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Journal of Plant Breeding and Crop Science

Table of Contents: Volume 7 Number 3 March, 2015

ARTICLES

Research Articles

- Crossability and germinability potentials of some cassava (*Manihot esculenta* Crantz) progenitors for selection** 61
Njoku Damian Ndubuisi, Ikeogu Ugochukwu Nathaniel, Ewa Favour and Egesi Chiedozie
- Stability of resistance to cassava brown streak disease in major agro-ecologies of Uganda** 67
Anthony Pariyo, Yona Baguma, Titus Alicai, Robert Kawuki, Edward Kanju, Anton Bua, Christopher A. Omongo, Paul Gibson, David S. Osiru, Dennis Mpairwe and Phinehas Tukamuhabwa
- Genotype by environment interaction of some faba bean genotypes under diverse broomrape environments of Tigray, Ethiopia** 79
Teklay Abebe, Yemane Nega, Muez Mehari, Adhiena Mesele, Assefa Workineh and Hadas Beyene

Full Length Research Paper

Crossability and germinability potentials of some cassava (*Manihot esculenta* Crantz) progenitors for selection

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Controlled crosses and selection of promising cassava genotypes were carried out at Cassava Breeding Programme of National Root Crops Research Institute (NRCRI), Umudike, Nigeria, in 2010. A 3 x 3 North Carolina mating design was used to generate nine F₁ population. Out of 7,044 seeds expected from all the pollinated flowers, only 1,102 seeds (15.6%) were harvested. Majority of the pollinated flowers did not develop into fruits due to abortion that happened shortly after pollination. Family TMS 98-0002 x TMS 05-0473 (B3) had the highest seed abortion rate of 75.5% whereas family TMS 98-0505 x TMS 01-1368 (B7) had the lowest seed abortion rate of 25.3%. Seeds generated from viability test were sown in pots and maintained for 28 days in the screen house. Seed germination ranged from 15.5 to 80.9% with a mean of 43.19%. The emerged seedlings were hardened and transplanted to the field alongside their parents. Four hundred and sixty four progeny survived and were evaluated 12 months after planting (MAP). There were very high levels of variability in the segregating F₁ progeny for all the traits studied that will be useful for cassava breeding in Nigeria. Subsequent evaluations of promising clones have reached advanced stage for release to farmers in Nigeria.

Key words: Cassava, botanical seed, germination, pollination, variation.

INTRODUCTION

Cassava is one of the leading staples in the world with a global total production of 252 million metric tonnes (FAOSTAT, 2012). Though with the current national estimate of 55 million cassava production in 2013 (NRCRI, 2013), the cassava genotypes grown by farmers in Nigeria are mostly local cultivars and few improved released varieties by NRCRI Umudike in collaboration with International Institute of tropical Agriculture (IITA), Ibadan (NRCRI, 2013). Improving the local cultivars

(landraces) that flower requires a hybridization programme to generate hybrid progeny for recombination and selection. Population improvement and recurrent selection in cross-pollinated crops such as cassava progressively increases the frequencies of genes for specific desirable traits. However, the success of a breeding programme depends largely on the choice of progenitors/parents used. Parental genotypes are usually selected on the basis of their performance or the

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Table 1. Description of parent cultivars used in a topcross mating design.

Selected parents	Source and morphological description
TMS 01-1368	NRCRI germplasm, resistant to CMD, CBB, CAD, CGM, bitter taste, broad leaf venation, profuse and early flowering (2-3 MAP) and moderate erect stem/architecture. Released in 2012.
TMS 05-1636	NRCRI germplasm, resistant to CMD, CBB, CAD, CGM, bitter taste, narrow leaf venation, profuse and early flowering (2.1 MAP) and low stem growth/architecture. Not released.
TMS 05-0473	NRCRI germplasm, resistant to CMD, CBB, CAD, CGM, bitter taste, broad leaf venation, profuse flowering (2.6 MAP) and moderate erect with branches. Not released.
TMS 98-0002	NRCRI germplasm, resistant to CMD, CBB, CAD, CGM, bitter taste, broad leaf, profuse flowering (2.4 MAP) and highly branching. Released in 2005.
TMS 97-2205	NRCRI germplasm, resistant to CMD, CBB, CAD, CGM, bitter taste, broad leaf, profuse flowering (2.3 MAP). Released in 2005.
TMS 98-0505	NRCRI germplasm, resistant to CMD, CBB, CAD, CGM, bitter taste, very broad leaf, many branches and profuse flowering (3 MAP). Released in 2005.

CMD (cassava mosaic disease), CBB (cassava bacterial blight), CAD (cassava anthracnose disease), CGM (cassava green mites), MAP (months after planting).

performance of their F₁ progeny (Ceballos et al., 2004). For instance, in maize, selection of parental genotypes to produce F₁ hybrids is usually based on the performance of their progeny (Derera et al., 2000).

Cassava breeders have traditionally used performance *per se* of parental genotypes (Ceballos et al., 2004). Also, in the current study, parental genotypes or progenitors were selected based on their performance *per se*. The selected local cultivars, though with low in pro-vitamin A (P_{VAC}) and susceptible to pests and diseases, have good root qualities and are popular with the farmers in the area of study (Njoku et al., 2014). The NRCRI improved varieties used to cross with the local cultivars are early bulking, high P_{VAC} and resistant to pests and diseases but lacked high dry matter and yield attributes acceptable to farmers (Njoku et al., 2014). It was assumed that crossing the two groups (local cultivars and NRCRI varieties), would result to new genotypes, which combine high P_{VAC} with other agronomic acceptable root qualities by Nigerian farmers.

In this study, the NC II mating design was used to generate the progeny from crosses between the two groups of parents (local cultivars versus improved varieties). Several researchers have used this breeding design, for example, in sugar cane (Hogarth et al., 1981), variety crosses in maize (Derera et al., 2000) and even feed conversion in broiler rabbits (Dedkova et al., 2002). In cassava, the design has been used to study resistance to cassava mosaic disease (Lokko et al., 2004).

However, experience has shown that cassava botanical seeds have low germination rate both in nursery and in the field (Ceballos et al., 2004; Kawano, 1978; Njoku et al., 2014) which eventually leads to loss of possible important genes, as well as wastage of land. Moreover, standard germination test by floatation do not always adequately predict seedling emergence under greenhouse and field conditions, and total percentage

loss has not yet been established. The aims of the study were to investigate the effect of cassava genotypes on seed set, seed germination, seed abortion, and to ascertain the level of variability among progenitors in cross combination.

MATERIALS AND METHODS

Evaluation site and germplasm source

Six cassava progenitors were selected and evaluated at National Root Crops Research Institute (NRCRI) Umudike research field, which lies within the humid rainforest agroecology of Nigeria (Njoku et al., 2014). They comprised three widely cultivated white root cassava cultivars and three yellow root cassava genotypes (one released in 2011 while others are yet to be released). The six cassava genotypes which were developed at NRCRI, Umudike have good agronomic, nutritional and disease resistance attributes. All the cultivars were selected based on their characteristics of good performance in terms of dry matter, pest and disease resistance, plant architecture, nutritional quality and flowering ability (Table 1).

The six selected progenitors were then planted in a crossing block at NRCRI, Umudike in May, 2010, in a 3 × 3 North Carolina mating design. Ridges were made with spacing of 1.0 m intra-row and 1.0 m inter-row. Hand weeding was performed as required. Controlled hand pollination was done according to the standard procedure described by Kawano (1980).

