EVALUATION OF CASSAVA (*Manihot esculenta* Crantz) GERMPLASM FOR RESISTANCE TO CASSAVA BROWN STREAK DISEASE AND VIRUS ELIMINATION USING *IN VITRO* TECHNIQUES

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A Thesis Submitted to the Graduate School in Partial Fulfillment of the Requirements for the Doctor of Philosophy Degree in Crop Protection of Egerton University

EGERTON UNIVERSITY

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DECLARATION AND RECOMMENDATIONS

Declaration

I declare that this thesis is my original work and has not been presented to any university for any degree or other award.

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DEDICATION

To my beloved daughter

Sheena

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ABSTRACT

Cassava brown streak disease (CBSD) is an important disease causing losses of up to 70% in the most susceptible cultivars. Its effects include: reduced root quality due to pitting, root constriction and necrosis, and reduced number and weight of tuberous roots. To contribute to CBSD management, a study was conducted to: (i) Screen East African cassava landraces and F_1 populations for CBSD resistance; (ii) Analyse symptom expression and virus accumulation in CBSV graft inoculated cassava varieties (iii) Eliminate virus from infected cassava using *in vitro* techniques. Two F1 populations were developed: Namikonga × AR37-80 and Pwani × AR37-80. Landraces and F1 population were screened for CBSD resistance in CBSD hotspot areas in Kenya and Tanzania. A partially balanced lattice design was used for the studies done in Tanzania, while a randomized complete block design (RCBD) was used for the study done in Kenya. Results showed that genetic makeup accounted for the largest variation observed (22.8 – 78.2%), followed closely by genotype by environment interaction (11.7 – 46.8%) in CBSD foliar symptoms, root necrosis, root necrosis incidence and usable roots. Similar observations were made on evaluated root traits including: root weight, number of roots per plant, dry matter content and harvest index, for genotype (22.5 – 84.2%) and genotype by environment interraction (10.5 – 44.7%). The results showed that although these traits are largely under genetic control, they can also be affected by environment to varying degree. The study identified new sources of CBSD resistance/tolerance among the landraces and F1 populations. In Tanzania, 28 resistant landraces were identified including: Chimaje, Chipanda, and Supa B. Additionaly, 27 tolerant landraces were identified including: Kikwada, Mbuyu, and Mreteta. In Kenyan, only tolerant landraces were identified including: Weite, Manchoberi, and Merry-go-round. Among the F1 populations, progenies categorized as resistant included: NAMAR050 NAMAR130, and NAMAR371 while those categorised as tolerant were PAR024, PAR057, NAMAR116 and NAMAR441. Different responses to CBSD inoculation were observed with Kaleso and Nase 1 showing the least symptoms and virus accumulation. Apart from Kaleso, Nase 1 was identified as a good CBSD progenitor in breeding CBSD resistant varieties. Some of the resistant landraces had high yield and could be used directly for cultivation and in cassava breeding programs for transfer of resistance to farmer preffered varieties. Thermotherapy at (35°C) for 2 weeks combined with subculturing into regeneration media can be used for production of virus free cassava since this treatment had the highest survival rate (77%) and success in virus cleaning (91%). The findings of this study will be important in future CBSD management.

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LIST OF ABBREVIATIONS AND ACRONYMS

ACMV	African cassava mosaic virus
cDNA	Complementary-sense DNA
CBSD	Cassava brown streak disease
CBSIs	Cassava brown streak ipomoviruses
CIAT	International Centre for Tropical Agriculture
CMD	Cassava mosaic disease
CBSVs	Cassava brown streak viruses
CBSV	Cassava brown streak viruses
CGM	Cassava green mite
CMGs	Cassava mosaic geminiviruses
CMBs	Cassava mosaic begomoviruses
CTAB	Cetyltrimethylammonium bromide
EACMV	East African cassava mosaic virus
EACMV-Ug	East African cassava mosaic virus – Uganda
EDTA	Ethylenediaminetetracetic acid
ESA	East and Southern Africa
G × E	Genotype by environment interaction
IITA	International Institute of Tropical Agriculture
MAS	Marker assisted selection
PCR	Polymerase chain reaction
qPCR	Quantitative PCR
qRT-PCR	Quantitative real time PCR
QTL	Quantitative trait loci
RT-PCR	Reverse transcription polymerase chain reaction
RNA	Ribonucleic acid
(+)ss RNA	Positive-sense single-stranded RNA
UCBSV	Uganda cassava brown streak virus
SSA	sub-saharan Africa

CHAPTER ONE
INTRODUCTION

1.1 Background

Cassava (*Manihot esculenta* Crantz) is the third most important source of calories in the tropics and the eighth most important food crop in the world after sugarcane, maize, rice, wheat, potatoes, soybeans and vegetables in terms of global annual production (FAOSTAT, 2018). It is a staple food for more than 800 million people world-wide (Raji *et al*., 2009) and its average yield in Africa is 9.0 t/ha, which is far below the genetic potential of 90 t/ha (Lebot, 2019; FAOSTAT, 2018). Cassava's edible roots are rich in carbohydrates, calcium, vitamins B and C, and essential minerals but a poor source of proteins (Motagnac *et al.*, 2009). Its nutrient composition however differs according to variety and age of the harvested crop, soil conditions, climate, and other environmental factors during farming (Motagnac *et al.,* 2009). The starchy storage roots of cassava are becoming increasingly important for processing into higher value products.

Cassava has multiple uses; it is consumed in many processed forms, in the industry and as a livestock feed (Lukuyu *et al.,* 2014). Cassava root meal, leaf meal and peels are nutritious with regards to high dry matter content, crude protein, sol protein, crude fibre, starch, and total sugars and have been widely used for feeding aquaculture species (Lukuyu *et al.,* 2014). Roots or leaves are ground into flours and other processed food products (Onyenwoke & Simonyan, 2014). Flours are of three types, yellow garri, white garri, or intermediate colour, with yellow garri considered the best product in Nigeria (Onyenwoke & Simonyan, 2014). Cassava is a raw material for making alcoholic beverage by conventional anaerobic fermentation of dried cassava before alcohol extraction (Ryosuke *et al.,* 2014). Additionally, it has medicinal properties and has been promoted as a treatment for bladder and prostate cancer due to the presence of antioxidants and anti-carcinogens in the plant (Mahbubur-Rahman & Akter, 2013; Montagnac *et al*., 2009). In Africa and Asia, the potential of cassava as a bio fuel crop has been exploited due to the rapid economic growth that led to a rapid rise in demand for energy, putting a concern on the national energy security (Marx and Nquma, 2013; Ogundari *et al.,* 2012; Jansson *et al.,* 2009). Cassava has a high conversion rate for ethanol hence is a suitable feed stock for bio ethanol apart from maize, wheat and sorghum which are the major feed stocks for bio ethanol production (Marx and Nquma, 2013; Ogundari *et al.,* 2012).

Cassava can grow well in marginal lands, requires low inputs, and is tolerant to pests and drought (Nweke *et al.,* 2002). The crop is not only strategically important as a food source and famine reserve but is also perceived as a pro-poor vehicle for economic development. In West Africa, particularly Nigeria, cassava has demonstrated its potential to replace most imported staples and cereal-based industrial raw materials. Efforts towards commercialization of cassava systems are currently transforming the subsistence-level operations into a private sector-led commercialized value chain (Abass *et al.,* 2013). The markets for cassava products in East and Southern Africa signal a possible high potential for growth in the food and starch sectors. Market signals serve as an inducement for investment by the private sector. Such industrialization has the potential to stimulate a "demand pull" and increase the potential for income growth for smallholders but the characteristic low efficiency and profitability of the sector serve as constraints to investment (Abass *et al.,* 2013). Therefore, a suitable approach to increase the efficiency and profitability of the sector is required. A combination of institutional arrangements and productivity-enhancing strategies, such as the use of improved varieties, fertilizer, optimum weeding mechanized production techniques, and improved agronomic practices, is required to increase production efficiency (Abass *et al.,* 2013).

Africa accounts for half of the total world cassava production while the rest is contributed by Asia and Latin America including the Caribbean. In Africa, the leading producers are Nigeria, Democratic Republic of Congo, Ghana, Angola and Mozambique with an average yield of 59.5, 30.0, 20.9, 8.7 and 8.5 million metric tonnes, respectively (FAOSTAT, 2018). Despite having larger production areas under cassava, Uganda and Tanzania have lower average yields of 5.3 and 5.7 t/ha respectively (FAOSTAT, 2018). Approximately 60%, 30% and 10% of cassava production in Kenya is in Western/lake basin, Coast and Eastern regions, respectively (Githunguri *et al.,* 2017). Traditional utilization of cassava in Kenya is limited to roasting and boiling of fresh roots for consumption. In Western Kenya, however, roots are also processed into flour for ugali in combination with flour from cereals i.e. maize (*Zea mays*) or sorghum (*Sorghum bicolor*) or both (Githunguri *et al.,* 2017). In the Coast of Kenya, cassava is the second main staple food (Githunguri *et al.,* 2017). The roots are mainly boiled or processed into fried chips, crisps and pure cassava flour besides young cassava leaves being used as vegetable.

Several biotic and abiotic factors constrain cassava production, the major ones being the cassava mosaic disease (CMD) and cassava brown streak disease (CBSD). CBSD is

caused by two distinct viruses: cassava brown streak virus (CBSV) and Ugandan cassava brown streak virus (UCBSV), both of which have (+)ss RNA genomes belonging to the genus *Ipomovirus* in the family *Potyviridae,* and generally producing similar symptoms in infected plants (Ndunguru *et al.,* 2015; Vanderschuren *et al.,* 2012; Legg *et al.,* 2011; Winter *et al.,* 2010). The disease causes economic losses resulting from damage to the aboveground parts characterized by leaf chlorosis & necrosis, elongated necrotic lesions on stems and secondary and tertiary vein chlorosis (Winter *et al.,* 2010; Hillocks & Jennings, 2003). Root spoilage occurs due to constriction caused by dry corky necrotic rot on starchy tissues and stunted growth on infected plants (Winter *et al.,* 2010; Hillocks & Jennings, 2003). Necrotic lesions and/or discoloration of the roots due to infection render the roots unpalatable and unmarketable, hence most of the quantitative and qualitative losses (Nichols, 1950). CBSD has been reported to cause up to 70% yield loss by reducing the root sizes and causing pitting and constriction on roots (Hillocks *et al.,* 2001).

CBSD has been endemic in the coastal regions of Kenya across the Tanzanian border and down as far as the Zambezi River in Mozambique and it is widespread around the shores of Lake Malawi (Mohammed *et al.,* 2012). These endemic areas are confined to altitudes below 1,000 meters above sea level (Hillocks *et al.,* 1999), but in the last few years, it has been reported at mid-altitude levels. Mixed infections of CBSV and UCBSV with high prevalence, incidence, and severity in the mid altitude areas (1181 – 1467 metres above sea level) of Western Kenya were reported by Osogo *et al*. (2014) and Mware *et al.* (2009). Ndunguru *et al*. (2015) detected both CBSV and UCBSV in low-altitude, mid-altitude, and high-altitude areas of Tanzania, disapproving the assumption that the viruses are limited by agro-ecological zones. These findings demonstrated a wide distribution of the disease in almost all cassava growing areas and confirming that other areas previously unaffected by CBSD are at risk of spread and increased prevalence of the disease.

Different approaches have been beneficial in CBSD management including phyto-sanitary practices (Tumwegamire *et al.,* 2018; Hillocks & Jennings, 2003) which play a significant role in limiting the spread of the disease through infected planting material. The most effective and realistic approach in reducing losses due to diseases is the use of host-plant resistance or the deployment of less-susceptible cultivars. Although natural sources of resistance for CBSD are available, introgression of the trait into farmer preferred cassava cultivars through conventional breeding has been challenging due to the difficulty of combining CBSD resistance with good root and harvest qualities (Jennings, 2003). Breeding

for resistance to CMD and CBSD started in 1935 at Amani, Tanzania (Hahn *et al.*, 1980). The most resistant variety developed from this programme was 46106/27, which was a third back-cross derivative from *Manihot esculenta* × *Manihot glaziovii* (Jennings, 2003; Nichols, 1947). It is undoubtedly the most successful product of the Amani research programme that is presently available to farmers and whose resistance to CBSD has persisted for many years in farmers' fields in Kenya where it is locally known as 'Kaleso' and in Tanzania as 'Namikonga' (Hillocks & Jennings, 2003). 'Namikonga' has the highest general combining ability for CBSD resistance (Kulembeka *et al.*, 2012).

1.2 Statement of the Problem

Cassava brown streak disease (CBSD) caused by cassava brown streak virus (CBSV) and Uganda cassava brown streak virus (UCBSV) has been an important biotic constraint to cassava production for the past 70 years. CBSD causes losses in production through reduced growth as well as spoilage of harvested roots due to necrotic rot. Quantitative assessments of yield losses have demonstrated that losses of up to 70% occur and can be higher in highly susceptible varieties. Apart from whiteflies, surveys have revealed that the transportation of infected materials to areas in which CBSD was previously absent has enabled the disease to spread from independent hotspots. This is because farmers exchange cassava stems used for vegetative planting material locally and over long distances. Deployment of resistant varieties is the most sustainable approach for controlling CBSD since they restrict both symptom development and virus accumulation but currently, variety 'Namikonga' is the only reliable source of CBSD resistance. New sources of resistance are therefore required to combat the newly emerged mixed infections of both CBSV and UCBSV. Some of the sources of resistance including Namikonga and Nase 19 have poor root and harvest qualities with significantly low harvest indices ranging between 0.15 – 0.26. This emphasizes the need of crossing with varieties having desirable root traits such as high yields and dry matter content to produce CBSD resistant farmer preferred varieties.

