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Full Length Research Paper

Assessment of large population of cassava accessions for resistant to cassava bacterial blight infection in the screen house environment

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Cassava bacterial blight (CBB) is caused by *Xanthomonas axonopodis* pv manihotis *(Xam)* a major cassava disease in all cassava growing area of the world. Resistance to the disease is found in *Manihot esculenta* and, in addition, has been introduced from a wild relative, *M. glaziovii*. We introduced a novel method of screening large population of cassava accessions in a screen-house environment using planting tray with a planting tree that can accommodate 68 different cassava stems cuttings in one planting tray. A total of 1,090 cassava accessions were screened with this method in the screen-house. The experimental design was augmented with randomized complete block design (ARCBD). Out of 490 land races cassava accessions that were assessed for susceptibility to CBB, 14.3 % were resistant to the disease. Land races populations were more susceptible to CBB infection having 30.1% highly susceptible and 12.3% susceptible cultivars. Six hundred cassava accessions of the improved and the new improved were screened in the greenhouse. Within the improved cultivars, 11.1% showed very strong resistance and 30.1% were resistant to the bacterial infections while 4.3% of these improved cassava accessions were observed as being highly susceptible to the disease and 36.6% were tolerant. Our results demonstrated that resistance to CBB is broadly distributed in cassava germplasm and for quick evaluation, the planting tray system should be employed.

Key words: CBB, *Xanthomonas axonopodis* pv manihotis, *Manihot esculenta,* resistance, susceptibility.

INTRODUCTION

Xanthomonas axonopodis pv manihotis causes bacterial blight, the most important bacterial disease of cassava in all cassava growing areas of the world (Lozano, 1986). Compared to the long history of cassava cultivation, the deployment of resistance cassava varieties to *X. axonopodis* pv manihotis in commercial cassava cultivars is relatively recent. The introduction of these resistance cassava cultivars correlated with a change in the pathogenic diversity of *X. axonopodis* pv manihotis populations, that is, new strains of the pathogen emerge and overcome deployed resistance (Dixon et al., 2002; Ogunjobi, 2005). These observations have stimulated much curiosity concerning the contribution of host genotype and other factors to the genetic diversity of the pathogen.

Molecular markers have been used in conjunction with virulence typing to evaluate the diversity and structure of *X. axonopodis* pv manihotis populations within and between countries in Africa (Assigbetsé et al., 1998; Ngeve, 1999; Dixon et al., 2002; Ogunjobi, 2005). In general, regionally defined pathogen populations in Africa were not found to be distinct (Wydra et al., 1998; Ogunjobi et al., 2001). Although populations within a region generally were similar, in some cases genetically similar strains were detected in different regions, suggesting the migration of strains between countries, (Assigbetsé et al., 1998; Wydra et al., 1998). Within Nigeria, significant differentiation in virulent populations was observed between different agro-ecosystems; that is, virulence of *X. axonopodis* pv manihotis in the rain forest and Southern Guinea Savannah agro-ecological systems in which improved cassava cultivars and land races varieties

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Plate 1. Screen house experimental layout showing cassava accessions planted in a plating tray. Each plating tray holds 68 cassava cultivars. The experimental design is augmented with randomised complete block design (ARCBD).

were grown together and were different from those in the Northern Sudan Savannah, where land races varieties dominated (Ogunjobi et al., 2001; Dixon et al., 2002).

To overcome the threat of CBB in cassava cultivation, there should be a strategy for screening large population of cassava accessions in other to determine resistant varieties for deployment to high disease pressure areas. Here we proposed a new method of screening large population of cassava in the green house to assist in quick identification of CBB resistant cultivars.

MATERIALS AND METHODS

The method of using cassava planting tree to screen cassava for CBB resistance was introduced and over 1000 cassava accessions held in International Institute of Tropical Agriculture (IITA) Ibadan were assessed for resistance to cassava bacterial blight with this method. This method is design to hold 68 cassava cultivars on a single tray of planting tree. The planting tree is made up of a long white polyethylene tube perforated at the lower end to allow water to drip out and the other upper side with an open end for in filling of the tube as shown in plate 1. The long white polyethylene tube (hereafter referred to as planting tree) in this case was filled with coco-peat which served as soil that supports the roots of the plants and the open end was tighten with a cupper wire. The polyethylene tubes were hung with an iron rod on an aluminum tray in such away that the perforated end was down. The design of the planting tray allow the entire set up to be covered with a covering net and also allow a holes pipe to be inserted at the top of the tube for irrigation as shown in plate 1.

