

Full Length Research Paper

Stability of resistance to cassava brown streak disease in major agro-ecologies of Uganda

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Received 21 November, 2014; Accepted 19 January, 2015

Cassava brown streak disease (CBSD) is the most devastating disease of cassava in southern, eastern and central Africa, and can cause up to 100% yield loss. Limited progress has been made in breeding for host plant resistance due to limited knowledge on the resistance variability to the disease. Reaction of promising cassava genotypes to CBSD in multi-environments are also unknown. Therefore, this study intended to: (1) Identify additional sources of resistance to CBSD; (2) Determine the stability of resistance to CBSD, and (3) mega-environments for screening resistance to CBSD. Field evaluation of 19 genotypes was conducted in RCBD with three replications at three agro-ecologies of Uganda for two cropping cycles. Additive Main Effects and Multiplicative Interaction (AMMI) and (GGE) biplot models were used to analyze genotype-environment interactions. Based on mean field reaction, the six best genotypes identified for resistance to CBSD were: TZ/06/140, TMS30572, TZ /06/130, N3/66/1, N3/58/1 with N3/104/3 and N3/66/1 being the most stable. While N3/66/1, N3/58/1 and N3/104/3, Mzungu and Kigoma Red were reported to be putative new sources of resistance to CBSD in Uganda. Genotypes (G), Environments (E), and GxE interactions were all significant, with no genotype exhibiting complete resistance. The significant result for GxE interaction to CBSD indicates the need for multi-environment screening and is suggestive of quantitative nature of CBSD resistance.

Key words: Cassava brown streak disease; resistance, GxE interaction, discriminatory ability.

INTRODUCTION

Cassava is the second most important staple crop in Uganda with a very high commercial potential, which can propel the country towards industrialization. However, the productivity of this crop is being threatened by cassava brown streak disease (CBSD), now rated as the most

important constraint to cassava production in Eastern and Central Africa (Mohammed et al., 2012; Hillocks and Jennings, 2003). The disease is caused by two distinct virus species, the coastal endemic virus, referred to as cassava brown streak virus (CBSV) and the highland

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endemic virus, referred to as cassava brown streak Uganda virus (UCBSV) (Mohammed et al., 2012). Both species belong to the genus *Ipomovirus*, family *Potyviridae* (Mbanzibwa et al., 2009a, b). Typical symptoms of the disease include; interveinal chlorosis on the leaves, necrotic streak on the stem and brown or grey corky root necrosis. The disease was first detected at a lower altitude (<1000 m.a.s.l) along the coastline areas of Kenya, Tanzania and Mozambique. Recently, the re-emergence of the disease was reported in Uganda (Alicai et al., 2007), which has an average altitude of >1,000 m.a.s.l and the environmental conditions are quite different from lowland areas where the disease has been endemic (Nichols, 1950).

Early research shows that symptom expression and resistance to the virus depend on environmental conditions, with severely diseased plants dying at high altitude (Nichols, 1950). In very susceptible varieties, severely diseased roots become completely destroyed and unfit for market or family use (Mohammed et al., 2012). CBSD root symptoms become more severe under unfavorable environmental conditions (Jennings, 1957). Higher incidence and severity of symptoms have been reported during low night temperatures (Jennings, 1957). Ironically, both stem and root symptoms may disappear or be reduced if conditions become more favorable to the growth of the plant (Jennings, 1957). It also suffices to note that, soil nutrient deficiencies, such as deficiency of Manganese and Zinc cause inter-veinal chlorosis similar to CBSD foliar symptoms. Phosphorus deficiency result in early abscission of leaves which interfere with disease assessment. When the environmental conditions cause symptoms similar in expression to the trait under evaluation, interpretation becomes more complicated. However, the consistent presence or absence of any particular symptom in any one infected clone is attributable to genetic factors (Nichols, 1950).

Steps in crop improvement begin with identification of sources of genetic variations for the target trait and suitable environment for its evaluation. The initial breeding for virus resistance was started by British scientists at the Amani Research Station, in the then called Tanganyika in 1937 (Jennings, 1957). Later, as the breeding program selected for both CMD and CBSD resistance, some genotypes segregated for both sources of resistance (Jennings, 1957). That is why the majority of the genotypes selected and evaluated in the current study had some pedigree related to the Amani breeding program. That was the hypothesis for the identification of additional sources of resistance to CBSD in Eastern Africa.

The re-emergence of CBSD in Uganda (Alicai et al., 2007) has created an urgent need for breeding for resistance as an effective management option. However, initiation of any breeding program must begin with identification of genetic variation and a suitable environment for the evaluation of the trait. No detailed

genotype by environment study has been conducted to understand the response of cultivars and / or breeding lines with regard to their CBSD reaction to the disease in Uganda. Recent studies conducted in Uganda focused mainly on screening of germplasm at one location and one season (Abaca et al., 2012a, b; Abaca et al., 2013), with limited emphasis on reaction grade. Studies conducted elsewhere, show that resistance to CBSD is inherited quantitatively, and therefore are likely to be influenced by environment, making multi-environmental evaluation necessary (Munga, 2008; Zacarias and Labuschagne, 2010; Kulembeka et al., 2012).

Recent studies on genetic diversity of cassava in Southern, Eastern and Central Africa regarding resistance to CBSD revealed significant genetic variation within the population in the region (Pariyo et al., 2013). However, the above diversity has not been evaluated in multi-locational trials to establish resistance stability. The results of such field evaluation would be useful in making decisions regarding breeding for resistance endemic (Allard, 1960). In that case, differences observed in disease resistance or susceptibility among genotypes can be associated with stability in performance (Xu, 2010). Early work on cassava also showed that wide variation in susceptibility to brown streak is a varietal characteristic and therefore presumably controlled by genetic factors (Nichols, 1950). Elsewhere, similar GxE studies have been conducted on cassava to identify suitable genotypes for various environments (Ngeve et al., 2005; Egesi et al., 2009; Aina et al., 2010). Comprehensive studies on GxE have not been conducted in Uganda since CBSD re-emerged. Furthermore, Knowledge of GxE interaction is important in germplasm exchange, especially for those with broad adaptation for resistance to the disease, identification of appropriate breeding environments, selection of parental genotypes for constitution of breeding combinations, and designing appropriate breeding approaches. Therefore, the specific objectives of this study were to (1) identify additional sources of resistance to CBSD, (2) determine stability of resistance to CBSD, and (3) characterize mega-environments for screening for resistance to CBSD.