Plants were observed for signs of flowering each morning. Pollination bags were used to enclose flowers about to open to prevent fertilization by stray pollen upon opening. Pollen from the corresponding male parent was collected in the morning (7 to 8 am) and pollination was done later in the day between 11 am and 2 pm after female flowers had opened by dusting pollen on the stigma of a matching female. One male flower was used to pollinate up to three female flowers. After pollination the female flowers were covered with the bags for one to two weeks. Each flower branch was marked by a tag indicating cross combination with female parent listed first and followed by date of pollination and pollinator. Developing fruits were covered with netting bags three to four weeks after pollination to catch the dehisced seeds (Figure 1). Seeds were collected after two months, sorted, labeled and stored



Figure 1. (a) Male flower, (b) Female flower, (c) Pollination, (d) Botanical seeds.



Figure 2. (a) Seedlings in trays, (b) Acclimatization/hardening.

till the time of sowing. Percentage seed set was determined by dividing total number of flowers pollinated with total number of seeds collected per cross. For pollinated fruits, it was assumed that every fruit had three ovules.

Germination of hybrid seeds

Seeds collected from each of the nine cassava populations were subjected to viability test and those that submerged were collected and sown in perforated plastic trays filled with unsieved sandy loam soil collected from an undisturbed field at western farm of NRCRI, Umudike. The plastic trays used were lined with cotton wool to prevent loss of soil particles through the holes underneath the trays. The soil in each tray was watered to field capacity and left overnight (24 h) before seeds were sown about 2 cm deep in the soil. The soil surface was covered with black polythene nylon to raise the soil temperature and thereby aid in germination of the seeds. The number of seeds from each population determined the number of trays used. The number of seeds that passed viability test per cross ranged between 45 and 385 seeds. Percentage germination and seed set in each population were used to determine crossability among the 6 parents used in the hybridization process. The trays were watered on a daily basis for 28 days when the emerged seedlings had reached 25 cm with a minimum of 3 leaves. In order to acclimatize the seedlings to vagary of weather, hardening of seedlings was done for 5 days by reducing watering and exposing them to ambient temperature before transplanting to the main field (Figure 2).

Transplanting of hybrids to field

Vegetative stem cuttings of 25 cm long each, about 4 cm thick and a minimum of 5 nodes in each stem cutting of the six progenitors (TMS 01-1368, TMS 05-1636, TMS 05-0473, TMS 98-0002, TMS 98-0505 and TMS 97-2205) were dipped into a bucket solution of 10 ml basudin (an emulsifiable concentrate insecticide containing 60% Diazinon) to 10 L of water for 5 min and allowed to dry overnight before planting to prevent termite attack. The progenitors were also used as checks in evaluating their hybrid progeny.

The seedlings (473) raised from hybrid seeds in each population were transplanted to the field alongside their parents, in 2010 at a spacing of 0.75 × 1 m for individual plant evaluation at NRCRI, Umudike. Because of late establishment of rainfall, the transplanted plants and their parents were watered twice daily (500 ml/plant) for 38 days to supplement the soil moisture. Hand weeding was carried out as required.

Data collection and analysis

The number of seedlings that emerged was recorded on weekly intervals (7, 14, 21 and 28 days after planting DAP) for each family until 28 days after planting (DAP) when there was no further emergence of seedling. The percentage germination for each hybrid population was calculated as emerged seedlings divided by the number of seeds sown multiplied by 100. Variability in percentage germination of seeds developed in different crosses involving each male or female parent was estimated. All data were

Table 2. Cross-pollination and abortion rate among nine cassava hybrid genotypes developed at NRCRI Umudike in 2009-2010.

Population	Cross combination	Number of female flowers pollinated	Expected number of seeds	Number of seeds collected	Abortion rate (%)
B1	TMS 98/0002 x TMS 01/1368	372	1116	201	54.0
B2	TMS 98/0002 x TMS 05/1636	268	804	74	27.6
B3	TMS 98/0002 x TMS 05/0473	511	1533	385	75.3
B4	TMS 97/2205 x TMS 01/1368	213	639	72	33.8
B5	TMS 97/2205 x TMS 05/1636	252	756	96	38.1
B6	TMS 97/2205 x TMS 05/0473	194	582	86	44.3
B7	TMS 98/0505 x TMS 01/1368	170	510	43	25.3
B8	TMS 98/0505 x TMS 05/1636	197	591	77	39.1
B9	TMS 98/0505 x TMS 05/0473	171	513	67	39.2
	Total	2348	7044	1101	

$$\% \text{ abortion} = \frac{\text{Number of seeds collected}}{\text{Number of female flowers pollinated}} \times 100$$

analyzed using Genstat Version 3 (Payne et al., 2008), SAS 9.2 Edition and SPSS 15.0. The REML linear mixed model was used to analyze family and progeny data at seedling stage. Mean, minimum, maximum, standard deviation and coefficient of variation (CV) were calculated for each hybrid population.

RESULTS AND DISCUSSION

Seed set and seed abortion

Table 2 shows the number of female flowers pollinated and number of seeds collected in each cross during the development of the hybrid populations at Umudike between November 2009 and February 2010. Out of 7, 044 seeds expected from all the pollinated flowers (2348) with three possible seeds in each trilocular fruit, only 1102 seeds (representing 15.6% of the maximum number of seeds expected) were collected. Majority of the pollinated flowers did not develop into fruits. This may be probably because of the late in pollination which was off-season (November to February, 2010) and a lot of seed abortions. However, less than one seed was obtained per pollination on average in this study. This is far below the expectation according to submission by Ceballos et al. (2004) who stated that one or two seeds are obtained per pollination on average. The highest percent seed set was recorded in the cross between TMS 98-0002 and TMS 05-0473 which were the best female and male parents in terms of seed set respectively. Population B3 had the highest abortion rate of 75.3%, followed by B1 (54.0%), while B7 had the lowest rate of 25.3%.

However, the dry season and harmattan aids drying of the seeds, hence, the drying period for the seeds was reduced. Opening of mature flowers and drying of matured fruits depend on environmental conditions (Ceballos et al., 2004; Olasanmi et al., 2014). Number of

seeds per female flower is genotype dependent; therefore, selection of highly fertile genotypes as female parents is a critical factor.

The number of parents used in this study may not reflect the wide genetic variations often found among clones used as parents in cassava breeding programme. However, large differences in coefficient of variation for seed set among the parents and significantly better performance of TMS 98-0002 and TMS 05-1636 in terms of seed set than other female and male parents respectively, suggest genetic differences in the crossability of these clones (Table 2). This is also in agreement with Olasanmi et al. (2014) who reported that both maternal effect and compatibility between male and female parents have influence on percent seed set in cassava, and it may be necessary to investigate the potential seed crossability of male and female parents used in the development of recombinant seeds in cassava breeding programmes. This will help cassava breeders to choose parental varieties with high seed set capability and also maximize the resources in terms of time, labour and funds needed.

Coefficient of variation (CV) for seed set was found to be low for TMS 97-2205, followed by TMS 98-0505 (Table 3). This suggests that seed set is more stable in these two parents. Although the number of parents analyzed may not reflect wide genetic variation often found among clones used as parents in breeding programmes, results based on CV indicate genetic differences in the crossability of these clones. There was no seed loss due to pest destruction because fruits were protected by the bags and the field was well maintained throughout the course of the hybridization programme. There was non-significant but very high positive correlation between number of female flowers pollinated and number of seeds harvested in each population ($r = 0.97$, $p = 0.4022$, $n = 9$). This shows that only 39.5% of the rate of seed set can be predicted using the number of female flowers pollinated. Also this non-significant correlation indicates that success of pollination programmes in cassava cannot be accurately predicted

Table 3. Variability in seed set among populations developed from each parent.