1.4.1 General Objective

Contribute to improved food security and livelihoods of smallholder cassava farmers through identification of germplasm resistant to cassava brown streak disease and virus elimination using *in vitro* techniques.

1.4.2 Specific Objectives

i) To screen for CBSD resistance in East African cassava landraces.
ii) To develop and screen F1 populations for CBSD resistance.
iii) Analyse symptom expression and virus accumulation in CBSV graft inoculated plants
iv) Assess *in vitro* chemotherapy and thermotherapy techniques for production of virus free cassava.

1.5 Null hypotheses

i) There are no sources of CBSD resistance among the East African cassava landraces.
ii) There are no CBSD resistant progenies among the F1 populations.
iii) Cassava varieties do not express CBSD symptoms or accumulate viruses.
iv) *In vitro* chemotherapy and thermotherapy techniques do not result into virus free cassava plants.

1.3 Justification

Several CBSD tolerant clones have been identified in East Africa including, Guzo, Nachinyaya and Kikwaha. However, they readily show foliar symptoms, with delayed or absent root necrosis unlike the resistant Kaleso which develops mild foliar symptoms with no root necrosis. Although CBSD tolerant varities produce sufficient yield, they spread the infection since they harbour high virus quantities. This leaves Kaleso as the only reliable source of resistance therefore the need for sourcing more resistant materials through field screening for CBSD resistance. Poor root and harvest qualities of most CBSD resistance progenitors stir the need for crossing with susceptible varieties and develop F1 progenies that have suitable agronomic traits. This will ensure production of farmer preferred CBSD resistant varieties. Owing to the enigmatic nature of CBSD symptoms and environmental influence on symptom expression, there is a possibility of escapes where a plant is not virus infected under field resistance evaluations. Graft inoculation and quantitative PCR comes in handy in ensuring that plants are inoculated, and virus quantities accumulated with time monitored. This enables the confirmation of resistance status of a variety. *In vitro* chemotherapy and thermotherapy provides an opportunity for mass propagation of virus free material which ensures availability of clean planting materials for cassava farmers. Planting virus free materials would ensure that CBSD does not spread beyond its currently confined distribution in eastern and southern Africa.

CHAPTER TWO
LITERATURE REVIEW

2.1 Cassava

2.1.1 Plant description

Cassava (*Manihot esculenta*), also known as manioc, tapioca, yuca, and mandioca, is a domesticated species of tuber, a root crop originally domesticated perhaps as long ago as 8,000–10,000 years ago, in southern Brazil and eastern Bolivia along the southwestern border of the Amazon basin. It is a perennial woody shrub of the Euphorbiaceae family that is grown in tropical and sub-tropical regions of the world for its edible starchy tuberous root (Li *et al.,* 2010, Figure 1). It is a tall semi woody perennial shrub or tree which can grow upto 7 meters high and has single to few stems (Alves, 2002). Cassava has dark green, palmately compound leaves that are 30cm or more across and have 5 - 9 lobes (Alves, 2002). Its long petioles (leaf stalks) are usually longer than the lamina and light green, or dark green, or blue-green, or red in colour. Its outer back is smooth and is light brown to yellowish green in colour. Cassava storage roots are not true tubers but are developed by secondary thickening storing starch within proliferated xylem parenchyma (Medina *et al.,* 2007). A cassava root is long and tapered, with a firm homogeneous flesh encased in a detachable rind, about 1 mm thick, rough and brown on the outside. Root size ranges from 2.5 – 10 cm in diameter and 20 – 37.5 cm in length, although roots upto 90 cm long have been found. The roots grow in outward pointing clusters from the base of the stem just below the soil surface.

2.1.2 Taxonomy

The cassava family, Euphorbiaceae, contains 28 wild species and its evolution is from inter-specific hybridisation among wild species (Lekha *et al*., 2011). It is an out crossing crop, therefore highly heterozygous and because of its out-crossing nature, 91.5% genetic variation exists in cassava germplasm. In a diploid cassava (2n = 36), DNA content is approximately 1.7 pg/cell nucleus and the haploid genome size is approximately 772 mega base pairs (Mbp) (Lekha *et al*., 2011).

2.1.3 Environmental requirements

Cassava is cultivated in all tropical and subtropical regions of the world between 30° N and S (Nassar, 2003). Cassava thrives well in areas where the annual precipitation ranges from 600 - 1200 mm and temperature range of 25 - 29°C with a photoperiod of 12 hours

(Alves, 2002). It is adapted to poor soils that allow acceptable harvest under marginal conditions where other crops cannot survive; cassava is able to survive longer dry periods (Siritunga & Sayre, 2003). During dry periods, the plant loses all its leaves and suspends growth even of the thick roots. When precipitation resumes, the plant regenerates without any major loss in yield. This ability adapts it to locations marked by indefinite and irregular precipitation regimes. Cassava has a flexible harvesting time, making it an excellent food security crop because the plants can be partially harvested and left growing in the ground until roots are next needed (Ihemere *et al.*, 2006; Nweke *et al.*, 2002).

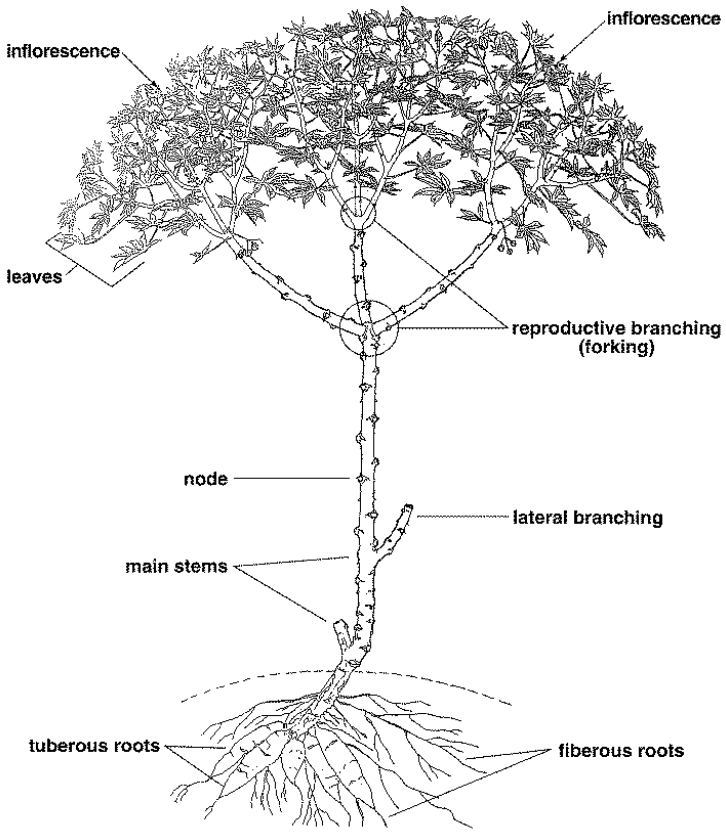


Figure 1: Morphology of a mature cassava plant (Ekanayake *et al.*, 1997).

2.1.4 Production and utilization

World production of cassava is around 278 million tonnes a year of which 57%, 32% and 11% are produced in Africa, Asia and Latin America respectively (FAOSTAT, 2018). Cassava is one of the major sources of farm income and is an important food security crop for the people of Africa. The resilience of cassava enables it to grow successfully under a wide range of agro-ecological zones where cereals and other crops cannot thrive, making it a

suitable crop for poor farmers to cultivate under marginal environments in Africa (Ntawuruhunga *et al.,* 2006). Cassava can be grown as an intercrop with other crops. Intercropping not only increases soil fertility but also production per unit area, thus improving household food security and income gneration (Tang *et al.,* 2020; Silva *et al.,* 2016).

Cassava serves as a raw material in the manufacture of processed foods, animal feed and industrial products (Aloys & Ming, 2006; Taiwo, 2006; Balagopalan, 2002). Cassava cultivation is transforming from subsistence to a more commercially-oriented farming enterprise, with increased prospects of starch as a source of ethanol for biofuels (Guira *et al.,* 2016; Nassar & Ortiz, 2010). The crop is gradually being transformed from a famine reserve crop and rural staple food to a cash crop for urban consumption (Guira *et al.,* 2016; Marx and Nquma, 2013; Ogundari *et al.,* 2012).). Consequently, cassava acreage has been increasing throughout Africa from 8.6 million Ha in 1990 to 11.0 million ha in 2000 and 24.6 million ha in 2018 (FAOSTAT, 2018).

2.1.5 Growth cycle

Cassava is mostly propagated from stem cuttings, but in nature and in the process of plant breeding, propagation by seed is common (Hillocks *et al.,* 2002). If the cuttings are planted in moist soil under favourable conditions, they produce sprouts and adventitious roots within a week. Flowering of the plant may start as early as sixth weeks after planting, although the exact time that flowering starts will depend on the cultivar and the environment (Alves, 2002). Once begun, flowering will continue intermittently for the rest of the plants life. Tuber formation begins by the eighth week after planting, if the environmental conditions (e.g. photoperiod) are favourable (Alves, 2002). The tubering process involves the onset of secondary thickening in some of the adventitious roots which were previously fibrous in nature. As the thickening progresses, the roots expand, and the bulk of the root comprises of fleshy tuber material (Alves, 2002). Usually, the secondary thickening begins at the proximal part of the root and progresses towards the distal portions. Thus, the tuber is fattest at the proximal part in which the thickening had occurred for a longer time, and tapers slowly towards the distal portions (Alves, 2002).

2.1.6 Reproductive biology

The cassava plant is monoecious; bearing separate male and female flowers on the same plant (Figure 2). The time interval from planting to flowering may vary from 1 to more than 24 months and depends on the genotype and environmental conditions (Byrne, 1984). A single branched panicle produces both male and female flowers. The female flowers are found at the base of the panicle while male flowers towards the tip. The flowers are small, with the male flower being about 0.5 cm in diameter, and the female flower slightly larger (Halsey *et al.*, 2008). Flowers usually begin to open around mid-day and remain open for about one day (Ceballos *et al.*, 2002). Cassava exhibits protogyny, where female flowers open first, and the male flowers follow 1 or 2 weeks laiter on a given branch. Thus, when male flowers open, the female flowers on the same branch will have undergone fertilization or aborted (Halsey *et al.*, 2008). However, because flowering on a single plant may last for more than two months, both self- and sib-fertilization may occur, with the proportion of each dependent on the genotype, the environment, and the presence of pollinating insects (Ceballos *et al.*, 2004; Jennings & Iglesias, 2002; Kawano, 1980).

Figure 2: Cassava flower. A – Male flower, B – Female flower, C – Floral branching (Byrne, 1984).

Environmental factors strongly influence flowering in cassava (Halsey *et al.*, 2008). A clone may produce no flowers in one environment, produce only aborted flowers or fail to produce viable seed in another environment (Halsey *et al.*, 2008). Cassava pollen grains are large and are sticky. Therefore, wind pollination appears to be of little consequence. Several species of wasp and honeybees (*Apis cerana, Apis mellifera*, etc.) are the main pollinators in different continents (Kawano, 1980). Cassava pollen shows size dimorphism, the larger grains being 130 to 150 microns in diameter, while the smaller grains range from 90 to 110 microns (Halsey *et al.*, 1991). In some varieties, the larger grains are more abundant, whereas

in other clones the smaller grains are more common. The larger grains are more abundant and have better germination percentages (60%) under in vitro conditions (2 hours at 40°C) than the smaller ones (Plazas, 1991).

Smaller grains typically germinate less efficiently than large ones and they may have less than 20% viability. Once shed, cassava pollen loses its' viability rapidly. Leyton (1993) has reported nearly 97% seed set with newly-collected pollen, 56% seed set with pollen stored for 24 hours at 25°C, and 0.9% seed set (one seed from 102 pollinations) after 48 hours of storage. In practice, breeders take care to perform pollinations within one hour after collection of pollen to avoid loss of viability. For some clones, induction of flowering appears to depend on long photoperiods up to 16-hours and temperatures of around 24ºC (Alves, 2002). Flowering is also dependent on plant habit. A flower bud typically forms when the plant branches, so that highly branched genotypes are more prolific than those with sparse branching habits (Halsey *et al.,* 2008). Apical branching precedes flower bud formation and it is a prominent visual indication of incipient flowering, and plants in the pre-flowering stage (Halsey *et al.,* 2008).

After pollination and subsequent fertilization, the Cassava ovary develops into young fruit. It requires 3 - 5 months after pollination for the fruit to mature (Hahn *et al.,* 1980). The mature fruit is capsule, globular in shape, with a diameter of 1 - 1.5 cm (Alves, 2002; Hahn *et al.,* 1980). The endocarp is woody, with three locules, each containing a single seed. When the fruit is mature and dry, the woody endocarp splits explosively to release and disperse the seeds (Hahn *et al.,* 1980). Cassava seed is ellipsoidal and 1 - 1.5 cm long. It has a brittle testa which is grey and mottled with dark blotches (Halsey *et al.,* 2008). There is a large caruncle at the micropylar end of the seed. Germination of the seed requires a long time, but the duration can be shortened by filing the micropylar end until the white embryo is just visible (Halsey *et al,* 2008). Fertilized seed is viable two months after pollination, and the fruit matures about one month later, or three months after pollination (Ceballos *et al.,* 2004). Dehiscence is explosive and the seed initially falls close to the mother plant, but ants can disperse them further. Ants usually carry an unknown percentage of the seed to their nests. Through these two mechanisms of autochory followed by myrmecochory, a seed may be dispersed up to several meters from its place of origin (Elias *et al.,* 2001).