MATERIALS AND METHODS

Experimental materials

Nineteen genotypes of diverse origin and all resistant to cassava mosaic disease (CMD) were used to avoid the confounding effect of CMD on the foliar evaluation for CBSD (Table 1). Ugandan adapted resistant genotype (MM96/4271) and susceptible genotype (TME14) were used as controls. The test genotypes used were categorized into five; (1) IITA introductions released in Uganda, (2) IITA introductions not officially released in Uganda, (3) Tanzanian landraces introduced in form of clones, (4) Tanzanian landraces introduced as sexual seeds and (5) S₁ progenies from TMS30572 as progenitor (Table 1). TZ/06/130, MM 96/4271 and MM96/0686 are reported to be tolerant to CBSD in Uganda but their relative

Table 1. Pedigree and origin of selected cassava cultivars used in the present study.

| Genotype | Genotype code | Pedigree | Genotype categories based on geographical or country of origin |
|-------------|---------------|----------------------------------|--|
| MH97/2961 | G1 | 58308 X OYARUGBA | IITA introduction released in Uganda as NASE13 |
| MM96/4271 | G2 | I92/0248 HS | IITA introduction released in Uganda as NASE14 |
| MM96/0686 | G3 | [81/0163X91/00454]HS | IITA introduction not officially released in Uganda |
| 95/SE00036 | G4 | OP unknown parents | IITA introduction not officially released in Uganda |
| TMS192/0067 | G5 | 91934 x TME 1 HS | IITA introduction not officially released in Uganda |
| Kigoma Red | G6 | Landrace clones | Tanzanian landrace to Uganda in TC plantlet |
| Mzungu | G7 | Landrace clones | Tanzanian landrace to Uganda in TC plantlet |
| N3/104/1 | G8 | TMS30572 S ₁ Clone | S ₁ progeny from TMS30572 |
| N3/104/3 | G9 | TMS30572 S ₁ Clone | S ₁ progeny from TMS30572 |
| N3/127/1 | G10 | TMS30572 S ₁ Clone | S ₁ progeny from TMS30572 |
| N3/514/4 | G11 | TMS30572 S ₁ Clone | S ₁ progeny from TMS30572 |
| N3/514/10 | G12 | TMS30572 S ₁ Clone | S ₁ progeny from TMS30572 |
| N3/58/1 | G13 | TMS30572 S ₁ Clone | S ₁ progeny from TMS30572 |
| N3/66/1 | G14 | TMS30572 S ₁ Clone | S ₁ progeny from TMS30572 |
| TZ/06/130 | G15 | NDL90/34 HS seeds | Selected from OP seed introduced from Tanzania |
| 95/NA00063 | G16 | 91934 X SULEJA-4 HS | IITA introduction released as NASE10 |
| TMS30572 | G17 | 58308 X BRANCA DE SANTA CATARINA | IITA introduction released in Uganda as NASE3 |
| TME14 | G18 | Abbey-lfe | IITA introduction not officially released in Uganda |
| TZ/06/140 | G19 | KIBAHA HS seeds | Selected from OP seed introduced from Tanzania |

Source: TC = Tissue culture plantlets; IITA = International Institute of Tropical Agriculture; G1-19 = Codes for the nineteen genotypes.

degree of resistance and stability are not known; *Kigoma Red* and *Mzungu* were reported to be tolerant to CBSD in Tanzanian coastal zone, but their reaction were not known in Uganda. While, N3/104/1, N3/104/3, N3/127/1, N3/514/4, N3/514/10, N3/58/1 and N3/66/1 (S₁ progenies from TMS30572); and 95/NA00063 were all of unknown reaction to CBSD. The last category of cultivars that is, TME14, MH97/2961, TZ/140, TMS192/0067, TMS30572 95/SE00036 were known to be susceptible but their degree of variability in susceptibility remained unknown.

All the planting materials for the test genotypes from Uganda were collected from CBSD symptomless plants; this was in Northern region of the country where CBSD pressure is extremely low. Stakes were virus tested prior to field establishment. Plant materials from Tanzania were received inform of virus indexed tissue culture and hardened in insect proof screen house prior to field establishment. This was to ensure that all the starting materials were free of viruses. Five plants of the test genotypes were then planted in single row plots without replication for one growing season (2010/2011) at Namulonge to generate sufficient planting materials for the multi-location trials. During the field multiplication, the test plants had uniform and adequate exposure to the CBSVS since the starting materials were disease free.

Test environments

Subsequent trials were conducted in two cropping seasons, 2011/2012 and 2012/2013, in three locations resulting in six test environments. Details of environmental conditions are presented in Table 2. These were, Bulindi, located at 1, 218 m.a.s.l, coordinates as 01°28'N and 031°26'E; Namulonge located at 1,144 m.a.s.l with coordinates as 00°52'N and 032° 62'E; and Ngetta located at 1,067 m.a.s.l with its coordinates as 02°17'N and 32°57'E. Basing on CBSD survey mapping, these areas were categorized by varied

levels of mean incidence of CBSD (Table 2). An analysis of the prevailing viruses in the test field at Namulonge and the surrounding cassava fields were done according to procedure described by Mbazibwa et al. (2010) prior to establishment of multi-environment trial (MET). This revealed that both species of CBSVs were present, with CBSV being predominant.

Experimental design for the main trial

The middle semi-woody portions of the stems were used as stakes for planting to ensure uniformity in the physiological state of the stakes and minimize variation during field establishment. The test plants were established in a randomized complete block design (RCBD) with three replications. Due to insufficient planting materials, single row plots of each genotype were established with five plants per row at spacing of 1 x 1 m. To ensure that no genotype escaped infection from the disease, a highly susceptible cultivar, TME204, was used as an infector row (Abaca et al., 2012, a, b). The infector plants were planted at the same spacing of the test plants but one month earlier to develop sufficient inoculum. The experimental plots were maintained weed free until harvest at 12 months under rain fed conditions without any soil amendments or pesticide applications.

Data collection

Disease assessment

Assessment of the response of the test genotypes to CBSD was based on evaluation of root necrosis (Nichols, 1950). At harvest all roots of test genotypes were harvested from each test plant and

Table 2. Prevailing conditions at the test environments.

| Parameter | Location / year | | | | | |
|-------------------------------|------------------|-------------------|------------------|-------------------|-------------------|-------------------|
| | 2011/2012 | | | 2012/2013 | | |
| | BUL ¹ | NAM ² | NGE ³ | BUL | NAM | NGE |
| R/Fall (mm) | 1306 | 1370 | 1452 | 1045 | 1435 | 1582 |
| Tem. Min (°C) | ND | 18.0 | 15.0 | ND | 15.7 | 14.8 |
| Tem. Max (°C) | 29.2 | 29.2 | ND | 28.9 | 28.7 | ND |
| District level CBSD Incidence | 7.7 | 50.2 ⁵ | 7.5 ⁴ | 26.8 ⁶ | 64.0 ⁵ | 15.8 ⁶ |
| Soil property | | | | | | |
| pH (4-8) | 5.60 | 6.00 | 5.80 | ND | ND | ND |
| OM (3%)* | 5.10 | 3.60 | 2.60 | ND | ND | ND |
| N (0.2%)* | 0.24 | 0.19 | 0.15 | ND | ND | ND |
| P (10.0 ppm) | 0.90 | 4.8 | 4.5 | ND | ND | ND |
| Ca (50.0 ppm) | 4933 | 3724 | 2379 | ND | ND | ND |
| Mg (14.3 ppm) | 1221 | 581 | 367 | ND | ND | ND |
| K (58..5 ppm) | 164 | 630 | 376 | ND | ND | ND |
| Zn (1.0 ppm)* | 0.70 | 4.10 | 0.6 | ND | ND | ND |
| B (0.2 ppm) | 0.02 | 0.02 | 0.02 | ND | ND | ND |
| Cu (5.0 ppm)* | 2.0 | 3.1 | 2.0 | ND | ND | ND |
| Fe (50 ppm) | 190 | 199 | 131 | ND | ND | ND |
| Mn (20 ppm)* | 189 | 156 | 187 | ND | ND | ND |

