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Tolerance to maize streak virus in local Burundi highland maize[1]

R.S. Zeigler* and A. Manirakiza**

Summary

Maize plants apparently tolerant to maize streak virus were selected in farmer fields during a maize streak epidemic in the Burundi highlands in 1983-84. Progeny of these selections were tested under natural infection in the lowlands, with tolerant plants then either open or self-pollinated. Progeny of these tolerant plants were tested in the highlands using mass-reared Cicadulina *vectors and a highland virus source, and ranged from very tolerant to susceptible. Inheritance of tolerance is consistent with its control by several genes and/or the presence of a number of modifying factors. A technique for mass rearing of vectors in a cool climate is presented.*

Résumé

Des plants de maïs tolérant à la striure du maïs (streak disease) furent sélectionnés dans les champs des agriculteurs de haute altitude au Burundi lors d'une épidémie de cette maladie en 1983-84. La descendance de ces plants fut testée sous infestation naturelle en basse altitude; les plants tolérants furent soit autofécondés soit fécondés au hasard, leur descendance fut alors testée un site de haute altitude après inoculation du virus local par le vecteurs Cicadulina élevé. Les plants de maïs furent classés de "très susceptibles" à "très tolérants" et l'héritage de la tolérance sembla être dû à l'action de plusieurs gènes et/ou facteurs influençants. Une méthode d'élevage des insectes vecteurs sous climat froid est proposée.

1. Introduction

Maize streak disease (MSD) is caused by a leafhopper (*Cicadulina spp.*)-transmitted virus. Symptoms are cream-colored spots which elongate and coalesce to form long streaks on leaves. The virus becomes systemic with symptoms appearing on juvenile and subsequently developing tissue. Very susceptible plants may become nearly 100% chlorotic. Symptoms distribution over the plant permits an estimation of the developmental stage of the plant at inoculation, with yield decline being most pronounced when very young plants are infected. The most feasible way to combat MSD is through varietal tolerance (9). Although various sources of tolerance have been available for many years (4, 5, 8), only recently have methods been developed for large-scale inoculation of breeding material. This has overcome the principal constraint of inadequate infection, preventing breeders from distinguishing late infection and escape from true tolerance.

In 1983-84 an epidemic of MSD caused serious losses in Burundi highland maize (12). MSD-tolerant lines from the International Institute of Tropical Agriculture (IITA) were tolerant in evaluations in lowlands and highlands, but were poorly adapted to highland conditions. Because of this poor adaptation, it was thought that if MSD tolerance could be selected in locally adapted maize an MSD-tolerant variety could be bred for the highlands sooner than if tolerance were introduced by backcross from IITA lowland material. This paper reports the results of the local selections and subsequent MSD evaluations. A methodology is presented for vector rearing and inoculation based on that developed at IITA, but suited to a highland environment, and within the capabilities of a small national program.

2. Materials and methods

2.1. Selection of tolerant maize on the farm

Farmer fields with high MSD incidence (> 50%) were visited in the highland areas affected by the epidemic to identify potentially tolerant (PT) maize individuals. Plants were considered as PT if they exhibited symptoms similar to MSD on the lower leaves but which either decreased in severity on subsequently developing foliage, or showed only little increase in severity. Plants showing symptoms on the upper leaves only could not be evaluated,

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* Present address: Rice Pathologist, Centro Internacional de Agricultura Tropical (CIAT), AA 6713, Cali, Colombia, S A

** Programme Maïs et Pois, Institut des Sciences Agronomiques du Burundi (ISABU), B.P 75, Bujumbura, Burundi

since it was not certain that complete symptoms expression had developed before all tissue was mature. As it was impractical to attempt self-pollination, the seeds from PT plants were open pollinated.

2.2. Field evaluation of tolerance

Seeds harvested from PT ears were planted in mid-May 1984, at an ISABU center on the Imbo plain (830 m), where plantings at this time of the year typically showed high levels of MSD (12). Test rows were alternated with susceptible rows (Igarama 4) and after each five test rows two susceptible rows and one row of an IITA tolerant variety were planted (Tlaltizapan 7844 SR). Fertilization was applied to maximize yield and the field was irrigated as required. Four weeks after emergence the alternating rows of Igarama 4, which were showing approximately 75% MSD incidence, were uprooted and left in the alleys to drive viriliferous vectors onto adjacent test rows, leaving one susceptible check and one tolerant check row every five rows. At flowering (eight weeks) the number of diseased plants and those showing tolerance and susceptibility were counted in each test line. Tolerant plants were marked, and self-pollinated when possible.