In this experiment, all the different cassava genotypes stem cuttings were well labeled with ribbon to differentiate them and they were planted on the tree of the planting tray. Ten planting trays were arranged with 600 different genotypes to be screened and 80 checks representing 8 checks per block in the greenhouse as shown in plate 1, and the experiment was done twice to avoid over crowding and repeated again for reproducibility. Augmented with completely randomized block design was used as the experimental design. The cassava cultivars were planted on coco-peat and were irrigated once in a week for the first three weeks and later they were irrigated every other day with 5 liters of water. The water drips into the planting tree through the 2 mm rubber holes connected from the water source to the tube. The excess water drained off from the perforated end of the tube (Plate 1). Isolates 131B, 119A and 115A of *X. axonopodis* pv manihotis that has been identified to be consistent in virulence across the six cassava genotypes (Ogunjobi, 2005) were used for screening experiment. The bacterial inocula were developed by washing 20 plates of 48 h old YGCA cultures with 500 ml sterile distilled water for each isolate. This was homogenized on a magnetic stirrer to obtain a uniformly dispersed bacterial suspension. Exactly 1000 ml of this bacterial cells suspension was made up to 20 litres with sterile distilled water to give 10^6 - 10^8 cfu/ml. About 50 ml of Tween 80 was then added and shaken together to facilitate adhesion of the inoculum on the under surface (abaxial surface) of the leaves.

Plate 2. Spraying cassava germplasm after fourth weeks of growth on the abxial surface of the leaves in the screen house with bacterial suspension using motorized sprayer (Made in Germany, Kleinmotore GMBH, Model: Solo TYP 422).

The cassava germplasms were sprayed, inoculated with the suspension of bacterial isolates after fourth weeks of growth on the abaxial surface of the leaves with motorized sprayer (Model: Solo TYP 422) shown in Plate 2. This was carried out very early in the morning before sunrise to facilitate condition for infection. The cassava plants were covered on the plating tray with the net designed to cover the tray as shown in Plate 1 for 24 h before and 48 h after inoculation to enhance maximum humidity. Also the floor of the screen house was kept wet with running water to increase the humidity and to reduce the greenhouse temperature. The symptoms manifestations were monitored and scored periodically for two months based on the responses of the cassava clones to the disease symptom development; a score on disease rating scale of 1 to 5 (where 1 is no symptom development and 5 is severe damage by the disease). The cassava accession were grouped as: (i) Resistant cultivars if they scored less than 2 on the scale; (ii) Mild resistant clones if score was between 2 and 2.5 on the disease scoring scale; (iii) Tolerant cultivars when score ranged from > 2.5 and 3.5 while (iv) Susceptible clones scored > 3.5 and 4. (v) Any cultivar that scored value that is greater than 4 was regarded as highly susceptible.

RESULTS

All the cassava germplasm populations of the International Institute of Tropical Agriculture Ibadan (which included landraces or local accessions, improved germplasm and new improved germplasm populations) screened for the inherent ability to withstand CBB attack in the screen-house condition, revealed different levels of susceptibility. A total 1,090 cassava cultivars were inoculated in the screen house using this method of planting as shown in Plate 1 and 2.

A total of 490 local cassava accessions were assessed for susceptibility to CBB. Out of these, 14.3% were resistant to the disease as shown Figure 1. The resistant cultivars were those that manifested symptoms less than or equal to 2.5 while the very resistant ones had disease symptom less than 2 on the rating scale. Majority of the land races population were susceptible to CBB infection, 30.1% were highly susceptible while 12.3% were susceptible to the disease. The susceptible cassava clones were those that developed disease symptom greater than 3.5 while the highly susceptible had symptom greater than 4 on the scale. The tolerant groups were those that developed the symptoms but resisted the spread of the disease beyond the initial blight stage that was scored 3 on the scoring scale, consequently 24.3% were tolerant to the infection among the local cassava accessions. Six hundred cassava accessions of the improved and the new improved were screened in the greenhouse. Of these, 11.1% showed very strong resistance and 30.1%