Source for critical values *Cadavid (2012). ¹Bulindi, ²Namulonge and ³Ngetta. The values in brackets are critical values for any crop and those without asterisks being specific for cassava. Rating of CBSD incidence in the surrounding of the experimental areas as; ⁴Low, ⁵Very High, ⁶Moderate, ND=Not determined.

slices made for a minimum of five times, depending on the length of the root, from the distal end in cross-section. Observations and scores for severity of the root necrosis was done on a 5 - point scale where; 1 = no apparent necrosis, 2 = less than 5% of root necrotic, 3 = 5 to 10% of root necrotic, 4 = 11 to 25% of root necrotic, mild root constriction and 5 = >25% of root necrotic, severe root constriction. Counts on the number of infected roots from each plant were used to compute the average plot CBSD incidence. The mean severity score for each plant was derived by averaging every root assessed which was then used to derive the plot mean severity score. Final disease assessment was based on plot means. To augment the observation on genotype response to CBSD disease, soil analysis was done with samples collected using the stratified sampling method according to procedure described by Hazelton and Murphy (2007) presented in Table 2.

Data analysis

Determination of response of test genotypes to CBSD infection

Genotypes were classified into four reaction categories on the basis of both the root severity and incidence. In the present study, resistance refers to the ability or the degree of the plant to suppress disease expression depending on the effectiveness of the protective mechanism. Basing on root severity, genotypes were classified as follows; mild severity score of 1.0 to 2.0, were considered as resistant; moderate severity score of 2.1 to 3.0, were considered as moderately resistant; moderately high severity score of 3.1 to 4.0, were referred to as moderately susceptible and genotypes expressing extremely high severity score of 4.1 to 5.0 were considered highly susceptible. While on basis of root incidence, genotypes were also categorized into four as: low disease incidence of 0 to 20%, were categorized as resistant; those with moderately low incidence of 21 to 40%, were referred to as

moderately resistant; while those with moderately high disease incidence of 41 to 60%, were categorized as moderately susceptible and those with very high incidence of 61 to 100%, were categorized as highly susceptible. Therefore, for a variety to be declared resistant based on root reaction, low incidence was considered along with low severity score (Hillocks and Jennings, 2003). Analysis from foliar assessment was considered unreliable due to complications from the nutritional deficiency symptoms that resemble CBSD symptoms (Hazelton and Murphy, 2007) and therefore not presented.

Analysis of variance

General analysis of variance (ANOVA) was used for detection of significance for genotype by environment interactions and the relative sizes of variations. ANOVA was combined over locations and years on the basis of plot means and pooled over locations and seasons using the generalized linear model procedures of the GenStat 13th Edition (2010). The general ANOVA was used to disaggregate the environment components into location, year and their interaction with the genotype based on mean squares.

Additive main effects and multiplicative interaction (AMMI) analysis

The AMMI model was considered important in the present study due to its power to compute the average genotype by environment means and genotype ranks across environments (Falkenhagen, 1996). During the analysis, the least squares fit for balanced data was obtained by fitting the additive part of the AMMI model with the ordinary analysis of variance and then applying the singular value decomposition of the matrix of the residual in order to obtain an estimation of the parameters of the multiplicative part.

Table 3. AMMI analysis of variance of response of 19 cassava Genotypes to CBSD evaluated at three locations for two years (2011/2012 – 2012/2013) in Uganda.

| Source of variation | DF | CBSD incidence | | | CBSD severity | | |
|---------------------|----|----------------|--------|------|---------------|--------|------|
| | | SS | MS | SS% | SS | MS | SS% |
| Genotypes (G) | 18 | 273072 | 15171* | | 209.1 | 11.61* | |
| Environment (E) | 5 | 23514 | 4703* | | 23.2 | 4.63* | |
| G X E | 90 | 95729 | 1064* | | 113.8 | 1.26* | |
| IPCA1 | 22 | 45322 | 2060* | 47.3 | 42.6 | 1.94* | 37.4 |
| IPCA2 | 20 | 18387 | 919* | 19.2 | 27.3 | 1.37* | 24.0 |
| Residual | 48 | 32020 | 667 | | 43.9 | 0.91 | |

*Interactions were significant at $p = 0.05$.

Genotype and genotype by environment interaction analysis

Genotype (G) and G by environment (E) GGE biplot analysis model was also used due to its statistical power to determine genotype stability, the discriminatory power of environments and characterization of the mega-environments (Yan and Kang, 2003). In this study environments were a combination of years (random effects) and location (fixed effects) and therefore treated as random effects. The GGE model was used to construct GGE bi-plots. The stability of a variety or environment was determined by the length of the vector from genotype marker to the average environment coordinate (AEC) abscissa. The vector which was closer to the AEC abscissa was considered to have less interaction effects and hence regarded as stable. A cultivar located at the origin would rank the same in all environments and is not at all responsive to the environments and therefore the most stable. The discriminatory power was detected by the length of the vector from the origin of the GGE biplot to the coordinate of the location. The longer the vector, the more discriminatory power.

RESULTS

Analysis of variance using additive main effects and multiplicative interactions (AMMI)

AMMI analysis of variance for responses of 19 genotypes to CBSD are presented in Table 3. The results indicate a significant genotypic effect, environmental effect and G x E interaction effects for both CBSD root incidence and severity (Table 3). The first bilinear interaction term of the AMMI analysis of the G x E for CBSD root incidence accounted for 66.5% of the sum of squares of the interaction term (PCA1 = 47.3% and PCA2 = 19.2%). Furthermore, in the analysis of CBSD root severity, the first two bilinear interaction terms accounted for 61.4% of the sum of squares of the available interaction (PCA1 = 37.4% and PCA2 = 24.0%) (Table 3).