Male parents	Female parents			Mean	CV (%)
	TMS 98-0002	TMS 97-2205	TMS 98-0505		
TMS 01-1368	18.1	11.3	8.4	12.6	39.5
TMS 05-1636	9.2	12.7	13.1	11.7	18.3
TMS 05-0473	25.1	14.8	13.1	17.7	36.7
Mean	17.5	12.9	11.5	13.9	
CV (%)	45.6	13.7	23.6		

CV (%) = Coefficient of variation.

Table 4. Number of seed sown and percentage germination of seeds in nine F₁ populations developed at NRCRI Umudike in 2009.

Populations	Cross combination	No. of seed sown	No. of emerged seedlings	Percentage germination (%)
B1	TMS 98-0002 x TMS 01-1368	184	125	67.9
B2	TMS 98-0002 x TMS 05-1636	68	55	80.9
B3	TMS 98-0002 x TMS 05-0473	337	159	47.2
B4	TMS 97-2205 x TMS 01-1368	61	44	72.1
B5	TMS 97-2205 x TMS 05-1636	80	45	56.3
B6	TMS 97-2205 x TMS 05-0473	81	18	22.2
B7	TMS 98-0505 x TMS 01-1368	32	5	15.6
B8	TMS 98-0505 x TMS 05-1636	58	12	20.7
B9	TMS 98-0505 x TMS 05-0473	58	10	17.2
Mean		106.6	52.6	44.5

$$\% \text{ germination} = \frac{\text{Number of emerged seedlings}}{\text{Number of seeds sowed}} \times 100$$

from the number of flowers pollinated (Ceballos et al., 2004; Olasanmi et al., 2014).

Hybrid seed germination

The number of seed sowed per cross ranged from 32 to 337 with a mean of 106.56, while the lowest number of seeds (32) was observed in family TMS 98-0505 x TMS 01-1368. The first sprouting was noticed and recorded four days after sowing (DAS) in population B3, followed by population B1 and B2 after 7 days. Also, sprouting ceased in populations B6 and B7 in 21 DAS but continued till 28 DAS in population B1, B2, B3, B4, B5, B8 and B9 respectively. There was a very high positive correlation between number of seeds sowed and number of emerged seedlings in each population ($r = 0.93$). Maximum temperature ranged between 31 and 34°C while the minimum temperature ranged between 22 and 24°C during hybridization, development and drying of the fruits and germination of seeds (November 2009-April 2010). The relative humidity ranged between 72 and 78% during the same period.

The highest germinability of 80.9 was recorded in B2

while B7 had the lowest value of 15.6% (Table 4). The lowest coefficient of variation (CV) of 14.6% for germinability found among the crosses involving TMS 98-0505 (Table 5) suggests that germinability is stable among the crosses. T-test results showed significant difference in the mean percent germinability among crosses developed from TMS 98-0505 and those from TMS 98-0002. However, there were no significant differences in percent germinability among crosses from TMS 98-0002 and those from TMS 97-2205 as well as among crosses from TMS 97-2205 and those from TMS 98-0505 (Table 5).

Generally, the differences observed in the rate of germination of hybrid seeds in the nine populations may also be another consequence of compatibility between the two parents used to generate each population since the germination process was carried out at the same time under the same weather conditions. This is also in agreement with Olasanmi et al. (2014) who suggested that one can predict to a large extent the number of seedlings expected in a breeding programme using the number of seed generated (Table 6).

Conclusion

Most breeding programs start with the evaluation of available genetic variability, and how easily this variability

Table 5. Variability in percentage germinability of seeds developed from each parent.

Male parents	Female parents			Mean	CV (%)
	TMS 98-0002	TMS 97-2205	TMS 98-0505		
TMS 01-1368	67.9	72.1	15.6	51.9	60.7
TMS 05-1636	80.9	56.3	20.7	52.6	57.6
TMS 05-0473	47.2	22.2	17.2	28.9	55.7
Mean	65.3	50.2	17.8	44.4	
CV (%)	25.9	50.8	14.6		

Table 6. T-test result of comparison among female parents for % germination.

Parents	Probability
TMS 98-0002 vs TMS 97-2205	0.45 ^{ns}
TMS 97-2205 vs TMS 98-0505	0.09 ^{ns}
TMS 98-0505 vs TMS 98-0002	0.008*

*significantly different; ns = not significantly different; CV (%) = Coefficient of variation.

can be fixed in the genotypes is very important. After evaluation of all the yellow fleshed roots and white fleshed roots cultivars in NRCRI cassava breeding programme, 6 cultivars were selected (3 white and 3 yellow) based on their per se performance in terms of high yielding, pests and diseases resistance, high dry matter, high carotene amongst others. About 90% of the progeny have higher beta-carotene than their parents and an appreciable quantity of dry matter content. This diversity can be utilized by breeders to develop beta-carotene rich cassava varieties and improve existing varieties. Apart from combating vitamin A deficiency amongst heavy cassava consumers in Nigerians, beta-carotene rich cassava varieties can also be used to supplement yellow maize in poultry feed.

Conflict of Interest

The authors have not declared any conflict of interest.

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Full Length Research Paper

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

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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 *Potyviriidae* (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 Gx E 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 Gx E have not been conducted in Uganda since CBSD re-emerged. Furthermore, Knowledge of Gx E 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

