



Statistical evidence linking the zigzag stem habit with tolerance to cassava brown streak disease

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Abstract

Cassava (*Manihot esculenta* Crantz) provides the cheapest source of food calories in Africa and forms the major staple for roughly 40% of Africans. More than 200 million people in sub-Saharan Africa derive over 50% of their carbohydrate intake from cassava. It has been recognised as a powerful poverty fighter with the potential to help alleviate poverty both through food security and commercialization.

The low average yields in eastern Africa are caused by many factors including susceptibility of commonly grown varieties to major diseases and pests including cassava mosaic disease (CMD) and cassava brown streak disease (CBSD). The most effective and realistic approach to reducing losses to CMD and CBSD is the use of host-plant resistance or deployment of less-susceptible cultivars.

Several CBSD tolerant local cultivars have been identified and recommended for cultivation in Kenya (Kaleso, Guzo, Gushe, Kibiriti Mweusi, Ambari and Kahoteli), Mozambique (Nikwaha, Chigoma Mafia, Nachinyaya, Xino Nn'goe, Likonde, Mulaleia and Badge) and Tanzania (Namikonga, Kiroba, Nachinyaya, Kigoma Mafia (Kigoma Red), Kitumbua, Kalulu, Mfaransa, Muzege, Kikombe, Gezaulole and Kibangameno). These are the main sources of resistance used in breeding for CBSD resistance.

In efforts to improve breeding efficiency, some researchers have investigated the correlation between morphological markers and resistance to CBSD but in vain. All the CBSD tolerant cultivars mentioned above in Tanzania and Mozambique are genetically heterozygous (Zz) for the stem habit trait. It has been reported that resistance or tolerance of cassava to CBSD was associated with the "z" allele controlling stem growth habit. This study confirms the reported association. The zigzag stem habit can therefore be used as a morphological marker in breeding for CBSD resistance. Furthermore, it will be possible for breeders to use the marker in pre-emptive breeding where the disease is not present. This is the first report of using a morphological marker in indirect selection for an economic important trait in cassava.

Introduction

Cassava (*Manihot esculenta* Crantz) provides the cheapest source of food calories in Africa and forms the major staple for roughly 40% of Africans. More than 200 million people in sub-Saharan Africa derive over 50% of their carbohydrate intake from cassava. It has been recognised as a powerful poverty fighter with the potential to help alleviate poverty both through food security and commercialization.

In Africa cassava productivity is on average nearly 10 t/ha (8.1 t/ha in DRC, 9.8 t/ha in Tanzania, 10.6 t/ha in Kenya and 12 t/ha in Uganda). However yields in some South Asian countries are much higher: China (16.2 t/ha), Indonesia (16.6 t/ha), Thailand (22.9 t/ha) and India (32.9 t/ha) (FAO, 2009). The low average yields in Eastern Africa are caused by many

factors including susceptibility of commonly grown varieties to major diseases and pests including cassava mosaic (CMD) and cassava brown streak disease (CBSD). CBSD which historically was confined to the coastal lowlands of Kenya, Tanzania and Northern Mozambique, is now considered the greatest threat to food security in the Great Lakes region.

The most effective and realistic approach to reducing losses to CMD and CBSD is the use of host-plant resistance or deployment of less-susceptible cultivars. Cassava breeding started in 1935 at Amani in Tanzania to develop cultivars resistant to both CMD and CBSD. CBSD was considered sufficiently important for resistance breeding to be included in the cassava improvement programme supported by the British Government during the 1940s and 50s under the auspices of the East African and Forest Research Organization (EAAFRO). The most resistant variety developed from this programme was 46106/27 and was a third backcross derivative from *M. esculenta* x *M. glaziovii*. This clone which has survived and remained popular to date is still one of the most CBSD tolerant clones now grown in Kenya under the local name Kaleso. Recently using molecular techniques (over 500 single nucleotide polymorphism (SNP) markers), we have shown that Kaleso is genetically identical to cultivar Namikonga which is grown in Tanzania.

Namikonga is popular in Mtwara and Lindi regions. Several CBSD tolerant local cultivars have been identified and recommended for cultivation in Kenya (Kaleso, Guzo, Gushe, Kibiriti Mweusi, Ambari and Kahoteli), Mozambique (Nikwaha, Chigoma Mafia, Nachinyaya, Xino Nn'goe, Likonde, Mulaleia and Badge) and Tanzania (Namikonga, Kiroba, Nachinyaya, Kigoma Mafia (Kigoma Red), Kitumbua, Kalulu, Mfaransa, Muzege, Kikombe, Gezaulole and Kibangameno). These are the main sources of resistance used in breeding for CBSD resistance.

In efforts to improve breeding efficiency, some researchers have investigated the correlation between morphological markers and resistance to CBSD but in vain (Nichols, 1947, Mahungu et al., 1999). Marker genes control the expression of traits which can be easily classified into distinct states, each controlled by a different allele, and whose expression is little influenced by the environment. They can be a tool for studying linkage groups and mating systems, and sometimes they are directly important as determinants of economically important characters. The zigzag stem growth habit is one of the marker genes identified in cassava. There is strong evidence to suggest that the zigzag stem is controlled by a single recessive gene (Hershey and Ocampo 1989).