Genotype rank and stability based on incidence of CBSD using GGE bi-plot

Significant GxE interaction for genotype responses to CBSD was observed through rank change based on disease incidence (Table 4). The best six genotypes had

low mean incidence (0 to 20%) and were referred to as resistant ; these included; N3/104/3, N3/58/1, N3/66/1, TZ/06/130, TMS30572 and TZ/06/140 (Table 4). Genotypes TZ/06/140 and TMS30572 had the lowest overall mean for disease incidence and were ranked the best in the three environments (Table 4). The genotypes N3/104/3, N3/66/1, TZ/06/130 and TMS30572 had short vector distance from AEC abscissa indicating relative stability while genotypes N3/58/1 and NAM/06/140 were unstable (Figure 1). In this category no genotype had a consistent leading rank across all the test environments.

The second category of genotypes considered were moderately resistant (21 to 40%) based on CBSD root incidence and consisted of four genotypes namely; MH97/2961, MM96/0686, Kigoma Red and Mzungu (Table 4). On the GGE bi-plot analysis, the two genotypes, Kigoma Red and Mzungu, of Tanzanian origin had the shortest vector distance from AEC abscissa indicating relative stability across all environments (Figure 1).

The third category of the genotypes considered in the ranking had moderately high incidence of CBSD (41 to 60%) which were referred to as moderately susceptible based on CBSD incidence and these consisted of four genotypes, N3/127/1, N3/514/4, N3/514/10 and TME14 (Table 4). On the GGE bi-plot analysis (Figure 1), the genotypes N3/514/4, N3/514/10 and TME14 had the longest vector distance from the AEC abscissa indicating relative instability for moderate susceptibility across all environments (Table 4). While genotype N3/127/1 had the shortest distance from the AEC and since it is located at the origin, it was considered the most stable in the category of moderate susceptibility.

The fourth category of the genotypes were of very high disease incidence (61 to 100%) which were referred to as highly susceptible based on CBSD root incidence (Table 4). These included five genotypes, MM96/4271, TMS192/0067, 95/SE00036, N3/104/1 and 95/NA00063 all registered across all the test environments. The worst two genotypes observed for disease incidence were 95/SE00036 and 95/NA00063. The GGE bi-plot analysis showed that all the genotypes laid close the AEC abscissa confirming their high stability for susceptibility in

Table 4. Mean CBSD incidence on roots of cassava genotypes evaluated at three locations for two years in Uganda.

| Genotype | Test environments | | | | | | Mean | SE |
|-------------|----------------------|----------------------|------------------------|------------------------|---------------------|---------------------|----------|------|
| | Bulindi 2011/2012 | Bulindi 2012/2013 | Namulonge 2011/2012 | Namulonge 2012/2013 | Ngetta 2011/2012 | Ngetta 2012/2013 | | |
| MH97/2961 | 7.9(4) | 12.0(6) | 72.0(13) | 31.7(8) | 12.4(4) | 8.5(8) | 24.1(8) | 10.2 |
| MM96/4271 | 90.5(17) | 59.2(14) | 83.9(14) | 60.5(13) | 79.4(16) | 56.7(16) | 71.7(16) | 6.0 |
| MM96/0686 | 1.9(2) | 7.3(5) | 90.8(15) | 32.5(9) | 3.2(1) | 6.1(6) | 23.6(7) | 14.2 |
| 95/SE00036 | 98.7(19) | 95.6(18) | 98.6(18) | 100.0(18) | 100.0(18) | 87.1(18) | 96.7(19) | 2.0 |
| TMS192/0067 | 72.3(15) | 65.9(16) | 98.6(18) | 76.6(17) | 75.0(13) | 61.0(17) | 74.9(17) | 5.3 |
| Kigoma Red | 55.5(12) | 14.5(8) | 59.9(11) | 17.2(6) | 34.9(8) | 15.5(9) | 32.9(9) | 8.4 |
| Mzungu | 7.3(3) | 43.2(11) | 51.0(9) | 61.6(14) | 40.1(10) | 30.0(10) | 38.9(10) | 7.7 |
| N3/104/1 | 73.1(16) | 57.8(12) | 94.7(16) | 66.7(15) | 69.7(12) | 54.5(15) | 69.4(15) | 5.8 |
| N3/104/3 | 35.3(8) | 0.0(1) | 51.0(9) | 4.8(1) | 16.6(5) | 0.2(4) | 18.0(6) | 8.6 |
| N3/127/1 | 53.3(11) | 38.3(10) | 62.2(12) | 44.4(10) | 52.2(11) | 33.5(11) | 47.3(11) | 4.3 |
| N3/514/4 | 63.5(13) | 59.4(15) | 31.6(4) | 57.8(12) | 77.9(15) | 47.8(12) | 56.3(12) | 6.4 |
| N3/514/10 | 45.7(10) | 69.2(17) | 19.2(4) | 71.5(16) | 80.7(17) | 51.6(14) | 56.3(12) | 9.1 |
| N3/58/1 | 35.8(9) | 12.5(7) | 3.5(1) | 9.0(5) | 35.3(9) | 5.4(5) | 16.9(4) | 6.0 |
| N3/66/1 | 19.1(7) | 0.0(1) | 41.6(6) | 7.8(4) | 11.9(3) | 0.0(1) | 13.4(3) | 6.4 |
| TZ/06/130 | 1.0(1) | 16.3(9) | 32.7(5) | 30.2(7) | 19.7(6) | 6.8(7) | 17.8 (5) | 5.1 |
| 95/NA00063 | 90.6(18) | 97.8(13) | 97.2(17) | 100.0(18) | 100.0(18) | 87.5(19) | 95.5(18) | 2.0 |
| TMS30572 | 8.6(5) | 0.0(1) | 41.9(7) | 7.1(3) | 5.0(2) | 0.0(1) | 10.4(1) | 6.5 |
| TME14 | 70.6(14) | 57.7(13) | 42.6(8) | 56.1(11) | 77.5(14) | 48.6(13) | 58.8(14) | 5.4 |
| TZ/06/140 | 18.9(6) | 4.2(4) | 11.6(2) | 6.9(2) | 20.8(7) | 0.0(1) | 10.4(1) | 3.4 |
| Mean | 44.7 | 37.4 | 57.1 | 44.3 | 48.02 | 31.6 | 43.9 | |
| SE | 7.5 | 7.5 | 7.0 | 7.2 | 7.6 | 6.8 | | |

SE = Standard Error of the mean, the number in brackets denotes the rank of the genotype.

all the test environments (Figure 1).

In all categories, no genotype had a consistent leading rank across all the test environments for CBSD incidence. However, the best two overall genotypes for low disease incidence and stability were TZ/06/130 and TMS30572 while the worst two genotypes for disease incidence were 95/SE00036 and 95/NA00063.

