Foreword

The Maize Streak Virus Workshop marked the culmination of the first phase of a collaborative effort spearheaded by ISAAA to gain a better understanding of maize streak virus (MSV) disease. Launched in 1996 in response to Kenya's need to stabilize yields, the project is providing national research groups in Africa with the data and information they need to combat MSV through improved resistance breeding. Prior to this project, scientists lacked basic information about how the virus was spread, what strains of the virus were most virulent, and what types of MSV resistance were most effective. To answer these questions, several MSV-resistant types of germplasm from Kenya and elsewhere were tested in multilocation trials for disease incidence, disease severity, and ecological adaptability. Using proprietary technologies donated by the John Innes Centre in the UK and the University of Cape Town in South Africa, scientists from Kenya were also able to characterize virus isolates and identify the best maize lines to use for breeding MSV-resistant varieties. This success has moved the project into its second phase, and work is now being directed towards developing high-yielding, MSV-resistant hybrids for the region's small-scale farmers. Local and international seed companies operating in Africa have also been involved for greater impact.

Held in Nairobi, Kenya in September 1999, the Maize Streak Virus Workshop brought together African scientists from different countries involved with MSV and related maize improvement programs, policymakers, and other key players from around the world. Its accomplishments, furthermore, testify to the power of the international partnerships that made its work possible. The donation of technology and training by institutions in developed countries, for example, provided Kenyan scientists with such cutting-edge biotechnology techniques as agro-inoculation, molecular marking, and DNA polymorphism, microsatellite analysis provided through the proprietary technology of Novartis in France to Kenya Agricultural Research Institute (KARI) scientists, is a remarkable achievement. And the collaborative efforts required to locate MSV-resistant germplasm involved not only institutions in industrialized countries, such as CIRAD (France) and Pannar (South Africa), but also institutions in developing countries such as KARI (Kenya), CIMMYT (Mexico), IITA (Nigeria), and others. Such South/South collaboration helped lay the groundwork for the current regionalization of the project; in fact, the Workshop Report already includes MSV status reports from Ethiopia, Malawi, and Uganda. Such regional cooperation builds confidence, increases the speed of research, and uses resources more efficiently.

We are pleased to disseminate the information presented at this Workshop to a larger audience. Both KARI and ISAAA hope that the scientific information contained here will be useful to others and that the collaborative structure of this project may serve as a model for similar efforts. We believe that partnerships between the North and South, between the private and public sectors, are the most effective way to transfer the benefits of agri-biotechnology to those who need them most: the world's resource-poor farmers. Finally, we would like to express our special thanks to The Rockefeller Foundation for funding this project and the Maize Streak Virus Workshop.

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Preface

Diseases, whether human or plant, have no fixed boundaries. They do not stop at a nation's borders. That is why I am pleased with the presence at this workshop of participants from this region and beyond.

The objectives of the Kenya National Food Policy Paper No.1 of 1994 and Kenya Agricultural Research Institute are to meet the need for food self-sufficiency and to raise the quality of life of Kenyans. Maize is the staple food throughout most of Kenya, and we all know that in Africa a lack of grain, especially maize, means famine. Yields of the crop are dangerously low. The current yield gap in Kenya and other African countries is wide: 2.3 to 4.7 tons lower per hectare than the crop's potential yield. This gap is exacerbated by a population growth rate that is even higher than the cereal crop production growth rate. A concerted effort is needed to reduce this yield gap, which we can achieve by eliminating or reducing the factors that are contributing to low yields.

Socioeconomic and infrastructure constraints are the primary issues that afflict farmers in Africa. However, there are also important biotic and abiotic factors, such as pests, diseases, weeds, and soil fertility problems. One such factor, maize streak virus (MVS), is endemic to Africa, appearing in Southern, Eastern, and Western Africa. Reports have indicated that in some instances MSV disease causes yield losses of 100%.

Here at KARI, tremendous achievements were recorded during the first phase of the MSV project, which focused on understanding the mechanism of MSV resistance in maize lines from Kenya and other parts of Africa. The project acquired several MSV-resistant types of germplasm and tested them in multilocation trials for disease incidence, disease severity, and ecological adaptability. We have detected variability in the MSV genome and in the virulence of the disease in different populations.

Transmission of the disease involves *Cicadulina* vectors, and we have identified various hosts. The team also acquired agroinoculation technology to screen for the disease, and they were trained in vector taxonomy and in the molecular (RFLP) comparison of maize genotypes. Due to the importance of some of their findings, the project found it necessary to share some of their experience and information with other scientists in the region before embarking on phase II.
The project has developed an interdisciplinary research approach that fosters team building within and between institutions. By strengthening the Institute's research capability through training and the acquisition of technology—particularly agroinoculation techniques—the project has made important advances in understanding MSV disease.

Ladies and gentlemen, your scientific efforts to manage this disease should focus on finding relevant, practical solutions that are farmer friendly. If our people's basic food, health, and education needs are not met, we will not join the ranks of developed nations. Real development requires not just independence and democratic participation but also solutions to poverty, hunger, and environmental destruction. These are some of the areas in which you scientists must play a vital role in. This practical vision of improving your fellow citizen's lives and protecting the environment should be your inspiration. Never forget that your work on MSV is making a real difference. It has the potential to double current maize yields through new cultivars that are tolerant to MSV, other maize diseases, and pests.

I am therefore pleased to announce that phase II of the MSV project has been developed and approved for funding. I wish to express my sincere thanks to the Rockefeller Foundation for having supported this project and for agreeing to finance its second phase. We at KARI are indeed very appreciative of the support that the Rockefeller Foundation has given and is still giving to this and other KARI projects. I thank them for their commitment to promote agricultural research in Africa and specifically for sponsoring this workshop.

Finally, it gives me great pleasure to express appreciation to all the participants, especially those from other countries in the region. Let me take this opportunity to thank all the other collaborators: ISAAA, for their tireless facilitation of phase I; and International Center for Insect Physiology and Ecology (ICIPE), the University of Cape Town, and the John Innes Centre, UK, for providing our researchers with facilities to do molecular studies. Lastly, I thank the MSV team for their good work.

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downy mildew, caused by *Peronoscelerospora sorhi* C.G. Shaw (Weston and Uppal), and gray leaf spot, caused by *Cercospora zea-maydis* (Tehon and Daniels).

Maize streak virus surveys. Uganda has also undertaken surveys on MSV and created a mass rearing system for the vector. Surveys conducted in four districts (Mubende, Masindi, Tororo, and Kumi) showed that MSV incidence ranges from 8% to 29.5% even in nonepidemic periods. It was, however, generally lower in the crop planted early in both the first and second rainy seasons. MSV was also more severe in the Mubende and Kumi districts.

The 1990 surveys carried out in these four districts also showed that *C. mbila*, *C. triangula*, *C. arachidis,* and *C. chinai* were common. Bioassays obtained by caging individual insects collected from the field on maize varieties susceptible to MSV disease showed that between 1.3% to ---% were already infected. Although the percentage seems low, on a large geographic scale it can result in many infective leafhoppers capable of causing widespread epidemics.

Data on insects collected in the June and October 1990 surveys indicated that the Masindi district, with a mean of 2.7%, had the highest percentage of naturally infective Cicadulina. Other studies carried out during 1990-93 include analyses of the comparative biology of Cicadulina species from various districts, host plant suitability, and adaptation to artificial rearing. Pearl millet was found to be the most responsive to the rearing system and was also the most prolific and efficient in virus transmission. Trials were also carried out in the screenhouse to simulate dry conditions to study what happens when host plants dry up and become unsuitable. CIMMYT was also involved in the collaborative MSV screening program initiated in Uganda.

2.2.3 MSV in Ethiopia (Dr. A. Yusuf)

Introduction

Begun in 1950, Ethiopian maize research is nowadays coordinated under a national network. Maize is grown in nearly 1 million ha. MSV was identified and recorded in 1967, and in 1980 the gemini virus was diagnosed using electron microscopy. MSV infestation reaches 80% at research stations and up to 20% in farmers' fields at Gambella in the southwest.

Maize Research

The Institute of Agricultural Research coordinates the maize research activities of a team of specialists in various fields like breeding, weed science, agronomy, physiology, entomology, pathology, soil and plant nutrition, farming systems research, and extension services. Other institutions involved include the Alamaya University of Agriculture, the Awasa College of Agriculture, nongovernment organizations (NGOs), and government organizations.

Field observations of the 1992-93 crop season at Gambella, which is 500 m above sea level, showed that the MSV infection rate was 80% at research stations and up to 20% in farmers' fields. Another field observation in 1996-97 was conducted in the north at Gojam, Tigray, which is 2,530 m above sea level. Researchers recorded MSV infection rates of 60%.

MSV vectors in Ethiopia include *C. mbila*, *C. bipunetella*, *C. ghauri*, *C. storeyi*, and *C. niger*.

MSV mid-season infected field causing poor comb set without grains.

The objectives of maize research include:

- Identify maize viruses in major regions of the country
- Establish a screening center
- Determine the most prevalent maize viruses of economic importance with particular emphasis on MSV in four regions
- Study vector dynamics, population, and immigration

Finally, we believe that the most promising way to control MSV is through developing MSV-resistant varieties.

3. The Ecology of MSV

3.1 Vectors

3.1.1 Cicadulina *spp. and MSV (Dr. V.A. Okoth)*

Overview and Introduction

Maize streak virus was first described as mealie variegation by Fuller in 1901. In 1925, Storey suggested that the infectious agent was a virus. MSV belongs to the gemini virus group and was first purified by Bock, Guthrie, and Woods in 1974. The virus was first recorded in South Africa where it occurred in maize (*Zea mays* L.), oats, and grasses (Fuller 1901). It also infects other grain crops like wheat, barley, finger millet, pearl millet, oats, and sorghum. Spread by leafhoppers, MSV does not replicate in the insect vector. In Kenya, 17 grass plants have been reported to serve as MSV hosts. Farmers in many countries have reported heavy yield losses during epidemics. MSV reduces plant height by 50% and yield losses reach 100%, especially if plants are infected before 7 weeks. High yield losses have been recorded in various parts of Africa, including Kenya (100%), Nigeria (100%), and Mauritius (91%) (Fajesmisin 1984).

MSV Transmission (Vector-MSV studies)

The virus is transmitted by several species of leafhoppers in the genus *Cicadulina (*Cicadellidae, Homoptera). *Cicadulina mbila* (Nude) is the main vector species (Rose 1983). Storey defined the virus cycle in the insect and showed that acquisition is very rapid (less than a minute) if the insect feeds on mesophyll or phloem in chlorotic areas of infected leaves (1933, 1938). The minimum latent period varies from 6-12 h depending on the temperature (Storey 1928). According to Storey, only active races of *C. mbila* can transmit the virus (1933). In other races, however, the virus cannot cross the gut wall. This permeability depends on a major sex-linked chromosome (Storey 1932). Based on the latency period and the persistence of transmission efficiency in spite of the very short acquisition access period, Storey postulated that MSV multiplied in the insect. This, however, was doubted by Bawden (1964).

