). A QTL was considered likely if the LOD score for the presence of a QTL exceeded 5.5. The LOD of 5.5 was arrived at by doubling the LOD score calculated from the formula of Lander and Botstein (1989) as advised by Mark Daly of MAPMAKER group (personal communication). This LOD of 5.5 was calculated at the 0.01 probability level having considered that we had a sparse map where consecutive markers were spaced in most cases (Lander and Botstein, 1989). This compares to that of Reiter et al (1992) and Wang et al (1994) who used a LOD of 6.0 with RIL population studies of maize and rice respectively.

Similar results to those with Knapp and Bridges analysis were obtained with MAPMAKER/QTL analysis (Table 11). Interval bnl5.62-bnl12.06 did not contribute to MSV variation in the Namulonge experiment as well as umc67-php20855. The latter was, however, significant in the Knapp and Bridges analysis. Thus only three genomic intervals were common in all analysis methods; bnl12.06-npi262, npi262-umc167, and umc167-umc67. The highest LOD scores and phenotypic variation explained were observed at the fourth rating, in each interval, and these data were
used to draw the QTL likelihood maps (Figure 6a,b,c). LOD scores ranged from 6.22 to 35.89 and the phenotypic variation explained from 29.9 to 95.9. Again these are very high values providing strong evidence for the presence of QTL associated with MSV tolerance. Lower LOD scores and percent variation explained, from analysis with MAPMAKER/QTL, have been reported in other host plant genetic studies. These include studies by Heun (1992) who reported 19.8% of the variation of powdery mildew resistance in barley explained by two QTLs. Variation in resistance to bruchids (*Collosobruchus chinesis*) in mungbean was explained by one QTL with LOD of 15.3 and 87.5% of the variation (Young et al., 1992). Bubeck et al (1993) reported multiple QTL models explaining variation in resistance to gray leaf spot in three populations over environments of 42% from 5 markers, 58% from 6 markers, and 24% from 2 markers respectively. Chen et al (1994) using QTL-STAT (B. Lieu and S.J. Knapp, unpublished) identified two genes conditioning resistance to barley stripe rust (*Puccinia striiformis* f. sp. *hordei*). One of them had a major effect accounting for 57% of the variation in disease severity, and the other with a minor effect accounting for 10%. 61.6% of the variation in soybean cyst nematode resistance was explained by two QTLs (Concibido et al., 1994). Two genomic regions strongly associated with bacterial wilt resistance in tomato accounted for 77% and 24% respectively when root inoculated (Danesh et al., 1994). When shoot inoculated the two regions accounted for 82%. Dirlewanger et al (1994) reported variation of 61% for Ascochyta blight resistance in pea explained by two QTLs. Three genomic intervals accounted for 29.5% for average number
of lesions per leaf, five intervals for 44.6% for average percent leaf tissue diseased (severity), and two intervals for 28.9% for average size of lesions for host plant response to northern leaf blight (*Exserohilum turcicum*) in maize (Freymark et al., 1994).
Table 11. Results from MAPMAKER/QTL analysis showing marker intervals on Chromosome 1 detecting significant variation, peak LOD and percent variation (% var.), for MSV tolerance.

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<tr>
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<th>Harare expt.2</th>
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<td>% var. c</td>
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a Loci in intervals listed in relative order on chromosome 1 (Coe, 1993).
b Maximum LOD between each interval.
c Phenotypic variation explained by respective loci. NS not significant.
AUDPC area under disease progress curve.
Figure 6. QTL likelihood maps indicating LOD scores, at the fourth rating, for MSV tolerance.

a) Namulonge.

b) Harare experiment 1.

c) Harare experiment 2.
Figure 6
Table 12 shows the LOD score and phenotypic variation explained at loci positions. The phenotypic variation values observed are identical with $R^2$ values (expressed in percentage) obtained from SFAOV method. Identical results from two analytical methods also has been reported by Paterson et al (1988), Stuber et al (1992), Bubeck et al (1993) and Wang et al (1994). By setting marker intervals equal to zero, one is effectively performing a linear regression analysis such as that performed with SFAOV (Darvasi et al., 1993).
Table 12. Results from MAPMAKER/QTL analysis showing LOD scores and percent variation (% var.) at marker loci on Chromosome 1 associated with MSV tolerance.