Genotype rank and stability based on mean severity of CBSD infection

The analysis of genotype by environment for CBSD root severity damage were significant and that was exhibited in rank change (Table 5). In the subsequent ranking of the genotypes, they were grouped into four categories based on severity scores as described in materials and methods section. The first category had six genotypes with mild severity (1.0 to 2.0) and were referred to as resistant and these included; TZ/06/140, TZ/06/130, TMS30572, N3/66/1, N3/58/1 and N3/104/3. This category consisted of two genotypes, TMS30572 and N3/104/3, with relatively high standard error (Table 5). However, an analysis of the corresponding GGE bi-plot showed that these genotypes, TMS30572, TZ/06/130 and N3/104/3, had the longest vector distances from the AEC

abscissa in the category indicating relative instability across most of the test environments. Contrariwise, TZ/06/140, N3/58/1 and N3/66/1 were the closest to the AEC meaning that they are relatively stable across the test environments.

In the ranking of the second category of genotypes, genotypes that had moderately severe infection (2.1 to 3.0) were regarded as moderately resistant and these included MM96/4271, MM96/0686, Kigoma Red, Mzungu, N3/127/1, N3/514/4, N3/514/10 and TME14 (Table 5). In this category, MM96/4271 had the lowest severity while Kigoma Red and Mzungu had the next lowest mean severity. The corresponding GGE bi-plot (Figure 2) analyses confirmed Kigoma Red and Mzungu had relatively short vector distance from the AEC abscissa suggestive of relative stability across all the test environments.

The third category of the ranking consisted of genotypes with moderately high severity (3.1 to 4.0) which were regarded as moderately susceptible. These included four genotypes; MH97/2961, TMS192/0067, N3/104/1 and 95/NA00063. Bi-plot analysis revealed that all genotypes were stable for susceptibility across all the environments (Figure 2).

In the fourth category of the ranking, genotypes with high severity (4.1 to 5.0) were regarded as highly

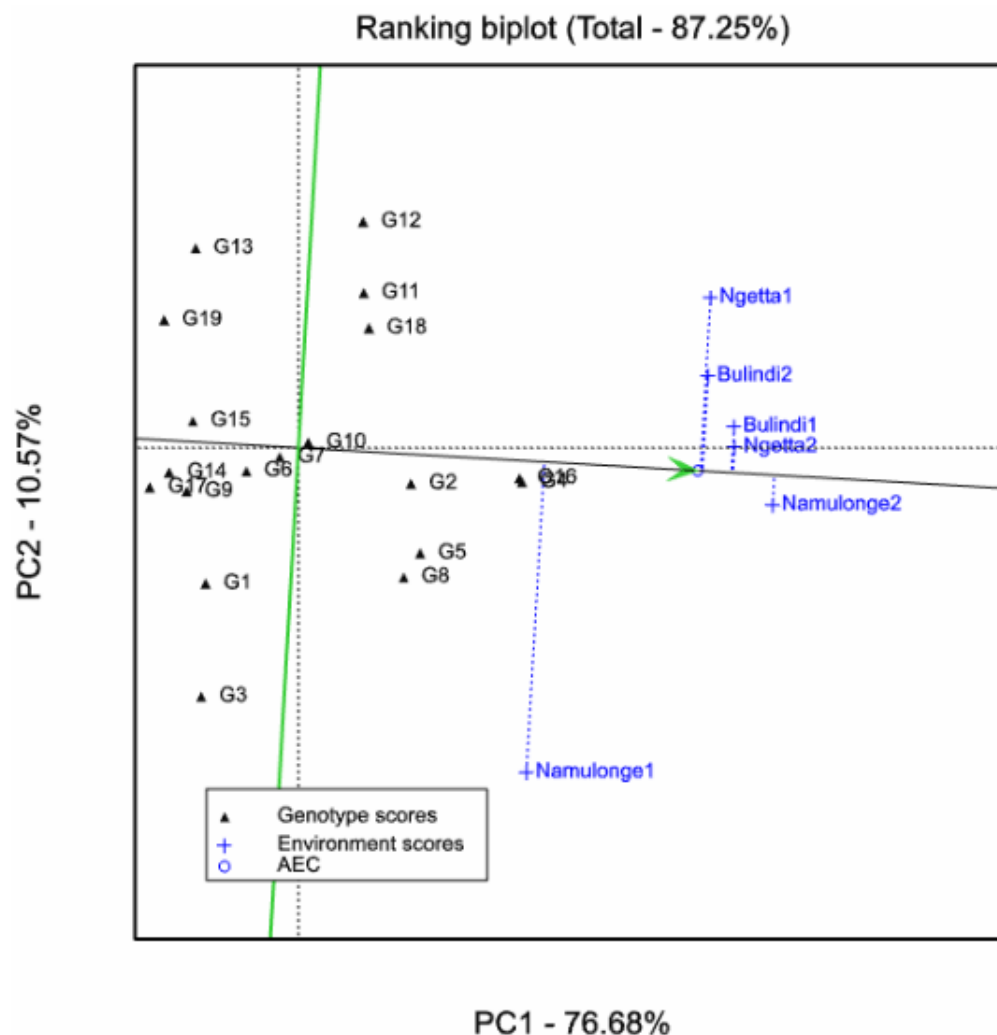


Figure 1. Illustration of genotype stability for resistance to CBSD based on CBSD root incidence using GGE bi-plot. 1 = MH97/2961, 2 = MM96/4271, 3 = MM96/0686, 4 = 95/SE00036, 5 = TMS192/0067, 6 = KIGOMA, 7 = MZUNGU, 8 = N3/104/1, 9 = N3/104/3, 10 = N3/127/1, 11 = N3/514/4, 12 = N3/514/10, 13 = N3/58/1, 14 = N3/66/1, 15 = TZ/06/130, 16 = 95/NA00063, 17 = TMS30572, 18 = TME14 and 19 = TZ/06/140. 1 & 2 in blue denote season 1 and 2 at each of the three locations respectively. AEC = Average Environment Coordinate.

susceptible and this consisted of one genotype; 95/SE00036 (Table 5). When the test genotype was compared to all the previous genotypes in the above categories, it had the lowest standard error and the GGE bi-plot indicated that it had the closest distance to the AEC indicating the highest stability for susceptibility across all the test environments (Figure 2). This genotype (95/SE00036) was consistently ranked the most susceptible across all the test environments. No genotype had a consistent leading rank for resistance based on root severity across all test environments suggestive of crossover interaction. However, overall, the best two genotypes with the lowest severity were TZ/06/140 and TZ/06/130 with TZ/06/140 being the most stable. On the other hand, the two genotypes with the highest, overall, severity were 95/NA00063 and

95/SE00036 which belonged to third and fourth categories respectively.