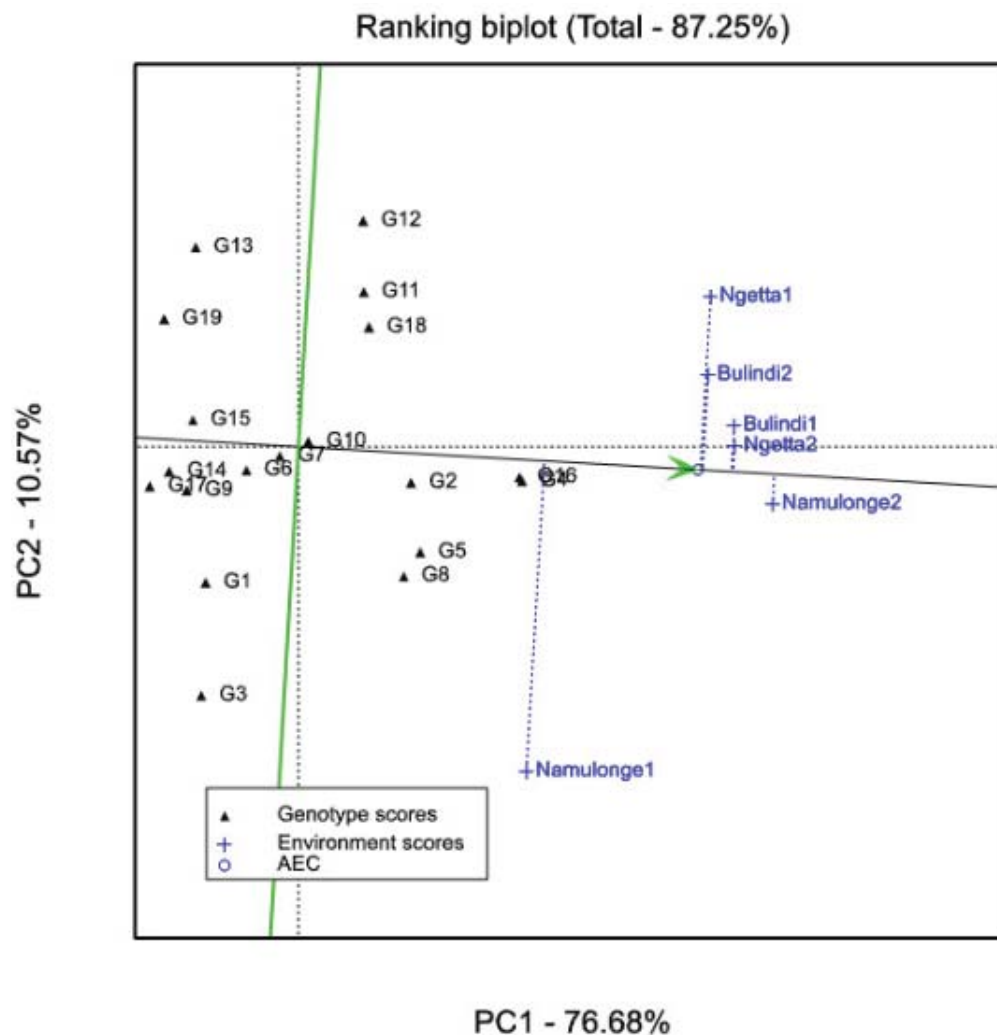


Figure 1. Illustration of genotype stability for resistance to CBSD based on CBSD root incidence using GGE bi-plot. 1 = MH97/2961, 2 = MM96/4271, 3 = MM96/0686, 4 = 95/SE00036, 5 = TMS192/0067, 6 = KIGOMA, 7 = MZUNGU, 8 = N3/104/1, 9 = N3/104/3, 10 = N3/127/1, 11 = N3/514/4, 12 = N3/514/10, 13 = N3/58/1, 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

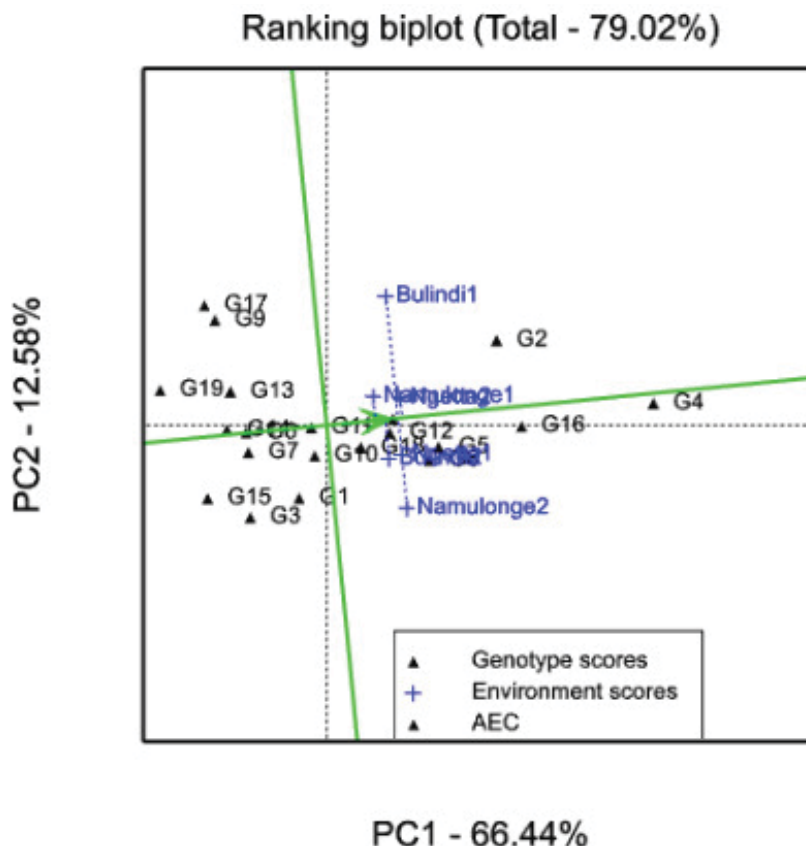


Figure 2. GGE biplot illustrating genotypic stability for resistance based on CBSD root severity. 1 = MH97/2961, 2 = MM96/4271, 3 = MM96/0686, 4 = 95/SE00036, 5 = TMS192/0067, 6 = KIGOMA, 7 = MZUNGU, 8 = N3/104/1, 9 = N3/104/3, 10 = N3/127/1, 11 = N3/514/4, 12 = N3/514/10, 13 = N3/58/1, 14 = N3/66/1, 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 S₁ progeny of TMS30572. The S₁ progeny had similar

performance like their S₀ 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 S₁ 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) S_1 s 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 S_1 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.

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Full Length Research Paper

Genotype by environment interaction of some faba bean genotypes under diverse broomrape environments of Tigray, Ethiopia

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Advanced breeding lines with acceptable resistance and tolerance levels to broomrape is an important way of decreasing yield loss. The objective of this research was to assess the yield stability of faba bean genotypes under diverse broomrape (*Orobanche crenata*) prone production environments. Six faba bean genotypes were tested across six environments. The AMMI analysis showed significant ($P < 0.01$) genotype, environment and genotype by environment interaction and the environment explained higher sum of square for the response variable grain yield. The AMMI one gives the best model fitness for the grain yield and broomrape number. Using the AMMI 1 biplot, polygon view of the GGE biplot and comparison of genotypes based on ideal genotype, the genotype ILB4358 was higher yielder and stable with lower Orobanche number followed by the genotype Sel.F5/3382/2003-4. Using the AMMI biplot analysis E3 (Adigolo, 2011) and E4 (Adigolo, 2012) were unfavorable environments, while, E1 (Awliegara, 2011), E2 (Awliegara, 2011), E5 (Kolatsihidi, 2011) and E6 (Kolatsihidi, 2012) were favorable testing environments.

Key words: AMMI, broomrape, environments, genotypes, GGE, faba bean.

INTRODUCTION

Legume crops represent an important component of agricultural food crops consumed in developing countries and are considered a vital crop for achieving food and nutritional security for both poor producers and consumers. Faba bean (*Vicia faba* L.) is one of the earliest domesticated food legumes in the world (Singh et al., 2013). Faba bean occupies around 2.44 million ha with annual production of 4.4 million tons (FAOSTAT, 2008). The main faba bean producers are China (1.65 Mt), Ethiopia (0.61 Mt), France (0.44 Mt), Egypt (0.29 Mt)

and Australia (0.19 Mt) (FAOSTAT, 2009). Faba bean is used as a source of protein (20-41%) in human diets, as forage crop for animals, and for available nitrogen in the biosphere (Crépona et al., 2010; Rubiales, 2010). Faba bean takes the largest share of the area and production of pulses grown in Ethiopia including Tigray region. It occupies close to 574,060 ha⁻¹ of land with annual production about 943,964 tones (CSA, 2013). However, in spite of these advantages faba bean acreage has declined due to low and unstable yields as well as

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Table 1. List of faba bean International *Orobanche* Nurseries (FBION-08) in Tigray.