| Locus\[a\] | Trait | Namulonge | | | Harare expt.1 | | | Harare expt.2 | | |
|-------------|-------|-----------|-----|-----|-----------|-----|-----|-----------|-----|
|             |       | LOD\[^{b}\] | % var.| LOD | % var. | LOD | % var. | LOD | % var. |
| bnl12.06    | date1 | NS        | -    | 8.39 | 39.5 | 8.01 | 37.0 |
|             | date2 | "         | "    | 10.84| 57.9 | 11.29| 55.8 |
|             | date3 | "         | "    | 9.03 | 46.5 | 12.27| 52.1 |
|             | date4 | "         | "    | 13.20| 67.4 | 12.72| 54.1 |
|             | average| "        | "    | 9.53 | 47.4 | 11.94| 51.5 |
|             | AUDPC | "         | "    | 9.31 | 46.5 | 11.89| 51.4 |
|             |       |           |       |      |       |      |      |      |
| npi262      | date1 | 5.63      | 26.6 | 15.06| 57.4 | 14.48| 54.4 |
|             | date2 | 10.44     | 43.1 | 21.43| 69.7 | 24.44| 73.7 |
|             | date3 | 8.61      | 37.0 | 14.31| 54.5 | 25.77| 75.7 |
|             | date4 | 10.64     | 43.6 | 24.30| 75.4 | 26.23| 77.2 |
|             | average| 10.48    | 42.9 | 15.55| 57.8 | 24.85| 74.5 |
|             | AUDPC | 10.13     | 41.1 | 15.43| 57.4 | 25.20| 74.9 |
|             |       |           |       |      |       |      |      |      |
| umc167      | date1 | 4.54      | 21.8 | 9.84 | 41.7 | 10.26| 42.7 |
|             | date2 | 8.29      | 36.8 | 12.69| 50.5 | 14.36| 54.4 |
|             | date3 | 6.22      | 29.8 | 9.50 | 42.7 | 14.79| 55.4 |
|             | date4 | 7.62      | 35.1 | 12.73| 50.2 | 13.67| 53.4 |
|             | average| 7.96     | 35.9 | 10.19| 43.5 | 14.40| 54.5 |
|             | AUDPC | 7.69      | 35.2 | 10.24| 43.9 | 14.73| 55.4 |
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<td>% var.</td>
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<td>% var.</td>
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<sup>a</sup> Loci listed in relative order on chromosome 1 (Coe, 1993).
<sup>b</sup> LOD score at locus position.
<sup>c</sup> Phenotypic variation explained by respective loci. NS not significant.
AUDPC area under disease progress curve.
GENERAL DISCUSSION

All loci identified by the three analytical methods span a distance of 39.9 centimorgans (cM) (Figure 5) on chromosome 1. These linkage distances were obtained by analysis with MAPMAKER/EXP, using LOD of 3.0. In this study bnl5.62 did not belong to the same linkage group with the other 6 loci. The most critical region highly associated with MSV tolerance (bnl1206 - npi262 - umc167 - umc67) spans a distance of 20.8 cM on the short arm of chromosome 1. These markers are tightly linked and may be considered as a single QTL. When two or more significant markers were found in the same linkage group, Paterson et al. (1991) considered them linked to independent QTLs if they were separated by more than 50 cM. In our studies the distances between the four loci are: bnl12.06--(11.6cM)--npi262--(6.2cM)--umc167--(3.0cM)--umc67.