Seed production and viability are variable, depending on the quality of the female parent (Kawano, 1980). Jennings (1976) reported that controlled pollination normally produces one viable seed per fruit, from a maximum of three in the trilocular ovary. A study

by Ceballos *et al.* (2004) indicated that handpollination produces one to two viable seeds. Newly harvested seeds are dormant, requiring 3 to 6 months of storage before they germinate (Jennings & Iglesias, 2002).

Cassava seeds are adapted to dispersal by ants through deep burial in soil and the large energy reserves allow a long dormancy period (Pujol *et al.,* 2002). Seeds can remain viable for up to 1 year, although germination percentages may decline considerably after 6 months (Rajendran *et al.,* 2000). Seed germination is favoured by dry heat and complete darkness. Ellis *et al.* (1982), working with two-dimensional temperature gradient plates, found that germination occurred most often when temperatures exceeded 30°C for part of the day, with a mean temperature of at least 24°C. He suggested that an alternating temperature regime of 30°C for 8 hours and 38°C for 16 hours for at least 21 days is the most appropriate for determination of cassava seed viability under laboratory conditions. Botanical seeds are not typically used for commercial propagation. Genetically, any cassava genotype is extremely heterogeneous, and propagation from sexual seed results in wide and unpredictable diversity of phenotypes, which is of interest to breeders but presents difficulties in propagation (Ceballos *et al.,* 2004).

2.2 Cassava Brown Streak Disease

Cassava brown streak disease (CBSD) is endemic in areas along the Indian Ocean Coast of Eastern Africa, from the north-eastern border of Kenya across the Tanzanian border down as far as the Zambezi River in Mozambique; it is also widespread around the shores of Lake Malawi (Mohammed *et al.,* 2012). In the endemic areas, CBSD was confined to altitudes below 1,000 metres above sea level (Hillocks *et al.,* 1999; Nichols, 1950; Storey, 1936). However, Jeremiah & Legg, (2008) and Alicai *et al.* (2007) reported at mid-altitude levels (1200 - 1500 meters above sea level) in Democratic Republic Congo, Uganda and the Lake zone areas of Tanzania (Jeremiah & Legg, 2008) which were not considered to be at risk previously. Ndunguru *et al.* (2015) reported twelve new whole genomes of CBSD causing viruses, including seven of CBSV and five of UCBSV, doubling the genomic sequences available in the public domain. These new sequences disprove the assumption that the viruses are limited by agro-ecological zones. This is raising concern because disease incidences of up to 100% have been recorded (Hillocks *et al*., 2001), and in sensitive varieties the disease causes rotting of tubers, reducing both the quality and quantity of tubers available for consumption (Hillocks *et al.,* 1996; Nichols, 1950; Storey, 1936). A moderate infection

by CBSD (10 - 30% damage to root surface area) decreases the market value of cassava tubers drastically by 90% (McSween *et al*., 2006) fetching under US $5 per tonne, as opposed to $55 for fresh healthy cassava roots.

2.2.1 Aetiology, transmission, host range and diagnosis of CBSD

Based on complete genome sequences, CBSD is caused by two distinct virus species. The coastal endemic virus known as cassava brown streak virus (CBSV) (Monger *et al.,* 2001), and the highland epidemic virus as Uganda cassava brown streak virus (UCBSV) (Mbanzibwa *et al.,* 2009a). Both virus species belong to the genus Ipomovirus, family Potyviridae (Monger *et al.,* 2001; Winter *et al.,* 2010) and CBSVs is the general term used when referring to them.

CBSD has been mostly affecting cassava (*Manihot esculenta*), and has had no known alternative crop or weed hosts. However, Mbanzibwa *et al.* (2011a) detected CBSV in the perennial species tree cassava (*Manihot glaziovii)* which revealed the first virus reservoir other than cassava. Recently, Amisse *et al.* (2019) reported CBSV for the first time in two non-cassava perennial wild plant species: *Zahna africana* (Radlk.) Exell and *Trichodesma zeylanicum* (Burm.f.) R.Br., that occur widely within and near casssava fields in Mozambique. Additionaly, CBSV and UCBSV were detected in *Manihot carthaginensis* subsp. *glaziovii* a wild cassava relative. The findings provided definitive evidence of a wider host range for CBSV and UCBSV indicating that these viruses are not restricted to cultivated cassava.

The aerial symptoms of CBSD in cassava include feathery chlorosis along the veins of leaves or sometimes circular chlorotic patches between the primary veins, brown necrotic streaks on the stem and stem die-back in severe cases (Jennings, 2003; Nichols, 1950). Symptoms in the tuberous roots consist of a brown corky necrosis of the starchy tissue, occasional radial constrictions, and a reduction in the content of starch and cyanide (Hillocks & Jennings, 2003; Nichols, 1950). The viral symptom phenotypes are variable depending on the virus isolate involved, variety of cassava, age of plant and the environmental conditions (Patil & Fauquet, 2014). Mohammed *et al.* (2012) characterized the symptoms produced by different isolates of CBSV and UCBSV, in both cassava and *Nicotiana benthamiana* under uniform conditions and identified contrasting levels of symptom severity produced by different isolates.

Several herbaceous plant species of different families can be artificially infected using mechanical transmission methods, and the variation in symptom phenotypes is more

pronounced in the model host *Nicotiana benthamiana* (Mohammed *et al.,* 2012; Ogwok *et al.,* 2010; Winter *et al.,* 2010; Mbanzibwa *et al.,* 2009b). Comparison of symptom severity between isolates of CBSV and UCBSV has shown that generally CBSV causes more severe symptoms, causing necrosis in *Nicotiana benthamiana*, whereas with UCBSV only mosaics and rugosity are induced in this host (Mohammed *et al.,* 2012; Mbanzibwa *et al.,* 2011b; Winter *et al.,* 2010). Grafting experiments in cassava cultivars also demonstrated higher virulence of CBSV than UCBSV, and the cuttings infected with CBSV showed significantly reduced sprouting, because of higher virus accumulation, compared with UCBSV (Mohammed *et al.,* 2012; Wagaba *et al.,* 2013). Using whole genome sequences from NGS data, Alicai *et al.* (2016) produced the first coalescent G based species tree estimate for CBSV and UCBSV. This led to the finding that CBSV had a faster rate of evolution when compared with UCBSV. Further, CBSV nonsynonymous substitutions were more predominant than synonymous substitution and occur across the entire genome. All comparative analyses between CBSV and UCBSV presented suggest that CBSV may be outsmarting the cassava immune system, thus making it more devastating and harder to control to UCBSV.

Under artificial conditions in *Nicotiana benthamiana*, UCBSV and cassava mosaic geminiviruses (CMGs) interact synergistically (Ogwok *et al.,* 2010). However, there are no reports of synergism in field-grown cassava. In the early 1930s, Storey & Nichols (1938) proposed the whitefly, *Bemisia tabaci* (Genn.) (Homoptera: Aleyrodidae), as a CBSVs vector, but this was not confirmed until many years later by Maruthi *et al.* (2005). Unlike CMGs, which *Bemicia tabaci* transmits in a persistent manner, CBSVs are transmitted semi-persistently, like other ipomoviruses, and are not retained for more than 24 hours (Maruthi *et al.,* 2017; Dombrovsky *et al.,* 2014). Early and accurate diagnosis of CBSVs in diseased plants remains a great challenge. Effective diagnostics will help to monitor and forecast disease outbreaks, giving enough time for the application of management strategies (Martin *et al.,* 2000; Miller *et al.,* 2009).

There are several methods available for detection and diagnosis of CBSD-causing viruses. Using ELISAs, it is possible to detect CBSD but not to distinguish between CBSV and UCBSV (Winter *et al.,* 2010). Several nucleic acid-based methods have been employed for diagnostics of CBSD viruses, such as reverse transcription (RT-PCR) (Abarshi *et al.,* 2012; Abarshi *et al.,* 2010; Mbanzibwa *et al.,* 2011a; Moreno *et al.,* 2011), real-time RT-PCR (Adams *et al.,* 2013) and more recently loop mediated isothermal amplification (Tomlinson *et*

al., 2013). These PCR-based approaches allow specific detection of CBSV and UCBSV, and when used with multiplexing primers, they may specifically and differentially amplify the target regions of CBSV and UCBSV (Abarshi *et al.,* 2012). With advances in sequencing technologies, hitherto-unknown viruses are being identified by deep sequencing of RNA extracts from virus-infected plants (Kreuze *et al.,* 2009). This approach has also been used for diagnosis of CBSVs (Monger *et al.,* 2010).

2.2.2 Current status of CBSD in Africa and effects on yield

The earliest report of CBSD was from northern coastal areas of Tanzania in 1935 (Storey, 1936) corresponding to the region where CMD was first observed (Warburg, 1894) and emphasizing the fact that Tanzania is a hot spot for biodiversity of cassava viruses (Ndunguru *et al.,* 2005). Early reports of CBSD noted that affected areas were almost entirely restricted to coastal areas of East Africa and the shores of Lake Malawi (Nichols, 1950). Although CBSD was observed in some parts of Uganda, for many years it was believed that the disease did not spread to altitudes above 1000 m above sea level (Nichols, 1950).

The first systematic countrywide assessment of CBSD was completed in 1994 in Tanzania (Legg & Raya, 1998) and the highest incidences were recorded from the southern lowland coastal districts of Mtwara (36.0%) and Masasi (25.2%), whilst the disease was virtually absent from the mid-altitude (800 masl.) region of north-western Tanzania. Significantly, however, small numbers of symptomatic plants were observed near Entebbe, in central/southern Uganda (~1200 masl) in 1994 and from Tabora in north-western Tanzania (~1200 masl) (Legg & Raya, 1998). However, the view that CBSD is a lowland disease remained unchanged until 2004, when the first report showed significant spread of CBSD in central/southern Uganda (Alicai *et al.,* 2007). Following these first reports from Mukono district, significant increases in the incidence and distribution of the disease were recorded in Uganda through to 2007, by which time approximately 10% of all fields included infected plants and overall incidence was 1.9% (Alicai *et al.,* 2007). Shortly after reporting CBSD spread in Uganda, similar observations were made in western Kenya. Mixed infections of CBSV and UCBSV were observed with high prevalence, incidence and severity in the mid-altitude areas of Kenya (1181 - 1467 meters above sea level) (Mware *et al.,* 2009; Osogo *et al.,* 2014) and mid-altitude and high-altitude areas of Tanzania (Ndunguru *et al.,* 2015).

CBSD incidence has been increasing in Tanzania and surveys of 19 districts within the north-western regions of Kagera, Mara, Shinyanga and Kigoma revealed a steady pattern

of increase in CBSD incidence from 5.9% in 2006 to 11.5% in 2007 and 31.6% in 2008 (Legg *et al.,* 2011). Greater levels of disease in districts in which CBSD was already present by 2006 (12 of 19 districts) contributed to most of the increase in incidence (Legg *et al.,* 2011). All these survey assessments, based primarily on visual assessments of leaf symptoms, are underestimates of the true level of infection, as cassava plants may not express CBSD leaf symptoms under unfavourable weather conditions Additional reports state that over the last 10 years, CBSD has spread to other countries in East, South and Central Africa such as Rwanda, Burundi, Congo, DR Congo, South Sudan and Zambia (Bigirimana *et al.,* 2011; Mbanzibwa *et al.,* 2011a; Mulenga *et al.,* 2018; Mulimbi *et al.,* 2012). Although CBSD like symptoms have been observed in tuberous roots of cassava plants harvested in Bas Congo Province in western DRC (Mahungu *et al.,* 2003), Mulanje Province in central Angola (Lava Kumar *et al.,* 2009) and parts of Madagascar, none of these reports has been verified despite extensive diagnostic efforts.

Understanding the absolute reasons behind for the sudden upsurge of CBSD incidence and geographical range is a challenging. Previous studies have shown that the spread of High disease pressure, planting susceptible genotypes and abundant whiteflies enhance CBSD (Katono *et al.,* 2015). While whiteflies can only disperse and amplify CBSD over short distances, the trade of infected planting materials spreads CBSD over both short and long distances (McQuaid *et al.,* 2017).

A study by Maruthi *et al.* (2005) confirmed the ability of *Bemicia tabaci* to transmit CBSV from infected to healthy plants under quarantine insectary and glasshouse conditions. Further studies indicated that whiteflies transmit CBSD viruses semi-persistently where they acquire the viruses in 5 - 10 min, retain them for up to 48 h and and finally transmitts them over relatively short distances of less than 17 m in a cropping season (Maruthi *et al.,* 2017). Critically, one of the key causes of the increases in both CMD and CBSD in the African Great Lakes region appears to be super-abundant numbers of whiteflies which can thrive at altitudes above 1000 m above sea level (Alicai *et al*., 2007; Jeremiah *et al*., 2015; Legg *et al.,* 2014, 2011). There has been a dramatic increase in populations of the whitefly vector in the region since the early 1990s and up to 100-fold increases have been recorded in CMD pandemic-affected regions of East and Central Africa, including Uganda, western Kenya, north-western Tanzania, Rwanda, Burundi, and eastern DRC (Legg *et al.,* 2006; Otim-Nape *et al.,* 1996). CBSD outbreaks occur from 3 to 12 years after increases in whitefly numbers (Legg *et al*., 2011). Evidence has also been presented for the association of specific

genotypes of *Bemicia tabaci* with the cassava virus pandemics of East and Central Africa (Legg *et al.*, 2014, 2002), although it has also been hypothesized that *Bemicia tabaci* population increases are a consequence of synergistic interactions with CMD-infected cassava host plants (Colvin *et al.*, 2006).