Bouton and Markham showed that the concentration of the virus in *C. mbila* decreases after transfer from infected maize to an immune plant (1986). Combined with the inability to detect replicate DNA forms of the virus in the insect, this led them to conclude that MSV did not multiply in *C. mbila.* Reynauld and Peterschmitt (1992) also noted that MSV does not multiply in *C. mbila*. They also observed that the transmission efficiency of *C. mbila* was subjected to 50-h acquisition access periods (AAP) and that there were more ELISA-positive insects after 50-h AAP than 3-h AAP. A mean value of 0.36 ng of MSV per *C. mbila* was found 3 days after the 50-h acquisition, whereas 14 days later there was 0.20 ng of virus per insect. When leafhoppers were kept on infected maize, however, they substantially accumulated MSV up to an average of 3.83 ng per insect after 35 days of continuous acquisition. The amount of virus detected in females was usually greater than the amount detected in males.

Studies carried out in Uganda from 1990 to 1993 showed that pearl millet was the most suitable host plant for rearing *Cicadulina* spp., while *C. triangulata* was the most prolific and efficient in transmitting the virus. MSV is exclusively transmitted by *Cicadulina* spp. Accordingly, researchers recommended cultural and chemical methods to reduce vector population density and migration into maize fields. Unfortunately, the main cultural method—rouging infected plants—actually increased MSV infection unless infected plants were sprayed.

3.1.2 Cicadulina Population Dynamics and Species Distribution (Drs. M. Gethi, K.A. Nyarko, and B.O. Odhiambo)

Overview and Introduction

MSV evolved with wild grasses and has been recorded in 18 wild grasses, most of them annual species (Onudi 1996). The size of the vector population mostly depends on the number of host grasses suitable for oviposition and nymphal development. The population, therefore, is lowest at the onset of the rainy season and highest at the beginning of the dry season. Seasonal abundance and aerial densities of trapped *Cicadulina* peak twice annually—in July-August and November-December. Relay cropping allows leafhopper populations to persist to the next season. The vector can also fly long distances, especially at the end of the rainy season.

Studies on the *Cicadulina* biology and field population of various species have indicated similarities in life cycle (Rose 1978). Recently, Asanzi et al (1995) indicated that survival and development of most *Cicadulina* species decreased as temperature increased, while longevity increased as temperature decreased.

The size of the hopper population mainly depends on the number of host grasses suitable for oviposition and nymphal development. These numbers are determined by rainfall patterns and result in decreased population at the onset of rains. They are highest at the beginning of the dry season (Okoth and Dabrowski 1987).

Fajemisin et al (1986), working in Nigeria, also demonstrated that there exists a relationship between weather conditions, leafhopper development, growth of grasses, and the rate of virus transmission to maize. Observations by Rose (1973) also indicated that *Cicadulina* can fly long distances particularly at the end of the rainy season. Dabrowski (1983) showed that *Cicadulina* leafhoppers existed in two morphological forms, long and short, depending on their mean flight duration.

In this study, field surveys were carried out at 10 sites in different ecological zones. It was noted that even resistant materials brought in after the 1988 MSV outbreak were more susceptible to infection and that there were indications of resistance breakdown.

Materials and Methods

Field surveys to determine leafhopper population and species diversity began in June 1997. Areas selected were spread over about 10 maize-growing sites in Kenya and are in the different ecozones described below:

1. Lake Region Ecozone (Mbita Point, Oyugis)
This area borders Lake Victoria, with altitudes of 1,000-1,200 m above sea level. Rainfall is bimodal (750-1,200 mm), which allows for two crops per year. Temperatures and relative humidity range from 30°C to 34°C, creating moisture deficits in some areas.

2. Western Region (Kiminini, Amagoro, and Kitale)
This area covers Kenya's western province and a part of the Rift Valley province. Altitude is about 1,500-2,500 m above sea level, and the rainfall is the country's highest (1,000-1,780 mm). Depending on the elevation, the area has either a bimodal or unimodal rainfall pattern.

3. Central Ecozone (Kagumo and Mitunguu)
This area covers some parts of the central and eastern provinces and is referred to as the highland east of the Rift Valley. The climate is influenced by both Mt. Kenya and the Aberdare Ranges. Rainfall is bimodal with an average 750 mm per annum. MSV is endemic to this area.

4. Coastal Ecozone (Mtwapa)
These are known as the coast lowlands, lying 20-125 m above sea level. Temperatures and relative humidity are relatively high: 26-35°C and 60-90%, respectively. The mean annual rainfall is about 700 mm spread over two seasons in a bimodal pattern. Most rainfall is received during the long rains; the short rains are unreliable.

Leafhopper sampling. Sites that were used for virus epidemiology studies were located in areas where maize was the main crop. These areas also had various grass species throughout the year. A modified cage as described by Okoth and Dabrowski (1987) was used to sample the leafhoppers. It measured 1.0 x 1.0 x 1.25 m, and was made of metal frame covered with a transparent nylon mosquito netting.

To trap the leafhoppers, the cage was gently lifted and swiftly placed to cover fresh grasses bordering the maize. A person then entered the cage through a side slit and shook the grasses to disturb the insects. All insects that respond to light would then attach themselves to the net side. The

Cicadulina leafhoppers were then aspirated individually. Twenty-five samples were taken at each site and the number of *Cicadulina* caught per square meter was recorded.

Male leafhoppers were separated from the females. Females were then caged on living plants grown on trays and then taken to the laboratory, where they were reared singly using PVC cages (5 x 25 cm). They were allowed to oviposit and the first generation progeny was then identified using the pygofer process and the aedeagus, which are morphologically different in *Cicadulina* species.

At Embu and Mbita Point, a Malaise trap obtained from the UK was set up to trap hoppers flying at least 1 m above the ground. At Mbita Point another trap known as MPFS pylon, a type of stationary yellow trap, was set. The sticky traps were mounted on poles using a pulley mechanism at 1-m intervals up to 12 m high.

The trap would monitor and determine the direction of insect flight to establish the importance of migration in MSV epidemiology. Leafhopper sampling on grasses was also carried throughout the year to establish other possible alternative hosts.

The spatial distribution of MSV and the percentage of infestation in maize fields surrounded by *Setaria sphacelata* was assessed at 2-week intervals using a quadrant and the efficiency of MSV transmission by different species of collected leafhoppers determined. Each species was replicated 30 times to determine transmission rates. Results indicated that species from different ecological zones might vary in transmission efficiency.

Results

***Cicadulina* population density.** Throughout the sampling period, *Cicadulina* population density varied in different locations. But at all sites there was a population buildup in January, which also marks the end of short rains (except for the Mtwapa site in the Coastal region, where the population peaked in April 1998).

This deviation from the normal trend was attributed to increased rainfall in the region, which preceded the El Niño rainfall. In Embu and Kagumo, the trend was similar to another population peak in May 1998 (1 to 1.3 leafhoppers/m^2). The two areas also experienced high rainfall.

At the Mituguu site, which is an irrigated area, the population increase followed the expected pattern—low population at the beginning of the long rains and a peak at the end of the rainy season (1.3 hoppers/m^2) in August. The population then declined until December 1998 and started rising again.

At Mtwapa in the coastal region, the trend was somehow oscillatory, with the highest density in the middle of the long rains in April, September, and December 1998. Population trends for Mwea started rising in January and peaked in February, which marks the real dry period. The population declined and had a small peak again in May. This meant that, as expected, the population increased during the rainy period.

At all sites, however, population trends were affected by increased rainfall due to El Niño. In some cases, flooding of sampling areas reduced insect numbers.

Species composition. Data on species composition and mean leafhopper density across different agroecozones are shown in Table 2. Six species of *Cicadulina* were recorded. These were *C. bipunclata, C. chinai, C. ghauli, C. mbila, C. latens,* and *C. storeyi. C. mbila* was found in all zones,

Table 2. Species composition and density of Cicadulina

Site	Species	Density/m^2
Lake Region		
Oyugis	*C. storeyi* *C. chinai*	0.2
Mbita	*C. chinai* *C. storeyi* *C. mbila*	2.0
HomaBay	*C. storeyi*	2.0
Western Region		
Kitale	*C. mbila*	0.2
Amagoro	*C. mbila*	0.7
Kimilili	*C. mbila*	0.8
Centralregion		
Mitunguu	*C. mbila* *C. ghauri* *C. bipunctata*	2.3
Jamaica	*C. mbila* *C. bipunctata*	2.9
Kagumo	*C. mbila* *C. bipunctata*	2.1
Embu	*C. mbila*	1.7
Nyeri	*C. mbila*	0.6

while *C. chinai* and *C. bipunctata* were only found in the Lake region (Table 2).

Samples from the Coast were not identified because only females were caught and they died before reaching the laboratory (Table 2). The mean population density for all species ranged from 0.2 hoppers/plant in the lake region (Table 2) to 2.9 hoppers/m^2 in the central zone (Jamaica site) (Table 2). *C. mbila* was the dominant species in all other areas except the lake region, where *C. chinai* was dominant.

The differing composition of species in all zones explains the different levels of MSV in different areas. The central region has the highest insect density and is a hot spot for MSV, which is not surprising since high *C. mbila* density is associated with high disease incidence (Table 2).

Relationship between maize invasion and alternative hosts. The spatial distribution of MSV-infected plants and the infestation percentage in maize fields surrounded by *Setaria sphacelata* was not correlated.

Efficiency of MSV transmission by vectors from different agroecozones. Results indicate that species from different ecologies might differ in their transmission efficiency. It is evident that in all tests conducted, the *C. mbila* and *C. storeyi* collected from the lake region (Oyugis and Mbita) were more efficient in transmission than the *C. mbila* collected from the western region (Kitale). Which further explains the disparity observed in streak incidence between the two areas.

Additional data on the alternative breeding hosts of *Cicadulina* spp. indicated that they inhabit different wild grasses. Nymphs were recorded on several grasses, *Setaria sphacelata,* var. *splendida, Echinochloa spyramidalis* (Lam), *Hyparrhenia rufa, Setaria incrassata* (Hochst), and *Sorghum vulgare.*

Discussion

Population dynamics of *Cicadulina* leafhoppers was dependent on the moisture available in different agroecological zones. The apparent decrease in leafhoppers at Mitunguu irrigated area during the usual wet season of April to May could be explained by the drought between the presumed long rains and the onset of irrigation.