Name	Pedigree	Origin	FAO status
ILB 4358	ILB 4358	Morocco	Designated
Sel.F5/3053/2003-3	HBP/DS0/2000	ICARDA	Undesignated
Sel.F5/3085/2003-4	HBP/DS0/2000	ICARDA	Undesignated
Sel.F5/3382/2003-4	HBP/DS0/2000	ICARDA	Undesignated
ILB 1814(Susc. check)	Syrian local large	Syria	Undesignated
Local check	-	Ethiopia	-

incidence of diseases worldwide (Stoddard et al., 2010) including Ethiopia (Nigussie et al., 2008). Meanwhile, the major constraint for faba bean cultivation in the Mediterranean area and west Asia (Pérez-de-Luque et al., 2010; Maalouf et al., 2011) and Ethiopia (Besufikad et al., 1999; Rezene and Gerba, 2003; Abebe et al., 2013) is broomrape infection. Broomrapes are root parasitic weeds which are completely dependent on the host due to the lack of chlorophyll and functional roots. Several broomrape species can infect faba bean, crenate broomrape (*Orobanche crenata* Forsk.) being the most damaging and widespread (Fernández-Aparicio et al., 2012; Rubiales et al., 2014). The damage caused by the parasite is significant and estimated yield losses were 7 to 80% depending on the level of infestation (Maalouf et al., 2011). In Ethiopia, the yield losses due to *O. crenata* reached 75 to 100% depending on host susceptibility, level of infestation and environmental conditions. Consequently, farmers in highly infested areas generally avoid growing food legume crops, resulting in substantial reductions to both the extent of cultivated areas and to food legume production (Besufikad et al., 1999; Rezene and Gerba, 2003; Abebe et al., 2013).

Ever since crenate broomrape is a menace in Ethiopia, efforts are being made to manage by different methods including hand weeding, crop rotation, fallowing, late sowing and chemical control. Nevertheless, none of these disease controls have been proved satisfactory. Therefore, breeding for resistance was considered to be the best form of control against broomrape (Rubiales et al., 2006). Producing varieties with high yielding ability have always been the first and the foremost among plant breeding objectives but, such high yielding varieties have to be characterized by relative resistance to biotic stresses in general and broomrape specifically. Many programs in the regions viz. Spain, Egypt, Syria, Morocco and Ethiopia have set up faba bean breeding programs to select broomrape-resistant varieties despite the complexity of resistance breeding for broomrape. As a result, only cultivars with moderate levels of resistance to *O. crenata* are available (Pérez-de-Luque et al., 2010; Maalouf et al., 2011; Gutiérrez et al., 2013). According to Maalouf et al. (2011), selection of genotypes with adequate broomrape resistance is also strongly affected by the genotype – environment interaction (GEI). This

makes it difficult to predict the behavior of the accessions in different situations reinforcing the need for multi-environmental testing of stability of disease resistance for faba bean crop. Genotype by environment interaction are important sources of variation in any crop and the term stability used to characterizes a genotype, which shows a relatively constant yield, independent of changing environment conditions (Sabaghnia et al., 2006). GGE biplot analysis has been previously proven useful to identify and characterize disease resistance and yield stability of breeding material in field trials (Fernández-Aparicio et al., 2012; Rubiales et al., 2012; Flores et al., 2013; Sánchez-Martín et al., 2014) taking advantage of the discrimination power versus representativeness view of the GGE biplot effective in evaluating test environments. In Ethiopia, sufficient information regarding stability parameters is not available in *Orobanche* resistance faba bean genotypes which could be used in further breeding programme. Hence, this investigation aimed at study genotype stability of some faba bean genotypes and select resistant and/or tolerant with high seed yield potentiality under different *Orobanche* infestation levels and environments.

MATERIALS AND METHODS

The present research was carried out in Ofla district, Tigray, Ethiopia, located at 12°31'N latitude and 39°33'E longitude. Five genotypes were introduced from International Center for Agricultural Research in Dry Areas (ICARDA) and local check were evaluated for *Orobanche* resistance in highly infested soils with *O. crenata* and tested at six environments and three locations. The pedigree and origin of genotypes were presented in Table 1. These genotypes and one local check were evaluated in complete randomized block design with three replications in each environment. Sowing was done during the first week of July each year. The plot size for each genotype consists of 6 rows of 3 m length, with 0.1 m intra-row and 0.5 m inter-row spacing. At planting, 100 kg DAP ha⁻¹ (46 kg P₂O₅ and 18 kg N) was applied. All the culture practices were applied during the whole growing environments to ensure good crop stand. Hand weeding of other than broomrape was carried out; herbicides were not applied to avoid interference with broomrape development. The valuable examined were yield and number of emerged broomrape shoots per plot was determined at crop maturity.

Combined analysis of variance was conducted to determine genotypic differences and the significant genotype x environment interactions for broomrape was studied by using the AMMI model

Table 2. Mean value of seed yield and *Orobanche* number under diverse locations and seasons.

Genotype	Yield Qt/ha						Orobanche number per ha ('000)					
	Adigolo		Awliegara		Kolatsihidi		Adigolo		Awliegara		Kolatsihidi	
	2011	2012	2011	2012	2011	2012	2011	2012	2011	2012	2011	2012
ILB 1814 (Susc. check)	14.54	4.36	15.81	12.2	19.07	22.16	214.2	534.2	402.2	408.9	30.8	538.3
Sel.F5/3053/2003-3	6.79	14.9	15.2	19.97	47.91	23.35	339.2	520	376.7	175	0	619.2
ILB 4358	21.24	21.54	39.00	38.57	50.46	41.42	404.2	383.3	145	90	0	188.3
Sel.F5/3382/2003-4	14.82	14.11	29.15	21.33	44.98	25.99	330.8	409.2	146.7	135	0	411.7
local check	6.53	0	19.05	12.9	18.11	11.85	351.7	435	276.7	171.7	1.7	774.2
Sel.F5/3085/2003-4	12.6	11.48	26.30	20.97	45.03	20.58	311.7	500.8	368.3	153.3	1.7	521.7

Table 3. Additive main effects and multiplication interaction (AMMI) for yield and Orobanche count over six environments.

Source	Yield			Orobanche count	
	df	MS	% Explained	MS	% Explained
Treatments	35	484**	86.59	132873**	89.02
Genotypes	5	1347.5**	39.77	22811*	2.45
Environments	5	1627.2**	48.03	788527**	84.78
Rep (environment)	12	44.4	3.15	4674	1.21
Interactions	25	82.7**	12.20	23755**	12.77
IPCA1	9	175.8**	76.54	55952**	84.79
IPCA2	7	46.3ns	15.67	11485ns	13.54
IPCA3	5	26 ^{ns}	6.29	1924 ^{ns}	1.62
IPCA4	3	10.3 ^{ns}	1.50	96 ^{ns}	0.05
IPCA5	1	0	0.00	0	0
Error	60	34.8		8625	

which proposed by Zobel et al. (1988). A biplot on the first main effect of the interaction IPCA-1 of both genotypes and environments simultaneously (Kempton, 1994). The analysis were done using the CropStat 7.2 software to compare the genotypes under different environments by GGE biplot analysis proposed by Yan et al. (2000) and the analysis was done using the software Genstat13.

RESULTS

Combined analysis variance

The combined analysis of variance for grain yield across different growing environment showed that the genotype ILB 4358 was consistently higher yielder. The genotype ILB 4358 was also recorded the lowest number of broomrape population than the remaining genotypes (Table 2). However, the genotype ILB 1814 (susceptible check) and local check had the lowest yield across locations and years.

AMMI analysis grain yield

The AMMI analysis of variance for the additive main

effect showed a significant difference ($P \leq 0.01$) for the genotype, environment and genotype by environment interaction (Table 3). The result showed that the environment captured the maximum sum of square 48.03% followed by the genotype 39.77% and the genotype by environment interaction sum of square was lowest 12.2%. The magnitude of the environment was 3.9 times greater than the genotype by environment interaction (Table 3). The significant genotype by environment interaction were decomposed in to principal component analysis and the first interaction principal component explained 76.54% and the second interaction principal component additionally explained 15.67% the two interaction principal component totally captured 92.21% of the genotype by environment interaction variation. The first (IPCA1) interaction principal component was significant (Table 3).