Apart from whiteflies, surveys have revealed that the transportation of infected material to areas in which CBSD was previously absent has enabled the disease to spread from independent hot spots (Legg *et al.*, 2011). This is because farmers exchange cassava stems used for vegetative planting material locally and over long distances. Further, one report concluded that contaminated cutting tools can infect healthy cassava plants. (Rwegasira & Chrissie, 2015); however, a similar study has shown that such practices do not result in the transmission of CBSVs (Maruthi *et al*., 2017). CBSVs are found only in Africa. Therefore, there is a perception that the viruses evolved within East Africa on an unknown species and subsequently jumped host into cassava in a new encounter situation (Monger *et al*., 2010). It is a possibility that there may be other hosts for CBSVs, which could serve as viral inoculum sources in the field (Monger *et al*., 2010). As an example, CBSV has been detected in the wild perennial species *Manihot glaziovii* (Mbanzibwa *et al*., 2011a) although the importance of this to CBSD epidemiology is not currently known.

Whichever is the case, the upsurge in CBSD prevalence is incontrovertible and whitefly vector and infected planting materials appears to be the key drivers of the new mid-altitude outbreaks of CBSD (Legg *et al.*, 2014, 2011). These recent changes in the dynamics and distribution of CBSD mean that there is great current concern about the threat of further westward spread within Africa towards the West (Legg *et al.*, 2014), which is currently the world's largest producer of cassava (FAOSTAT, 2018).

CBSD causes losses in production through reduced growth as well as spoilage of harvested roots due to necrotic rot (Nichols, 1950). There have been few quantitative assessments of yield losses. The first was conducted in southern coastal Tanzania (Hillocks *et al.*, 2001) and demonstrated that losses of up to 70% occur in the most susceptible cultivars. It was also noted that root symptoms become increasingly severe as plants mature. Therefore, farmers harvest their cassava crop early to prevent root spoilage. In Malawi, variable effects of CBSD on cassava roots were reported (Gondwe *et al.*, 2002). These included reductions in the quality of roots caused by pitting, constrictions, and root necrosis, as well as effects on the productivity of plants, which included reductions in the number and weight of tuberous roots. Since cassava is widely grown as a subsistence crop, yield losses due to CBSD threaten

food security for millions of households. In addition, the economic development of smallholder farmers and larger producers is constrained, with yield losses estimated to be more than 750 million US dollars annually across the worst affected countries in Eastern Africa alone (Tomlinson *et al.,* 2017; Maruthi and Hillocks, 2015). Although some progress has been made, the diverse and complex effects of CBSD on cassava plants and the people who grow, process, and consume cassava products are only partially characterized. Substantial additional research on this topic is therefore merited.

2.2.3 Management of CBSD through phytosanitary practices

Like other viral diseases of vegetatively propagated crops, phyto-sanitary practices can play a significant role in limiting the impact and spread of CBSD (Hillocks & Jennings, 2003; Storey, 1936). In view of the cryptic symptoms of CBSD, where symptoms are typically mild and typically confined to lower leaves, it can be difficult to distinguish between healthy and infected plants. This has the consequence that infected planting material readily propagates CBSVs. Additionally, the semi-persistent transmission of these viruses' means that they are retained for relatively short periods of time, limiting the distance over which they can be carried by their whitefly vector (Maruthi *et al.,* 2017). CBSD therefore appears to be spread by vectors over relatively short distances but readily carried longer distances through transport of planting material. This contrasts with the CMGs causing CMD, which whiteflies can carry over long distances but are less likely to be propagated through planting material as their symptoms are much more obvious (Legg *et al.,* 2011).

In view of these biological characteristics, phyto-sanitation is of much greater importance for CBSD than it is for CMD. Major components of CBSD control programmes, therefore, include: (1) The production of 'clean' stocks of planting material, including virus indexing of parent material in tissue culture, systematic virus testing in isolated pre-basic germplasm multiplication and regular rouging of symptomatic plants in the propagation field. (2) Collective action at a community level to encourage groups of farmers growing cassava near one another to co-operate in implementing phyto-sanitary measures, including the sourcing of 'clean' planting material and its maintenance through rouging and selection of healthy stems for replanting. (3) Large-scale initiatives are currently being implemented in parts of eastern and southern Africa that are using these approaches to constrain both local and regional spread of CBSD. In addition, the importance has been emphasized for national and sub-regional-level quarantine authorities to enforce effective controls on intra- and inter-

continental movements of cassava germplasm. This will ensure that CBSD does not spread beyond its currently confined distribution in Eastern and Southern Africa (Legg *et al.*, 2014).

2.2.4 Breeding for CBSD resistance and sources of resistance

The most effective and realistic approach in reducing losses due to diseases is the use of host-plant resistance or the deployment of less-susceptible cultivars. A variety is considered resistant when it has minimal CBSD foliar and root necrosis symptoms and root necrosis incidence (Hillocks & Jennings, 2003). Further, resistant varieties have accumulate low virus quantities (Kaweesi *et al.*, 2014; Maruthi *et al.*, 2014a). The term tolerance is used to describe a host that can be infected by a virus which causes symptoms without significantly diminishing the plant growth or yield (Cooper and Jones, 1983). With regards to CBSD, tolerant varieties express moderate to severe foliar symptoms but minimal or no visible root symptoms thus have 100% utilisable roots (Hillocks *et al.*, 2001). Although tolerant varieties produce sufficient yield, harbour high virus quantities and can disseminate the virus (Maruthi *et al.*, 2014a). Nzuki *et al.* (2017) reported two QTLs located on chromosome 1 and 12 to be significantly associated with CBSD root necrosis while four other QTLs located on chromosome 2, 4, 6 and 17 controlling foliar severity. The study suggested some degree of independence in genetic control for CBSD resistance implying that resistance to CBSD foliar and root symptoms could be governed by different genes. CBSD susceptibility on the other hand describes a host plant with high virus titres, severe symptoms both on leaves and roots and thus significant yield loss (Maruthi *et al.*, 2014).

Breeding in cassava is a major challenge, as it is a cross-pollinated and a highly heterozygous crop (Ceballos *et al.*, 2012). Breeding for resistance to both CMD and CBSD began in 1935 at Amani, Tanzania (Hillocks and Jennings, 2003; Hahn *et al.*, 1980; Jennings, 1960). Failure to identify reliable sources of CMD resistance from a worldwide collection of cassava cultivars led to the lengthy process of transferring genes for both CMD and CBSD resistance from related species (Jennings, 2003; Nichols, 1947). The most resistant variety developed from this programme was 46106/27, which was a third back-cross derivative from *Manihot esculenta* × *Manihot glaziovii* (Jennings, 2003; Nichols, 1947). It is probably the most successful product of the Amani research programme that is presently available to farmers and whose resistance to CBSD has persisted for many years in farmers' fields in Kenya where it is locally known as Kaleso (Hillocks & Jennings, 2003). More than 500 single nucleotide polymorphism (SNP) markers have recently been used to show that Kaleso is genetically identical to cultivar Namikonga, which is grown in Tanzania and is considered

as the best source of CBSD resistance (Pariyo *et al.*, 2013). These SNP markers have been placed on an integrated SNP-simple sequence repeat genetic linkage map, which are used for quantitative trait locus (QTL) detection of tolerance to CBSD (Pariyo *et al.*, 2013; Ferguson *et al.*, 2012; Kulembeka *et al.*, 2012; Rabbi *et al.*, 2012). Generating a mapping population of 60 F1s from a cross between CBSD-tolerant cultivar Namikonga and a susceptible cultivar Albert, led to the identification of QTLs associated with CBSD resistance (Kulembeka, 2010). The availability of the cassava genome sequence (Wang *et al.*, 2014; Prochnik *et al.*, 2012) should help in identifying genes controlling CBSD resistance, as well as novel markers associated with CBSD resistance.

Efforts are going on to identify CBSD resistance genes by RNA sequencing analysis and transcriptome profiling of CBSD resistant and susceptible cassava cultivars. RNA sequencing is a technology that uses the capabilities of next-generation sequencing for whole-transcriptome shotgun sequencing to study the gene expression at a given moment of time. Three varieties of cassava – Kaleso (Highly resistant to CBSD), Kiroba (moderately resistant to CBSD) and Albert (highly susceptible to CBSD) – were challenged with CBSD and then subjected to Illumina RNA sequencing (Maruthi *et al.*, 2014a). Sequence analysis showed over expression of more than 700 genes in CBSD-resistant Kaleso in comparison with Albert. Although virtually none of the over expressed genes resembled known resistance gene orthologues, some genes encoded enzymes or factors involved in hormone signalling pathways and secondary metabolites, both of which are linked to disease resistance (Maruthi *et al.*, 2014a).

Several CBSD-resistant clones have been identified in Kenya (Kaleso, Guzo, Gushe, Kibiriti Mweusi and Ambari), Mozambique (Nikwaha, Chigoma Mafia, Nanchinyaya, Xino Nn'goe, Likonde, Mulaleia and Badge) and Tanzania (Namikonga, Kiroba, Nanchinyaya, Kigoma Mafia, Kitumbua, Kalulu, Mfaransa, Muzege, Gezaulole and Kibandameno) (Patil *et al.*, 2015). Some of these clones are former Amani hybrids that are no longer recognized as such, as they have been given local names. Most of them are better described as 'tolerant', as they readily show foliar symptoms, but root necrosis is delayed or absent (Hillocks & Jennings, 2003). The exchange of virus-resistant cassava germplasm is one of the principle activities of the International Institute of Tropical Agriculture led project 'New Cassava Varieties and Clean Seed to Combat CMD and CBSD' funded by the Bill and Melinda Gates Foundation, which was initiated in 2012 and will run through to 2016 (Tumwegamire *et al*., 2018). The project aims to ensure that farmers have access to diverse disease-free improved

varieties with combined resistance to CBSD and CMD, as well as preferred end-user characteristics. These are now being used extensively in breeding programmes as sources of resistance to generate new improved clones. Inter-crossing among them will concentrate resistance genes and allow recessive genes to be expressed (Hillocks & Jennings, 2003). Some of the F1 progeny remain symptom free after being challenged with the virus or show a low incidence of infection and reduced symptom severity. Both additive and non-additive genetic effects have recently been reported to be important in the expression of CBSD resistance, and in studies of these effects, Kaleso (Namikonga) had the highest general combining ability for resistance to CBSD (Kulembeka *et al.*, 2012; Mtunda, 2009; Munga, 2008).

Recent surveys undertaken in Tanzania, Kenya, Uganda, and Malawi show that farmers themselves may have recognized that some varieties show tolerance to CBSD and that they are less predisposed to to root necrosis (Hillocks & Maruthi, 2015). However, most of the varieties grown are prone to infection by CBSVs and their widespread propagation maintains virus inoculum at high levels. The long-term solution is based on developing varieties with true resistance to CBSD in which plants restrict symptom expression and virus replication both on leaves and roots. This type of resistance has been observed in Kaleso (Masumba *et al.*, 2017; Kulembeka *et al.*, 2012; Nichols, 1947) and it is important to exploit this resistance in addition to identifying more sources of CBSD resistance and their underlying mechanisms and genetics.

CHAPTER THREE

SCREENING EAST AFRICAN CASSAVA LANDRACES FOR RESISTANCE TO CASSAVA BROWN STREAK DISEASE

3.0 Abstract

Cassava brown streak disease (CBSD) caused by cassava brown streak virus (CBSV) is an important viral disease of cassava in Africa and is a major threat to food security. CBSD causes rotting of edible roots and deployment of resistant varieties is the most cost-effective and sustainable way of managing the devastating effects of the disease. This study aimed at screening and identifying cassava landraces resistant to CBSD. Sixty three and thirteen landraces were collected in Tanzania and Kenya, respectively. The experiments were set up in a partially balance lattice and randomized complete blocked design with three replicates in CBSD hotspot areas in Tanzania and Kenya, respectively. Data were collected on CBSD foliar and root symptoms severity and yield related traits including root weight, number of roots per plant, dry matter content and harvest index. ANOVA revealed significant differences among the landraces for the traits evaluated. Apart CBSD foliar symptoms at 6 and 9 MAP in the Kenyan trial, the largest sum of squares (SS) was attibuted to genotype (40.0 – 83.0 %) followed by genotype by environment interaction (11.7 – 44.7%) and finally environment (0.01 – 26.8%) for all traits evaluated. This showed that although trait expression was largely genetically controlled, it was influenced by genotype by environment interaction and environment in a few landraces. The Kenya trial identified CBSD tolerant landraces including: Weite, Merry-go-round, Nyakasamuel, and Manchoberi which had low root weights of ≤ 10.0 t/ha but high dry matter content ranging from 31.0 – 41.0%. They had low root weights probably due to CMD infections and this emphasizes the need for deployment of varieties resistant to both CBSD and CMD. In the Tanzanian trial, resistant landraces with high root weight (≤ 20.0 t/ha) and high dry matter content (≤ 30.0 %) included Benny, Katewanya, Limbanga, Mombasa, Musa Said, Mweda, Simanyu, Supa B and Supa Jangwa. These could be used directly for cultivation and in cassava breeding programs for transfer of resistance to farmer preffered varieties. Tolerant high yielding varieties were also identified including: Mreteta, Mdimbe, Nyoka, and Vicent and could be taken through virus cleaning so that farmers can have access to clean planting materials for these particular landraces.

Key words: Cassava, Landraces, CBSD resistance, Genotype × Environment interraction, Yield related traits.