Results from Mwea rice irrigation area indicated that the draining of water during harvest also reduced leafhopper population. Wild grasses, which could serve as alternative hosts also dried up due to lack of paddy water. Rose (1972 and 1973) suggested that leafhoppers migrate at the onset of the dry season to areas with fresh grasses.

The study also confirmed reports by Rose (1978) and Webb (1987) that *C. mbila, C. chinai,* and *C. bipunctata* occur in Kenya. *C. mbila*, which is the most effective transmitter of MSV, formed the largest percentage except in the lake region, while the central highlands had the highest density. *C. storeyi* was the dominant species in the lake region.

Field testing of F_3 parental lines from crosses made using MSV-resistant and local MSV-susceptible germplasm and *Cicadulina vector*.

The average density was between 0.2 to 2.9 hoppers/m^2.

Future Plans for Vector Ecology and Epidemiology

Factors contributing to vector and MSV outbreaks are not yet well understood, and there is a need for more studies on the spatial and temporal distribution of leafhoppers. More data are also needed on environment variables, cropping pattern, vector density, disease incidence, severity, and prevalence.

There is also a need to clearly establish the relationship between vector migration and disease outbreaks, while the use of DNA markers will help in detecting genetic variability. Future plans also include detecting other viral diseases and vectors, documentation of alternative reservoirs, and developing MSV prediction models.

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3.1.3 Discussions, Unanswered Questions

Prof. Peter Markham gave two presentations: "Leafhopper or vector feeding behavior" and "MSV transmission and agroinoculation." His papers left participants with more questions than answers, which indicates just how much more there is to learn about the virus, its vector, and maize crops.

In the first presentation he reminded the participants that only a handful of insect species are involved in disease transmission. Also, when it comes to MSV, which is indigenous to Africa, we may need to look at indigenous crops that are resistant to MSV, such as sorghum, to better understand resistance mechanisms.

He then described how the virus is transmitted. MSV creates "ideal" conditions that facilitate its spread by the vector *Cicadulina*. It uses the insect as a vehicle, hijacking the bug for a lift from plant to plant. In fact, MSV-infected maize even tends to have a yellowish color that attracts the vector: "It is like the virus is calling the insect vector to come and pick it up. It is as if the insect is under the influence of the virus because it is delivered in the maize plant's phloem where it can be easily circulated into plant cells or various appropriate tissues." The insect picks up the virus from the mesophyll, which it then infects via the phloem. Within 6 h it is ready to be delivered. Everything happens so quickly that within 6 h the virus can be transmitted again by the insect vector. The effects, as everyone knows, are very bad: maize plants attacked at the sixth leaf or earlier have significantly reduced leaf area and plant height, while grain yields decline by 90%.
He explained that the vector picks up many MSV viruses and not individual or single ones. Since MSV does not replicate in the insect—as declining viral titers indicate—the amount of virus taken up by the insect tends to last a lifetime.

The relationship between MSV and the host or insect vector, *Cicadulina* spp., is very complex and there is much more to learn. We must go forward in a zigzag course and change our ideas when necessary. For now, not answers but questions structure the field. Why are MSV epidemics increasing? What is tolerance? Is tolerance in other plants funneling the virus into maize plants? Should we be looking for immunity? What obstacles are there to finding immunity? We know that maize did not come from Africa, yet it has genes that are resistant to MSV. Are there some overlapping resistance genes in cereals or grains? What can we learn about this in the last 60 years of plant research? Does it provide the information that breeders are looking for?

Dr. Markham explained that the Green Revolution left all genes for resistance to insects untouched and that there was considerable potential here. In the meantime, he suggested that we try to better understand the insect vector. Can we stop the insect from feeding on maize crops? What is the source of the insect? Can we predict an outbreak or when it is preparing to migrate by looking for changes in its physiology, morphology, or chemistry? Which species move locally or migrate long distances? What are the prime ecological zones that the insect vector favors?

He said that when the project was conceived it tried to do something more ambitious, something sustainable. MSV is an African problem that Africans are going to solve. The infrastructure benefits are too great and the potential technologies too valuable for such research to be a "pack and go" type of activity.

Finally, Dr. Markham praised the project's attempts to improve sustainable agriculture and regional collaboration. As he observed, when individuals work together they can obtain answers to shared questions and find missing links in research more quickly while avoiding the pursuit of dead-end inquiries. Everyone benefits from less waste and faster research. Collaboration also makes it easier to seek more support or funding. He urged all to work carefully but boldly, to identify questions whose answers will make an impact, and to think of what could be done with available methodologies. Much training and technology has been transferred, and it must not go to waste. Although progress has been made, we still face the challenge of predictably controlling MSV. Furthermore, there is a large pool of viruses out there ready to mutate, and in the future even immune lines may break down. Our work in biotechnology training has been a success, but this is no time to sit on our laurels. We must all strive to keep up with new developments and opportunities.

3.2 Disease and Epidemiology

3.2.1 Introduction and Overview

MSV disease is characterized by chlorotic lines along veins of maize leaves. In highly susceptible varieties, chlorotic streaks tend to coalesce into uniformity, which frequently results in death. The disease has been observed in many weeds and crops belonging to the family *Graminae*. In a study entitled "Variation of MSV in Kenya," Dr. Jackson Njuguna studied and assessed levels of MSV resistance in maize germplasm for use in national maize breeding programs.

Dr. Njuguna and his team studied the biological difference of MSV isolates in the highlands east of the Rift Valley with bimodal rainfall, the highlands west of Rift Valley with a unimodal rain pattern, the Lake Basin with its bimodal rain pattern, and the Coastal lowlands with bimodal rainfall. Their work showed that:

- Both CIMMYT and CIRAD have maize germplasm with very high resistance to MSV.

CIRAD390 and OSU23I from CIMMYT demonstrated a high level of MSV resistance. These were immune to MSV in most locations. The two, however, also succumbed to MSV in some locations.
- There are at least two MSV biotypes in Kenya. The different MSV biotypes are not confined to specific ecology but appear to be spread all over.
- PANNAR hybrid 5195 appeared to perform very well in most locations in its MSV resistance and adaptability.
- Glasshouse screening matched field results.

Dr. Njuguna recommended that farmers grow PANNAR hybrid 5195 to avoid heavy losses (see tables provided in Dr. Njuguna's paper, section 3.2.2). He also pointed out that there is 98% homology in the genomes of MSV isolates from Nigeria, South Africa, and Kenya. But Dr. Martin Darren from the University Cape Town urged workshop participants to remember that in the MSV replication process the best gene or gene products can serve the whole community: "A virus does not use only its own gene product. It also uses those of all the other viruses in the cell, and one extremely good gene product may, for example, encapsulate all other viruses." He stated that a difference of 2.5% in the genome of different virus strains was insignificant.

3.2.2 Studies on the Variation of Maize Streak Virus in Kenya (Dr. J.G.M. Njuguna)

The primary purpose of this study was to examine the biological differences of MSV in Kenya's different ecological zones. Four different ecological zones were identified:

1. Highlands east of Rift Valley in a bimodal rainfall pattern
2. Highlands west of the Rift Valley with unimodal rainfall pattern
3. Lake Basin with bimodal rainfall pattern.
4. Coastal lowland ecology with a bimodal rainfall pattern.

The other purpose was to assess levels of MSV resistance in maize germplasm gathered from diverse breeding programs for potential use in national maize breeding programs.

KARI, CIMMYT, and Rockefeller Foundation representatives at a multilocation trial using MSV-resistant lines.

Materials and Methods

Streak-resistant maize germplasm was obtained from five different programs: the Summer Grains Institute, CIMMYT, IITA, CIRAD, and PANNAR. One or more maize germplasm specimens from these institutes were planted in a national multilocation trial. A total of 18 maize types were planted in each of the 12 locations in the four agroecological zones (Table 3). Of these genotypes, two were susceptible to MSV. The remaining 16 were resistant or tolerant to MSV (see Table 4 for details about the maize germplasm and their sources of origin). During the planning of the experiment, it was hoped that a significant MSV epidemic would break out at each of the 12 locations and that one or more types of the streak-resistant maize germplasm would serve as a differential among locations. This could help reveal whether MSV varied in different locations.

Table 3. Description of multilocation trial sites (12)

Name	Description
Jamaica	Cool wet highlands bimodal rainfall
Mucatha	Cool wet highlands bimodal rainfall
Githunguri	Cool wet highlands bimodal rainfall
Kagumo	Cool wet highlands bimodal rainfall
Kitale	Cool wet highlands unimodal rainfall
Kiminini	Cool wet highlands unimodal rainfall
Amagoro	Lake basin bimodal rainfall
Oyugis	Lake basin bimodal rainfall
Mtwapa	Hot humid coastal lowlands
Mtepeni	Hot humid coastal lowlands
Nyeri (FTC)	Cool wet highlands

Table 4. Description of the 18 maize genotypes selected for the multilocation trial

Pedigree	Description	Source
VHCY	OPV	S. Africa
VHCW	OPV	S. Africa
AO89	Inbred line	PANNAR
AO76	Inbred line	PANNAR
HASR	OPV	Burundi
CML 202	Inbred line	CIMMYT
CML 197[MSRXPOOL 9]	Inbred line	CIMMYT
CIF2-205-1)osu23l)	Inbred line	CIMMYT
Pool 9A BSAF3	Population	CIRAD
[MSR/Pool 9A]C2F5	OPV	CIMMYT
Tzi3	Inbred line	IITA
KU144SR/(Tzi35)	Population	IITA
ACR TZE-MSR-W	Population	IITA
TZE MSR-W-F	Population	IITA
Embu 11	Population	Kenya
CIRAD390	Inbred line	CIRAD
PANNAR 5195	Hybrid	PANNAR
Local check	Hybrid	Kenya

The local check varied from location to location and was usually a Kenyan commercial hybrid popular in the specific trial area. These included the following hybrids: Hybrid 614, Hybrid 512, Hybrid 511, Hybrid 622, and Pwani Hybrid.

All local checks were known to be streak-susceptible germplasm. The local check was also planted around the experimental plots to serve as guard and MSV spreader rows. The experimental design used in each location was a randomized complete block design with two replicates. The experimental units were 3-m-long single rows, with 75 cm between rows and 30 cm within rows. Two seeds were planted per hill and later thinned to one plant per hill. Diammonium phosphate (DAP) fertilizer was applied (250 kg/ha) during planting. Fields were also topdressed with calcium ammonium nitrate (CAN) at 250 kg/ha 7 weeks after planting.

The crop was exposed to natural sources of MSV inoculum and other diseases. Records were taken on the incidence and severity of MSV at each location. Notes were taken on the adaptability of the exotic streak-resistant germplasm and their reactions to other diseases.

