SELECTED AND EVALUATING LOCAL TOLERANCE TO STEAK VEIN DISEASE IN BURUNDI RICARDO HATARE

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Abstract

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

1. Introduction

As in most countries represented at this workshop, maize streak disease (MSD) is a problem in some Burundi environments. Prior to 1983 MSD was a problem only in the lowlands (4,189m); however 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 field evaluations in lowlands and highlands, but were very 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 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.

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2. Methods

2.1 Selection of tolerant maize on-farm

During our efforts to assess the distribution and impact of the highland MSD epidemic we noticed, at very low frequency, 
\((1 \times 10^3 - 10^4)\) plants which showed symptoms similar to those of MSD on the lower leaves with little subsequent symptom development. These plants were considered to be potentially tolerant (IT) and we decided to try to exploit this apparently indigenous, highland source of tolerance. Farmers with fields containing PT plants were contacted and purchase of ears from PT plants negotiated (usually at \(4-5\times\) market price). Plants were marked and symptom development followed, if possible. As it proved impractical to attempt self-pollination, the seed from PT plants was open pollinated.

2.2 Field evaluation of tolerance: lowland virus

Seed from harvested PT ears were planted in mid-May, 1984 at an IASABU center on the Imbe plain (330m), where plantings at this time typically show high levels of MSD (12). Test rows were alternated with susceptible rows (Igarama 4) and after each five test rows were planted two susceptible rows and one row of an ITA tolerant variety (Taltizapan 75/43R). Four weeks after emergence the alternating rows of Igarama 4, which were showing approximately 7% 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 plants diseased and those showing tolerance and susceptibility were counted for each test line. Tolerant plants were marked, and, when possible, self-pollinated.

2.3 Controlled inoculation field evaluation: highland virus

2.3.1 First failures

Initial attempts to inoculate large numbers of plants in the field using 1-2 insects per mini-cage and leaving them on the leaves for 1 hour proved unsatisfactory. While the time period
was adequate for transmission under warm conditions, the insects tended not to feed in cool morning temperatures. High mortality and only about 20% active vectors hampered the effort as well. Because of the cumbersomeness of the method and the amount of labour required, we decided that even for our small program, a mass screening approach was needed.

2.3.2. Vector rearing and virus acquisition

Vectors were reared following closely the techniques developed at IITA (2, 3, 6). However, due to environmental conditions at the high elevation Kisazi station (2090 m), and budgetary considerations, several modifications were introduced. Low ambient temperatures demanded that rearing be done in a structure with double walls of clear plastic sheeting and supplemental heating initially from a kerosene burner and electric space heaters. The frame of the rearing house was of locally fabricated brick and wood. Frequent cloudy conditions required that supplemental lighting be supplied by 40W fluorescent tubes and that cages have clear plastic tops and sides facing the windows. Insects were collected using a 12V, 180W automobile vacuum cleaner and a collection cup. The cup, made from a common plastic medicine bottle, had a removable top and nozzle, and with fine screening towards the vacuum. CO₂ gas for calming the insects during transfers and field inoculations was obtained from CO₂ fire extinguishers, as it is not otherwise available in Burundi.

Individuals of C. stercorii China and C. milde Haude were placed in cages fabricated from locally available materials 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 MSV-affected maize plants and allowed to feed for four days. Oviposition, incubation, nymphal development, and transmission data were obtained from single females placed on a maize leaf in 2 cm$^3$ mini-cages. Cages were cut from plastic 10 cc pipettes with a leaf-slit and leaf-attachment pin added.

2.3.3. Controlled field inoculations

Progeny of plants selected from the field inoculation trial in the lowlands were planted in an ear-to-row, manner at Kisozi in October, 1964, replicated twice, and fertilized to maximize yield. A susceptible check, Igarasa 4, was planted after each nine test rows. When the 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 placed 4-5 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 leaves 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 were either harvested by the farmers before they could be saved, or 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).
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's fields, 12 produced plants with a rating of less than three at eight weeks 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, and the rest were open-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. The variability was probably due to the rather wide range of temperature. Maintaining a mixture of two 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 of MSD rating of 2 or less (table 1), although these came from only two of the original selections. Differences in families and tolerant symptom expression are shown in Figure 1. Without exception tolerant plants were found in both replications of lines producing tolerant plants. No susceptible check plants were rated less than 4.

4. Discussion

The MSD tolerance observed in highland farmers' field was maintained in some cases, over two successive challenges. The rather low frequency of good tolerance, even in this selected sample, is consistent with observations of others. It is possible that the lowland evaluation was so severe that otherwise tolerant highland plants were rendered susceptible by their extreme non-adaptation 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, perhaps, by less virulent strains of MSV (1). That only 15% of progeny from self-pollinated plants showed good tolerance is in close agreement with Soto et al. (9) who found only 21% tolerant progeny in their first self-pollination of tolerant plants, although this increased to 100% after three selfings. The self-pollinated PT plants in Imbo were a progeny of PT plants open-pollinated in fields of virtually 100% susceptible plants, thus, segregation is to be expected.

In the thousands of MSD plants we examined, only very few showed heritable tolerance of interest. That tolerance to MSV should be found in Burundi highland maize at very low frequency is 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 statement as to the mode of inheritance of this MSD tolerance. However, the genetic factors involved do not appear to behave in a simple dominant fashion. The distribution of tolerant and susceptible progeny suggest the involvement of a number of genes; either as modifying factors, or directly controlling 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 this tolerance into agronomically useful maize has begun. Populations may be improved rapidly for MSD tolerance and 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. In the normal highland season, progeny of the intercrossed plants from the lowlands may be selected for agronomic performance following MSV challenge 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 seed, those who have suffered losses to MSD have expressed a willingness to purchase MSD-tolerant varieties. Many have indicated their willingness 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.

Some remarks on our methodology and philosophical approach are in order. First, those of you who may have had the opportunity to visit the IITA MSD screening facilities may have been a bit overwhelmed by the modern facilities and, despite the enthusiasm and encouragement of Dr. Dabrowski, concluded that such an effort was beyond the means of your program. However, we assure you, that with our modest facilities and very limited resources (both human and material) if we were able to succeed in rearing Cicadulina, virtually any program can. After establishing the methodology two technicians spend about 20-40% of their time tending the insects. The principal requirements are reasonably clear picture of the method (available after visits to IITA and studying the various publications), flexibility to construct equipment with what is at hand, identifying limitation (in our case cool temperatures and low light) and most importantly willingness to persevere in the face of repeated setbacks.

Philosophically, one may ask why, given the infrastructure already in place at IITA, should a national program invest in a MSD screening procedure? It should be clear that for programs saving highland regions there is little choice. While there is excellent SR material available for the lowlands, there is nothing for the highlands and this situation will continue for some time. Even using SR material for conversion of local material will require an inoculation method, for low and middle altitudes which predominate in the region, it is unlikely that an SR variety will be ideally suited for release. With no SR screening method in place, it is likely that given multigenic SR control and segregation observed in SR lines that after local improvement and multiplication there will be a decline in MSD tolerance. Released MSD-tolerant varieties may then show more MSD than acceptable to farmers paying a premium for "resistant" seed.
5. Acknowledgements

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References

Table 1. MSD rating, following controlled inoculation, of progeny of open-pollinated (O) and self-pollinated (S) individuals of the two best families from the lowland field evaluation.

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