3.1 Introduction

Cassava brown streak disease (CBSD) is one of the most dangerous threats to cassava (Manihot esculenta Crantz), which is Africa's most important food security crop. The disease causes losses to cassava root production and quality. Cassava brown streak virus (CBSV) (Monger *et al.,* 2001) and Ugandan cassava brown streak virus (UCBSV) belonging to the genus Ipomovirus, family Potyviridae cause CBSD (Mbanzibwa *et al.,* 2009a). Both viruses, together called cassava brown streak ipomoviruses (CBSIs), have a positive-sense single stranded RNA genome (Ndunguru *et al.,* 2015; Winter *et al.,* 2010). CBSD symptoms include foliar chlorosis and necrosis, brown streaks on stems, constrictions and dry corky necrotic rot of roots and stunted plant growth (Hillocks & Jennings, 2003; Vanderschuren *et al.,* 2012; Winter *et al.,* 2010). CBSIs are mostly spread by the propagation of infected cassava cuttings by farmers, although the insect vector whitefly *Bemicia tabaci* (Gennadius) also transmit the viruses in a semi-persistent manner (Maruthi *et al.,* 2017; Mware *et al.,* 2009; Maruthi *et al.,* 2005).

Early reports on CBSD distribution indicated that the disease was mostly restricted to the East African coast and the shores of Lake Malawi (Nichols, 1950). For many years, it was believed that the disease does not spread at altitudes 1000 masl (Hillocks *et al.,* 1999; Nichols, 1950). However, in the last few years, outbreaks of CBSD have been reported at mid altitude levels (1200–1500 m above sea levels) in Uganda, western Kenya and Tanzania, Mozambique, Rwanda, Burundi, and in isolated parts of the Democratic Republic of Congo (Ndunguru *et al.,* 2015; Osogo *et al.,* 2014: Jeremiah and Legg, 2008; Alicai *et al.,* 2007; Mahungu *et al.,* 2003). This new spread poses a major threat to the major cassava-growing regions of West Africa.

The genetic factor of a plant is by far the biggest contributor to CBSD symptom determination and severity (Kulembeka *et al.,* 2012; Munga, 2008; Nichols, 1950). CBSD symptoms are variable in terms of severity, onset of symptom expression, parts of the plant affected, viral isolate involved in causing symtoms, genotype of variety (resistant or susceptible), environmental conditions (temperature, rainfall and altitude), and age of infected plant (Mohammed *et al.,* 2012; Nichols, 1950). This variability makes diagnosis difficult for farmers (Nichols, 1950) and can result in farmers being unaware that their crop is affected until they harvest storage roots (Legg & Kanju, 2015). The difficulty in the diagnosis of CBSD has meant that infected stems have been transported to areas in which CBSD has previously been absent and used for planting material. Symptom variability has also

hampered epidemiological studies, as the disease can go unnoticed in an area for lengthy periods.

In terms of control, the most economically viable method for CBSD management is the use of host-plant resistance. Cassava breeding for disease resistance began at the East African Cassava Research Institute at Amani in northern Tanzania (Hillocks & Jennings, 2003; Nichols, 1950). The search for resistance led to breeders' introgressing disease resistance through inter-specific crosses with wild cassava Manihot spp. (Jennings, 1957). Crosses with *Manihot glaziovii* backcrossed three times and intercrossed with resistant hybrids produced inter-specific hybrids that were rated as highly resistant to another major cassava disease called cassava mosaic disease (CMD) but moderately resistant to CBSD (Jennings, 1957).

Some of the best-known intercrosses at Amani included cultivars 46106/27, 5318/34 and 5543/156 (Jennings, 1994). Hybrid 46106/27, also known as Amani in Tanzania showed high levels of field resistance to CBSD. 46106/27 is closely related to, but not identical to, a Tanzanian local cultivar called Namikonga (Kulembeka *et al.,* 2012; Pariyo *et al.,* 2013). Namikonga was, therefore, considered to be an inter-specific hybrid from the Amani program that was subsequently adopted by the farming communities and given a local name. Namikonga has been consistently resistant to CBSD for many years and has the highest general combining ability for disease resistance (Masumba *et al.,* 2017; Kulembeka *et al.,* 2012; Nichols, 1947). Some of the present day so-called "local cultivars" in Tanzania, especially the few which have proved to be resistant or tolerant to CBSD, including Kigoma Red, Kigoma Mafia and Kiroba are also likely to have some pedigree related to the Amani breeding program (Bredeson *et al.,* 2016; Kanju *et al.,* 2010; Mahungu *et al.,* 2003; Masumba *et al.,* 2017; Nzuki *et al.,* 2017; Pariyo *et al.,* 2015). The best CBSD resistance genotypes are likely to have survived in farmer fields as landraces in Tanzania from the Amani research program. Research has also shown that some of these CBSD-resistant genotypes have performed well in multiple locations, adapting to different agro-ecologies and disease pressures (Pariyo *et al.,* 2015; Abaca *et al.,* 2012). For example, Kigoma Red a local landrace is resistant to CBSD in both Tanzania and Uganda. Identifying and saving such germplasm is therefore important for controlling the disease in the affected African countries.

At present, Namikonga still expresses field resistance to CBSD and is used as one of the best sources of CBSD resistance in conventional breeding programs (Masumba *et al.,* 2017; Maruthi *et al.,* 2014a; Pariyo *et al.,* 2013; Kanju *et al.,* 2010). Bredeson *et al*. (2016)

reported Namikonga, TMS130572, KBH2006/18, Mkombozi, TMS 1972205 and Akena to have a common *Manihot glaziovii* haplotype on chromosome 1 designated as the 'Amani haplotype'. For a long time Kiroba was perceived as an Amani hybrid but Nzuki *et al*. (2017) reported that its *Manihot glaziovii* haplotype is different from that of Namikonga and it has a close parent-offspring relationship with tree cassava. When the Amani program ceased in around 1958, it is thought that some of the inter-specific crosses found their way into farmer's fields in Tanzania and have been incorporated as farmer varieties (Kanju *et al.,* 2003). The clones may have lost their identities and farmers are growing by them under different local names. Although cassava breeders have identified some inter-specific hybrids that show strong levels of resistance/tolerance to CBSD, there are still many unidentified clones which potentially are good sources of CBSD resistance. This work aimed at addressing this gap with the intention of identifying best CBSD resistant cassava germplasm particularly focusing on those that do not develop root necrosis (Legg *et al.*, 2011). New and diverse sources of resistance are required to combat the newly emerged threat by CBSD in which mixed infections of both CBSV and UCBSV are common both in mid and low altitude areas of Eastern and Southern African regions (Ndunguru *et al.,* 2015; Osogo *et al.,* 2014; Adams *et al.,* 2013).

3.2 Materials and methods

3.2.1 Collection of germplasm

In Kenya, 13 cassava landraces including improved varieties were collected from Migori and Kakamega counties. Landraces included: Amakuria, Merry-go-round, Weite, Manchoberi, Nyakasamuel, Obaro dak, and Matuja and improved varieties were: Migyera, MM96/4466, MM96/9308, MM96/2480, MM98/3567, and Agric I. Nyaboda was used as a CBSD susceptible control while improved variety MH95/0183 was used as a tolerant control. In Tanzania, 63 cassava landraces were collected from farmer's fields in Tandahimba, Mtwara rural, Newala, Masasi, Lindi urban, Lindi rural, Kilwa, Mtwara, Urban, Rufiji and Mkuranga Districts in the Southern and Eastern zones of Tanzania. Cassava varieties Kiroba (CBSD tolerant) and Albert (CBSD susceptible) were used as controls during for CBSD resistance screening.

3.2.2 Screening locations

The screening study was carried out in the 2014/2015 and 2015/2016 cropping seasons in Kenya and 2014 and 2015 cropping seasons in Tanzania. In Kenya, the study was carried out at the Kenya Agricultural Livestock & Research Organization (KALRO-Alupe). KALRO-Alupe is in Busia County and lies at latitude N0° 30.26′, longitude E 34° 07′ and an elevation of 1151 m above sea level. Alupe falls in LM1 (Lower midland zone 1) and receives a bi-modal rainfall of 840 mm in the first season (March - July) and 620 mm during the second season (September - November) (Jaetzold *et al.,* 2012). The mean minimum and maximum temperatures are 15.8°C and 28.6°C, respectively. Soils at Alupe are moderately deep to shallow and poorly drained. The soils are classified as ferallo-orthic ACRISOL in petro ferric phase (FURP, 1987). KALRO-Alupe is optimal for CBSD screening since it has both high disease pressure and whitefly populations. Besides, both species of cassava brown streak (UCBSV and CBSV) are available (Osogo *et al.,* 2014).

In Tanzania, screening was conducted at the Tanzania Agricultural Research Institute (TARI)-Naliendele, which is a hot spot for CBSD infection (Masumba *et al.,* 2017; Nzuki *et al.,* 2017). Naliendele lies on the coastal belt of the Indian Ocean and is located at 10° 22' 20"S, 40° 10' 34"E and 111 m above sea level. Naliendele receives rainfall from December-May with scattered showers in August-October (TMA, 2009). The soils are very deep, well drained, weak structured, dark reddish brown loamy sand topsoil over a reddish brown moderately structured sandy loam to sandy clay loam subsoil (Mugogo and Njapuka, 2007).

3.2.3 Field layout

In Kenya, the experiment was laid out in a randomized complete block design (RCBD) with three replicates. Single row plots of 13 plants per landrace were planted at a spacing of 1.0 m × 1.0 m. CBSD infected plants of the highly susceptible landrace Nyaboda was used as a spreader to ensure high CBSD pressure prevails in the screening blocks. The spacing of one meter by one meter was maintained for the spreader rows planted after every five rows of test plants and all around the blocks.

A partially balanced lattice design with three replicates was used for screening of Tanzanian cassava local landraces. Fifteen cassava cuttings (about 25 cm long and having 4 to 5 nodes with viable buds) from each of the landraces and controls (Kiroba and Albert) were planted at spacing of 1.0 m x 1.0 m. To increase CBSD inoculum pressure, cuttings from a known susceptible and infected variety Albert were also planted after every ten rows

of the test landraces to act as a disease spreader. In addition, the first and last rows were also planted with infected Albert cuttings. This configuration ensured that every plant is exposed to similar high inoculum pressure and no plant escapes infection. In both experiments, neither fertiliser nor irrigation were applied, and the field was rain fed throughout the growing seasons. The field was also kept weed free by hand weeding throughput the growing seasons.

3.2.4 Data collection

Data was collected on foliar severity at 3, 6 and 9 MAP and was scored according to Hillocks *et al*. (1996) and Hahn *et al.* (1989) (Figure 3). CBSD foliar severity was recorded following a scale of 1 - 5 where 1 = no apparent symptoms, 2 = slight foliar mosaic, no stems lesions, 3 = foliar mosaic, mildstem lesions and no die back, 4 = foliar mosaic, pronounced stem lesions and no die-back, and 5 = defoliation with stem lesions and pronounced die-back.

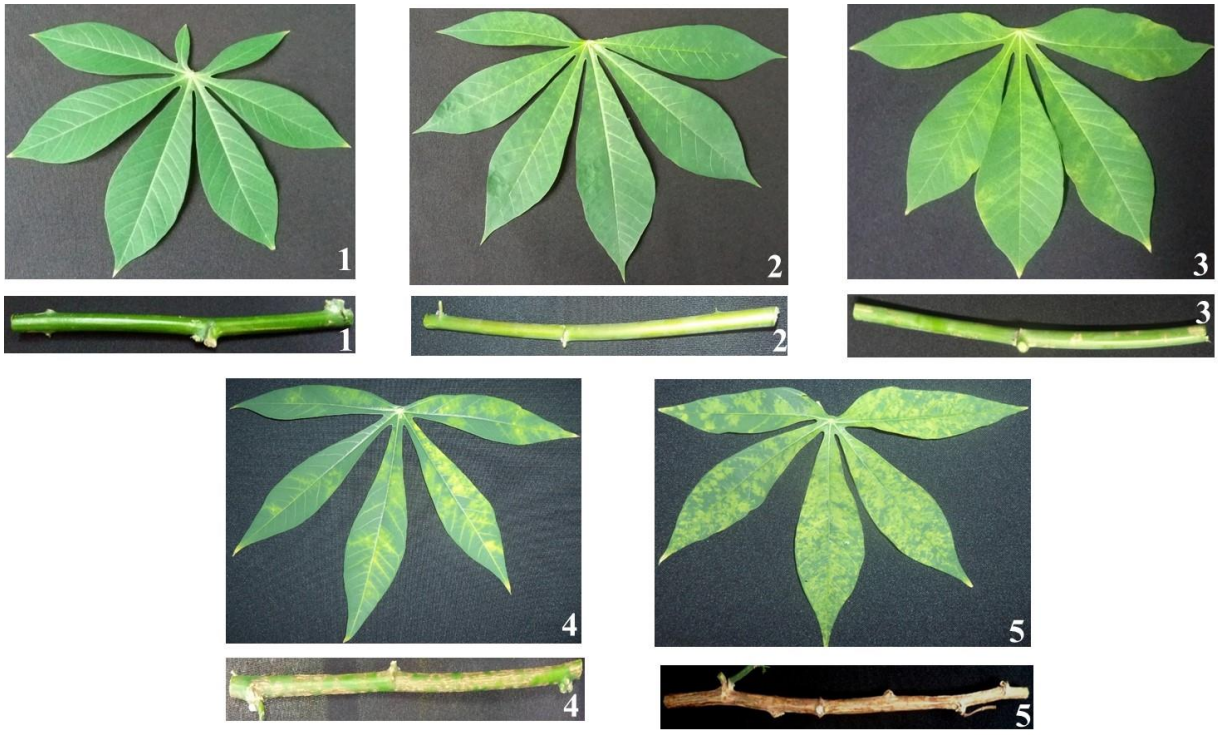


Figure 3: CBSD foliar symptom severity scale of 1 – 5 .