A parallel experiment was set up in the glasshouse to gain a better understanding of the level of MSV resistance in various genotypes listed in Table 4. Viruliferous *Cicadulina mbila* was used to screen the exotic germplasm by determining MSV resistance levels in a controlled environment.

Nonviruliferous *C. mbila* was caged on healthy pearl millet *(Pennisetum americunum*) in an insect-proof glasshouse maintained at approximately 25 °C. Pearl millet was planted in a steam-sterilized potting mixture contained in plastic pots 12 cm in diameter and 12 cm in height. When the pearl millet dried up or became unsuitable for oviposition, it was gradually replaced with younger seedlings, 4-6 in tall. Maize seedlings for glasshouse screening were similarly raised. Five seeds were planted per plot and then inoculated soon after emergence.

Acquisition access period (AAP). At 48 h, nonviruliferous leafhoppers (about 30-40) were given AAP on MSV-infected maize or wild grasses. In most cases, detached MSV-infected leaves, rather than an intact plant were used as the virus

source. The distal portions of leaves were inserted into a glass cage 2.5 cm in diameter and 20 cm in height. The distal end of the glass cage was sealed with fine muslin cloth (secured with a rubber band) to allow air circulation. MSV-infected maize leaves (Muguga isolate) were usually secured at the other end of the cage by a cotton wool plug. The basal parts of MSV-infected leaves were immersed in a beaker of water to maintain turgidity during AAP.

Inoculation access period (IAP). Five viruliferous leafhoppers were given a 7-day IAP on each lot of test maize seedlings. Cellulose acetate cages (10 x 30 cm) were used for IAP because they allowed five or more maize seedlings to be inoculated with five leafhoppers. Records were made on the incidence and severity of MSV in each maize genotype 30 days postinoculation period (PIP). A graded scale of 0-5 was used to assess the severity in each maize genotype: 0 = no streaking, 1 = very few streaks, 2 = light streaking, 3 = moderate streaking, 4 = severe streaking on at least 60% of leaf area and 5 = severe streaking on 75% of leaf area or more plus stunting (Mesfin et al 1992).

Results

Maize streak disease incidence was very low during the three seasons in which the experiment was carried out. The exception was Jamaica (location #1), one of four sites in the highlands east of the Rift Valley. Table 5 summarizes disease incidence in each of the 10 locations over the three seasons. Of the 10 locations planted in the first season (1997 long rain) there was streak disease incidence at four sites but very little in four others. At Mitunguu (location #11), disease incidence was recorded in only five genotypes. Reasonable MSV disease was recorded in Jamaica; incidence ranged from 100% to 0% (Table 5). Although an MSV incidence of 0% was recorded in the maize genotype CIRAD 390 at Jamaica (location #1), disease incidences of 3% and 1% were recorded in the same genotype at Muchatha (location #2) and Githunguri (location #3), respectively.

While no MSV incidence was recorded in the genotype [MSRxPooL 9]CIF2-205-1(OSU23I) at Jamaica (location #1), during the 1997 long rain season, disease incidence of 1% was recorded in the same genotype at Mitunguu in the same season.

During the 1997 short rain season, the multilocation experiment was conducted in five locations. MSV incidence was recorded at all sites, but the highest MSV disease incidence was recorded at Jamaica, ranging from 88% in the local check to 0% in CIRAD 390. But disease incidences of 2% and 6% were recorded in the same genotype (CIRAD 390) at Kagumo (location #4) and Oyugis (location #8), respectively. During this season MSV disease incidences of 1% and 4% were recorded on genotype [MSRxPooL 9]CIF2-205-1(OSU23I) at Jamaica and Kagumo (Table 5).

During the 1998 long rain season, the multilocation experiment was planted at nine locations. There was no MSV incidence in two locations (Table 5). While MSV disease incidence increased at Muchatha (location #2), it decreased in Jamaica. Disease severity in the 18 entries of Jamaica (Table 6) is typical of other locations, except that CIRAD 390 was rated immune at this location with a score of 0 and incidence of 0%. In at least four locations, however, CIRAD 390 succumbed to an MSV biotype, although MSV symptoms on CIRAD 390 were very mild (a score between 1.5 and 2.0). Similarly, in three locations where [MSRxPooL 9]CIF2-205-1(OSU23I) succumbed to the MSV biotype, symptoms were mild (scores between 1.5 and 2.0).

Glasshouse screening was routinely conducted using a Muguga isolate of MSV. In cases where the test material proved immune, MSV isolates from the Coast and Lake Basin regions were used to reconfirm findings. Results of glasshouse screening presented in Table 7 appear to confirm the data from multilocation trial site # 1 (Jamaica): the two immune genotypes were not escapes but indeed immune to the local MSV biotype. The immune line from CIMMYT [MSRxPool 9]CIF2-205-1(OSU23I) was later screened in the glasshouse with MSV isolates from Oyugis, Amagoro, and Mtwapa. The isolates from Oyugis and Mtwapa caused mild streak symptoms (score 1.5). The isolate from Amagoro, however, did not induce streak symptoms on this genotype.

Other diseases recorded in the multilocation experiment included sugarcane mosaic disease, common rust caused by *Puccinia sorgi, Turcicum blight* caused by *Exserohilum turcicum*, common smut caused by *Ustilago maydis*, and head smut caused by *Sphacelotheca reiliana*. Sugarcane mosaic virus (SCMV) was the most severe on C390 (CIRAD 390) in all locations. Common rust and polysora rust (in the coast) were common in most genotypes except in CIMMYT inbred lines CML202

Table 5. Disease incidence (%) in 12 locations

Entry	1	2	3	4	5	6	7	8	9	10	11	12
1997 long rains												
VHCY	82.8	0.0	0.0	-	0	0	0	0	0.0	-	0.0	0
VHCW	76.0	0.0	0.0	-	0	0	0	0	0.0	-	3.0	0
AO89	98.3	0.0	0.0	-	0	0	0	0	0.0	-	0.0	0
AO76	88.4	0.0	0.0	-	0	0	0	0	0.0	-	0.0	0
HASR	91.7	0.0	0.0	-	0	0	0	0	1.3	-	0.0	0
CML202	91.3	0.0	0.0	-	0	0	0	0	0.0	-	0.0	0
CML197	100.0	0.0	0.0	-	0	0	0	0	0.0	-	0.0	0
[MSRxPool 9]CIF2-205-1 (OSU23I)	0.0	0.0	0.0	-	0	0	0	0	0.0	-	1.3	0
Pool 9A BSAF3	6.0	0.0	0.0	-	0	0	0	0	0.0	-	1.3	0
[MSR/Pool 9A]C2F5	48.0	0.0	0.0	-	0	0	2	0	0.0	-	0.0	0
Tzi3	94.0	0.0	0.0	-	0	0	0	0	0.0	-	0.0	0
KU144SR/(Tzi35)	100.0	0.0	0.0	-	0	0	0	0	0.0	-	0.0	0
ACR TZE-MSR-W	91.1	0.0	0.0	-	0	0	0	0	0.0	-	0.0	0
TZE MSR-W-F	93.6	0.0	0.0	-	0	0	0	0	0.0	-	3.0	0
Embu11	98.0	0.0	0.0	-	0	0	0	0	0.0	-	1.1	0
CIRAD 390	0.0	3.0	1.0	-	0	0	0	0	0.0	-	0.0	0
PANNAR 5195	93.8	0.0	0.0	-	0	0	0	0	0.0	-	0.0	0
Local check	98.5	0.0	0.0	-	0	0	0	0	2.0	-	0.0	0
1997 short rains												
VHCY	15.3	0.0	-	14.5	-	-	1.4	1.3	-	-	0.0	-
VHCW	19.7	0.0	-	3.6	-	-	0.0	9.2	-	-	0.0	-
AO89	11.5	0.0	-	11.2	-	-	0.0	4.6	-	-	0.0	-
AO76	21.5	0.0	-	5.7	-	-	3.1	0.0	-	-	0.0	-
HASR	31.6	0.0	-	16.6	-	-	3.9	3.0	-	-	0.0	-
CML202	18.8	0.0	-	2.2	-	-	1.4	3.1	-	-	0.0	-
CML197	59.5	0.0	-	1.8	-	-	5.0	9.2	-	-	0.6	-
[MSRxPool 9]CIF2-205-1 (OSU23I)	1.1	0.0	-	3.7	-	-	0.0	0.0	-	-	0.0	-
Pool 9A BSAF3	3.0	0.0	-	0.0	-	-	0.0	0.0	-	-	0.0	-
[MSR/Pool 9A]C2F5	17.2	0.0	-	0.0	-	-	9.0	2.7	-	-	0.0	-
Tzi3	16.7	4.1	-	6.9	-	-	0.0	3.3	-	-	0.0	-
KU144SR/(Tzi35)	27.2	0.0	-	13.3	-	-	0.0	2.8	-	-	0.0	-
ACR TZE-MSR-W	20.2	0.0	-	27.3	-	-	0.0	3.0	-	-	0.0	-
TZE MSR-W-F	31.5	0.0	-	9.2	-	-	0.0	7.5	-	-	3.0	-
Embu11	85.1	1.2	-	12.6	-	-	7.1	10.4	-	-	0.0	-
CIRAD 390	0.0	3.0	-	2.0	-	-	0.0	5.7	-	-	0.0	-
PANNAR 5195	10.0	0.0	-	6.2	-	-	0.0	3.1	-	-	0.0	-
Local check	88.2	0.0	-	15.2	-	-	9.5	11.0	-	-	0.6	-
1998 long rains												
VHCY	7.7	0.9	-	0.0	0	0	0.0	0	3.9	-	0.0	-
VHCW	6.1	0.0	-	1.1	0	0	0.0	0	0.0	-	2.3	-
AO89	8.1	48	-	0.0	0	0	4.5	0	0.0	-	2.0	-
AO76	3.1	2.9	-	0.0	0	0	1.4	0	0.0	-	0.0	-
HASR	6.5	0.0	-	3.0	0	0	4.3	0	0.0	-	0.0	-
CML202	0.0	1.0	-	3.7	0	0	1.4	0	0.0	-	0.0	-
CML197	4.1	0.93	-	16.9	0	0	0.0	0	39.2	-	0.0	-
[MSRxPool 9]CIF2-205-1 (OSU23I)	0.0	0.0	-	0.0	0	0	0.0	0	0.0	-	2.5	-
Pool 9A BSAF3	0.0	0.0	-	0.0	0	0	0.0	0	0.0	-	2.5	-
[MSR/Pool 9A]C2F5	2.8	1.9	-	2.1	0	0	1.5	0	0.0	-	0.0	-
Tzi3	8.9	14.7	-	5.9	0	0	8.9	0	0.0	-	0.0	-
KU144SR/(Tzi35)	17.0	0.0	-	3.9	0	0	0.0	0	11.8	-	0.0	-
ACR TZE-MSR-W	12.6	1.0	-	7.6	0	0	1.3	0	5.1	-	0.0	-
TZE MSR-W-F	13.1	1.0	-	0.0	0	0	0.0	0	9.8	-	3.0	-
Embu11	13.2	12.6	-	23.9	0	0	4.0	0	62.5	-	1.1	-
CIRAD 390	0.0	0.0	-	2.2	0	0	0.0	0	0.0	-	0.0	-
PANNAR 5195	6.6	0.96	-	28.3	0	0	0.0	0	31.5	-	0.0	-
Local check	23.3	59.0	-	1.6	0	0	10.1	6.8	28.6	-	0.0	-