AMMI 1 biplot grain yield

Genotype or environment located to the right side of the perpendicular line had the high yielding with favorable environment and when the genotype and environment are located to the left of the perpendicular line (grand

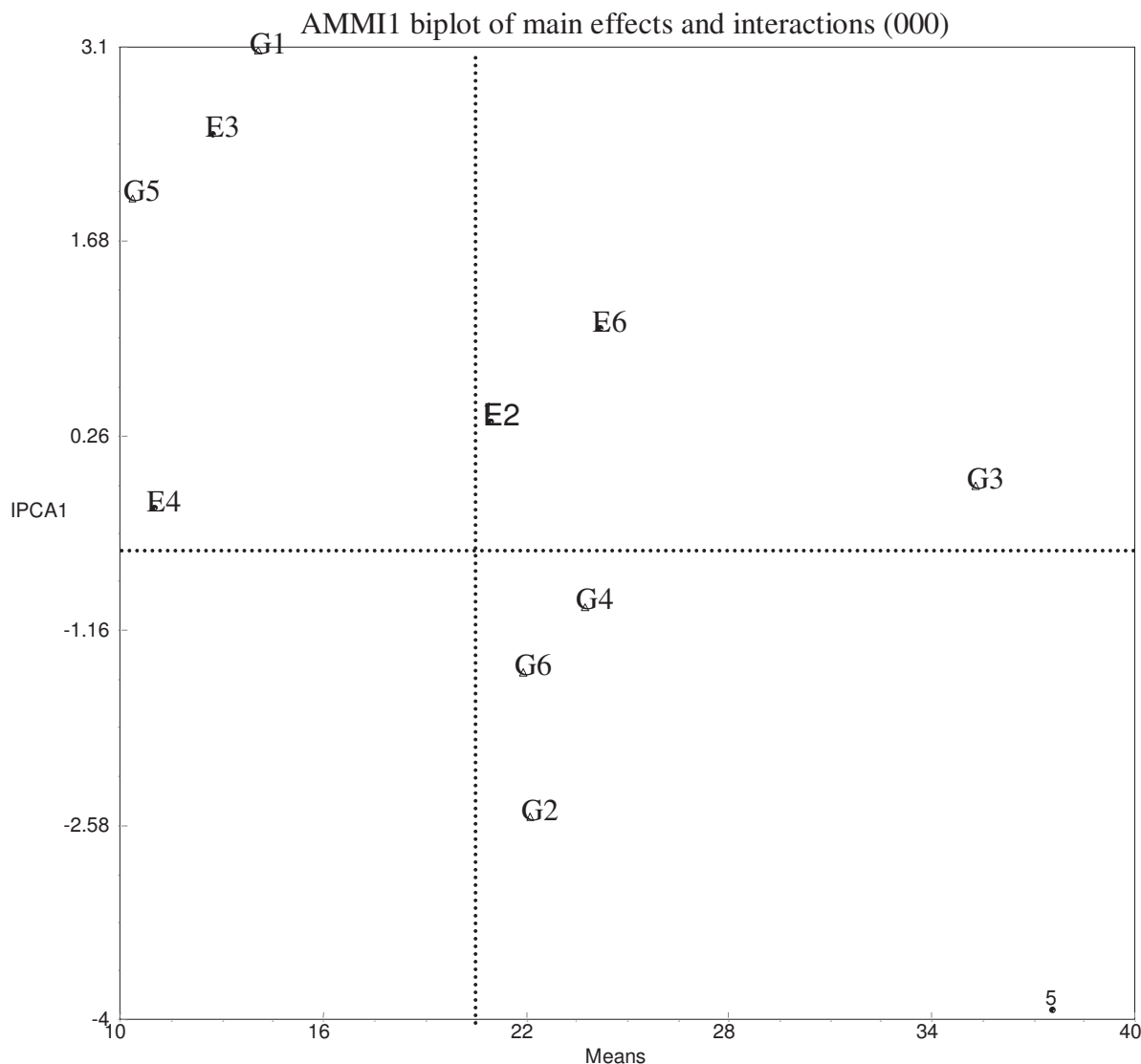


Figure 1. AMMI biplot for grain yield of six faba bean genotypes in six environments of Tigray. The genotypes abbreviated as G1, G2... and environment abbreviated as E1, E2... G1 (ILB 1814), G2 (Sel.F5/3053/2003-3), G3 (ILB 4358), G4 (Sel.F5/3382/2003-4), G5 (local check) and G6 (Sel.F5/3085/2003-4). E1 = Awliegara, 2011, E2 = Awliegara, 2012, E3 = Adigolo, 2011, E4 = Adigolo, 2012, E5 = Kolatsihidi, 2011 and E6 = Kolatsihidi, 2012.

mean) the reverse is true. Genotype or environment nearly placed to the origin (horizontal line) is stable genotype while, the genotype or environment disparaged from the origin the reverse is true.

The two genotypes; G1 (ILB 1814 or susceptible check) and G5 (local check) were lower in yield than the other genotypes and located to the left of the grand mean. The three genotypes; G2 (Sel.F5/3053/2003-3), G3 (ILB 4358), G4 (Sel.F5/3382/2003-4) and G6 (Sel.F5/3085/2003-4) were higher in yield (Figure 1). The two genotypes; G3 (ILB 4358) and G4 (Sel.F5/3382/2003-4) were stable nearly placed to the origin. G1 (susceptible check) was interactive genotype with unstable performance across testing environment.

The E3 (Adigolo, 2011) and E4 (Adigolo, 2012) were unfavorable environments while, E1 (Awliegara, 2011), E2 (Awliegara, 2012), E5 (Kolatsihidi, 2011) and E6 (Kolatsihidi, 2012) were favorable testing environments (Figure 1).

AMMMI analysis *Orobanche* count

The additive main effect of the AMMI analysis of variance revealed that genotype by environment interaction and environment were significant at level ($P \leq 0.01$) where as the genotype was significant at level ($P \leq 0.05$). The significance effect of the genotypes indicated that the