The most damaging aspect of the CBSD syndrome is root necrosis and at 12 MAP, plants from each landrace were harvested and roots examined for root symptoms. Roots from each landracewere chopped longitudinally and transversely to check for the presence of necrotic patches on the starch bearing tissues. Root necrosis severity scores were based on the

standard five-point scoring scale for CBSD where 1 = no apparent symptoms, 2 = <5% of root necrotic, 3 = 5 - 25% of root necrotic, 4 = 25 - 50% root necrotic & mild root constriction, and 5 = >50% of root necrotic with severe root constriction (Gondwe *et al*., 2002) (Figure 4). Root necrosis incidence (%) was calculated by expressing the number of symptomatic roots as a percentage of the total number of roots per plant.

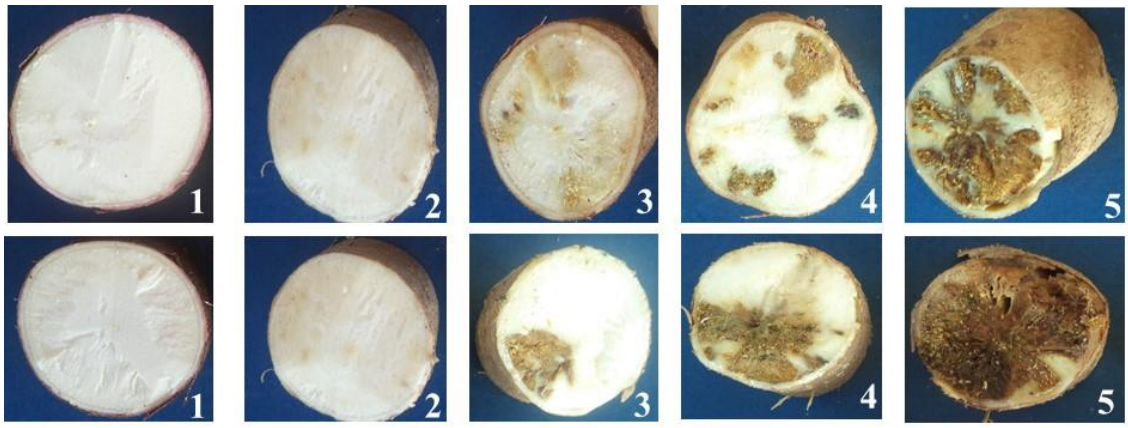


Figure 4: CBSD root necrosis symptoms severity scale 1 - 5

All roots with necrosis score of ≤2 were considered usable as only tiny spots of root necrosis were observable at this score. Severe root necrosis affects root quality, therefore reducing the quantity of usable roots. Usable roots were determined by peeling and cutting out necrotic potions of the roots. The necrotic portions were regarded as unusable. Each category was weighed separately, and usable roots (%) was calculated by expressing the weight of the unusable roots (t/ha) as a percentage of the total root weight (t/ha). Further, data was collected on root weight (t/ha), number of roots per plant, dry matter content and harvest index. Root weight in tonnes per hectare (t/ha/) was estimated according to Kamau *et al.* (2011) (Equation 3.1), root dry matter content using the specific gravity method (Kawano, 1987) (Equation 3.2) and harvest index according to Hühn, (1990) (Equation 3.3).

$$Root\ weight(t/ha) = \frac{root\ weight(kg/m2) \times 10000}{1000} \quad (3.1)$$

$$Dry\ matter\ content\ = 158.3 \times \left[\frac{weight\ of\ roots\ in\ air}{weight\ of\ roots\ in\ air - weight\ of\ root\ in\ water} \right] - 142 \quad (3.2)$$

$$Harvest\ index = \frac{weight\ of\ roots\ (kg)}{total\ plant\ weight\ (kg)} \quad (3.3)$$

3.2.5 Data analysis

Means were obtained by taking the average of the three replications for CBSD leaf symptoms severity at 3; 6 and 9 MAP, root necrosis, root necrosis incidence, usable roots (%), root weight in tonnes per hectare, number of roots per plant, root dry matter content and harvest index of different landraces and subjected to analysis of variance (ANOVA) as described in section. Combined ANOVA was carried out for the two cropping seasons and treatment means separated using Dunnett's method to compare each landrace against a control (MH95/0183 for the Kenyan landraces) and Kiroba (for the Tanzanian landraces), at 95% confidence level. Spearman's correlation analysis was carried out to determine the correlation between CBSD symptoms, root weight, number of roots per plant, dry matter content, and harvest index as described in section.

Landraces were classified into three categories based on foliar and root necrosis severity scores where;

1.0 – 2.0 were considered resistant,

2.1 – 3.0 were considered tolerant, and

3.1 – 5.0 were considered susceptible.

Landraces were also classified based on root necrosis incidence;

0 – 10% were categorized as resistant,

11 – 40% were categorized as tolerant, and

41 – 100% were categorized as susceptible.

These two sets of data were used together for classifying the landraces as described previously by Hillocks & Jennings (2003). Acccording to Hillocks & Jennings (2003) and Hillocks *et al.* (2001), resistant varieties show low foliar symptoms while tolerant ones readily express foliar symptoms but with low root necrosis severity. On the other hand, susceptible varieties show severe foliar and root symptoms with elevated levels of disease incidences.

3.3 Results

3.3.1 CBSD foliar symptoms

The mean CBSD foliar severity increased throughout the growing seasons for the Kenyan cassava landraces. On a scale of 1 to 5, the mean foliar severity was 1.7 at 3 MAP, 2.0 at 6 MAP, and 2.4 at 9 MAP across all the landraces tested (Table 1). At 3 MAP, most of the landraces had low mean foliar severity ranging from 1.3 – 1.9. However, MM98/3567, Nyaboda, and Obarodak had significantly ($P \leq 0.05$) higher mean foliar severity ranging from 2.2 – 3.1. At 6 MAP, MH95/0183, Agric I, Amakuria, Manchoberi, Matuja, Merrygo round, MM96/2480, MM96/9308, MM98/3567, and Nyakasamuel had a moderate mean foliar severity ranging from 1.7 – 2.4. Although Nyaboda and Obarodak had significantly higher mean foliar severity of 2.8 and 2.7 respectively, Migyera and MM96/4466 had significanlty lower mean foliar severity of 1.3 and 1.5 respectively. At 9 MAP, MH95/0183 had a mean foliar severity of 3.0, that was not significantly different from that of Amakuria, Matuja, Merrygoround, MM96/2480, MM96/4466, MM96/9308, MM98/3567, Nyaboda, Nyakasamuel, and Obarodak. Weite, Migyera, Manchoberi, and Agric I however, had significantly lower mean foliar severity ranging from 1.0 – 2.3 (Table 1).

Comparable results were observed for Tanzanian landraces and the CBSD mean foliar severity increased throughout the growing seasons. The mean foliar severity was 1.4 at 3 MAP, 1.8 at 6 MAP, and 1.9 at 9 MAP (Table 2) across all the landraces tested. At 3 MAP, most landraces had low mean foliar severity ranging from 1.0 – 1.9, which was not significantly ($P \leq 0.05$) different from the tolerant control Kiroba, whose mean foliar severity was 1.3. Kigoma Red, Kitumbua, Mbuyu, Nachinyaya, Ntonto, and Nyankagile had significantly ($P \leq 0.05$) higher mean foliar severity ranging from 2.4 – 2.8. At 6 MAP, Kiroba had a mean foliar severity of 2.1, and several landraces had significantly lower mean foliar severity ranging from 1.2 – 1.5. In contrast, Kigoma Red, Kikombe, Kitumbua, Mbuyu, Ntonto, and Nyankagile had higher mean foliar severity ranging from 2.7 – 3.1. At 9 MAP, Kiroba had a mean foliar severity of 2.1, while Benny, Chimaje, Katewanya, Likonde, Limbanga, Liumbukwa, and Mnacho had significantly lower mean foliar severity ranging from 1.1 – 1.3. Both tolerant (Kiroba) and susceptible (Albert) controls had mean foliar severities that were not significantly different from each other at 3, 6, and 9 MAP (Table 2).

Table 1: Means of CBSD symptoms and yield related traits for Kenyan cassava landraces

Genotype	Foliar 3	Foliar 6	Foliar 9	Root N	Root NI (%)	Usable R (%)	Root W	Root No.	Dry M (%)	H Index
MH95/0183	**1.3**	**2.2**	**3.0**	**1.4**	**19.1**	**94.6**	**16.8**	**5.5**	**31.7**	**0.65**
Agric I	1.7	1.8	2.3*	5.0***	100.0***	0.0***	3.3***	2.8**	18.4**	0.47**
Amakuria	1.5	2.4	2.5	2.6***	58.8***	67.0***	1.1***	3.5	17.5***	0.25***
Manchoberi	1.3	2.2	1.2***	1.1	3.2***	99.7	4.0***	3.6	39.6	0.38***
Matuja	1.6	2.1	2.8	1.8	45.8***	75.3***	2.8***	3.4	29.0	0.42***
Merry-go-round	1.9	2.2	2.7	1.2	11.0	97.9	3.6***	3.1*	37.6	0.38***
Migyera	1.2	1.3***	1.0***	1.7	30.3*	79.8***	7.6***	4.0	36.1	0.48**
MM96/2480	1.7	2.0	3.2	1.6	40.6***	87.6	10.5***	8.1**	31.4	0.55
MM96/4466	1.7	1.5*	2.8	3.0***	50.0***	50.0***	6.9***	4.3	30.9	0.38***
MM96/9308	1.3	2.1	2.5	1.8	42.3***	80.7***	15.6	6.5	37.7	0.58
MM98/3567	2.3***	1.7	2.3	3.1***	56.6***	49.8***	7.5***	4.6	27.5	0.33***
Nyaboda	3.1***	2.8*	3.1	2.7***	79.5***	41.2***	12.1***	5.5	32.6	0.53
Nyakasamuel	1.3	1.8	2.5	1.6	28.3	80.3***	5.7***	3.4	31.3	0.42***
Obaro dak	2.2***	2.7*	2.5	2.2***	38.9***	77.8***	2.6***	3.9	40.3	0.30***
Weite	1.8	1.8	2.0***	1.2	11.6	96.8	6.3***	5.3	38.6	0.35***

Table 1 contd: Means of CBSD symptoms and yield related traits for Kenyan cassava landraces

Genotype	Foliar 3	Foliar 6	Foliar 9	Root N	Root NI (%)	Usable R (%)	Root W	Root No.	Dry M (%)	H Index
Mean	1.7	2.0	2.4	2.1	41.1	72.0	7.1	4.5	32.0	0.43
Mean (2014)	1.4	1.5	1.5	1.8	30.2	80.5	7.7	5.1	31.6	0.52
Mean (2015)	2.0	2.5	3.3	2.5	51.9	63.3	6.5	4.0	32.4	0.35
Dunnett	0.6	0.5	0.6	0.6	8.9	7.6	2.3	2.2	10.3	0.15
CV	20.9	17.6	18.5	16.0	16.0	9.2	14.9	13.6	20.2	10.2

-Means separation done by dunnett test (MH95/0183 is the control)

-Statistical significance: * = $P \leq 0.05$, ** = $P \leq 0.01$, *** = $P \leq 0.001$

-Mean (2014) and (2015) are means for cropping season one and two respectively.

-Dunnet significant difference value

-CV - coefficient of variation

-Foliar 3, 6, 9 – foliar symptoms at 3, 6, 9 months after planting; Root N – Root necrosis; Root NI (%) – Root necrosis incidence; Usable R (%) – Usable roots; Root W – Root weight (t/ha); Root No. – Number of roots per plant; Dry M (%) – Dry matter content; H Index – Harvest index

Table 2: Means of CBSD symptoms and yield related traits for Tanzanian cassava landraces

Landraces	Foliar 3	Foliar 6	Foliar 9	Root N	Root NI (%)	Usable R (%)	Root W	Root No.	Dry M (%)	H Index
Kiroba	**1.3**	**2.1**	**2.2**	**1.0**	**0.0**	**100.0**	**22.4**	**5.4**	**27.2**	**0.47**
Albert	1.3	1.9	1.8	3.4***	99.2***	53.2***	12.7***	4.3	24.2	0.46
Azoa	1.1	1.2***	1.8	1.1	3.8	98.3	15.8*	3.6*	29.2	0.36***
Bangi	1.4	1.7	1.4**	1.4*	5.5	92.2	21.4	5.8	28.5	0.50
Benny	1.1	1.3***	1.2***	1.2	2.3	99.0	19.5	5.1	33.2**	0.52
Binamuli	1.3	1.7	1.8	1.1	7.6***	98.7	13.3***	5.6	37.3***	0.46
Binti Ally	1.1	1.7	2.0	1.4*	14.3***	94.9	18.6	5.2	26.5	0.43
Binti Juma	1.4	2.0	2.0	1.1	8.5***	89.5**	10.0***	3.2***	29.0	0.36***
Binti Pindi	1.3	1.5*	1.8	1.0	1.4	98.5	14.7***	4.1	29.8	0.54
Chidubwa	1.8	2.4	2.2	1.2	2.3	99.0	15.4**	4.6	32.2*	0.37***
Chimaje	1.3	1.3***	1.1***	1.0	0.0	100.0	13.6***	3.8	30.4	0.47
Chipanda	1.3	1.3***	1.7	1.0	0.0	100.0	17.1	5.4	33.2**	0.41
Cosmas	1.8	2.1	2.4	2.1***	54.3***	69.9***	9.9***	3.9	27.0	0.45
Hamad Rashid	1.1	1.3***	1.6	1.1	3.8	97.3	4.7***	3.5**	27.3	0.12***
Hingawali	1.1	1.7	1.8	1.6***	20.4***	84.2***	14.0***	4.8	26.8	0.53
Kalinda	1.5	2.3	2.2	1.8***	26.6***	82.3***	12.1***	4.5	31.8*	0.40
Katewanya	1.4	1.5*	1.2***	1.1	3.5	98.3	23.5	4.3	35.3***	0.48
Kibangameno	1.0	1.9	2.3	1.5***	14.7***	90.0*	13.2***	2.9***	26.7	0.44