In many host-pathogen systems, phenotypic variation can be categorized as resistant versus susceptible and coded like molecular marker data (Timmerman et al., 1993). Mapping this type of resistance can be accomplished by linkage analysis. In this study, the fourth rating data of each experiment were used to classify the RILs as tolerant or susceptible. RILs were considered tolerant if they were more tolerant or statistically not significantly less tolerant than the tolerant parent, TZi4, and were considered susceptible when significantly less tolerant than TZi4. Tolerant and susceptible RILs were coded B and A, respectively, and the locus was named 'msv1'. The data obtained fitted well a chi-square test for single
locus goodness of fit to the 1:1 Mendelian ratio at the 0.05 probability level indicating presence of a single gene. Multipoint linkage analysis with MAPMAKER/EXP. 3.0, using LOD score of 3.0, the locus 'msv1' was located within the same linkage group already identified with MSV tolerance (Figure 7). The order was: bnl12.06--(10.4cM)--msv1--(3.1cM)--npi262--(6.8cM)--umc167--(3.1cM)--umc67. This confirms why RFLP marker npi262 explains the largest proportion of phenotypic variation for it is the closest marker to 'msv1' locus (3.1 cM). I conclude that tolerance to MSV in TZi4 is under control of one gene, designated \( Msv1 \) (maize streak virus tolerance, gene 1) which is bracketed by RFLP markers bnl12.06 and npi262. This gene was dominant in the Harare experiments and exhibited partial dominance in the Namulonge experiment. Kim et al (1982) reported that MSV tolerance in IB32 was under control of 2-3 genes. IB32 was developed from TZ-Y population which was the donor for resistance to the population from which TZi4 was developed. The difference between our estimates in gene numbers may be attributed to precision of different methods used and/or several generations of inbreeding could have led to fixation of only one gene in TZi4.
Figure 7. Chromosome 1 showing loci associated with MSV tolerance.
In order to explain for the transgressive segregation for higher tolerance, in terms of symptoms observed, a two-factor analysis of variance using SAS Proc. GLM between npi262, the marker strongly associated with MSV tolerance, and the rest of the markers was performed. A significance level of $P < 0.01$ was used to declare a significant interaction. A marginally significant interaction was obtained between npi262 and npi285b (found on Chromosome 10) only at the second and fourth ratings in the two Harare experiments. $R^2$ values obtained were 73%, and 75% for experiment 1 and 77% and 78% for experiment 2. These $R^2$ values are not much different from those already obtained with npi262 alone (69% and 73% for expt. 1 and 74% and 76% for expt. 2). Moreover, transgressive segregation was observed at all rating times in the two experiments. The occurrence of transgressive segregation thus remains unexplained. For disease symptoms to become apparent a virus has to multiply and spread (van Loon, 1987); however, the host genotype can modify expression of symptoms independently from virus multiplication (Kuhn et al., 1981).

The association of the four RFLP markers, bnl12.06, npi262, umc167, and umc67 with MSV tolerance in TZi4 can be explained from the observation that these markers constitute one linkage block and hence the appearance of a single major QTL in the likelihood maps. In the Harare experiments the interval bnl5.62-bnl1206 was associated with MSV tolerance (Tables 10, 11). This interval includes RFLP marker bnl5.62 which was not significant in the SFAOV method and does not belong to the same linkage group. It is most likely that the significant effect
observed is due to RFLP marker bnl12.06, which is next to it on the maize genetic map (Coe, 1993).

In the Namulonge experiment, interval php20855-umc128 is of marginal significance and is not considered to contribute to MSV tolerance. The two intervals, bnl5.62-bnl12.06 and umc67-php20855, which border the major QTL region were not significantly associated with MSV tolerance in the Namulonge experiment in both interval mapping approaches. The marked phenotypic differences in disease severity, which were higher in the CIMMYT experiments, could have made detection of these regions more likely than in the Namulonge experiment. Marker-assisted detection of QTL is enhanced when marked differences in the trait exist between genotypes.

Despite markedly different responses in disease severity between Namulonge and Harare, the same gene was found to confer tolerance to MSV in TZi4. This implies that there is no MSV variability between the locations with respect to the locus 'msv1' and the responses observed could be due to genotype by environment interaction. The consistency of the same gene being effective in the two environments is of importance to breeding for tolerance to MSV for it reduces costs involved in testing cultivars in different environments. Virus tolerance is simply inherited in many virus-host genetic systems (Fraser, 1992) and Msv1 may be considered as an addition to the list of tolerance being under control of one gene.
The observation of different symptom severity classes in response to MSV infestation is an example wherein quantitative differences between phenotypes are not necessarily linearly related to quantitative differences in gene numbers (Fraser and Gerwitz, 1987). After all, by definition the difference between quantitative and qualitative traits resides in the relative magnitudes of allele substitution effects at a genetic locus (Comstock, 1978). If the effect of substituting one allele for another is large relative to total phenotypic variation, the trait is generally classified as qualitative such as results indicate in this study. When such substitution effects are small, the trait is considered to be quantitative.

The identification of RFLP markers tightly linked to a gene, Msv1, conferring tolerance to MSV will be useful in marker-assisted selection and for broadening the germplasm base amenable to stand MSV pressure in cultivation. MSV is obligately transmitted by Cicadulina leafhoppers and this increases the difficulty in screening for MSV tolerance/resistance. This establishment of MSV association with RFLP markers can reduce the need for insect-mediated screening of each breeding cycle. This can also result in a more effective and expedient improvement of MSV through the 'crash conversion' of germplasm through backcrossing as suggested by Pratt et al (1992). This technology, although not yet available to National Maize programs in Africa, can benefit the latter through cooperation with International Centers such as CIMMYT, Mexico, where it is being used. A question remains whether this gene is consistent over different genotypes. Further research in understanding the genetics of tolerance to MSV using molecular markers is being carried out on a
different MSV-tolerant genotype (Hoisington, D.A., personal communication). The identification of other genes conditioning tolerance/resistance to MSV will be of great importance in gene deployment for MSV control. For marker assisted selection, the association of this genomic region bracketed by markers bnl12.06 and npi262 with other desirable agronomic traits should be investigated.
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Table 13. MSV data of the RILs for the four ratings of each experiment.