Table 6. Maize streak disease incidence and severity on 18 maize genotypes planted in Jamaica, 1997 long rains

Entry	Source	Plants established (no.)	% MSV incidence	Severity score
VHCY	S. Africa	58	82.8	3.0
VHCW	S. Africa	68	76.0	3.0
AO89	PANNAR	59	98.3	3.0
AO76	PANNAR	43	88.4	2.0
HASR	Burundi	60	91.7	1.5
CML202	CIMMYT	46	91.3	1.5
CML197	CIMMYT	35	100.0	2.5
[MSRxPool 9]CIF2-205-1 (OSU23I)	CIMMYT	70	0.0	0.0
Pool 9A BSAF3	CIRAD	66	6.0	1.0
[MSR/Pool 9A]C2F5	CIMMYT	75	48.0	2.5
Tzi3	IITA	67	94.0	3.0
KU144SR/(Tzi35)	IITA	56	100.0	3.5
ACR TZE-MSR-W	IITA	76	91.1	2.0
TZE MSR-W-F	IITA	63	93.6	2.0
Embu11	Kenya	64	98.0	5.0
CIRAD 390	CIRAD	43	0.0	0.0
PANNAR 5195	PANNAR	64	93.8	3.0
H614	Kenya	68	98.5	5.0

Table 7. Glasshouse screening of 18 maize genotypes intended for multilocation trial using the Muguga isolate of MSV

Entry	Source	Plants established (no.)	% MSV incidence	Severity score
VHCY	S. Africa	24	8.3	4.0
VHCW	S. Africa	24	42.6	3.0
AO89	PANNAR	21	80.9	3.0
AO76	PANNAR	14	71.4	3.0
HASR	Burundi	11	81.8	2.5
CML202	CIMMYT	-	-	-
CML197	CIMMYT	17	100.0	3.0
[MSRxPool 9]CIF2-205-1 (OSU23I)	CIMMYT	20	0.0	0.0
Pool 9A BSAF3	CIRAD	23	34.8	2.0
[MSR/Pool 9A]C2F5	CIMMYT	20	35.0	2.5
Tzi3	IITA	19	100.0	2.0
KU144SR/(Tzi35)	IITA	-	-	-
ACR TZE-MSR-W	IITA	17	29.4	3.0
TZE MSR-W-F	IITA	16	93.8	3.0
Embu11	Kenya	-	-	-
CIRAD 390	CIRAD	60	0.0	0.0
PANNAR 5195	PANNAR	-	-	-
H614	Kenya	-	-	-

and [MSRxPool 9]CIF2-205-1(OSU23I). The PANNAR hybrid was well adapted in most locations. Perhaps the most important finding is that all the streak-resistant maize germplasm from all five sources were indeed resistant to MSV in Kenya, but their level of resistance varied from one genotype to the other.

Discussion and Conclusions

The data indicated that MSV disease was actually absent in many of the 12 locations. However, conclusions can be drawn from Jamaica (location #1) and two others. Both maize genotypes CIRAD 390 and [MSRxPOOL 9]CIF2-205-1(OSU23I) demonstrated high levels of MSV resistance. They

were immune to MSV in most locations. This agrees with data from glasshouse screenings using a Muguga isolate of MSV. Since these two genotypes succumbed to MSV in some locations but not in others, it can be concluded that at least two biotypes of MSV occur in Kenya, one more virulent than the other. This observation is similar to that of Bock (1975) and Njuguna (1996). The glasshouse screening appears to agree with the field data, suggesting that MSV isolates from some locations were more virulent than others. For example, both Amagoro and Oyugis in the lake ecology had MSV isolates of differing virulence. But contrary to earlier reports by these two authors, the different MSV biotypes are not confined to specific regional ecologies but appear throughout Kenya.

Both CIRAD and CIMMYT germplasm can be made available to the national maize breeding program at no cost to MSV resistance breeding. This will be our best source of resistant germplasm. Finally, with regard to its MSV resistance and adaptability, PANNAR hybrid 5195 appeared to perform very well in most locations. For this reason, Kenyan farmers should be encouraged to grow this variety to avoid heavy losses associated with the MSV epidemic.

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3.3 Breeding and Biotechnology

3.3.1 Overview

This session's presentations also attracted much discussion. Maize breeder Dr. J. Ochieng spoke on "Breeding for MSV resistance as a priority in Kenya." He argued that reducing losses in maize productivity through MSV-resistant/tolerant varieties should be the farmers' first line of defense. He reported that the Kenyan team has developed a strategy that harnesses multidisciplinary synergies to produce multiple disease-resistant varieties of maize. This strategy relies on such biotechnologies as marker-assisted selection (MAS), agroinfection, and maize genome transformation.

Spanning a decade, their long-term plan is to use genome mapping and transformation techniques to produce MSV-resistant populations for future inbred lines. Their medium plan (5-10 years) uses agroinoculation to produce MSV-resistant open-pollinated varieties (both synthetics and composites). In the short term, the project will use MAS on MSV-resistant **VC, DC, TWC, TC, and SC** hybrids. The team is awaiting the results of mapping studies to implement MAS and other supportive techniques.

Dr. Ochieng listed several questions for which scientific answers are urgently needed. Why has MSV infection escalated over the years? Are more virulent types linked to mutation, recombination, or migration? Is the emergence of more virulent types linked to the increase in the number of susceptible maize varieties being grown? Dr. Ochieng concluded by praising the efforts of his research team and by reminding the audience of how desperately

Africa's farmers need MSV-resistant maize varieties (for Dr. Ochieng's complete study results, see section 3.3.3).

Workshop participants also eagerly listened about the work of Dr. Jane Ininda and her team on "DNA polymorphism among sources of MSV resistance and disease expression in their F_2/F_3-derived lines." Several participants went on a field trip to see her work, especially at the Muguga Research Center. Dr. Ininda explained that eight varieties of MSV-resistant maize were obtained. CIMMYT provided CML202, CML197, and OSU23I. IITA provided Tzi3 and Tzi35; PANNAR Seeds provided A076. CIRAD supplied C390 and the Grain Crops Institute supplied VHCY.

Her results showed that the highest percentage of highly resistant lines was in the F_3 lines that originated from CIMMYT's OSU23I. At least 46% of OSU23I had high resistance to MSV vs. 26% for CIRAD's C390. Only 2% of IITA's Tzi3 lines was found to be highly resistant. This dropped to 0.01% for CML202 lines from CIMMYT.

Other highlights of Dr. Ininda's work included the findings that 69% of OSU23I lines was resistant vs. 62% of C390 lines. In addition, 42% of CML02 and 40% of Tzi3 lines were grouped as MSV resistant. Finally, trials at Alupe in the Lake Victoria region showed that 38% of OSU23I lines, 28% of C390 lines, 14% of CML202 lines, and 4% of Tzi3 lines were highly resistant (see section 3.3.2 for Dr. Ininda's compete study results).

3.3.2 DNA Polymorphisms among Sources of MSV Resistance and Disease Expression in F_2 and F_3-derived Lines (J. Ininda, J. Snape, and R.O. Opondo)

Introduction

The basic objectives of this study are (1) to evaluate restriction fragment length polymorphism (RFLP) and microsatellite polymorphism among sources of MSV and (2) to evaluate the expression of MSV disease symptoms among F_3 lines of different parental sources. It is hoped that identifying MSV-resistant maize lines will assist Kenya's breeding efforts to fight MSV. This is a top priority program because the country's most popular maize hybrid lacks resistance to MSV.

Resistance against MSV has been identified in various sources. In the early 70s, **IRAT** and KARI researchers discovered that the cultivar "Revolution" from Reunion Island possessed MSV resistance. Today, however, breeding programs have access to and use different sources of resistance. At CIMMYT's Harare station, for example, resistance has been developed from a population ZSR 923 BULK, while the West African-based IITA relies on TZ-Y. France's CIRAD breeders use the CVR-C3 population that goes back to IRAT 297, and in South Africa an unpublished source of resistance has been used to develop various MSV-resistant hybrids.

Our work takes advantage of new technologies to begin to map and characterize different sources of resistance. Various parental sources of resistance have been acquired, and F_3 populations have been derived from them using molecular-mediated mapping.

Materials and Methods

Eight sources of MSV resistance were supplied by various institutions. CIMMYT provided CML202, OSU23I, and CML197. IITA supplied Tzi3, and Tzi35; CIRAD supplied C390. The Grain Crops Institute provided VHCY and PANNAR Seeds donated AO76. These lines were chosen because they were traced back to different sources of MSV resistance. In addition, Kenya provided the susceptible check EM11-133 (Table 8).

In September 1997, initial crosses were made between EM11-133 and each of the sources of MSV resistance. In May 1998, parents were grown in glasshouses at the JIC for DNA extraction.

DNA extraction and RFLP. Fragments produced after DNA digestion with restriction enzymes in any one locus vary in size or length for different individuals. These differences are designated RFLP. In this experiment, equal quantities of leaf tissue from each inbred line were harvested and then lyophilized at –80°C. Plant genomic DNA was extracted using the CTAB technique. All parental lines were analyzed for 36 RFLP probe-enzyme combinations using three restriction enzymes (EcoR1, EcoRV, and HindIII) and 64 pairs of microsatellite primers (see Tables 9 and 10). RFLP probes were obtained from the University of Missouri, Columbia, and were initially chosen to map four chromosomes of the maize genome. Separate digests were performed with EcoR1, EcoRV, and HindIII and then separated on 0.7%

Table 8. Stage of inbreeding and agronomic descriptions of 10 maize lines used to assess RFLP and microsatellite polymorphism

Source	Line	Generation	MSV[a]	Rust[a]	Maturity	Grain color
CIMMYT	CML202	S_4	T	T	Late	White
	CML197	S_4	T	R	Late	White
	OSU23I	S_4	I	T	Mid	White
IITA	Tzi3	S_2	T	R	Mid	Yellow
	Tzi35	S_2	T	R	Late	White
PANNAR	A076	-[b]	T	T	Mid	White
GRAIN	VHCY	-	T	R	Early	Yellow
CIRAD	C390	-	I	R	Early	Yellow
Kenya	EM11-133	S_4	S	R	Late	White
Kenya	E12-210	S_4	S	R	Mid	White

[a] T = tolerant, S = susceptible, I = immune, and R = resistant.
[b] Inbreeding level not available.