Table 2 contd: Means of CBSD symptoms and yield related traits for Tanzanian cassava landraces

Landraces	Foliar 3	Foliar 6	Foliar 9	Root N	Root NI (%)	Usable R (%)	Root W	Root No.	Dry M (%)	H Index
Kifuu cha nazi	1.0	1.9	2.1	1.2	11.7***	99.7	11.9***	3.9	26.5	0.47
Kigoma Red	2.7***	3.1***	2.7	3.4***	89.8***	37.6***	16.1*	5.4	28.2	0.25***
Kikombe	1.7	2.7**	2.9	2.0***	45.0***	67.9***	9.6***	3.5**	32.7**	0.50
Kikwada	1.9	2.2	2.3	1.0	0.0	100.0	11.3***	4.0	29.0	0.47
Kitumbua	2.4***	2.8***	2.9*	1.7***	20.8***	87.7***	12.2***	4.4	32.9**	0.45
Kiwinda	1.5	1.8	2.0	1.0	0.0	100.0	13.3***	3.4***	32.5**	0.45
Likonde	1.1	1.0***	1.1***	1.2	11.3***	94.0	11.0***	4.6	31.0ns	0.28***
Likonde II	1.7	2.0	2.2	2.0***	24.7***	81.8***	21.7	6.2	25.2ns	0.47
Limbanga	1.1	1.1***	1.2***	1.0	0.0	100.0	23.0	5.6	33.4**	0.44
Liumbukwa	1.0	1.0***	1.2***	1.9***	19.5***	83.9***	17.0	5.3	36.4***	0.35***
Makame	1.0	1.6	1.9	1.1	2.3	92.8	15.5**	4.4	29.7	0.41
Mbuyu	2.8***	3.0***	2.9	1.0	2.2	100.0	15.7*	4.0	27.7	0.44
Mdimbe	1.4	2.4	1.9	2.1***	35.2***	69.7***	20.7	5.6	28.5	0.36***
Mfaransa	1.5	1.3***	1.4**	1.0	0.0	100.0	6.8***	2.1***	31.0	0.18***
Mkwanyule	1.2	1.2***	1.6	1.2	1.6	99.3	13.1***	3.8	22.6*	0.51
Mnacho	1.2	1.6	1.4**	1.1	2.0	97.4	16.2*	4.9	31.1	0.44
Mnondodya	1.4	2.0	1.6	3.1***	59.8***	41.5***	11.8***	4.2	30.8	0.47
Mombasa	1.0	1.0***	1.5*	1.2	10.0***	92.6	22.5	5.2	32.6**	0.48

Table 2 contd: Means of CBSD symptoms and yield related traits for Tanzanian cassava landraces

Landraces	Foliar 3	Foliar 6	Foliar 9	Root N	Root NI (%)	Usable R (%)	Root W	Root No.	Dry M (%)	H Index
Mreteta	1.2	2.2	2.0	1.1	2.3	98.6	22.8	5.9	31.6	0.49
Musa Said	1.0	1.6	2.0	1.2	1.6	99.0	26.9	4.8	29.8	0.45
Mweda	1.3	1.5*	1.6	1.2	5.3	97.8	35.0***	6.8	31.2	0.51
Mwendo-wa-loya	1.0	1.3***	1.5*	1.2	7.2**	99.1	10.1***	5.5	32.3*	0.42
Mzigo-wa-mwizi	1.2	1.2***	1.8	1.9***	17.3***	80.8***	15.3**	4.3	27.2	0.48
Nachinyaya	2.4***	2.1	2.2	1.2	4.1	98.6	14.9**	5.5	24.5	0.41
Nakuchima	1.0	1.6	1.7	1.5***	6.4**	94.9	8.4***	3.9	34.3***	0.26***
Nalilekuchumba	1.4	1.9	1.7	1.5***	14.3***	91.1*	13.6***	4.5	24.2	0.48
Namanjongonda	2.0*	2.0	2.2	1.7***	29.0***	73.8***	17.3	4.6	32.5**	0.48
Namkola	1.5	1.7	1.4**	1.1	3.8	97.9	14.8***	5.9	27.4	0.42
Nanjeja	2.0**	2.4	2.8	1.6***	23.7***	74.4***	13.8***	3.9	35.8***	0.51
Nanjenjeha	1.1	2.1	1.9	1.3	6.1*	96.7	19.6	4.6	25.9	0.43
Ndanda	1.5	1.8	1.6	1.0	0.0	100.0	17.2	4.5	28.5	0.32***
Nkutiao	1.0	1.1***	1.5**	1.1	0.6	99.6	7.5***	6.6	31.8*	0.42
Ntara	1.2	2.7*	1.6	1.1	1.0	99.0	25.8	5.2	25.1	0.47
Ntonto	2.5***	2.8***	2.8	2.7***	57.1***	42.6***	31.3***	4.5	25.4	0.51
Nyankagile	2.4***	2.7**	2.4	1.1	1.2	99.4	15.1**	4.4	28.8	0.45
Nyoka	1.8	2.6*	1.9	1.0	0.0	100.0	21.9	6.3	32.8**	0.46

Table 2 contd: Means of CBSD symptoms and yield related traits for Tanzanian cassava landraces

Landraces	Foliar 3	Foliar 6	Foliar 9	Root N	Root NI (%)	Usable R (%)	Root W	Root No.	Dry M (%)	H Index
Sakada	1.5	1.5*	1.4**	1.2	3.3	98.7	6.5***	2.1***	25.8	0.37***
Salanga	1.2	2.0	1.4**	1.0	0.6	100.0	18.4	5.2	31.7	0.48
Sheria	1.4	1.8	1.8	2.3***	42.7***	54.5***	7.7***	3.2***	22.2*	0.54*
Simanyu	1.3	1.4*	1.7	1.2	3.9	97.7	23.9	7.0	31.9*	0.43
Sumu-ya-panya	1.7	1.8	2.0	2.1***	46.2***	68.2***	23.9	5.0	24.4	0.43
Supa	1.2	1.9	2.1	1.0	1.3	99.6	19.5	3.4**	29.6	0.44
Supa B	1.1	1.3***	1.4**	1.0	0.0	100.0	24.9	6.1	38.4***	0.50
Supa Jangwa	1.0	1.4**	1.5*	1.1	1.6	99.9	20.7	4.4	32.2*	0.54*
Vicent	1.5	1.7	2.1	1.0	0.0	100.0	21.8	5.0	30.4	0.45
Victory	1.3	2.5	2.0	1.4***	30.6***	86.5***	15.4**	5.0	27.8	0.44
Mean	1.4	1.8	1.9	1.4	14.3	89.7	16.5	4.7	29.7	0.43
Mean (2014)	1.4	1.9	2.0	1.5	14.5	91.0	18.7	4.8	27.1	0.42
Mean (2015)	1.4	1.7	1.7	1.3	14.1	88.5	14.0	4.5	32.4	0.45
Dunnett	0.6	0.5	0.6	0.3	5.8	8.5	5.9	1.7	4.5	0.1
CV	20.0	15.2	18.4	11.6	21.5	4.8	18.2	17.2	7.7	8.0

Means separation done by dunnett test (Kiroba is the control); Statistical significance: * = $P \leq 0.05$, ** = $P \leq 0.01$, *** = $P \leq 0.001$; -CV - coefficient of variation; Mean (2014) and (2015) are means for cropping season one and two respectively; Dunnet significant difference value; Foliar 3, 6, 9 – foliar symptoms at 3, 6, 9 months after planting; Root N – Root necrosis; Root NI (%) – Root necrosis incidence; Usable R (%) – Usable roots; Root W – Root weight (t/ha); Root No. – Number of roots per plant; Dry M (%) – Dry matter content; H Index – Harvest index

3.3.2 CBSD root symptoms

The mean root necrosis severity was 2.1 for the Kenyan cassava landraces (Table 1). MH95/0183, Manchoberi, Matuja, Merry-go-round, Migyera, MM96/2480, MM96/9308, Nyakasamuel, and Weite had the lowest mean root necrosis severity ranging from 1.1 - 1.8. However, Agric I, Amakuria, MM96/4466, MM98/3567, Nyaboda, and Obaro dak had significantly ($P \leq 0.05$) higher mean root necrosis severity ranging from 2.2 – 5.0. Comparable results were observed for root necrosis incidence where MM95/0183 had mean root necrosis incidence of 19.1%, that was not significantly ($P \leq 0.05$) different from that of Merry-go-round (11.0%), Weite (11.6%), and Nyakasamuel (28.3%) (Table 1). Manchoberi (3.2%) had a significantly low mean root necrosis severity, while Agric I, Amakuria, Matuja, Migyera, MM96/2480, MM96/4466, MM96/9308, MM98/3567, Nyaboda, and Obaro dak had significantly high root necrosis incidence ranging from 30.3 – 100.0%. MH95/0183, Merry-go-round, MM96/2480, Weite, and Manchoberi had low root necrosis severity hence, high quantities of usable roots ranging from 87.6 – 99.7%. On the contrary, Agric I, MM96/4466, MM98/3567 and Nyaboda had significantly lower quantities of usable roots ranging from 0.00 – 50.6.7%.

The mean root necrosis severity was 1.4 across all the Tanzanian landraces evaluated. Kiroba, Azoa, Benny, Chimaje, Chipanda, Kikwada, Likonde, Mombasa, Nyoka, and Supa B had low mean root necrosis severity of 1.0 (Table 2). On the other hand, Albert, Bangi, Cosmas, Hingawali, Kalinda, Kigoma Red, and Sumu ya Panya had significantly higher mean root necrosis severity ranging from 1.4 – 3.4. Comparable results were observed for root necrosis incidence. Kiroba, Azoa, Bangi, Benny, Chidubwa, Chimaje, Katewanya, Limbanga, Mfaransa, and Nachinyaya had the lowest mean root necrosis incidence ranging from 0.0 – 5.5% and had minimal root necrosis. Albert had the highest mean root necrosis incidence of 99.2%. Landraces including Cosmas, Kigoma Red, Kikombe, Mnondodya, and Ntonto had significantly higher mean root necrosis incidence than Kiroba that ranged from 45.1 – 89.8% (Table 2). Kiroba exhibited the least root necrosis symptoms with all roots being usable. Kiroba, Chimaje, Kiwinda, Limbanga, Azoa, Benny, Mweda, Nakuchima, and Simanyu exhibited the least root necrosis symptoms and had >95% usable roots (Table 2). Seriously affected landraces with lower quantity of usable roots included Mnondodya (41.5%), Ntonto (42.6%), Sheria (54.5%), Kigoma red (37.6%%), Sumu ya Panya (68.2%), and Cosmas (69.9%), which were all comparable to the susceptible control Albert (53.2%).

3.3.3 Cassava related yield traits

The mean root weight for the Kenyan cassava landraces was 7.1 t/ha (Table 1). MH95/0183 and MM96/9308 had the highest root weight of 16.8 t/ha and 15.6 t/ha, respectively. Most of the local landraces including Amakuria, Manchoberi, Matuja, Merry-go-round, Nyakasamuel, Obaro dak, and Weite had significantly lower root weights ranging from 1.1 – 6.3 t/ha. Contrastingly, most of the improved varieties including MM96/2480, Migyera, MM96/4466, and MM98/3567 registered higher root weight ranging from 6.9 – 10.5 t/ha. MH95/0183 had the highest dry matter content at 31.7%, compared to the other varieties whose dry matter contents ranged from 17.5 – 18.4%. The highest harvest index of 0.65 was also recorded in MH95/0183 compared to 0.55 from MM96/2480, 0.58 from MM96/9308 and 0.53 from Nyaboda (Table 1). The other landraces had significantly ($P \leq 0.05$) lower harvest indices ranging from 0.25 – 0.48.

The mean root weight for Tanzanian cassava local landraces was 16.5 t/ha. The highest mean root weight was recorded in Mweda at 35.0 t/ha, and it was significantly ($P \leq 0.05$) higher than that of Kiroba at 22.4 t/ha (Table 2). Kiroba's root weight was not significantly different from other landraces except Binti Juma, Cosmas, Hamad Rashid, Mwendo wa Loya, Nkutiao, Sakada, and Sheria, which had low root weights ranging from 4.7 - 15.8 t/ha (Table 2). The highest mean number of roots per plant was recorded in Simanyu (7.0), Supa B (6.1), Nyoka (6.3), Nkutiao (6.6), Mweda (6.8), and Likonde II (6.2) (Table 2). Although Kiroba (5.4) had a lower mean number of roots per plant, it was not significantly different from the other landraces with the highest number of roots. On the other hand, Azoa, Binti Juma, Hamad Rashid, Kibangameno, Kikombe, Kiwinda, Mfaransa, Sakada, Sheria, and Supa had significantly ($P \leq 0.05$) lower mean number of roots ranging from 2.1 - 3.6 (Table 2). The highest dry matter contents were recorded in Supa B (38.4%), Binamuli (37.3%), and Liumbukwa (36.4%). Additionally, these landraces had significantly ($P \leq 0.05$) higher dry matter content compared to Kiroba at 27.2% (Table 3). Significantly low dry matter content was recorded in Sheria (22.2%) and Mkwanyule (22.6%). The highest harvest index was recorded in Sheria (0.54) (Table 3). Kiroba had a harvest index of 0.47 and was not significantly different from other landraces which had harvest indices ranging from 0.41 – 0.53. Landraces with significantly ($P \leq 0.05$) lower harvest indices ranging from 0.12 – 0.37 included Hamad Rashid, Sakada, Ndanda, Nakuchima, Mfaransa, Mdimbe, and Liumbukwa.

