

Breeding cassava for brown streak resistance: Regional cassava variety development strategy based on farmer and consumer preferences

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Abstract. Cassava is an important food crop in the East African coastal lowlands. However, yields are low due to various reasons amongst which are pests and diseases. Cassava brown streak virus disease (CBSD) is one of the major diseases of economic importance in Kenya, Tanzania, Mozambique and Malawi. Yield losses attributed to CBSD ranging from 49 – 74% have recently been reported in some coastal areas of Tanzania. It is estimated that in Tanzania alone, CBSD causes economic losses of more than USD 16,000,000 annually. The wide adoption of resistant varieties is the best sustainable control strategy. However, only a hand full of resistant/tolerant varieties is available in each of the severely affected countries. In order to increase the number of resistant varieties available to farmers, a seedling nursery was raised at Kibaha, Tanzania. About 513 seedlings were selected and cloned for further evaluation. At Kibaha, CBSD disease pressure and spread was very high therefore, 88% of the harvested seedlings were infected. This indicated that this site was ideal for screening cassava genotypes for resistance to CBSD. Although the vector is still unknown, it was very active and infective at this site. Because the seedlings were raised from botanical seeds, ruling out the chance of vegetative plant material transmission,

these results prove that vector transmission can account for a large percentage of plants infected in a field. Families with a high percentage of number of seedlings selected (PNSS) included Kiroba, 71762 and I88/00188. The importance of marking diseased seedlings during the growing season is discussed. Furthermore, massive introduction of germplasm through open quarantine, the establishment of crossing blocks to generate improved genotypes and the use of a decentralized participatory evaluation procedure have been initiated as important strategies towards availing farmers with a wider choice of improved varieties.

Introduction

Cassava (*Manihot esculenta* Crantz) is an important food crop in the East African coastal lowlands. As a food security and famine reserve crop, it is produced mainly through subsistence farming with use of low inputs, rudimentary technology, large post-harvest losses and minimal processing. Excluding Uganda, cassava yields in all the countries of Eastern and Southern Africa are lower than the world's average, estimated at 10.5 MT per hectare (FAO 2001). The major causes for the low yields are: pests and diseases, poor

agronomic practices, poor soil fertility, drought and use of cultivars with poor genetic potential. The major pests are cassava green mites and termites. However, mealybugs, variegated grasshoppers and white scales can sporadically cause serious damage in some parts of these countries. The major diseases are cassava mosaic disease (CMD), cassava brown streak disease (CBSD) and cassava bacterial blight (CBB).

CBSD first reported from Amani, Tanzania in 1936, (Nichols, 1950) is now considered to be one of the major biotic constraints to cassava production especially in the coastal lowlands of Eastern and Southern Africa (Mahungu *et al.*, 1999). Seriously affected countries are Kenya, Tanzania, Mozambique and Malawi. An *Ipomovirus* of the family *Potyviriidae* (Hillocks and Thresh, 1998) whose vector is yet to be positively identified causes CBSD. However, because cassava is normally vegetatively propagated, the use of infected cuttings readily introduces the disease into newly planted areas. CBSD reduces total yields as well as rendering the roots useless for human consumption due to the necrosis it causes to the starch storage tissues (Hillocks *et al.*, 2001). Yield losses ranging from 49 – 74% have recently been reported in some coastal districts of Tanzania (Muhanna and Mtunda, 2002). The coastal lowlands of Tanzania contribute to about 50% of the national annual cassava production, estimated at 5,650,000 MT (FAO, 2001). Taking a conservative average yield loss estimate of about 40%, CBSD causes annual economic losses amounting to USD 16,500,000 (price of fresh cassava estimated at USD 15 per MT). The figures will be higher for Mozambique, where the disease has in recent years become very devastating and thus seriously threatening household food security in the three major cassava producing provinces of Nampula, Zambezia and Cabo Delgado.