All the CBSD tolerant cultivars mentioned above in Tanzania and Mozambique are genetically heterozygous (Zz) for the stem habit trait. For Kenya, Kaleso and Kahoteli are also heterozygotes whereas, Guzo, Gushe, Kibiriti Mweusi and Ambari are of unknown genetic constitution (they have just



recently been reported by Munga, 2008 to be CBSD tolerant). There is a need to find out the genetic basis of their stem habit characteristic.

Kanju et al., 2007 reported that resistance or tolerance of cassava to CBSD was associated with the "z" allele controlling stem growth habit. This study was carried out to estimate some genetic parameters among CBSD tolerant cultivars. However, the data were used to find out whether there is statistical evidence to support the aforementioned hypothesis.

Methodology:

Open pollinated seeds obtained from seven CBSD tolerant parents (NDL 90/34, Kigoma Mafia/Red, Namikonga, Nachinyaya, Kiroba, Kitumbua and Kalulu) planted in a polycross block established at ARI Naliendele during the 2004/05 season were used to raise F1 seedling clones for use in the genetic analysis of CBSD and CMD tolerance or resistance, fresh root yield and dry matter content. All the seedlings which established were cloned at harvest without selection to establish the Clonal Evaluation Trial (CET) during the 2005/06 season. The CET was arranged in a check plot design with NDL 90/34 (improved check) planted after every 10 clones. Each clone was represented by five plants in a plot spaced at 1m x 1m. Harvesting was done 12 months after planting (MAP). At harvest CBSD root necrosis was scored from

each plant using a scale of 1 – 5 where 1 indicated no visible symptoms and 5 indicated very severe damage. The highest score was used to distinguish between resistant/tolerant (score 1 – 2) from susceptible clones (score 3 – 5).

Since all the parents used in this study were CBSD tolerant and heterozygous for stem habit (Zz), we expect that mating among them (including selfing) will result in a 3:1 (resistant/tolerant to susceptible) segregation ratio if the above mentioned hypothesis is true. The Chi-Square test was used to test this hypothesis. Chi-Square is a statistical test commonly used to compare observed data with data we would expect to obtain according to a specific hypothesis. It was estimated by using the GraphPad Software Inc., 2005 as follows: $\chi^2 = \sum [(O - E)^2 / E]$ where O = number of observed resistant/tolerant or susceptible clones and E = number of expected resistant/tolerant or susceptible clones.

Results and Discussion

The reaction of the clones from the seven families to CBSD (root necrosis) is summarized in Table 1. The $\chi^2 = 13.274$ with 13 degrees of freedom was obtained. The two-tailed P value equals 0.4269. By conventional criteria, this difference is considered to be not statistically significant. This implies that the data were sampled from the distribution we expected (GraphPad Software Inc., 2005).



Zigzag main stem habit (zz) on the left and normal stem habit (ZZ or Zz) on the right



Table 1: Reaction of clones from seven families to CBSD at ARI Naliendele, Mtwara, 2004/05 season

Family	No. of Clones evaluated	No. of clones observes to be resistant*	No. of clones expected to be resistant	No. of clones observes to be susceptible	No. of clones expected to be susceptible
Naliendele-OP	97	77 (78%)	72.75	20	24.25
Kiaoma Red-OP	62	49 (79%)	46.5	13	15.5
Namikona-OP	89	80 (90%)	66.75	9	22.25
Nachinyaya-OP	163	124 (76%)	122.25	39	40.75
Kiroba-OP	107	83 (78%)	80.25	24	26.75
Kitumbua-OP	134	100 (75%)	100.5	34	33.5
Kalulu-OP	116	91 (78%)	87	25	29
Total	768	604 (79%)	576	164	192

OP = Open pollination in a polycross design; * Figures in bracket are the number of tolerant clones expressed as a percentage of the total number of clones evaluated.

We have noted that it is phenotypically difficult to distinguish between heterozygous (Zz) and homozygous dominant (ZZ) clones. Homozygous recessive clones (zz) can very easily be distinguished because they have very pronounced zigzag stem and branches (Caption above). From these results we expect that the majority of the “zz” F1s should be tolerant/resistant to CBSD. These can be used as resistant parents to cross with susceptible cultivars (which have good agronomic or other consumer preferred traits) in isolated crossing blocks. This will ensure that all the F1s from the crossing blocks will be “Zz”, the majority of which will be tolerant/resistant to CBSD. This approach can be used in pre-emptive breeding for CBSD tolerance in the absence of the disease e.g. in West Africa. This work can be initiated if the CBSD resistant parents are available to breeders. If this is not possible due to quarantine issues, the crossing blocks can be established in Tanzania and the seeds sent to the breeders. CBSD is not known to be seed-borne. The breeders can then select F1s with zigzag stems (zz) and use them as parents. This will be the first report of using a morphological marker in indirect selection for an economic important trait in cassava.

We have so far not known any CBSD resistant/tolerant clone that is normal for the stem habit (ZZ) trait. However, we have obtained some Zz and zz clones that were susceptible to CBSD. This implies that the resistance gene is not very tightly linked to the marker gene to rule out false positives. This does not rule out the use of this marker since the majority of clones (75 to 90%) from each family were resistant/tolerant to CBSD (Table 1).

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