2.3. Controlled inoculation field evaluation

2.3.1. Vector rearing and virus acquisition

Vectors were reared following closely the techniques developed at IITA (2, 3, 6). However, due to environmental conditions at high elevation of the Kisozi station (2090 m) and budgetary constraints, several modifications were introduced. Low local temperatures required that rearing be done in a structure with double walls of clear plastic sheeting and supplemental heating from a kerosene burner and electric space heaters, when electric power was available. The frame of the rearing house was made from local bricks and wood. Frequent cloudy conditions required supplemental lighting supplied by 40 W fluorescent tubes and cages with clear plastic tops and sides facing the windows. Insects were collected, using a 12 V, 180 W automobile vacuum cleaner and a collection cup. CO_2 gas for calming the insects during transfers and field inoculations was obtained from CO_2 fire extinguishers, as it is not otherwise available in Burundi.

Individuals of *C. storeyi* China and *C. mbila* Naude were placed in cages containing young maize and pearl millet (*Pennisetum typhoides*). After several generations adults were transferred to cages containing fresh plants. The original populations were left to develop, providing a continuous supply of adults over a period of several months.

Adults were collected from the cages through a zipper door by draping a black cloth over the cage to cover all but the observation window, with the insects attracted to the light. For virus acquisition, adults were then placed in cages containing MSD-affected maize plants, and were allowed to feed for four days. Oviposition, incubation, nymphal development, and transmission data, were obtained from single females places on a maize leaf in 2 cm^3 mini-cages.

2.3.2. Controlled field inoculations

Progenies of plants selected from the field inoculation trial in the lowlands were planted in an ear-to-row manner at Kisozi in October 1984, replicated twice, and fertilized to maximize yield. A susceptible check, Igarama 4, was planted after each nine test rows. When plants reached the 3-5 leaf stage, they were inoculated using viruliferous vectors. Insects from acquisition cages were collected, transported to the field, calmed with CO_2, and 4-5 individuals were placed in the whorl of each plant. After five days, plants were examined on a daily basis to fix the time after inoculation for the appearance of symptoms. Tolerance was evaluated just prior to flowering, with only those plants that showed symptoms at least six weeks earlier considered for evaluation. The evaluation scale used was similar to that proposed by Soto *et al.* (9): 0 = no symptoms (considered escape); + = one or very few spots on only one or two leaves; 1 = spots distributed over several leaves, gradually disappearing on upper leaves; 2 = spots coalescing to form streaks, but not forming appreciable chlorotic areas; 3 = streaking and chlorosis on less than 50% of affected leaved with some stunting; 4 = streaking and chlorosis on 50 - 75% of affected leaves with marked stunting; 5 = chlorosis on 75 - 100% of affected leaves, severe stunting, premature tasseling, and plant death.

3. Results

3.1. Field selections

One hundred sixty one PT plants were identified and marked during the survey. Of these, one hundred were harvested. Those lost showed subsequent symptom development inconsistent with true tolerance. It was not always clear whether weak symptom expression was due to MSV tolerance or to other virus-induced problems (11). The frequency of PT plants when they were present was estimated to be $1{:}10^3 — 1{:}10^4$.

3.2. Field evaluation under natural infection

Six weeks after emergence 71 % of test plants were showing MSD symptoms. Of the 100 open-pollinated lines collected from highland farmer fields, 12 produced plants with a rating of less three at eight week after emergence. Eight of these lines contained plants whose tolerance continued at an acceptably high level until after flowering, when symptom development ceases. Most resistant checks had a rating of 2 or less, although a few were rated 3. All susceptible checks were rated 4 or 5, with most plants dead before flowering, or producing sterile ears. A total of 26 ears were harvested from the most tolerant plants of the remaining eight lines. Of these, 10 were self-pollinated. As the tolerant check lines were 2 - 3 weeks later maturing than the test lines there was little chance of contamination of the open-pollinated lines.

3.3. Controlled field inoculations

3.3.1. Vector rearing

Under the conditions created in the rearing house and cages (15 - 38° C, 16 hr photoperiod) nymphs began emerging from eggs after 17 days and completed their life cycle three to five weeks later. Maintaining a mixture of two *Cicadulina* species in the same cages did not appear to have an adverse effect on fecundity.

3.3.2. Field evaluation

Beginning five days after inoculation, 89.1 % of the test and check plants showed MSD symptoms. Even when only one or two spots were visible, they were so characteristic that it was not difficult to fix the first day of symptom expression. No difference was found in the number of plants showing symptoms among check and test lines, or within test lines. Likewise, no significant differences in incubation period was detected among lines. Eighteen lines from open- and self-pollinated individuals from the lowland screening had some plants with an MSD rating of 3 or less, and 14 lines had plants with MSD rating 2 or less (table 1), although these resistant lines came from only two of the original selections. Differences in symptom expression between tolerant and susceptible plants are shown in figure 1. Without exception tolerant plants were found in both replications for the lines producing such tolerant plants. No susceptible check plants were rated less than 4.