Early workers in East Africa appreciated the use of resistant varieties for the control of both CMD and CBSD. Breeding for resistance to CBSD went hand in hand with breeding for

resistance to CMD, which started at Amani, Tanga (Tanzania) in the 1930s. The wide variability in susceptibility to CBSVD was observed to be a varietal characteristic and therefore presumably genetic. Their efforts led to the development of resistant varieties popularly known as the Amani hybrids. Intraspecific crosses among the few varieties, which possessed some resistance to CMD, resulted in successful hybrids. However, few of the varieties possessed resistance to CBSD except *Aipin Valenca*. *Macaxeira Aipin*, though susceptible, contributed factors for CBSD resistance, which were evident in the progeny (Jennings, 1957). F1 interspecific hybrids produced from the cross *Manihot glaziovii* (Ceara rubber) x cassava although tree like, were however, mildly infected by CMD in field trials. In the first backcross to cassava, improved resistance to CMD was achieved and one clone in particular, No. 4032, remained free from both CMD and CBSD when tested in a field trial and by grafting (Jennings, 1957). Hybrids from subsequent crosses proved to be the most promising agronomically, and therefore were the most widely distributed (Jennings, 1957). Many of IITA's most popular varieties (including TMS 30572, TMS 4 (2) 1425) were developed with a CMD resistant clone (58308) as one of their parents. This clone was a selection from third backcross derivatives of the interspecific cross between cassava and ceara rubber from Nichol's work at Amani, in the 1940s (Asiedu *et al.*, 1994).

Correlation between morphological traits and resistance to CBSD has been investigated by a few researchers but has not been detected (Nichols, 1947; Mahungu *et al.*, 1999). However, there is mounting evidence that it might be associated with the zigzag stem habit (Kanju *et al.*, 2003).

Some of the present day so-called "local cultivars" especially in Tanzania could have been distributed from Amani (Mahungu *et al.*, 1999), more especially the few which have proved to be tolerant/resistant to CBSD (*Kigoma Mafia*, *Kiroba*, *Namikonga*, and *Kitumbua*) and CMD (*Kibaha*, *Namikonga*

and *Kitumbua*). In each of the seriously affected countries, only a few cultivars have been identified to be tolerant/resistant to CBSD. These are: Kiroba, Nanchinyaya, Kigoma Mafia, NDL 90/134, Kitumbua, Namikonga (Tanzania); Nikwaha, Mulaleia, Chigoma Mafia, Nachinyaya, Likonde, Macia 1, MZ 89/001, MZ 89/105, MZ 89/186, and MZ 89/192 (Mozambique); Kaleso and Guzo (Kenya); CH 92/077 and CH 92/112 (Malawi).

When resistant varieties are not available, tolerant varieties are the best option especially if disease-free planting material can be availed from isolated multiplication plots where crop hygiene is practiced. This is the short-term control strategy being implemented in both Tanzania and Mozambique. These are now being multiplied for wide distribution in the respective countries. The dangers of growing genetically uniform cultivars over wide areas cannot be overemphasized. Moreover, resource-poor farmers in unfavourable environments are credited for maintaining and managing crop genetic diversity. This diversity is of two types: First is the agromorphological diversity which is largely in response to use and preferences, e.g. colours, flavours, plant architecture and crop types. Second is the diversity in terms of a crop's adaptive characteristics, e.g. adaptation to microenvironments, environmental stresses or biological hazards such as pests (Eyzaguirre and Iwanaga, 1996). This project was therefore initiated to contribute to addressing the above two mentioned issues. The project's objective was therefore to develop new cassava varieties which combines multiple resistance to economic important diseases and pests, with farmers'/consumers' preferred traits. Consequently, farmers in the CBSD affected countries will have access to a wider range of improved cassava varieties.

Materials and Methods

Seedling trial. During the 2001/2002 planting season, a seedling nursery was established at the Sugarcane Agricultural Research Institute (SARI), Kibaha, Tanzania. The

seedlings belonged to 124 open-pollinated families of which 119 were from IITA Ibadan, four were from Malawi and one was from the local CBSD tolerant cultivar, *Kiroba*. Seedlings were spaced at 50 cm between and within rows in the field. Diseases and pests were monitored at three-month intervals. Seedlings showing disease symptoms were painted on their stems using waterproof paint. Different colours were used for different diseases. Harvesting was done 12 months after planting (MAP).

At harvest seedlings among each family that were painted red (CBSD symptoms) were heaped together, and those not red painted (no CBSD symptoms) were heaped separately. The number of seedlings in each group was recorded. All plant parts of the CBSD free seedlings were inspected closely for any disease symptoms. Roots were cut thoroughly piece-by-piece to make sure that only seedlings with no any CBSD symptoms (class 1) were selected for further evaluation. Roots of the seedlings painted red were also chopped to look for symptoms in the storage tissues (root necrosis). The number of seedlings that showed root necrosis in the two groups was also recorded.