Table 9. RFLP probes used in assessing polymorphism

Probe (locus name)	Source type	Insert size(bp)	Polymorphism
bn18.01	UMC[a]	Genomic	2400 abc[b]
Csu3	UMC	CDNA	1200 abc
Csu21	UMC	CDNA	600 abc
Npi262	UMC	Genomic	490 abc
Npi268	UMC	Genomic	670 abc
Npi297	UMC	Genomic	1000 abc
Npi304	UMC	Genomic	1000 abc
Umc10	UMC	Genomic	1100 abc
Umc11	UMC	Genomic	890 abc
Umc29	UMC	Genomic	990 abc
Umc105	UMC	Genomic	610 abc
Umc167	UMC	Genomic	620 abc

[a] UMC, University of Missouri, Columbia.
[b] abc means polymorphism was observed between EM11-133 and CML202, Tzi3, and OSU23I for at least one enzyme.

Agarose gel. Southern blotting, filter hybridization with ^{32}P-labeled probes, and autoradiographing were described by the Cereals Department at JIC. Data were collected for all the probe-enzyme combinations and for those bands that both yielded a clear hybridization signal and showed a clear detection of a band. RFLP bands in the autoradiographs were scored visually on a scale of 1 or 0, where 1 = presence of an allele and 0 = absence of an allele.

Microsatellite (SSR) analysis. Microsatellites are short DNA repeats of two or three base pairs. The alleles in the different genotypes are amplified by PCR and differentiated by the size of the repeat. The size of the microsatellite is a function of copy number of the basic repeat. A search was conducted for maize microsatellite markers, and at least 100 pairs of SSR reverse and forward primers were obtained from Research Genetics, Inc. in Huntsville, Alabama (Table 10). The SSR procedure was a 20 ul PCR reaction mix, containing 20 ng of genomic DNS, 0.1 uM of each primer, 1x reaction buffer, 1 u Boe Taq DNA polymerase, and 2 mM each of dGTP, dTTP, dATP, and dCTP. Samples were covered with 1 drop of mineral oil and subjected to 30 thermal cycles. They were then denatured (1 min, 94°C), annealed (1 min, 56 °C), and extended (1 min, 72°C), which was followed by a final extension step (4 min, 72°C) using a DNA thermal cycler. PCR products were denatured for 5 min at 95°C and then separated on 6% denaturing acrylamide gel with 8M urea at 80W of constant power for 1 h and 15 min. The silver stunning protocol was used and autoradiographs were exposed and scored.

Table 10. Microsatellite primers used in analyzing parental polymorphism, their expected sizes, and annealing temperatures

Locus	Expected size cbp)	Anneal (°C)	Polymorphism[a]
MAC.E00BO3	148	56	ab
MAC.T03B03	158	56	x
MAC.E01CO8	123	56	x
MAC.E01F06	131	56	ab
MAC.T02B10	107	56	a-
MAC.T02H12	138	56	-
MAC.1A01	110	56	xb
MAC.E01C01	95	56	ab
MAC.E0G01	137	56	ab
MAC.T02B08	112	56	ab
MAC.T02E01	147	56	ab
MAG.1A03	104	56	ab
MAC.E01A03	167	56	ab
Bngl107	-	58	-
Bngl108	-	58	ax
Bngl119	-	58	xx
Bngl127	-	61	-
Bngl131	-	61	-
Bngl143	-	61	-
Bngl149	-	61	-
Bngl153	-	61	-
Bngl161	-	55	ab
Bngl166	-	55	ab
Bngl176	-	55	xx
Bngl182	-	55	ab
Bngl236	-	55	-
Bngl238	-	55	ab
Bngl240	-	55	ab
Bngl279	-	55	xx
Bngl603	-	55	-
Bngl609	-	58	xx
Bngl653	-	58	xx
Bngl107	-	58	ax
Bngl391	-	58	-
Bngl426	-	56	ab
Bngl434	-	58	ab
Bngl572	-	58	-
Bngl657	-	58	xx
Bngl119	-	58	-
bngl669	-	58	a-
bngl127	-	58	ax
bngl1292A	-	58	-
bngl430	-	58	-
bngl469A	-	58	a-
bngl619	-	58	-
bngl153	-	58	-
bngl236	-	58	-
bngl640	-	58	a-
bngl420	-	58	-
bngl667	-	58	ab
bngl389	-	58	ab
bngl565	-	58	ab
bngl572	-	58	-
bngl589	-	58	xx

[a] a = polymorphism was observed between CML202 and EM11-133, b = polymorphism was observed between Tzi3 and EM11-133, x = not polymorphic, - = did not amplify.

Degree of polymorphism and levels of heterozygosity. All probes and microsatellite primers were initially screened for all 10 parents to detect the degree of polymorphism between sources of MSV resistance and susceptible parents.

Mapping populations and assessment of disease severity. Of the eight resistant parents, four were chosen to develop mapping populations. By selfing individual F_2 plants, 104 F_2:F_3 lines were obtained from the cross EM11-133/OSU23I and 156 lines from EM11-133/Tzi3. All the F_3 lines were planted in three locations in Kenya. Artificial infestations with viruliferous leafhoppers carrying the Coast virus isolate were done at Mtwapa, Alupe, and Muguga.

MSV severity was rated on 10 individual plants per plot using a scale of 1-5, where 1 = no symptoms on leaves, 2 = light streaking on young and old leaves, 3 = moderate streaks on leaves, 3-4 = moderate streaks and some stunting, 4 = severe streaking on 60% of leaf area, 4-5 = severe streaking of 75% of leaf area, 5 = severe streaking on >75% of leaf area.

Results and Discussion

RFLP and Microsatellite Polymorphisms among Sources of Maize Streak Virus Resistance

Genetic variation for RFLPs among parents. An autoradiograph was obtained after analyzing the parents for polymorphism with three radioactive labeled probes. Polymorphism was observed for at least one probe/enzyme combination for any two parents. Some probe-enzyme combinations showed more RFLP variants than others. For example, umc167 showed only 4 alleles while umc10 showed polymorphism between EM11-133 and CML202, Tzi3, C390, and OSU23I (Fig. 1). This suggests that the use of EcoR1 may expose greater polymorphisms among parents.

Genetic variation for SSRs among the parents. Genetic variation for microsatellites (Figs. 2 and 3) shows two extreme patterns obtained for two different loci amplified. A total of 54 microsatellite primers were assayed, and 35 gave detectable PCR (polymerase chain reaction) products and/or polymorphisms (Table 10). Unfortunately, difficulties in establishing the right PCR conditions for DNA amplifications in our genotypes limited work progress. But our study

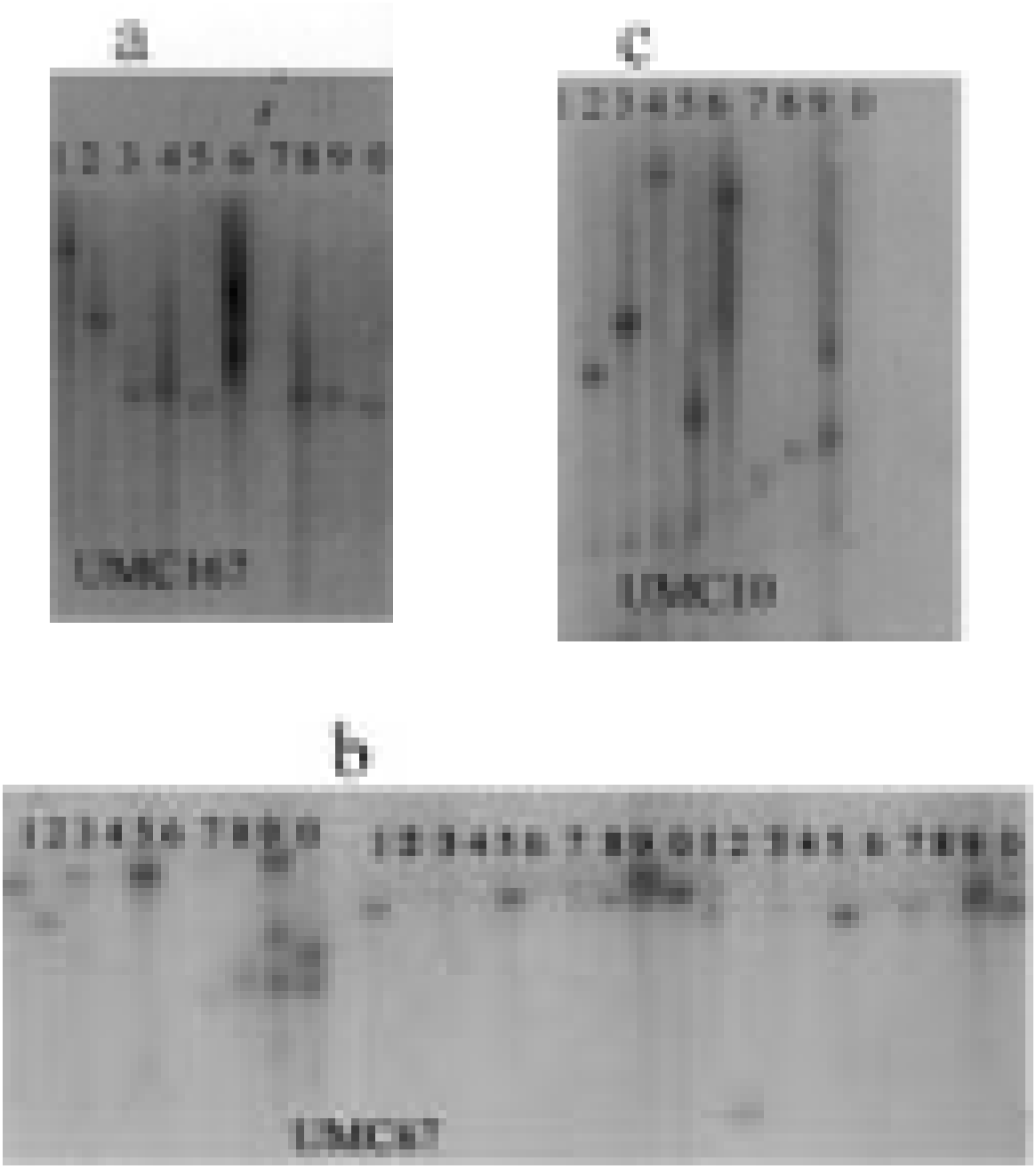

Fig. 1. Pattern of RFLP bands obtained from 10 parents by labeling probes with radioactive ^{32}P