Figure 1 : A. Leaves from susceptible (right) and tolerant (left) maize infected with maize streak virus Each leaf is the 9th to develop following first symptom expression. B Susceptible (right) plants, and Tolerant (left) each inoculted with MSD at the 3rd leaf stage

TABLE 1

MSD rating, following controlled inoculation of the progenies of open-pollinated (0) and self-pollinated (S) parents of the best families from the lowland field evaluation.

Parent	Cross	# Plants in MSD Rating Class[a]					
		±	1	2	3	4	5
Igarama 4	Check					59	56
1254 — 15	O		1		4	19	4
— 25	O	2		3	2	11	9
— 9	O				1	15	5
— 24	O		1		5	23	7
— 4	O		2	2	4	21	1
— 16	O	1		3	3	18	6
— 11	O	1	3	3	7	18	1
— 0	O	1	2	7	2	3	
— 17	S			1		26	
— 13	S				2	15	12
— 19	S				1	16	16
— 20	S		4	4	6	13	2
— 26	S			2	5	15	16
— 5	S	2	1	2	4	5	
— 18	S	1		7	3	14	2
— 12	S	3	4	3	8	5	
1223 — 22	O					7	33
— 10	O				1	22	15
— 7	S	3		2	1	18	10

4. Discussion

MSD tolerance observed in farmers' fields was maintained, in some cases, over two successive challenges. The rather low frequency of good tolerance is consistent with observations of others. Soto *et al.* (9) found only 3 % tolerant plants in the population "Revolution" considered as highly tolerant (7). It is possible that the lowland evaluation was so severe that otherwise tolerant plants in highlands were made susceptible by their extreme unadaptation to the lowland environment. Loss of resistance due to environmental stress is not unusual. It is possible that some PT selections were affected by other viruses or by less virulent strains of MSV (1). That only 15 % of the progenies from self-pollinated plants showed good tolerance is in close agreement with Soto *et al.* (9) who found only 21 % tolerant progeny in the first self-pollination of tolerant plants, although tolerance increased to 100 % after three selfings. Self-pollinated PT plants in Imbo were progenies of PT plants open-pollinated in fields of virtually 100 % susceptible plants so that segregation was to be expected.

Within the several thousand MSD plants examined, only a few showed heritable tolerance of some interest. That tolerance to MSV should be found at very low frequency in Burundi highland maize was to be expected. As maize, with several different known sources of tolerance, is exotic to Africa, where MSV is endemic, it is likely that genes conferring MSV tolerance are present in many, if not all American maize populations. There is no reason to believe that some of these may not have been introduced into Burundi among the many maize introductions this century.

The data presented here do not permit a definitive conclusion as to the mode of inheritance of MSD tolerance. However, the genetic factors involved do not appear to behave in a simple dominant fashion. The distribution of tolerant or susceptible progeny suggest the involvement of a number of genes, either as modifying factors or direct inducers of tolerance. Storey and Howland (10) found the tolerance identified in South Africa to be controlled by a single gene, neither dominant nor recessive, whose expression was under the influence of a number of modifying factors. Thus, the range of tolerance and susceptibility in the progeny of the self-pollinated plants may well reflect a similar situation, rather than suggest that a large number of recessive genes are responsible for tolerance.

Incorporation of MSD tolerance into agronomically useful maize has begun. Populations may be improved rapidly for MSD tolerance and other agronomic traits by alternating selections between highlands and lowlands. Concentration of tolerance genes can be achieved by planting in the lowlands during the "streak" season and selecting and intercrossing only those plants showing acceptable tolerance. Little selection for agronomic traits is undertaken as the plants are out of their target environment. During the normal highland maize season, progeny of the intercrossed plants from the lowlands may be selected for agronomic performance, following MSV inoculation using reared vectors. This permits two seasons per year in a region where typically only one season of maize is grown.

Varieties for release with this tolerance are expected to be available in 2 - 3 years. Because tolerance is apparently lost when plants are crossed with susceptible individuals, farmers will have to renew their seed every year or two. Although Burundi farmers generally prefer to save their own maize seeds, those who have suffered losses from MSD have expressed a willingness to purchase MSD-tolerant varieties. Many have indicated they are willing to pay a premium of 25 % for such seed. Release of an MSD tolerant variety may be a tool for increasing the practice of renewing maize seed, and serve as a vehicle for improving highland maize for other agronomic characters.

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R.S Zeigler: American Ph. D. (Plant Pathology) International Development Research Centre (Ottawa, Canada).

A. Manirakiza: Burandais, Ir Agr. (Bujumbura, Burundi)