|   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| G | R | N | N | N | N | A | A | H | H | H | H | A | A | H | H | H | H | A | A |
| H | I | 1 | 2 | 3 | 4 | V | D | 1 | 1 | 1 | 1 | V | D | 2 | 2 | 2 | 2 | V | D |
| L | N | N | 1 | 2 | 3 | 4 | H | 1 | 1 | 2 | 2 |

|    |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| 2  | H3f | 2.8 | 4.0 | 4.0 | 4.0 | 3.7 | 3.88 | 3.8 | 3.8 | 4.0 | 4.0 | 3.9 | 3.87 | 3.3 | 3.3 | 3.5 | 3.8 | 3.4 | 3.9 |
| 3  | H5f | 2.3 | 3.3 | 3.5 | 3.5 | 3.1 | 3.31 | 2.8 | 3.0 | 3.3 | 3.0 | 3.0 | 3.03 | 2.0 | 2.3 | 2.8 | 2.8 | 2.4 | 2.43 |
| 4  | H6f | 2.5 | 3.5 | 3.0 | 2.3 | 2.8 | 2.94 | 2.8 | 3.0 | 2.5 | 2.5 | 2.7 | 2.72 | 2.0 | 2.5 | 2.3 | 2.2 | 2.5 | 2.20 |
| 5  | H7f | 3.0 | 4.0 | 5.0 | 5.0 | 4.3 | 4.53 | 3.8 | 4.0 | 4.3 | 4.3 | 4.1 | 4.07 | 3.8 | 3.8 | 4.3 | 4.5 | 4.1 | 4.01 |
| 6  | H8f | 2.5 | 4.3 | 4.3 | 4.5 | 3.9 | 4.14 | 4.5 | 4.3 | 5.0 | 5.0 | 4.7 | 4.64 | 4.0 | 4.0 | 4.8 | 5.0 | 4.4 | 4.37 |
| 7  | H10f| 2.8 | 4.0 | 5.0 | 5.0 | 4.2 | 4.50 | 4.8 | 4.8 | 5.0 | 5.0 | 4.9 | 4.87 | 4.0 | 4.3 | 4.8 | 5.0 | 4.5 | 4.46 |
| 8  | H11f| 2.3 | 3.8 | 4.0 | 3.8 | 3.4 | 3.71 | 4.8 | 4.8 | 5.0 | 4.9 | 4.9 | 4.87 | 4.3 | 4.0 | 4.8 | 5.0 | 4.5 | 4.41 |
| 9  | H12f| 3.3 | 4.0 | 4.5 | 4.5 | 4.1 | 4.24 | 4.5 | 4.5 | 4.8 | 5.0 | 4.7 | 4.65 | 3.0 | 3.5 | 4.0 | 4.0 | 3.6 | 3.64 |
| 10 | H14f| 2.5 | 3.8 | 4.0 | 3.8 | 3.5 | 3.74 | 2.8 | 3.5 | 3.8 | 3.5 | 3.4 | 3.44 | 3.5 | 3.8 | 4.3 | 4.0 | 3.9 | 3.9 |
| 11 | H15f| 3.0 | 4.0 | 4.0 | 4.5 | 3.9 | 4.01 | 4.3 | 4.5 | 5.0 | 5.0 | 4.7 | 4.69 | 3.8 | 4.0 | 5.0 | 5.0 | 4.4 | 4.41 |
| 12 | H16f| 3.5 | 5.0 | 5.0 | 5.0 | 4.6 | 4.86 | 5.0 | 5.0 | 5.0 | 5.0 | 5.0 | 5.00 | 4.5 | 4.8 | 5.0 | 5.0 | 4.8 | 4.82 |
| 13 | H17f| 2.0 | 3.3 | 4.0 | 4.3 | 3.4 | 3.65 | 2.3 | 2.8 | 3.3 | 3.3 | 2.9 | 2.89 | 2.0 | 2.3 | 3.0 | 3.0 | 2.6 | 2.55 |
| 14 | H21f| 3.5 | 4.3 | 5.0 | 5.0 | 4.4 | 4.64 | 4.5 | 4.8 | 5.0 | 5.0 | 4.8 | 4.82 | 4.0 | 4.5 | 5.0 | 5.0 | 4.6 | 4.64 |
| 15 | H22f| 2.5 | 3.3 | 3.5 | 3.3 | 3.1 | 3.28 | 3.5 | 3.5 | 3.8 | 3.8 | 3.5 | 3.54 | 3.3 | 3.0 | 3.0 | 3.1 | 3.0 | 3.04 |
| 16 | H23f| 3.0 | 3.8 | 4.3 | 4.3 | 3.8 | 3.99 | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 4.00 | 3.5 | 3.3 | 3.5 | 3.5 | 3.4 | 3.41 |
| 17 | H25f| 2.5 | 3.5 | 3.5 | 4.5 | 3.5 | 3.62 | 3.5 | 3.8 | 3.8 | 3.8 | 3.7 | 3.70 | 3.8 | 3.5 | 3.5 | 3.5 | 3.6 | 3.54 |
| 18 | H26f| 2.5 | 2.8 | 2.8 | 2.5 | 2.6 | 2.67 | 3.3 | 2.8 | 2.8 | 2.8 | 2.9 | 2.9 | 2.88 | 2.0 | 2.0 | 2.3 | 2.3 | 2.0 | 1.99 |
| 20 | H28f| 3.0 | 4.0 | 4.8 | 4.5 | 4.1 | 4.32 | 4.5 | 5.0 | 5.0 | 5.0 | 4.9 | 4.91 | 4.0 | 4.3 | 5.0 | 5.0 | 4.6 | 4.55 |
| 21 | H29f| 3.0 | 4.0 | 4.3 | 5.0 | 4.1 | 4.22 | 4.5 | 4.8 | 5.0 | 5.0 | 4.8 | 4.82 | 3.8 | 4.5 | 5.0 | 5.0 | 4.6 | 4.60 |
| 22 | H30f| 2.5 | 3.3 | 3.5 | 3.3 | 3.1 | 3.28 | 3.3 | 3.5 | 3.5 | 3.3 | 3.4 | 3.42 | 3.3 | 3.0 | 3.0 | 3.1 | 3.04 |
| 23 | H31f| 2.8 | 4.0 | 3.5 | 3.5 | 3.4 | 3.58 | 4.3 | 4.5 | 5.0 | 5.0 | 4.7 | 4.69 | 3.8 | 4.0 | 4.5 | 4.5 | 4.2 | 4.18 |
| 24 | H32f| 2.3 | 3.3 | 4.5 | 4.5 | 3.6 | 3.93 | 3.8 | 4.0 | 4.3 | 4.3 | 4.1 | 4.07 | 3.0 | 3.5 | 3.8 | 3.8 | 3.5 | 3.53 |
| 25 | H35f| 2.8 | 4.5 | 4.0 | 4.0 | 3.8 | 4.03 | 4.5 | 4.5 | 5.0 | 5.0 | 4.8 | 4.73 | 4.0 | 4.0 | 5.0 | 5.0 | 4.5 | 4.45 |
Table 13 (continued),