1 = A076, 2 = C390, 3 = CML197, 4 = Tzi3, 5 = CML202, 6 = EM11, 7 = EM12, 8 = OSU23I, 9 = VHCY, and 10 = Tzi35
a: Nearly monomorphic bands of umcl167 for DNA digested with HindIII, b: Polymorphic bands of umc67 for digests of EcoR1, EcoRV, and HindIII, c: Polymorphic bands of umc10 for DNA digested with HindIII

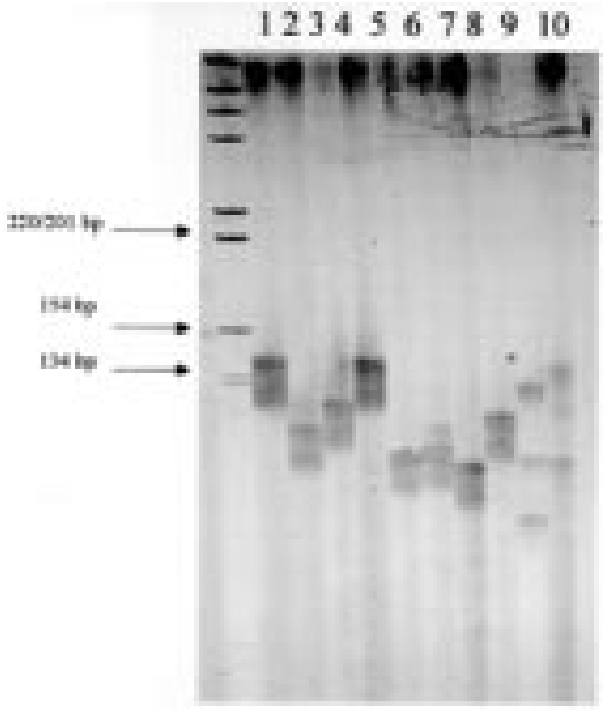

Fig. 2. Pattern of microsatellites showing polymorphism among 10 parents by using silver staining

1 = A076, 2 = C390, 3 = CML 197, 4 = Tzi3, 5 = CML202, 6 = EM11-133, 7 = EM12-210, 8 = OSU23I, 9 = VHCY, and 10 = Tzi35

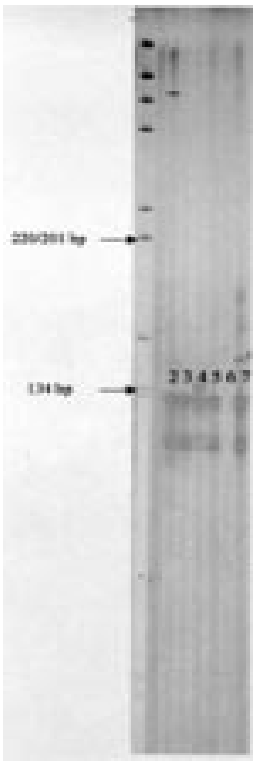

Fig. 3. Pattern of microsatellite locus showing nearly monomorphic bands in 5 lines

2 = C390, 3 = CML197, 4 = Tzi3, 5 = CML202, and 7 = EM12

also showed that there is adequate polymorphism for both RFLP and microsatellite markers between at least two sources of MSV resistance (CIMMYT and IITA) and the susceptible Kenyan inbred lines of EM11-133 to enable screening of the mapping population for DNA markers.

Expression of Disease Symptoms among F_3 Lines of Different Parental Sources of MSV Resistance

Results indicate that the probability of obtaining highly resistant MSV lines is higher when the source of resistance used for conversion possesses immunity to MSV (Table 11). The highest percentage of highly resistant lines was found in F_3 lines originating from OSU23I. In this population, 46% of the lines had high resistance to MSV at Muguga, while lines possessing C390 as a source of resistance had 26% highly resistant lines. In addition, 69% of the lines from OSU23I, 62% from C390, and 42% from CML202 were classified as

Table 11. Percentage of lines in each disease category for each mapping population

MSV Score/ Class	EM11-133/ C390	EM11-133/ OSU23I	EM11-133/ CML202	EM11-133/ Tzi3
Muguga				
1.0-2.0	26.3	46.2	0.01	2.2
2.1-3.0	35.3	23.1	42.0	38.0
3.1-4.0	26.3	14.4	46.4	55.5
4.1-5.0	12.1	16.3	11.6	4.3
Alupe				
HR[a]	28.2	37.3	13.7	4.4
R	24.3	32.5	73.9	42.2
S	47.5	29.2	11.8	53.3

[a]HR = highly resistant, R = resistant, and S = susceptible.

resistant. CML202 and Tzi3 with high resistance to MSV were fewer: 0.01% and 2.2%, respectively. But a greater percentage of their lines fell in the middle category. Lines with the OSU23I and CML202 sources were found to be adapted and resistant to other common diseases, particularly leaf rust, gray leaf spot, and *turcicum* blight at Muguga (data not shown). This suggests that OSU23I or CML202 have stronger resistance to MSV.

The same pattern of segregation was observed at Alupe using the Lake region isolate. A larger percentage of lines from OSU23I and C390 were in the highly resistant class compared with the other two sources (Table 11). However, 74% of the lines derived from the MSV source CML202 fell in the resistant or tolerant category. Furthermore, 42% of the lines from the Tzi3 source also fell in this category. In both Muguga and Alupe, the largest number of lines that showed susceptibility to MSV were from Tzi3: 56% of the lines were susceptible to MSV at Muguga and 53% were susceptible at Alupe.

3.3.3 Breeding for MSV Resistance as a Priority in Kenya (J.A.W. Ochieng)

A survey conducted on maize farms in 34 districts in Kenya by Ray Louie in 1978 revealed low and sporadic incidence of maize streak virus disease (MSVD). A repeat survey 10 years later by Theuri, however, indicated a remarkable (4-80-fold) increase in MSD incidence in five districts of Kenya's Central Province (Table 12). Similarly, a 1998 survey of 10 farms in southwest Kenya showed a high incidence of 80-100%.

Some pertinent questions to ask about these changes are: Why has there been an escalation in MSD incidence over the years? Was it due to new and more virulent MSV pathotypes coming to the fore? Or was it a case of more MSD-susceptible maize varieties being grown by farmers? If it is a case of new MSV strains, are these pathotypes the result of (gene) mutation, migration, or genetic recombination? It is hoped that research scientists elsewhere have unraveled these intricacies. If not, then Kenyan breeders will have to figure out the causes of the haphazard reactions of maize genotypes (varieties) to MSD over time and space. We urgently need this information to plan more viable breeding programs that will develop useful products (cultivars) for our target farmers.

Table 12. Distribution of maize streak virus (MSV) disease (% incidence), Kenya in 1988

Province	District	1977	1978	1988	
				Site	
				1	2
Central	Nyandarua	0.0	0.0	-	-
	Nyeri	11.6	8.2	36	1
	Kirinyaga	1.0	7.3	80	0
	Muranga	-	1.3	82	5
	Kiambu	1.6	9.6	87	13

Source: J.M. Theuri (1988).

Breeding strategies. Past research efforts to develop MSV-resistant maize varieties in Kenya were based on crossing and backcrossing using "Hickory King" and "Reunion Revolution" varieties as putative sources of resistance. But there were little tangible benefits from this early research effort. Recent reports show an increasing incidence of MSVD in Kenya, with infection rates as high as 70-80% in the Central highlands (Theuri and Njuguna 1988).

A conventional breeding solution is envisaged in the Coordinated Ecosystem Breeding (CEB) strategy launched by the maize breeding team of KARI in 1999 (Table 13). The breeding plan is based on backcrossing, followed by selection. It uses reputed exotic sources of MSV resistance in crosses with adapted breeding stocks and capitalizes on genetic complementarity. The CED strategy is designed to harness multidisciplinary synergy to develop multiple disease-resistant varieties of maize.

Even biotechnological approaches, such as using marker-assisted selection, agroinfection via *Agrobacterium tumefaciens*, and maize genome transformation are seen as candidate methodologies. Supporting research thrusts include MSD epidemiology, characterization of MSV isolates from various parts of Kenya, and studies on insect feeding behavior. Susceptibility reactions among maize genotypes will aid in crafting viable breeding plans. The overall goal is to incorporate resistance to MSV and other economically important disease pathogens into maize varieties with desirable agronomic traits.

So far, the MSV Project team has assembled both a large array of very good sources of MSV resistance and genetic stocks (accessions) with good agronomic traits. These are now ready to be filtered into the mainstream breeding program. In

Table 13. Genetic breeding strategy for developing maize streak virus disease varieties

	Short-term<5 yr	Medium-term: 5-10 yr	Long-term: >10 yr
Germplasm available	Inbred lines: (Local and exotic)	Breeding populations	Populations/Landraces
Methodology envisaged	Recycling (Conversion):- · Crossing /Backcrossing	Backcross-cum-recurrent selection	Recurrent selection schemes
	· Pedigree selection	Formation of synthetics and composites	Formation of composites
	· Sister line development		
Commercial (products)	(Marker-assisted selection)	(Agroinoculation)	(Genome mapping and transformation)
	MSV-resistant VC, DC, TWC, TC, and SC hybrids	MSV-resistant OPVs: (synthetics, composites)	MSV-resistant populations as source of future inbred lines

the meantime, results of mapping studies are being obtained so that MAS and other supportive techniques can be applied. Crosses have been made between the exotic MSV resistance sources and adapted local breeding stocks. In doing this, caution has been taken to "respect" heterotic patterns. Whatever breeding plan is put into action, breeders should not lose sight of the need to sort out heterotic patterns and develop hybrids (for the formal seed sector) and open-pollinated varieties (for the informal seed sector).

Reference

Njuguna, J.G.M., C.J. Kedera, L. Muriithi, S. Songa, and B. Odhiambo. 1990. Overview of maize disease in Kenya. In: Proceedings of a Workshop on Review of the National Maize Research Program. KARI/ISNAR Management Training Linkage Project. 19-23 November 1990. Kakamega, Kenya.

3.4 Private Sector–Maize Seeds and MSV

3.4.1 Kenya Seed Company and MSV (F. Ndambuki)

Introduction

MSV infection is very high in Kenya's Central Province, particularly in the Kiambu district. MSV occurs in Nyanza, the Coast region, and isolated parts of the Eastern Province. In addition to MSV, other diseases such as lowland blight (*H. maydis*), lowland rust (*P. polysora*), ear rot, striga, and maize stalk borer are rampant in the coastal lowlands and around Lake Victoria.