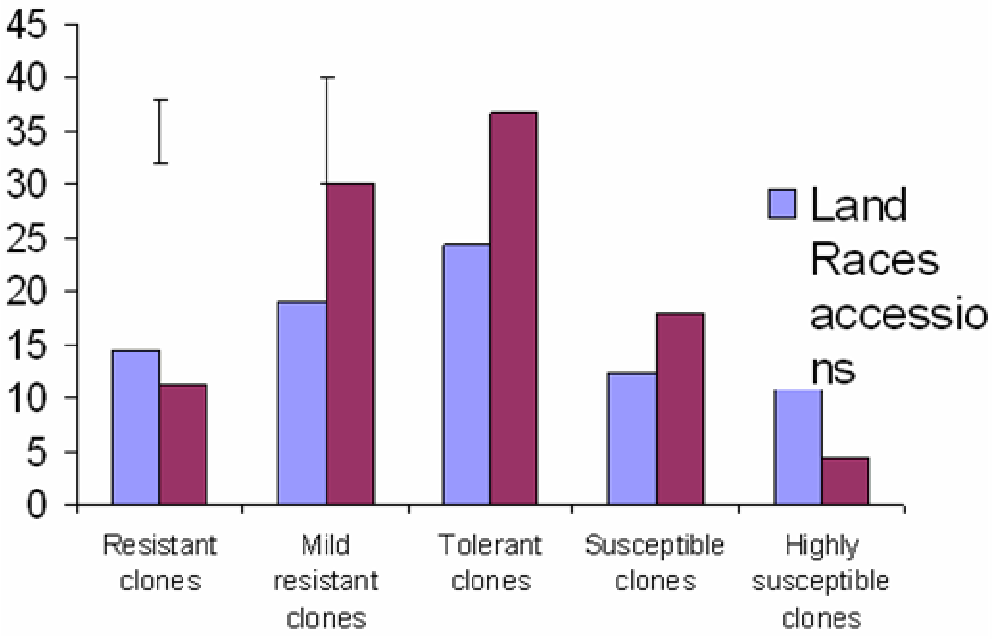

Figure 1. Susceptibility level of improved and local cassava accessions to cassava bacterial blight in International Institute of Tropical Agriculture (IITA) cassava germplasm collection

were resistant to the bacterial infections while 4.3% of these cassava accessions were observed as being highly susceptible to the disease while 36.6% were tolerant. The method showed very efficient way of screening large population of cassava cultivars within a very short period in a control environment.

DISCUSSION

The cassava populations were grouped into three categories based on their susceptibility level to this disease caused by Cassava Bacterial Blight (CBB). The resistant cassava varieties were the fewest (4.3%) and the most numerous were the tolerant varieties (36.6%) among the improved genotypes. The tolerant clones cannot be relied on for either planting in high disease pressure areas or in breeding for disease resistant cassava since they might revert to being susceptible in another environment. Some of the clones that exhibited strong resistance to CBB infection could be developed for mass deployment to areas where there is high level of disease pressure, although caution must be exercised in such deployment since it has been established that some cassava varieties show regional adaptability (Ngeve, 1999) and most cassava adaptability is narrow (Dixon et al., 1994; Dixon and Nukenine, 1997; Dixon et al., 2002). Such resistant cassava cultivars should be examined in the areas where the deployment is intended before mass production could be embarked upon.

Close observations showed that some of these cassava cultivars such as TME 9 and TME 3 that were tolerant in the screen-house were susceptible to the disease from the field record, such cultivars could be deceptive if it is deployed to area with high disease pressure. According to Dixon et al. (2002), Ibadan and Ubiaja were identified as locations with high levels of disease pressure. These two locations in Nigeria are suitable for screening cassava genotypes for resistance to CBB because genotypes that show resistance to CBB at either of these locations could as well be able to resist the disease at other locations where the disease pressure is not as high. The screening carried out in this study was done in Ibadan, one of the locations identified as having high disease pressure with one of the most aggressive isolates (strain 131B) originating from Ibadan. This was in line with the report of Johnson (1984) who proposed that the most powerful test for durability of resistance in plants was for the plant to be grown in an environment favouring the disease. Another advantage of screening all the cassava accessions was to suggest possible cassava cultivars that could be useful in molecular mapping for resistant markers and marker-assisted selection, characterizing resistance to CBB in the cassava population was also to assess the likely effects of selection for this character on the overall genetic diversity.

Host plant resistance has been used extensively for disease control in many crop species. The deployment of resistant varieties is also the major method of CBB control in cassava. Resistance has been found in *M. esculenta* and in its wild relative *M. glaziovii* (Hahn, 1978). Resistance in cassava is said to be polygenic and

additively inherited (Lozada, 1990), but the genes involved in the resistance response have not yet been identified (Verdier et al., 1997). Many types of resistance, however, are not long-lasting as a result of rapid changes in pathogen populations (Vera Cruz et al., 2000). Changes in the population structure of *X. axonopodis* pv manihotis has been reported in Colombia where the predominant haplotype has changed (Restrepo et al., 2004). Durability of resistance (R) genes is thus central to sustainable disease management. To date, it has been possible to assess the durability of an *R* gene only after its deployment for a long time over a large area (Johnson, 1984). The capacity to predict the durability of *R* genes would be highly desirable for making sound investments in breeding and genetic engineering. Although, variation in the aggressiveness of *Xam* strains has been reported (Maraite and Meyer, 1975; Restrepo and Verdier, 1997), and in recent study, Africa population of the bacteria was reported to be heterogynous indicating that the population *Xam* is not static in Africa (Assigbetsé, et al., 1998; Ogunjobi et al., 2006, 2007). Also, no clear interactions between cassava cultivars and *Xam* strains have yet been established. The most limiting factor in such studies has been the lack of a suitable way of choosing the cassava varieties that can be used for establishing an appropriate set of host differentials. Since there are very large populations of cassava accessions available in the world cassava collection held at CIAT (Sánchez et al., 1999).

However, with this method of screening large population of cassava cultivars at once in a control environment, it is now possible to establish clear interaction between cassava cultivars and *Xam* strains. This method of screening also is of immense advantage because of the environmental safety regulation that prohibits the release of pathogenic organisms in the environment. This had since being a very serious restriction to massive screening of cassava cultivars in this country for deployment to other neighbouring African countries. Although, the method is labour intensive, it is a task that must be done if we are going to survive the onslaught of Cassava bacterial blight and ensure food security in Africa.

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