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|   | H91f | 3.3  | 5.0  | 5.0  | 4.6  | 4.84 | 5.0  | 5.0  | 5.0  | 5.00 | 4.3  | 4.8  | 5.0  | 5.0  | 4.8  | 4.78 |
|---|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|
| 63| H92f | 1.8  | 3.8  | 4.0  | 3.5  | 3.3  | 3.62 | 3.0  | 3.5  | 3.5  | 3.4  | 3.41 | 2.8  | 3.5  | 3.0  | 3.1  | 3.14 |
| 64| H94f | 3.3  | 5.0  | 5.0  | 4.6  | 4.84 | 5.0  | 5.0  | 5.0  | 5.00 | 4.0  | 4.8  | 5.0  | 5.0  | 4.7  | 4.73 |
| 65| H95f | 2.3  | 4.0  | 3.8  | 3.4  | 3.4  | 3.68 | 3.3  | 3.5  | 3.3  | 3.4  | 3.42 | 2.8  | 2.8  | 3.0  | 2.8  | 2.83 |
| 66| H97f | 2.8  | 4.0  | 4.5  | 3.9  | 4.19 | 4.8  | 5.0  | 5.0  | 4.9  | 4.95 | 4.0  | 4.5  | 5.0  | 4.6  | 4.64 |
| 67| H98f | 3.0  | 5.0  | 5.0  | 4.5  | 4.82 | 5.0  | 5.0  | 5.0  | 5.00 | 3.8  | 4.8  | 5.0  | 4.6  | 4.69 |
| 68| H102f| 3.0  | 4.3  | 5.0  | 4.3  | 4.60 | 4.3  | 4.5  | 5.0  | 4.7  | 4.69 | 3.8  | 4.3  | 5.0  | 4.5  | 4.50 |
| 69| H105f| 2.5  | 4.3  | 4.8  | 4.1  | 4.40 | 4.8  | 5.0  | 5.0  | 4.9  | 4.95 | 4.0  | 4.8  | 5.0  | 4.7  | 4.73 |
| 70| H107f| 2.8  | 4.0  | 5.0  | 4.2  | 4.50 | 4.3  | 5.0  | 5.0  | 4.8  | 4.86 | 3.8  | 4.3  | 5.0  | 4.5  | 4.50 |
| 71| H109f| 2.3  | 4.0  | 4.0  | 4.3  | 3.6  | 3.89 | 2.8  | 3.0  | 3.3  | 3.0  | 3.03 | 2.5  | 2.3  | 2.8  | 2.6  | 2.52 |
| 72| H110f| 2.8  | 3.3  | 3.3  | 2.8  | 3.1  | 3.17 | 2.5  | 2.8  | 3.0  | 3.0  | 2.82 | 2.8  | 3.0  | 3.0  | 2.9  | 2.96 |
| 73| H111f| 3.3  | 4.5  | 5.0  | 4.4  | 4.69 | 4.8  | 5.0  | 5.0  | 4.9  | 4.87 | 4.5  | 4.3  | 5.0  | 4.7  | 4.63 |
| 74| H113f| 2.5  | 3.8  | 3.5  | 3.5  | 3.3  | 3.48 | 1.5  | 1.5  | 2.3  | 1.8  | 1.77 | 1.8  | 1.5  | 2.3  | 1.9  | 1.88 |
| 75| H116f| 3.0  | 4.8  | 5.0  | 5.0  | 4.4  | 4.67 | 5.0  | 4.5  | 5.0  | 4.9  | 4.82 | 4.3  | 4.8  | 4.8  | 5.0  | 4.7  | 4.70 |
| 76| H119f| 2.5  | 3.5  | 4.5  | 4.3  | 3.9  | 4.13 | 4.5  | 4.5  | 5.0  | 4.8  | 4.73 | 4.0  | 4.3  | 4.8  | 5.0  | 4.5  | 4.46 |
| 77| H123f| 2.0  | 3.8  | 4.0  | 4.0  | 3.4  | 3.74 | 3.0  | 3.3  | 3.3  | 3.1  | 3.08 | 2.5  | 2.5  | 2.8  | 2.6  | 2.61 |