Current Commercial Maize Varieties and their Survival with MSV

There are no MSV-resistant maize varieties in the highlands of Kenya. This includes the four varieties H614, H625, H626, and H627 that were developed for the highland region (over 1,700 m asl), where MSV was formerly rare. The two hybrids—H622 and H623—which are suited for the medium- to high-altitude zones (1,200-1,700 m), are also not resistant to MSV.

Two hybrids, H511 and H513, are common in the mid-altitude zone. H511 is susceptible to MSV, although H513 is fairly tolerant. Furthermore, DH01, DH02, and the newly recommended release—DH194 (DH03)—are partially resistant to MSV. In the lowlands there are CCM, PHI, and PH4 maize varieties. PHI and PH4 are fairly resistant to MSV, but CCM is susceptible.

Managing Maize Streak Virus

The private sector has developed long- and short-term strategies for controlling MSV. The long-term goal is to develop MSV-resistant varieties, which can be achieved by breeding for multiple disease resistance. In the short term, the goal is to integrate cultural with chemical practices to decrease MSV losses.

An off-season nursery in Sigor, West Pokot, where the temperature oscillates around 28°C, provides an excellent site for MSV screening. To increase the number of leafhoppers, either finger millet or bulrush millet is planted around the maize plot. Field data collection, particularly for MSV, was prevented during variety testing by the erratic

occurrence of the disease, which greatly depends on the population dynamics of the vector *Cicadulina* spp. In some years, damage is insignificant; in others, epidemics wipe out farmers' crops.

Collaboration
Working with KARI provides the Kenya Seed Company with both useful information and germplasm. We use KARI's Muguga facilities to screen varieties in National Performance Trials (NPT). By screening maize lines from CIMMYT and other sources, we are able to use some of them into our breeding program. Advanced trials of very promising hybrids will be entered into NPTs next year.

Sources of Resistance
Efforts to introduce MSV resistance into commercial varieties have not been completely successful, but there are hopes that some varieties may soon prove useful. These include IB32, which belongs to CIMMYT's population lines. It is from these CIMMYT populations (TZMSR, 8549SR, 9725SR, 8730SR, etc.) that we extracted inbred lines to combine with local germplasm. Other sources include HASR from Burundi, which unfortunately is not very stable, and CML lines, which are proving useful for resistance.

The work has faced several constraints, such as:
- Difficulties in obtaining uniform field infestation, which results in unreliable field screening.
- Impossibility of detecting field escapes.
- Lack of cheap, uniform screening methods.
- Glasshouse and leafhopper rearing facilities are expensive. A virus infection method that does not require the vector would be helpful.
- Lack of suitable MSV-resistant germplasm/lines with stable resistance to other diseases.
- Available sources for resistance have grain yield potentials that are too low to be useful, particularly in the highland region, which has a 10-15 t/ha yield potential.

Cultural MSV Crop Management
The short-term approach combines various recommended aspects of crop management:

- Early planting in all areas to avoid vector buildup later in the season.
- Crop rotations with a nonhost crop where and when possible to reduce disease incidence.
- Avoiding relay planting of maize.
- Roguing out diseased plants, which is important because the major source of the virus is infected maize.
- Intercropping, which makes it more difficult for male and female leafhoppers to mate.
- Chemical control. The Company has decided to seed-dress certified seed maize sold in MSV-prone areas and maize seed produced in MSV-prone sites with Gaucho.

3.4.2 Seed Production and MSV in Zimbabwe

Seed production is mostly conducted in high rainfall areas with optimum environments for both summer and irrigated winter crops. Seed growers are farmers with irrigation facilities and good maize production skills. To maximize their annual incomes, most of the seed growers also grow irrigated winter wheat. During the dry winter, irrigated wheat is usually the only green crop, and so it harbors high populations of insect pests such as the MSV vector, *Cicadulina mbila*. Furthermore, the winter wheat crop usually dries up just as irrigated, early planted maize crops germinate. Since winter is a dry season in Zimbabwe, these early-planted maize crops are the only green plants in the field once the wheat crops have been removed. Naturally, the MSV vector and other insects migrate to early planted maize crops.

Because of this scenario, MSV infection is common and widespread in Zimbabwe. About 10% of the early planted crops are seed maize crops, and yield losses from MSV infection are reported to be as high as 43% (Mzira 1984). The MSV vector feeds on the sap of young maize plants, and the critical period for infection is the first 8 weeks from planting. Generally, the earlier the stage of infection the higher the yield losses. In addition to the winter wheat crops, other conditions favorable to the development of MSV infection include: close rotation of graminaceous crops, poor control of feed and susceptible volunteer plants, a late preceding rainy season, and neighboring irrigated pastures or graminaceous crops.

Control Measures
Control options include seed dressings with insecticides and avoiding the vector by not planting seed crops adjacent to winter cereal crops, not planting too early or too late, and/or clearing a 10-m-wide perimeter around the crop. Removing the source of infection requires roguing out infected plants, removing weeds and alternative hosts, and eradicating volunteer plants. Resistant parentals are most desirable. But farmers' only MSV-resistant options are C5051 and products from CIMMYT Harare, for direct use in crosses and backcrosses to elite lines.

4. Achievements, Results, and Recommendations

Research results and achievements during the first phase of the MSV project are divided into four areas:

4.1 Studies on MSV-Resistant Maize Lines

- Results show that the highest percentage of highly resistant lines is in F_3 lines originating from OSU23I provided by CIMMYT. At least 46% of OSU23I had high resistance to MSV.
- At least 26% of CIRAD's C390 had high resistance to MSV. Only 2% of Tzi3 lines from IITA was found to be highly resistant.
- 69% of OSU23I lines, 62% of C390 lines, 42% of CML02 lines, and 40% of Tzi3 lines are resistant.
- Trials at Alupe in the Lake Victoria region showed that 38% of OSU23I lines, 28% of C390 lines, 14% of CML202, and 4% of Tzi3 are highly resistant.
- Considerable information was gathered from field nursery materials and bulking seeds about agronomic traits of interest. This was shared during the workshop.
- Jane Ininda studied molecular markers at the John Innes Centre, and reports of her considerable, well-documented achievements were made available.

4.2 Studies on the MSV Vector

- Aerial densities of trapped *Cicadulina* indicate two annual population peaks: July-August and November-December.
- *C. chinai* was found to be the most efficient in MSV transmission (70%), followed by *C. mbila* (60%) and *C. storeyi* (40%). But species in different ecologies differed in their transmission rates.
- The progenies of *Cicadulina* breeding in maize crops are not important sources for disease spread to maize planted later.
- An ICIPE/KARI team began epidemiological work that provides valuable information, especially about the rearing of more aggressive vector species to challenge resistance in the field.

4.3 Studies on MSV

- MSV isolates in Kenya and the relevant gene constructs were analyzed at UCT and transferred to JIC for agroinfection studies. The system is used for preliminary screening to challenge MSV resistance from inbred lines. Agroinoculation/infection studies were started and optimized at JIC for future work at KARI by both Dr. Ininda and B. Odhiambo. This work continues in phase II.
- MSV isolates of different biological and molecular properties were detected, which means that mixed MSV populations do exist. This is important information for resistance breeding.
- Zones or regions prone to MSV outside of the peak periods of MSV disease infestation were identified.
- Preliminary evidence for virulence variability in MSV populations within zones and maize crops was established.

4.4 Collaborative Achievements and Benefits

- African scientists received advanced training and learned sophisticated biotechnology skills in DNA diagnostics and agroinoculation, such as the development of appropriate molecular markers for resistance breeding.
- Member African countries suffering similar MSV problems were provided information and research results. In addition, institutions that offered MSV-resistant germplasm, such as IITA, CIMMYT, CIRAD, PANNAR, and others, gained from the information and data generated by the project.
- Technology transfer and training improved Kenya's national capacity to breed and adapt MSV-resistant maize.
- The project's technical advisors to Kenya, Dr. Peter Markham and Prof. Ed Rybicki with their assistants, Dr. Jina Banks (JIC) and Martin Darren (UCT), visited as planned and provided project guidance.
- Closer links were created between specialists—plant breeders, pathologists, and entomologists—who have been used to working in isolation even at national research institutes like KARI.
- As part of the project's training component, Dr. Jane Ininda and Dr. Benjamin Odhiambo went to JIC, and Dr. Jackson Njuguna and Benjamin Odhiambo went to UCT. A joint KARI/ICIPE workshop on new molecular technologies for the project was also held at ICIPE.
- KARI received much-needed information through regional networking with other countries involved in similar or related kinds of research. This increased KARI's opportunities for collaborating with others in the public and private sectors at regional and global levels.
- Regional maize breeding efforts were refocused from improving yields to disease and pest resistance.

- Experts from JIC and UCT continue to visit MSV-infected maize fields in Kenya, which reflects their commitment to the project's success and allows for continued technology and information exchange.

The workshop was part of an effort to ensure that Africa reaps the benefits of new advances in agricultural technology. It focused on maize because effectively controlling or eliminating the various factors that reduce maize yields in Africa will help contribute to greater self-sufficiency and stability in the region.

The MSV project will improve the household food security and socioeconomic status of resource-poor farmers, especially women, who produce 75% of food crops. The project will make its impact, moreover, through sustainable agricultural practices. Institutions from the North and South—including the private sector—are working together to combat MSV and to stabilize maize yields and prices. Through this collaboration, KARI has already developed a multidisciplinary team of researchers who have access to some of the latest biotechnology techniques: agroinoculation, molecular markers, DNA polymorphism analysis, and other related skills. This technology and expertise is providing a better understanding of the pathology of MSV and of the genetic basis of resistance. Phase II is essentially a more focused effort to determine the location of MSV resistance genes. According to KARI's Dr. Jane Ininda, mapping to determine MSV resistant loci in five maize varieties ended in August.

4.5 Recommendations

The participants agreed on the need for a standard MSV Sample Data Sheet. The Sheet should include the following information: date, location, ecozone, streak incidence, genotypes, stage of infection, streak rating, and the area's infection history. Other notes could indicate comments about grass samples (infected or harboring MSV).

Participants also agreed that the exchange of germplasm should include appropriate and basic information, such as inbred lines, population, S2 lines, etc. Seed companies should also be appropriately involved in product development and promotion by assisting with various aspects of intellectual property rights or licensing agreements.

Finally, researchers emphasized the need to focus more on farmers and their needs, as well as on the constraints or limitations of their agricultural practices.

MSV-resistant germplasm nursery; sources include IITA, CIMMYT, CIRAD, PANNAR-South Africa, the Grain Crops Institute, and KARI.

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