Seedlings among the CBSD free heap that did not have any CBSD symptoms or severe CMD symptoms were tagged and given a number. Then the following data were recorded from each: Number of roots per plant, plant height, branching height, inner root skin colour, root pulp colour, number of stems. These traits will be followed up in subsequent trials to determine whether their expression at seedling stage correlated with their clonal stages.

The Percentage Number of Seedlings with Foliar Symptoms (PNSFS) was calculated by dividing the number of seedlings selected from each family by the number of harvested seedlings multiplied by one hundred. Foliar symptoms were taken as synonymous with above ground symptoms i.e. symptoms on stems and leaves. The Percentage Number of Seedlings with Root Symptoms (PNSRS) was calculated by dividing the number of

seedlings with root symptoms (necrosis) in each family by the number of seedlings with foliar symptoms (PNSFS).

Clonal Evaluation Trial (CET). Clones selected from the seedling trial were cloned and planted at three sites: Kibaha, a hot spot for both CMD and CBSD but poor soils (513 clones), Alavi estate, 10 km from Kibaha, where an entrepreneur (Mohamed Enterprises – MeTL) intends to establish a large cassava starch factory (480) and Zanzibar (170 clones). Planting was done in December 2002. Three cuttings per clone were planted. A check plot design was used where a local check was planted after every ten clones. The spacing of 1.0m x 1.0m was used. Plant height, pest and disease scores were taken at three months intervals up to harvesting. Painting their stems marked diseased plants. Red paint was used to mark CBSD affected plants, whereas black paint was used for CMD. Diseases and pests were subjectively scored on a scale of 1 – 5, where class 1 indicated no symptoms and class 5 very severe symptoms.

Open Quarantine Facility (OQF). In order to increase the number of resistant cultivars available to farmers, about 457 clones were introduced into the OQF (established at Kibaha, Tanzania in March 2003, Fig. 1) from the East African Root crops Research Network (EARRNET) breeding program at KARI Mtwapa, near Mombasa, Kenya. Mtwapa is a CBSD hot spot. Only clones, which did not show any CMD and CBSD symptoms, were introduced. The clones were also selected on the basis of their high yielding ability and high dry matter content. One stem per clone was taken. At Kibaha, each stem was cut into four cuttings and planted in the OQF. Planting was done on 4th May 2003. The spacing of 0.5m x 0.5m was used. After sprouting, the plants were closely inspected (weekly) for any disease symptoms (both CMD and CBSD). Plants showing any symptoms were recorded and then rouged and buried. After every two months, inspectors from the post-entry quarantine station based

at Arusha inspected the plants to make sure that only apparently disease free plants will be multiplied after the one-year compulsory confinement in the OQF. The OQF is located in isolation (at least 200m from the nearest cassava crop), fenced and guarded.

Crossing block. In order to generate new genotypes, which combine resistance to both CMD and CBSD, a crossing block was established at ARI Kibaha, Tanzania. The following cultivars were used: Kiroba, Amani 46106/27, NDL 90/34, Namikonga, Kigoma Red, Kitumbua (CBSD tolerant/resistant); Kibaha and TMS 4(2) 1425 (CMD resistant). Planting was done in April 2003. A randomised complete block design was used, with four replications. One row of ten plants constituted a plot. The spacing of 1.5m x 1.5m was used. A full diallel mating system will be used to generate F1 seeds for genetic studies.

Results

Out of 12,341 seedlings transplanted to the field 9,949 were well-established three months after planting (MAP). However, at harvest only 4,351 seedlings from 118 families were large and vigorous enough to be considered for further evaluation (Table 1). Hereafter, these will be referred to as the number of seedlings harvested.

The procedure of marking diseased plants with paint significantly made the harvesting and selection work much easier. Above ground CBSD symptoms may disappear due to leaf-fall and therefore, infected plants might look symptom-free at a later time of inspection.

About 88% of the harvested seedlings showed above ground CBSD symptoms, (mostly on leaves), among which 83% had root necrosis (Table 1). Only symptom-free seedlings were selected for further evaluation. A total of 513 such seedlings were selected (Table 1). Among the families which had a reasonably high number of seedlings harvested (more than 30), the following families had high PNSS (>50%): Kiroba (65%), 71762 (70%), and I88/00188 (60%).