| 78| H124f| 2.0  | 2.3  | 4.5  | 4.5  | 3.3  | 3.62 | 1.5  | 1.5  | 2.3  | 1.8  | 1.58 | 2.5  | 2.8  | 3.0  | 2.8  | 2.82 |
| 79| H126f| 2.5  | 4.8  | 5.0  | 5.0  | 4.3  | 4.70 | 4.8  | 4.8  | 5.0  | 4.9  | 4.87 | 4.0  | 4.5  | 5.0  | 4.6  | 4.64 |
| 80| H127f| 1.8  | 3.3  | 4.5  | 4.5  | 3.5  | 3.88 | 3.3  | 4.0  | 4.8  | 5.0  | 4.25 | 3.8  | 4.0  | 4.5  | 5.0  | 4.3  | 4.25 |
| 81| H130f| 2.0  | 2.8  | 3.8  | 4.0  | 3.1  | 3.35 | 3.0  | 3.5  | 3.5  | 3.3  | 3.37 | 2.8  | 3.0  | 3.5  | 3.1  | 3.02 |
| 82| H133f| 2.3  | 3.5  | 5.0  | 5.0  | 3.9  | 4.31 | 4.3  | 4.5  | 5.0  | 4.7  | 4.69 | 4.0  | 4.5  | 5.0  | 4.6  | 4.64 |
| 83| H136f| 2.0  | 2.5  | 2.5  | 2.5  | 2.4  | 2.45 | 2.8  | 3.0  | 3.3  | 3.0  | 3.03 | 2.3  | 2.8  | 3.0  | 2.8  | 2.75 |
| 84| H137f| 2.5  | 4.0  | 4.0  | 4.0  | 3.6  | 3.86 | 5.0  | 5.0  | 5.0  | 5.00 | 4.5  | 4.5  | 5.0  | 4.8  | 4.73 |
| 85| H138f| 3.0  | 3.8  | 4.3  | 4.3  | 3.8  | 3.99 | 4.0  | 4.0  | 4.0  | 3.5  | 3.93 | 3.5  | 3.5  | 3.3  | 3.4  | 3.47 |
| 86| H140f| 2.5  | 5.0  | 5.0  | 5.0  | 4.4  | 4.77 | 5.0  | 5.0  | 5.0  | 5.00 | 4.3  | 4.3  | 5.0  | 4.6  | 4.59 |
| 87| H141f| 2.8  | 4.8  | 4.8  | 4.8  | 4.57 | 4.8  | 5.0  | 5.0  | 4.9  | 4.95 | 4.5  | 4.8  | 5.0  | 4.8  | 4.82 |
| 88| H143f| 2.8  | 4.0  | 5.0  | 5.0  | 4.2  | 4.50 | 4.3  | 5.0  | 2.5  | 2.9  | 2.82 | 4.0  | 4.5  | 5.0  | 4.6  | 4.64 |
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GH greenhouse row number. RIL recombinant inbred line.
N1 - N4 first to fourth MSV ratings in the Namulonge experiment.
AVN average of the four ratings in the Namulonge experiment.
ADN AUDPC values of MSV in the Namulonge experiment.
H11 - H14 first to fourth MSV ratings in the Harare experiment 1.
AVH1 average of the four ratings in the Harare experiment 1.
ADH1 AUDPC values of MSV in the Harare experiment 1.
H21 - H24 first to fourth MSV ratings in the Harare experiment 2.
AVH2 average of the four ratings in the Harare experiment 2.
ADH2 AUDPC values of MSV in the Harare experiment 2.
Table 14. Agronomic traits of RILs taken at Namulonge and Agronomy Farm, Columbus, 1993.