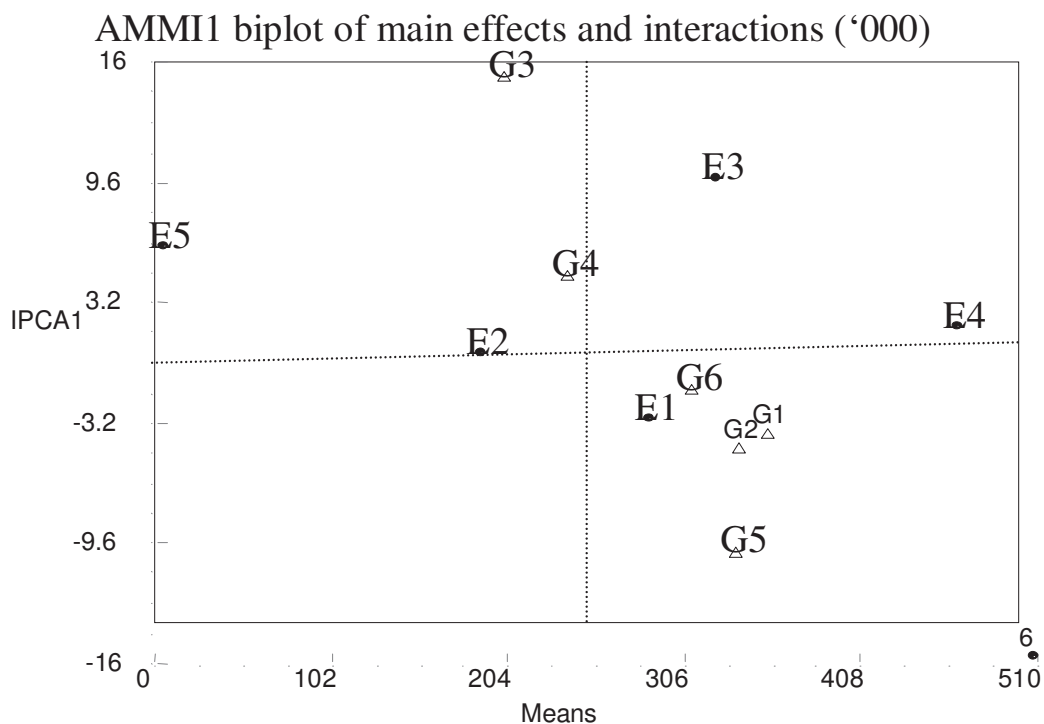


Figure 2. AMMI biplot for Orobanche count of six faba bean genotypes in six environments. The genotypes abbreviated as G1, G2... and environment abbreviated as E1, E2,...G1 (ILB 1814 or susceptible check), G2 (Sel.F5/3053/2003-3), G3 (ILB 4358), G4 (Sel.F5/3382/2003-4), G5 (local check) and G6 (Sel.F5/3085/2003-4) E1 = Awliegara, 2011, E2 = Awliegara, 2012, E3 = Adigolo, 2011, E4 = Adigolo, 2012, E5 = Kolatsihidi, 2011 and E6 = Kolatsihidi, 2012.

differential of the genotypes in tolerating the Orobanche infestation (Table 3). The multiplicative effect of the AMMI analysis was further classified into interaction principal components and the first interaction principal component (IPCA1) explained 84.79% and the second interaction principal component (IPCA2) was 13.54%. Grossly they explained 98.33 of the genotype by environment data. The F-test showed that only the first interaction principal component (IPCA1) was significant (Table 3).

AMMI 1 biplot Orobanche count

The two genotypes; G3 (ILB 4358) and G4 (Sel.F5/3382/2003-4) had lower number of Orobanche count located to the left of the perpendicular line. The two environments; E2 (Awliegara, 2012) and E5 (Kolatsihidi, 2011) were with lower *Orobanche* count (Figure 2).

Polygon view of the GGE biplot analysis

The four genotypes; G1 (susceptible check), G3 (ILB 4358), G5 (local check) and G2 ((Sel.F5/3053/2003-3),) were located on the vertices of the polygon performed

either the best or the poorest in one or more environments (Figure 3). The genotype G3 (ILB 4358) was the best adapted in all testing environments. The remaining vertex genotypes were not adapted to specific environment.

Evaluation of genotypes based on ideal environment

The genotype G3 (ILB 4358) was located in the first concentric circle and with short vector length as a result it was an ideal genotype with the highest yield and stable in performance in all environments. The two genotype; G1 (susceptible check) and G5 (local check) were undesirable genotypes according to distant from the first concentric circle and the lowest yield located under the grand mean level (Figure 4).

DISCUSSION

The AMMI analysis for grain yield indicated that the percentage explained by the genotypes was similar with that of the environment and this implying that the faba bean genotypes had differences in tolerance to Orobanche.

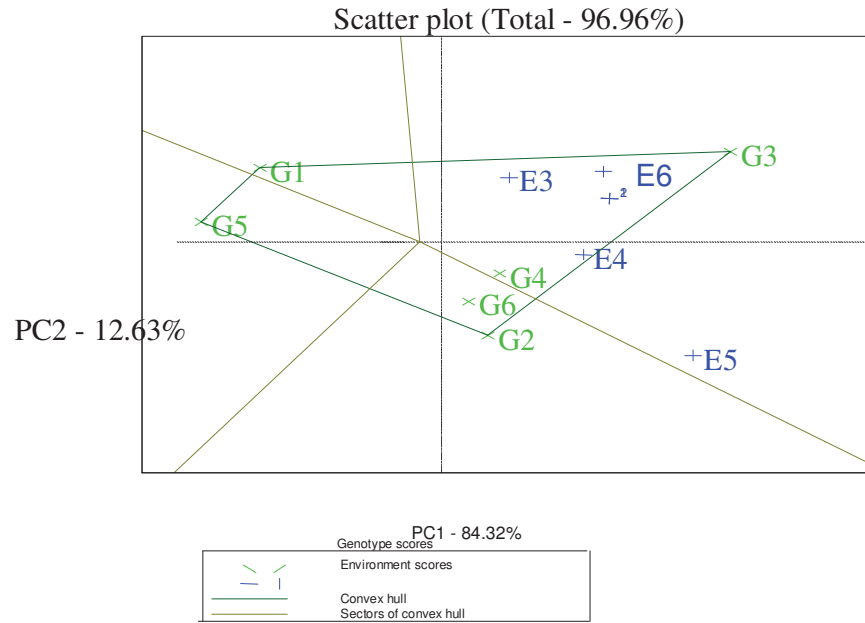


Figure 3. Polygon view of the GGE biplot of six faba bean genotypes across six environments. G = Genotypes and E = environment G1 (ILB 1814 or susceptible check), G2 (Sel.F5/3053/2003-3), G3 (ILB 4358), G4 (Sel.F5/3382/2003-4), G5 (local check) and G6 (Sel.F5/3085/2003-4) E1= Awliegara, 2011, E2 = Awliegara, 2012, E3 = Adigolo, 2011, E4 = Adigolo, 2012, E5 = Kolatsihidi, 2011 and E6 = Kolatsihidi, 2012.

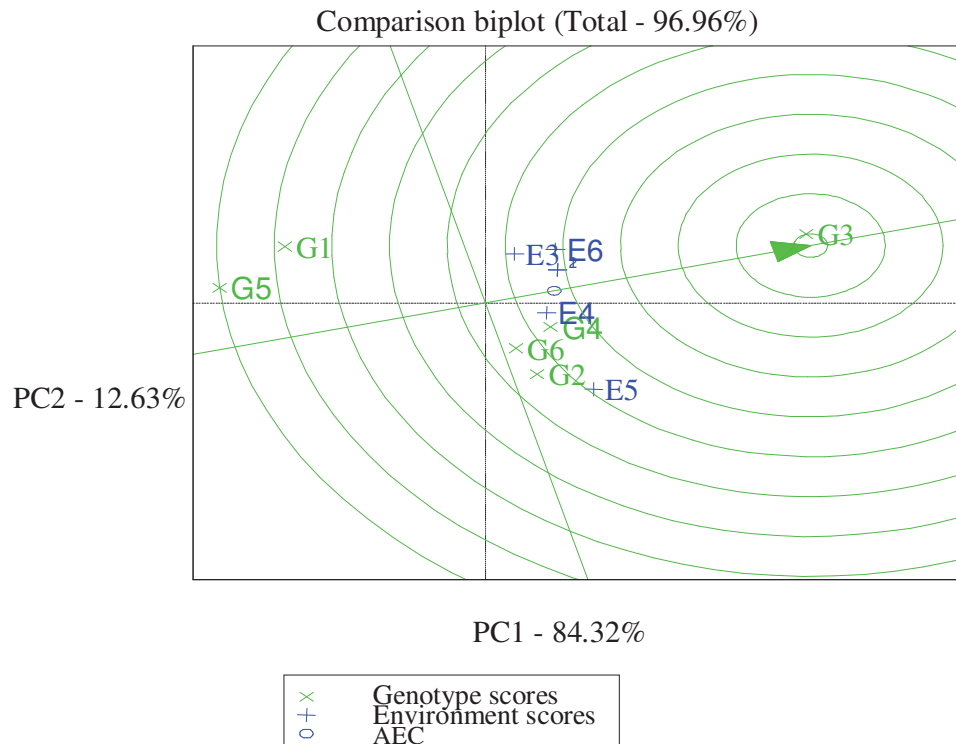


Figure 4. Evaluation based on the ideal genotypes of six genotypes over six environments. G = Genotypes and E = environment, G1 (ILB 1814 or susceptible check), G2 (Sel.F5/3053/2003-3), G3 (ILB 4358), G4 (Sel.F5/3382/2003-4), G5 (local check) and G6 (Sel.F5/3085/2003 4) E1 = Awliegara, 2011, E2 = Awliegara, 2012, E3 = Adigolo, 2011, E4 = Adigolo, 2012, E5 = Kolatsihidi, 2011 and E6 = Kolatsihidi, 2012.