Characterization of test environments for genotype response to CBSD

The rainfall and temperature data were collected from the three trial sites during the two seasons of experimentation and presented in Table 2. The rainfall data pattern indicated that the mean annual rainfall were relatively lower at Bulindi and Namulonge than Ngetta in both cropping seasons. The maximum average temperatures were relatively uniform across all the sites in both cropping seasons. While The lowest annual minimum temperatures were recorded at Bulindi while

Table 5. Mean CBSD severity on roots of cassava genotypes evaluated at three locations for two years in Uganda.

| Genotypes | Test environments | | | | | | Mean | SE |
|-------------|----------------------|----------------------|------------------------|------------------------|-------------------|---------------------|---------|------|
| | Bulindi 2011/2012 | Bulindi 2012/2013 | Namulonge 2011/2012 | Namulonge 2012/2013 | Ngetta 2011/12 | Ngetta 2012/2013 | | |
| MH97/2961 | 4.6(18) | 3.2(17) | 4.1(18) | 3.2(13) | 3.6(15) | 3.5(18) | 3.7(17) | 0.22 |
| MM96/4271 | 1.4(3) | 2.1(10) | 1.7(1) | 2.9(12) | 2.5(8) | 1.7(8) | 2.1(7) | 0.23 |
| MM96/0686 | 1.2(1) | 2.0(9) | 4.0(16) | 2.5(9) | 3.5(14) | 1.6(4) | 2.5(10) | 0.45 |
| 95/SE00036 | 4.7(19) | 4.5(19) | 4.6(19) | 4.9(19) | 4.8(19) | 4.4(19) | 4.6(19) | 0.08 |
| TMS192/0067 | 2.9(12) | 3.0(15) | 3.7(15) | 3.5(16) | 3.7(17) | 2.8(15) | 3.3(16) | 0.17 |
| Kigoma Red | 2.0(5) | 1.7(6) | 2.9(10) | 2.0(6) | 2.5(8) | 1.6(4) | 2.1(7) | 0.20 |
| Mzungu | 2.0(5) | 1.9(8) | 2.2(6) | 2.4(7) | 2.3(5) | 1.8(9) | 2.1(7) | 0.09 |
| N3/104/1 | 2.6(10) | 3.0(15) | 3.5(13) | 3.6(17) | 3.6(15) | 2.7(14) | 3.2(15) | 0.18 |
| N3/104/3 | 3.6(16) | 1.5(3) | 2.1(3) | 1.3(3) | 1.5(1) | 1.9(10) | 2.0(3) | 0.34 |
| N3/127/1 | 2.1(7) | 2.2(11) | 3.2(12) | 2.6(10) | 3.0(12) | 2.0(11) | 2.5(10) | 0.20 |
| N3/514/4 | 2.7(11) | 2.3(12) | 2.2(6) | 2.8(11) | 2.5(8) | 2.3(12) | 2.5(10) | 0.10 |
| N3/514/10 | 3.1(14) | 2.9(14) | 2.7(8) | 3.4(14) | 3.0(12) | 2.8(15) | 3.0(14) | 0.11 |
| N3/58/1 | 2.3(9) | 1.5(3) | 2.9(10) | 1.6(4) | 2.2(4) | 1.6(4) | 2.0(3) | 0.23 |
| N3/66/1 | 1.9(4) | 1.6(5) | 2.8(9) | 1.8(5) | 2.4(7) | 1.5(3) | 2.0(3) | 0.20 |
| TZ/06/130 | 1.3(2) | 1.7(6) | 2.0(2) | 2.4(7) | 2.3(5) | 1.4(2) | 1.9(2) | 0.19 |
| 95/NA00063 | 3.6(16) | 3.6(18) | 4.0(16) | 4.0(18) | 4.1(18) | 3.4(17) | 3.8(18) | 0.12 |
| TMS30572 | 3.2(15) | 1.1(1) | 3.5(13) | 1.0(1) | 1.9(3) | 1.6(4) | 2.0(3) | 0.48 |
| TME14 | 2.9(12) | 2.8(13) | 2.1(3) | 3.4(14) | 2.8(11) | 2.6(13) | 2.8(13) | 0.17 |
| TZ/06/140 | 2.1(7) | 1.1(1) | 2.1(3) | 1.2(2) | 1.6(2) | 1.3(1) | 1.6 (1) | 0.19 |
| Means | 2.6 | 2.3 | 3.0 | 2.6 | 2.8 | 2.2 | 2.6 | |
| SE | 0.230 | 0.206 | 0.196 | 0.248 | 0.197 | 0.193 | | |

SE = Standard error; Lower values indicate higher resistance; the number in brackets denote the genotype rank.

Namulonge had the highest annual minimum temperatures when compared to Ngetta.

Meanwhile, a separate survey report on CBSD prevalence in the surrounding of the experimental areas indicated that the highest incidence was recorded at Namulonge in both cropping seasons, with higher incidences observed in the second cropping season (NARO, 2014, Unpublished). Soil analyses results indicated that, all the test sites were moderately acid and slightly above the critical values but within the range for cassava production. The levels of organic matter at Namulonge and Bulindi were above the critical value for any crop production and the value at Ngetta was below the critical value. The percentage of total Nitrogen at Namulonge and Ngetta were below the critical value for crop production. The values for P were below the critical values at all the sites with Bulindi having the lowest value. Values for Ca, Mg and K were all above the critical values for crop production (Table 2). Whereas, the values of micro-nutrients such as Zn, B, and Cu were all below the critical values required by any crop, the lowest values were recorded at Bulindi and Ngetta (Table 2). Fe values were approximately three to four times the critical values whereas, the values for Mn were about seven to nine times higher than the critical values.

The highest environmental mean based on CBSD

incidence was recorded in first year (2011/2012) across all locations (Table 4). Namulonge registered the highest disease incidence during both cropping seasons, followed by Bulindi and then Ngetta. Likewise for CBSD severity, mean severities were higher at Namulonge in both cropping seasons, followed by Ngetta and then Bulindi (Table 5). Of the six environments, Namulonge had the longest length of vector from the origin to the location marker indicating that it had the strongest discriminative ability for CBSD incidence in both cropping seasons followed by Ngetta and Bulindi that had similar lengths of the vector (Figure 1). However, for the CBSD severity, Ngetta in season II (2012/2013) had the longest vector distance giving it the strongest discriminatory power followed by Namulonge and Bulindi in the same season (Figure 2). However, relatively shorter vector distance for CBSD severity was recorded in the first season, thus the season with the least discriminatory power.