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5 H7f 35 13 102 76 77 13 15 13 104 140 104
6 H8f 20 12 50 79 76 11 15 8 103 66 41
7 H10f 76 13 140 72 75 10 18 12 116 198 122
8 H11f 65 11 93 75 75 12 17 14 104 157 112
9 H12f 78 13 126 75 73 10 25 12 103 206 140
10 H14f 70 12 119 72 74 14 22 13 104 183 117
11 H15f 55 12 95 - 75 14 16 11 - 170 127
12 H16f 61 11 121 73 76 12 15 11 113 183 117
13 H17f 75 12 111 71 73 13 19 14 109 155 104
14 H21f 67 12 121 73 75 11 14 11 - 170 130
15 H22f 61 14 117 74 75 13 22 12 110 178 107
16 H23f 58 13 119 74 72 18 15 14 103 165 91
17 H25f 66 13 114 73 74 10 18 13 104 152 91
18 H26f 51 14 79 69 71 12 14 15 104 147 96
20 H28f 60 13 105 - 75 11 17 11 111 168 86
21 H29f 53 10 81 72 74 9 18 10 103 168 117
22 H30f 64 13 117 72 74 13 18 13 101 183 96
23 H31f 58 13 115 74 77 10 17 12 106 196 124
24 H32f 57 12 102 72 75 11 16 11 101 147 91
25 H35f 66 14 125 - 78 14 21 12 116 142 107
27 H38f 55 12 104 70 73 10 16 11 114 165 91
28 H39f 65 13 112 72 71 12 19 13 101 157 91
29 H40f 65 12 126 69 70 13 15 10 106 203 127
31 H44f 63 12 117 65 70 13 16 12 113 193 122
33 H46f 59 13 107 81 80 10 - 8 113 203 122
35 H48f 63 13 109 73 74 10 17 11 98 208 147
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39 H53f 79 14 122 72 76 13 18 12 98 188 127
40 H54f 51 11 90 73 78 12 15 10 100 183 122
Table 14 (continued),