At 9 MAP the majority of clones in the CET were apparently disease free both at Kibaha and Alavi estate (Table 2). Only twenty clones at Kibaha and 10 at Alavi estate had plants with maximum scores of class 3 for CBSD, which implies they are moderately susceptible to the disease. For CMD, 77 clones at Kibaha and 69 at Alavi estate had plants with maximum scores of class 4 – 5, indicating that they are highly susceptible to the disease (Table 2). Many clones at both sites showed high susceptibility to green mites, most probably due to the severe drought experienced this season. About 127 and 135 clones at Kibaha and Alavi estate respectively had plants, which showed high susceptibility to green mites (maximum scores of class 4 – 5).

Plant establishment in the OQF was very excellent due to watering. By the end of October 2003 (5 MAP), 169 (37%) clones out of the 457 introduced into the OQF had all their plants rouged. Out of the 288 (63%)

clones remaining, only 97 (21%) had all plants free of disease symptoms.

Discussion

Ruling out the possibility of seed-borne transmission (of which there is no evidence), the very high percentage of seedlings showing CBSD symptoms proves that at ARI Kibaha, high disease spread by the yet unknown vector occurred. Therefore, there is a high chance that, seedlings that did not show any CBSD symptoms and were therefore selected for further evaluation were not escapees. When you have a high NSH per family, and where the disease spreads easily and widely, PNSS can be a very good selection criterion for resistance to CBSD. Resistant varieties have been reported to remain symptom-free, and when symptoms did occur they were usually mild and frequently confined to the roots (Jennings, 1960). Families with a high PNSS could have a

Table 1: Summary of the data recorded during harvesting of cassava seedlings at Kibaha, 2001/02 season.

No. of seedlings harvested	No. of seedlings selected	No. of seedlings with foliar symptoms	No. of seedlings with root symptoms	Families with high PNSS	Families with low PNSS
4,351	513	3838 (88%)	3179 (83%)	Kiroba (65%) 188100188 (60%) 71762 (30%)	Kiroba (26%) 71762 (30%)

Table 2: Reaction of clones to CMD, CBSD and green mites evaluated in the CET at Kibaha and Alavi estate, 9 MAP during the 2002/03. season.

Maximum score	CMD		CBSD		Green mites	
	Kibaha	Alavi	Kibaha	Alavi	Kibaha	Alavi
Class 1	298	158	386	284	0	1
Class 2	69	45	75	34	158	192
Class 3	37	56	20	10	196	107
Class 4	48	51	0	0	88	28
Class 5	29	18	0	0	39	0
Total	481	328	481	328	481	328

high general combining ability for resistance to the disease.

The fact that the majority of clones in the CET remain free from both CMD and CBSD symptoms 9 MAP imply that they might be resistant to the diseases. The trial will be harvested in December 2003. Farmers will be invited to select clones, which they prefer. Researchers will document the selection criteria used by the farmers. The clones, which the farmers have selected, will be multiplied using rapid multiplication techniques so as to have enough planting material of each for on-farm evaluation the following season. Fast tract evaluation of the clones will be done both on station and on-farm using a decentralised, farmer participatory approach. This will ensure that the clones, which will perform well, on-farm, will be widely adopted, because they will possess traits that combine farmers' preference with agro-ecology specific adaptation. Farmers in these areas will within a short time (4–5 years instead of 8–10 years by conventional breeding scheme) therefore, have access to a wider range of improved varieties.

The fact that 79% of the clones introduced into the OQF had primary infection (cutting infection) highlights the difficulty of visual selection for clean planting material. Since the plants were regularly closely inspected for any disease symptoms at Mtwapa, it seems both of the two virus diseases remained latent in some clones and symptoms were expressed when grown at the new environment.

Conclusions

- Evidence of the spread of CBSD by the unknown vector has been obtained from this study
- Preliminary results indicates that the locally adapted CBSD tolerant cultivar “Kiroba” might be a good parent for use in breeding for CBSD resistance
- The majority of clones evaluated in the CET seem to be resistant to both CMD and CBSD because they remained free of symptoms 9 MAP

- The use of the OQF has facilitated the quick and efficient introduction of improved germplasm
- Visual selection for disease free planting material is a very unreliable method

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