The GGE characterization of the test environments based on CBSD incidence separated the target environment into two mega environments with Namulonge season one (2011/2012) as a distinct environment from the rest of the other five environments (Figure 1). Conversely, environment characterization based on CBSD root severity clustered all environments

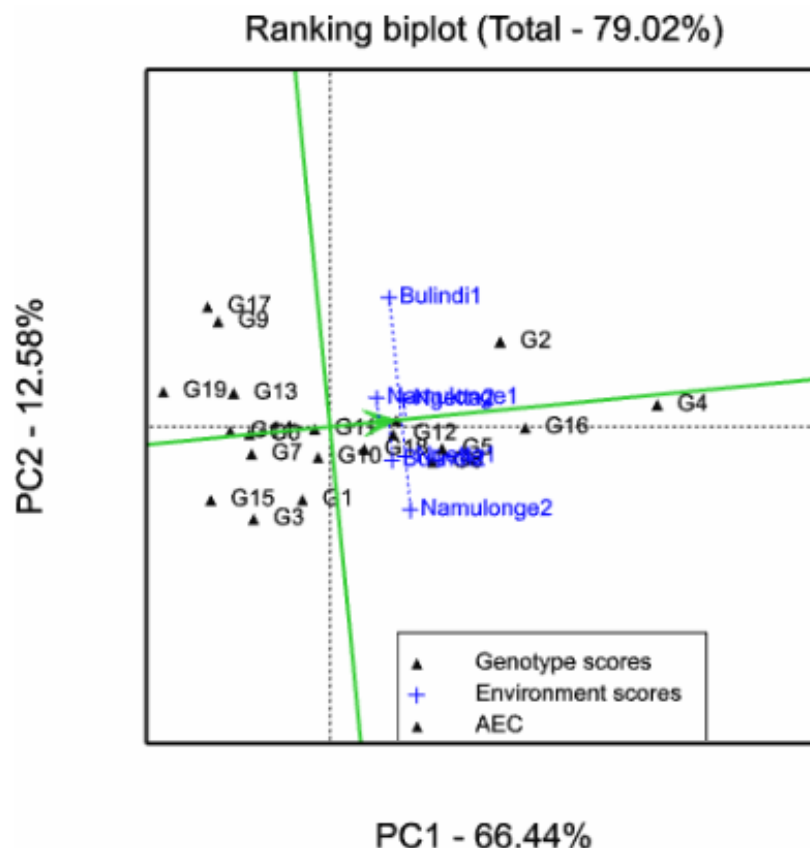


Figure 2. GGE biplot illustrating genotypic stability for resistance based on CBSD root severity. 1 = MH97/2961, 2 = MM96/4271, 3 = MM96/0686, 4 = 95/SE00036, 5 = TMS192/0067, 6 = KIGOMA, 7 = MZUNGU, 8 = N3/104/1, 9 = N3/104/3, 10 = N3/127/1, 11 = N3/514/4, 12 = N3/514/10, 13 = N3/58/1, 14 = N3/66/1, 15 = TZ/06/130, 16 = 95/NA00063, 17 = TMS30572, 18 = TME14 and 19 = TZ/06/140. AEC = Average Environment Coordinate.

into one group (Figure 2). The term mega environment used here is defined as a portion of crop species' growing region with a fairly homogeneous environment that causes similar genotypes to perform best (Xu, 2010). There was no genotype specifically associated with any one particular mega environment for both CBSD root severity and CBSD root incidence.

DISCUSSION

Variability in the response of cultivars to a disease is dependent on G and G x E interaction. G x E interaction is a major factor in the study of quantitative traits because it significantly affects the interpretation of genetic data and makes predictions difficult (Kearsey and Pooni, 1996). It is worse when dealing with breeding populations particularly when genotypes are evaluated and selected in one environment and utilized in another. The present

study was conducted to determine the degree of response of cassava genotypes to CBSD in Uganda. Here we discuss the causes of the observed response, resistance stability; GxE interactions and implications for crop improvement are discussed.

Genotype stability and variability in reaction to CBSD

The significant differences observed between genotypes for reaction to CBSD indicate wide genetic diversity among the genotypes for resistance to the disease. No genotype expressed immune responses to the disease in any environment. Furthermore, the significant interaction between the genotypes and environment for reaction to CBSD is suggestive of the behavior of a quantitative trait. Genotypes TZ/06/140, TMS30572, TZ/06/130, N3/66/1, N3/58/1 and N3/104/3 were categorized as resistant because of their relatively low CBSD root incidence and

severity. Test genotype N3/66/1 exhibited significant stability for both incidence and severity while, TZ/06/140 only exhibited stability for root severity and NAM/06/130 exhibited stability for root incidence only. Genotypes, TZ/06/130 and TZ/06/140, were part of the half-sib population from Tanzanian released variety, *Naliendele* and *Kibaha*, landrace, respectively. These genotypes were introduced to Uganda in form of botanical sexual seeds. The progenitors of these half-sib seeds were known to be tolerant to CBSD in Tanzania (Dr. Edward Kanju, IITA Tanzania, personal communication) and were the reason for their inclusion in the resistance evaluation in Uganda. An earlier effort in screening for resistance to CBSD conducted at Namulonge, identified TZ/06/130 to be tolerant and TZ/06/140 was considered susceptible based on folia incidence (Abaca et al., 2013). However, in that study, assessment of resistance was based only on disease incidence where a genotype was considered resistant at <15% root and folia incidence and any genotype with root and foliar incidence of >60% was categorized as susceptible. However, the study was only conducted at one location and did not consider systematic reaction gradient for both disease incidence and variation in severity. It should be noted that, one environment study is not sufficient to study stability pattern of a trait. The present study employed a reaction gradient in the assessment of both incidence and severity, in which both TZ/06/140 and TZ/06/130 were included in the resistant category. The result showed that resistances in both genotypes exhibited differential stability with cultivar TZ/06/140 being more stable for root severity and TZ/06/130 was more stable for root incidence. The reported instability of resistance indicates low levels of resistance to the disease which is an indication of quantitative resistance. It can be speculated that genotypes with genetic background of Tanzanian landraces might have acquired resistance during co-evolution with the virus since its introduction to that geographical location.

The elite genotype, TMS30572, a popular variety among most African farmers, is a progeny from IITA breeding program and the pedigree indicates that, one of the parents, 58308, is from Amani breeding program and the other, BRANCA DE SANTA CATARINA, from South American germplasm. This genotype has been officially released in Uganda with the name NASE3. In both the previous (Abaca et al., 2013) and the present study, TMS30572 was reported to be resistant to CBSD but the present study demonstrates that it is only stable to root incidence. Therefore, these genotypes, TMS30572, TZ/06/130 and TZ/06/140, can be used to broaden the genetic base for breeding through development of new recombinants that can be better in resistance than parents. Genotypes N3/66/1, N3/58/1 and N3/104/3 exhibited resistance to CBSD and are considered putative new sources of resistance identified from S_1 progeny of TMS30572. The S_1 progeny had similar

performance like their S_0 parent. However, N3/66/1 was more stable than the parent indicating the possibility for genetic progress for resistance through inbreeding. However, all the newly identified resistance sources outperformed the resistance checks (MM96/4271 and MM96/0686) used in the present study previously identified to be resistant (Abaca et al., 2013). The expression of resistance in S_1 is an indication that resistance to CBSD is also likely to be recessively inherited. The resistant partial inbred lines identified in this study have unlocked the potential of selfing in cassava as a new strategy for breeding for resistance to CBSD. The CBSD resistance genes derived from *Manihot glaziovii* may also be present in cassava germplasm distributed by International Institute of Tropical Agriculture (IITA) to African countries including Uganda and Tanzania. Previously, cassava seeds from advanced Amani hybrids were sent to IITA and was the most important source of CMD resistance genes when the breeding program began there in the 1970s (Hillocks and Jennings, 2003; Beck, 1982).