According to Romagosa and Fox (1993), the variation in Orobanche number was mainly attributed to the magnitude of the environment in multi locations yield trials the variation captured by the environment is 70%, genotype 10% and genotype by environment interaction explained 20%. The resistance of these genotypes against *O. crenata* was characterized by several indexes such as low number of parasite attachments per host plant, poor performance or stand of the weed, delay of Orobanche establishment and tolerance/resistance (Rubiales et al., 2006; Maalouf et al., 2011). Orobanche number was taken in this study and be considered as simple and efficient measure of resistance or tolerance to the parasitic weed in different breeding programs in agreement with Cubero and Hernandez (1991) and Maalouf et al. (2011). The genotype ILB 4358 showed greater tolerance to Orobanche and better yield stability than the remaining genotypes followed by the genotype Sel.F5/3382/2003-4, similar results obtained by (Maalouf et al., 2011). The two genotypes (Sel.F5/3053/2003-3) and Sel.F5/3085/2003-4) recorded high infestation level of the parasitic weed but they gave acceptable levels of yield under infested soils. In contrast, the highest level of broomrape infestation was recorded/ possessed on the susceptible check (ILB 1814) and local check with non-acceptable yield (lower yield).

The multiplicative effect of the AMMI model for the response of the two variables grain yield and Orobanche count, the interaction principal components explained that one of them had the most source of variation. The most adequate model for analysis of genotype by environment interaction mainly depend on the type of crop, vegetation cover and the magnitude of genotype by environment interaction but generally the two interaction principal component analysis can identify suitable genotype by reducing systematically noise (Gauch and Zobel, 1988; Yan et al., 2000). Using the AMMI 1 biplot, polygon view of the GGE biplot and comparison of genotypes based on ideal genotype the genotype ILB 4358 was higher yielder and stable with lower Orobanche count than other genotypes. According to the results which obtained by Maalouf et al. (2011) identified that ILB 4358 had potential candidate with higher, stable yield and lower infested by Orobanche. Similarly, genotypes which had combining stability and higher mean grain yield are acceptable over wider range of environment and the most favorable (Annicchiarico, 2007).

The genotype could be used as a bench mark for screening of resistance to parasitic weeds in food legumes breeding programs. Generally using the ideal genotypes as a bench mark for selection can be made and the genotypes which are distant from the ideal genotype can be discarded in the early breeding cycle and the genotype that had great proximity with the ideal genotype can be further evaluated (Yan and Kang, 2002). Generally, the present study revealed that the level of resistance to Orobanche is not very high and stable

across all environments (Table 2). Therefore, integrated broomrape management using high yielding potential lines with good levels of tolerance and/or resistance is recommended (Perez-de-Luque et al., 2010).

Conflict of Interest

The authors have not declared any conflict of interest.

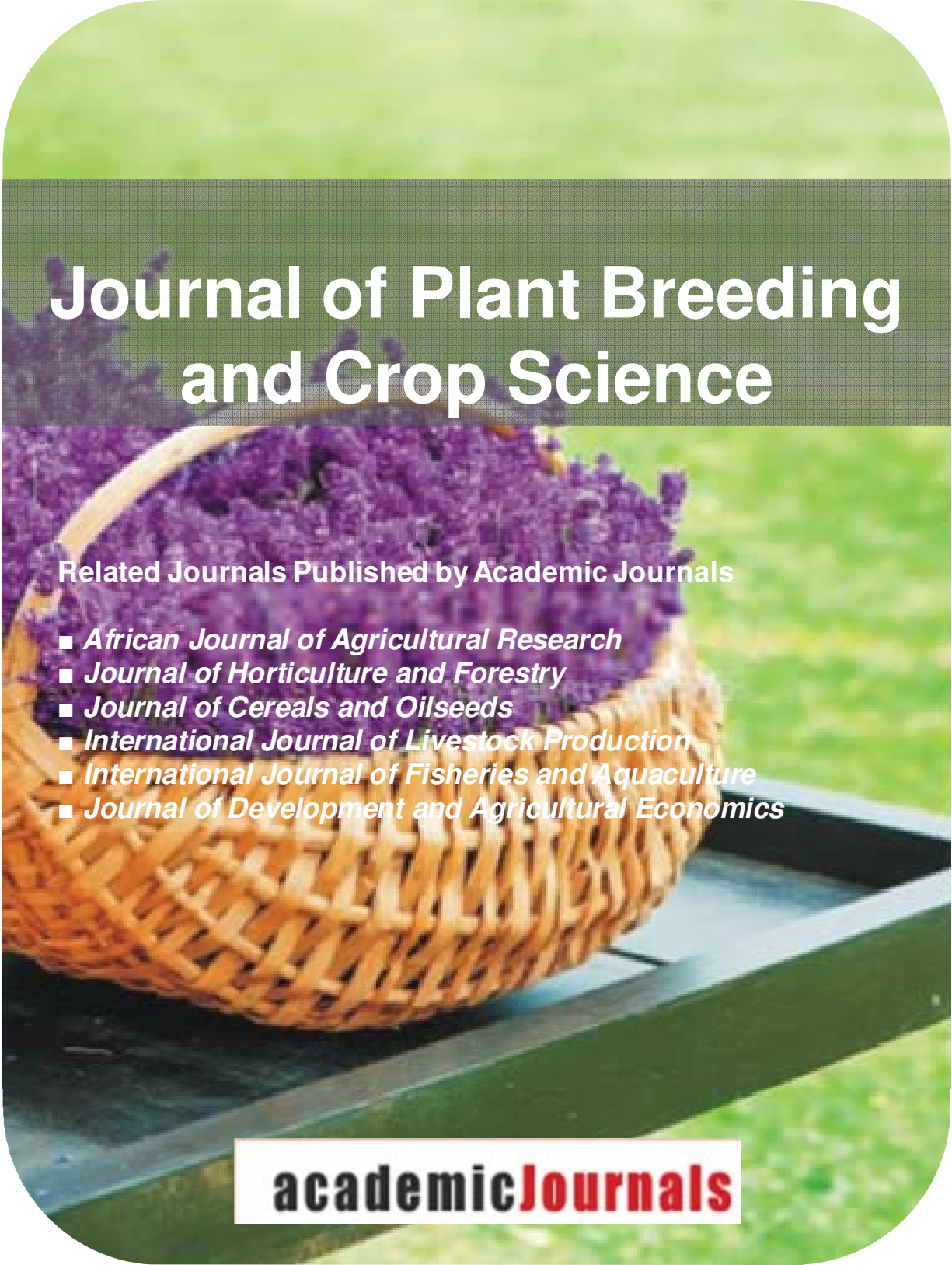
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