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| 41 | H55f | 83 | 15 | 164 | 78 | 79 | 12 | 22 | 12 |   | 188 | 124 |
| 42 | H56f | 60 | 13 | 106 | 72 | 73 | 10 | 19 | 13 | 101 | 188 | 117 |
| 43 | H60f | 59 | 12 | 89 | 72 | 73 | 8 | 15 | 10 | 102 | 168 | 119 |
| 44 | H61f | 65 | 11 | 111 | 68 | 72 | 11 | 16 | 11 | 109 | 218 | 150 |
| 45 | H62f | 64 | 12 | 118 | 69 | 71 | 12 | 13 | 15 | 102 | 185 | 132 |
| 46 | H63f | 73 | 12 | 116 | 76 | 75 | 12 | 18 | 13 | 101 | 168 | 102 |
| 47 | H64f | 67 | 11 | 111 | 68 | 72 | 9 | 15 | 12 | 98 | 188 | 124 |
| 48 | H67f | 64 | 12 | 102 | 73 | 73 | 11 | 20 | 11 | 98 | 193 | 130 |
| 49 | H69f | 71 | 13 | 131 | 75 | 75 | 11 | 21 | 11 | 106 | 226 | 142 |
| 51 | H72f | 63 | 14 | 122 | 71 | 71 | 10 | 19 | 11 | 100 | 203 | 107 |
| 52 | H73f | 70 | 14 | 120 | 73 | 77 | 12 | 21 | 11 | 104 | 193 | 112 |
| 54 | H76f | 53 | 14 | 95 | 74 | 74 | 10 | 21 | 12 | 100 | 142 | 91 |
| 56 | H78f | 56 | 13 | 100 | 75 | 74 | 13 | 15 | 13 | 101 | 147 | 86 |
| 57 | H81f | 59 | 13 | 111 | 75 | 77 | 14 | 19 | 12 |   | 208 | 132 |
| 58 | H82f | 64 | 15 | 110 | 76 | 76 | 12 | 25 | 13 | 108 | 157 | 96 |
| 59 | H83f | 55 | 13 | 114 | 75 | 76 | 12 | 23 | 10 | 102 | 188 | 107 |
| 60 | H84f | 62 | 13 | 112 | 76 | 75 | 9 | 23 | 9 | 103 | 188 | 117 |
| 61 | H85f | 62 | 12 | 121 | 72 | 75 | 12 | 17 | 13 | 102 | 178 | 102 |
| 62 | H88f | 53 | 12 | 104 | 74 | 75 | 12 | 21 | 13 | 116 | 180 | 127 |
| 63 | H91f | 52 | 12 | 113 | 73 | 79 | 13 | 14 | 13 | 113 | 203 | 107 |
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| 65 | H94f | 77 | 13 | 133 | 71 | 72 | 10 | 12 | 14 |   | 180 | 140 |
| 66 | H95f | 82 | 14 | 134 | 73 | 76 | 11 | 19 | 13 | 104 | 198 | 137 |
| 67 | H97f | 67 | 10 | 115 | 71 | 74 | 11 | 15 | 12 | 113 | 198 | 130 |
| 68 | H98f | 48 | 13 | 112 | 73 | 75 | 10 | 19 | 14 |   | 198 | 117 |
| 69 | H102f | 67 | 10 | 101 | 64 | 67 | 10 | 17 | 13 | 100 | 168 | 117 |
| 70 | H105f | 66 | 14 | 123 | 74 | 75 | 14 | 14 | 14 | 100 | 190 | 112 |
| 71 | H107f | 50 | 11 | 100 | 70 | 76 | 12 | 18 | 11 |   | 168 | 122 |
| 72 | H109f | 67 | 14 | 115 | 72 | 72 | 14 | 16 | 14 | 114 | 180 | 114 |
| 73 | H110f | 69 | 14 | 132 | - | 74 | 16 | 16 | 13 | 113 | 178 | 112 |
| 74 | H111f | 51 | 12 | 98 | 73 | 75 | 10 | 12 | 9 | 103 | 127 | 81 |
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| 78 | H123f | 53 | 12 | 107 | 74 | 75 | 13 | 24 | 10 | 103 | 147 | 104 |
| 79 | H124f | 53 | 12 | 116 | 76 | 79 | 11 | 17 | 9 | 116 | 150 | 89 |
| 80 | H126f | 58 | 13 | 102 | 75 | 76 | 9 | 20 | 11 | 114 | 122 | 91 |
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GH greenhouse row number. RIL recombinant inbred line.
NEHT ear height (cm) at Namulonge.
NLF# total number of leaves at Namulonge.
NPHT plant height (cm) at Namulonge.
NP50 days to 50% pollen shed at Namulonge.
NSK50 days to 50% silking at Namulonge.
NHSK# number of husk leaves at Namulonge
NWT100 100 kernels weight (gm) at Namulonge.
NKROW of kernel rows at Namulonge.
*CSK50 days to 50% silking date at Agronomy Farm, Columbus.
*CPHT plant height (cm) at Agronomy Farm, Columbus.
*CEHT ear height (cm) at Agronomy farm, Columbus.

* Columbus data was recorded by Mark A. Casey.