In the category with moderate resistance, only MM96/0686 had both low incidence and severity to the disease and was identified to be resistant although, unstable. In previous study (Abaca et al., 2013), MM96/4271 had been used as resistant genotype based on one environment evaluation. However, the present multi-environment study reveals that MM96/4271 on average, can develop high disease of incidence with an average of low levels of severity across the six environments which further confirms its instability to the disease. Whereas, MH97/2961 had low incidence, it had mean severity above the prescribed scale for the category and could not qualify for the category of moderate resistance. Therefore, the only genotypes that reliably qualify for the category of moderate resistance included MM96/0686, Kigoma Red, and Mzungu. Genotype MM96/0686 was a half sib clone from IITA breeding population introduced and evaluated in Uganda for resistance to CMD. In the previous study (Abaca et al., 2013), it was reported as resistant to CBSD.

However, Kigoma red and Mzungu, are other putative new sources of resistance that have been identified to be the most stable but with moderate resistance across the six test environments in Uganda. These genotypes, Kigoma and Mzungu; and TZ/06/130 and TZ/06/140 all have Tanzanian landrace background for resistance to CBSD. Surveys conducted in Tanzania and Mozambique indicated that some local cultivars showed resistance or tolerance to CBSD and it is likely that unintentional selection might have occurred in areas of high disease pressure (Hillocks and Jennings, 2003). Therefore, these genotypes could have developed resistance as a co-evolutionary selective process that might have acted on their progenitors. Varieties with possibly different sources of resistance to CBSD offer a future for pyramiding resistance to CBSD through recurrent selection.

The third category of genotypes MH97/2961, TMS192/0067 and N3/104/1 presented the most difficult situation for categorization and constituted the most unstable and most inconsistent genotypes switching ranks between category two and three. These are not useful in breeding for resistance to and / or the disease useful as controls in evaluation studies.

The fourth category includes genotypes 95/SE00036 and 95/NA00063 as the most susceptible and relatively stable. These performed worse than the previously known susceptible checks; MH97/2961, TME14 and TMS192/0067. These are therefore being recommended as susceptible checks for CBSD field screening. The finding of the present study agrees with the earlier observation that various sources of resistance to CBSD naturally exist in East and Central Africa (Pariyo et al., 2013).

Significance of environment in breeding for resistance

Both the AMMI and general ANOVA indicated that, a high proportion of the variation was explained by the genotypic variances for both CBSD incidence and severity. This suggests that resistance to CBSD is genetically controlled. However, the relatively high proportion of the variation was partitioned to other environmental sources suggesting that the resistance is also influenced by environment in describing a quantitative trait. Recently, both additive and non-additive genetic effects have been reported to be important in the expression of CBSD resistance (Munga, 2008; Zacarias and Labuschangne, 2010; Kulembeka et al., 2012). The presence of two species UCBSV and CBSV in Uganda could partly explain this significant GxE effects. Further, the highly significant genotype by environment interaction implies that genotypes have to be evaluated in multiple environments to achieve reliable resistance to CBSD.

The highest disease incidence and severities were registered at Namulonge in both cropping seasons. Coupled with its strong discriminatory power for disease incidence, Namulonge has been confirmed as the most suitable location for field evaluation of resistance to CBSD. The observation could be attributed to the higher average daily temperatures at Namulonge during both cropping cycles. Higher temperatures enhance the vector activity in transmission and spread of the virus among the host plants. The relatively stronger discriminatory ability of the environment in the second season could be due to the carry over effect of the virus accumulation from the first season which had a stronger symptom expression in the second season. This is in agreement with the earlier report by Jennings (1957) that many genotypes that showed good brown streak resistance in their first season, succumbed completely in their second season.

The analyses for environment characterization

identified Namulonge in season I as a different mega environment with high disease incidence. Whereas, in the second season, the disease incidence equilibrated with other environments.

While Bulindi had very low levels of phosphorus, which could have contributed to the poor root development. Severe Zinc deficiency symptoms observed at Bulindi could have contributed to the interveinal chlorosis and thus interfering with the photosynthetic surface. The greatest stress experienced at Namulonge by cassava plants was due to the infection from CBSD. However, the strong discriminatory power exhibited by Bulindi in season I (2012/2013) and Namulonge in season II (2011/2012) suggests that those environments are suitable for screening for abiotic and biotic stresses respectively.

CONCLUSIONS AND RECOMMENDATIONS

New sources of field resistance to CBSD have been identified, a few with stable reaction. The identified resistance sources can be categorized by pedigree into three: (1) S₁s from TMS30572, (2) the Tanzanian landraces, and (3) the Amani clones with the component of the wild genome of cassava. Based on field reaction, genotypes for resistance to CBSD were categorized as: resistant genotypes, TZ/06/140, TMS30572, TZ/06/130, N3/66/1, N3/58/1, N3/104/3 with N3/66/1 being the most stable. The genotypes with moderate resistance included; MM96/0686, Kigoma Red and Mzungu with the latter two being the most stable and new genotypes in Uganda. Whereas, the two genotypes, 95/SE00036 and MH97/29616 have been identified as the most stable susceptible genotypes. The genotypes N3/66/1, N3/58/1 and N3/104/3 have been identified as completely new sources of putative resistance from among the S₁ progeny of TMS30572.

Namulonge has been empirically confirmed as the most suitable environment for CBSD resistance screening while Ngetta was found to be the most suitable environment for exploiting yield potentials. Bulindi was found to be a suitable site for screening for nutrient use efficiency.

Further research to obtain higher levels of resistance to CBSD, can be conducted through intercrossing to identify more resistant recombinants. Additional studies on mechanisms of resistance to CBSD can be initiated to further elucidate the mechanisms of observed field resistance. An assessment for brown streak foliar symptoms expression needs to be conducted in an environment with adequate Phosphorus, Zinc and Manganese to prevent the confounding effect on foliar assessment of CBSD due to its high symptom similarity to the nutrient deficiency symptoms. The dynamics of the influence of environment on the virulence of the two viruses on the reaction of cassava genotypes are not

known and a detailed study is also recommended. To improve on selection for resistance to CBSD, there is need to develop a disease assessment index for resistance which should combine root severity and incidence; leaf severity and incidence, stem severity and incidence for a more universal classification.

Conflict of Interest

The authors have not declared any conflict of interest.

ACKNOWLEDGEMENTS

The authors are grateful to Millenium Science Initiative of the World Bank for funding the study through Uganda National Council for Science and Technology. We are also indebted to Dr. Edward Kanju of International Institute of Tropical Agriculture based in Tanzania and Dr. Geoffrey Mkamilo of the National Root Crops Research Program of Tanzania for the kind identification and provision of cassava genotypes of Tanzanian origin for the present study.

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