3.3.4 Correlation analysis

Correlation analysis for both the Kenyan and Tanzanian landraces revealed a positive correlation between CBSD foliar symptoms severity at 3 MAP and 6 MAP ($P \leq 0.001$, $r = 0.54$) (Table 3 and 4). A significant positive correlation was observed between foliar symptoms severity at 3 MAP and 9 MAP of ($P \leq 0.001$, $r = 0.52$) for the Kenya landraces as was for the Tanzania landraces ($P \leq 0.001$, $r = 0.43$). A highly positive correlation was observed betweeen foliar symptom severity at 6 MAP and 9 MAP ($P \leq 0.001$, $r = 0.61$) for both Kenyan varieties and Tanzanian landraces (Table 3 and 4). These results showed that approximately 50% of the plants with foliar symptoms at 3 MAP also had symptoms at 6 and 9 MAP.

In the Kenyan landraces, positive a correlation was observed between foliar symptoms and root necrosis at 3 MAP ($P \leq 0.001$, $r = 0.48$), between foliar symptoms at 3 MAP and root necrosis incidence ($P \leq 0.001$, $r = 0.44$), while a negative correlation was observed between foliar symptoms at 3MAP and usable roots ($P \leq 0.001$, $r = -0.41$) (Table 3). Similarly, a positive correlation was observed between foliar symptoms at 6 MAP and root necrosis ($P \leq 0.001$, $r = 0.33$), between foliar symptoms 6 MAP and root necrosis incidence ($P \leq 0.001$, $r = 0.33$), with a negative correlation between foliar symptoms at 6 MAP and usable roots ($P \leq 0.001$, $r = -0.30$). Additionaly, there was a positive correlation between foliar symptoms at 9 MAP and root necrosis ($P \leq 0.001$, $r = 0.45$), between foliar symptoms 9 MAP and root necrosis incidence ($P \leq 0.001$, $r = 0.46$), and a negative correlation between foliar symptoms 9 MAP and usable roots ($P \leq 0.001$, $r = -0.40$).

Similar results were observed in Tanzanian landraces where positive correlation was observed between foliar symptoms at 3 MAP and root necrosis ($P \leq 0.05$, $r = 0.11$), between foliar symptoms 3 MAP and root necrosis incidence ($P \leq 0.01$, $r = 0.13$), and a negative correlation between foliar symptoms at 3 MAP and usable roots ($P \leq 0.001$, $r = -0.15$) (Table 4). Likewise, positive correlation was observed between foliar symptoms at 6 MAP and root necrosis ($P \leq 0.001$, $r = 0.22$), between foliar syptoms at 6 MAP and root necrosis incidence ($P \leq 0.001$, $r = 0.21$), and a negative correlation between foliar symptoms at 6 MAP and usable roots($P \leq 0.001$, $r = -0.23$). Additionaly, there was a positive correlation between foliar symptoms at 9 MAP and root necrosis ($P \leq 0.001$, $r = 0.22$), between foliar symptoms at 9 MAP and root necrosis incidence ($P \leq 0.001$, $r = 0.20$), and a negative correlation between foliar symptoms at 9 MAP and usable roots ($P \leq 0.001$, $r = -0.22$). In comparison the Kenyan landraces had more severe CBSD symptoms than the Tanzanian landraces.

Table 3: Correlation coefficient values of CBSD symptoms and yield related traits for Kenyan cassava landraces

	Foliar 3	Foliar 6	Foliar 9	Root N	Root NI	Usable R	Root W	Root No.	Dry M	H Index
Foliar 3	1	0.54***	0.52***	0.48***	0.44***	-0.41***	-0.08	-0.03	-0.05	-0.28**
Foliar 6		1	0.61***	0.33***	0.33***	-0.30***	0.03	-0.04	0.12	-0.15
Foliar 9			1	0.45***	0.46***	-0.40***	-0.09	-0.15	-0.24*	-0.24*
Root N				1	.95***	-0.94***	-0.07	-0.16	-0.36***	-0.15
Root NI					1	-0.96***	-0.02	-0.12	-0.34***	-0.12
Usable R						1	0.03	0.14	0.34***	0.11
Root W							1	0.87***	0.38***	0.78***
Root N								1	0.01	0.67***
Dry M									1	0.28**
H Index										1

-Spearman's correlation used

-Statistical significance: * = $P \leq 0.05$, ** = $P \leq 0.01$, *** = $P \leq 0.001$

-Foliar 3, 6, 9 – foliar symptoms at 3, 6, 9 months after planting; Root N – Root necrosis; Root NI (%) – Root necrosis incidence; Usable R (%) – Usable roots; Root W – Root weight (t/ha); Root No. – Number of roots per plant; Dry M (%) – Dry matter content; H Index – Harvest index

Table 4: Correlation coefficient values of CBSD symptoms and yield related traits for Tanzanian cassava landraces

	Foliar 3	Foliar 6	Foliar 9	Root N	Root NI	Usable R	Root W	Root No.	Dry M	H Index
Foliar 3	1	0.54***	0.43***	0.11*	0.13**	-0.15***	-0.08	-0.03	-0.05	-0.06
Foliar 6		1	0.61***	0.22***	0.21***	-0.23***	0.04	-0.01	-0.15***	0.02
Foliar 9			1	0.22***	0.20***	-0.22***	0.06	-0.01	-0.15***	-0.04
Root N				1	0.90***	-0.88***	-0.07	0.01	-0.15***	0.09
Root NI					1	-0.88***	-0.14**	-0.02	-0.07	0.10
Usable R						1	0.12*	0.02	0.06	-0.13
Root W							1	0.58***	-0.10*	0.16
Root N								1	0.01	0.17
Dry M									1	0.17
H Index										1

-Spearman's correlation used

-Statistical significance: * = $P \leq 0.05$, ** = $P \leq 0.01$, *** = $P \leq 0.001$

-Foliar 3, 6, 9 – foliar symptoms at 3, 6, 9 months after planting; Root N – Root necrosis; Root NI (%) – Root necrosis incidence; Usable R (%) – Usable roots; Root W – Root weight (t/ha); Root No. – Number of roots per plant; Dry M (%) – Dry matter content; H Index – Harvest index

High positive correlations of more than 0.90 were observed between root necrosis and root necrosis incidence in both the Kenyan and Tanzanian landraces (Table 3 and 4). On the other hand high negative correlations of more than -0.88 were observed between root necrosis and usable roots, and between root necrosis incidence and usable roots in both Kenyan and Tanzanian landraces. These results showed that plants with severe root necrosis, also had high root necrosis incidence and subsequently low quantity of usable roots.

Athough CBSD symptoms were mostly negatively correlated with root weight, the correlations were not signifciant in both the Kenyan and Tanzanian landraces (Table 3 and 4). A significant negative correlation was however observed between root weight and root necrosis incidence ($P \leq 0.01$, $r = -0.14$), and a significant positive correlation ($P \leq 0.05$, $r = 0.12$), between root weight and usable roots in theTanzania landraces. Similarly, CBSD symptoms were negatively correlated with number of roots per plant, but the correlations were not signifciant in both Kenyan and Tanzanian local landraces. High positive correlation was observed between root weight and number of roots ($P \leq 0.001$, $r = 0.87$) in Kenyan landraces, and between root weight and number of roots ($P \leq 0.001$, $r = 0.58$) in Tanzanian landraces.

CBSD symptoms were negatively correlated with dry matter content. In the Kenyan landraces, a negative correlation was observed between dry matter content and foliar symptoms at 9 MAP ($P \leq 0.05$, $r = -0.24$), between dry matter content and root necrosis ($P \leq 0.001$, $r = -0.36$), and between dry matter content and root necrosis incidence ($P \leq 0.001$, $r = -0.34$) (Table 3). Simillarly for the Tanzanian landraces, there was negative correlation between dry matter content and foliar symptoms at 6 MAP ($P \leq 0.001$, $r = -0.15$), between dry matter content and foliar symptoms at 9 MAP ($P \leq 0.001$, $r = -0.15$), and between dry matter content and root necrosis ($P \leq 0.001$, $r = -0.15$) (Table 4).

Harvest index was mostly negatively correlated with CBSD symptoms in the Kenyan landraces (Table 3). However, a significant negative correlation was observed only between harvest index and foliar symptoms at 3 MAP ($P \leq 0.001$, $r = -0.28$), and between harvest index and foliar symptoms at 9 MAP ($P \leq 0.05$, $r = -0.24$) (Table 3). A high positive correlation was observed between harvest index and dry matter content ($P \leq 0.01$, $r = 0.28$), between harvest index and root number per plant ($P \leq 0.001$, $r = 0.67$), and between harvest index and root weight ($P \leq 0.001$, $r = 0.78$). This was similar in Tanzanian landraces although the correlations were not significant. Landraces like Kitumbua, Mbuyu, and Nanjeja had high

foliar symptoms upto 3.0 which would have contributed to a reduction in shoot weight and consequently higher root weight to shoot weight ratio (harvest index).

3.3.5 Mean squares and sum of squares for evaluated traits

The ANOVA for the Kenyan cassava landraces revealed a large percentage of total sum of squares (SS) of 62.2% attributed to the cassava genotypes in CBSD foliar symptoms at 3 MAP (Table 5). However, at 6 and 9 MAP, the highest SS was due to environment ranging from 49.3 – 55.1%. The findings showed CBSD foliar symptom expression was largely influenced by environment particularly at 6 and 9 MAP. Although variation due to genotype by environment interaction (G×E) was low ranging from 11.7 - 21.6%, the mean squares were significant ($P \leq 0.001$) indicating that G×E interaction influenced the foliar symptom expression of some landraces. Root necrosis, root necrosis incidence and usable roots had the highest SS due to genotype (55. – 60.5%) followed by G×E interaction (30.9 – 35.1%) and finally environment (6.5 – 12.0%). Likewise, a larger percentage of total sum of squares (SS) ranging from 39.5 - 63.7% was attributed to the cassava genotypes in other traits tested including root weight, number of roots per plant, dry matter content and harvest index (Table 6). Additionally, the mean squares were highly significant ($P \leq 0.001$) (Table 6). Genotype by environment interaction was second with an SS percentage ranging from 27.4 – 44.7% and with a highly significant ($P \leq 0.001$) mean squares. The environment accounted for the least variation recorded with ranges of 0.27 – 24.0% but still with highly significant mean squares ($P \leq 0.001$).

Comparable results were observed in Tanzanian local landraces where ANOVA revealed a larger percentage of total sum of squares (SS) ranging from 42.2 - 83.0% attributed to the cassava genotypes in CBSD foliar symptoms at 3, 6 and 9 MAP; root necrosis severity; root necrosis incidence; usable roots; root weight; number of roots per plant, dry matter content and harvest index (Table 7 and 8). The mean squares were also highly significant ($P \leq 0.001$). G×E interaction was second with a SS percentage range of 11.7 - 29.4% and with highly significant ($P \leq 0.001$) mean squares (Tables 7 and 8). The Environment accounted for the least variation recorded with ranges of 0.01 - 9.5% but still with highly significant mean squares ($P \leq 0.001$). All the above differences were analysed for their effect on CBSD leaf symptoms severity at 3, 6, and 9 MAP; root necrosis severity; root necrosis incidence; usable roots; root weight; number of roots per plant, dry matter content and harvest index.

Table 5: Means squares and sum of squares for CBSD foliar symptoms for Kenyan cassava landraces

Source of Variation	df	Foliar symptoms at 3 MAP			Foliar symptoms at 6 MAP			Foliar symptoms at 9 MAP		
		MS	SS	SS (%)	MS	SS	SS (%)	MS	SS	SS (%)
Total	89	-	33.1	-	-	48.2	-	-	146.4	-
Environment	1	8.53***	8.53	25.8	23.8***	23.8	49.3	80.7***	80.7	55.1
Replicate	2	0.04	0.08	0.24	0.07	0.14	0.29	0.21	0.43	0.29
Environment (Replicate)	2	0.00	0.00	0.00	0.05	0.10	0.20	0.15	0.30	0.20
Genotype	14	1.47***	20.6	62.2	0.99***	14.0	29.1	2.39***	33.4	22.8
Genotype*Environment	14	0.28*	3.87	11.7	0.73***	10.2	21.2	2.26***	31.6	21.6
Error	56	0.13	7.28	-	0.13	7.09	-	0.20	11.3	-

-df, MS, SS - degrees of freedom, mean squares, and sum of squares, respectively

-Statistical significance: * = $P \leq 0.05$, ** = $P \leq 0.01$, *** = $P \leq 0.001$

Table 5 contd: Mean squares and sum of squares of CBSD root symptoms for Kenyan cassava landraces

Source of Variation	df	Root necrosis severity			Root necrosis incidence			Usable roots		
		MS	SS	SS (%)	MS	SS	SS (%)	MS	SS	SS (%)
Total	89	-	152.6	-	-	107091.4	-	-	100980.4	-
Environment	1	10.0***	10.0	6.55	10512.7***	12854.6	12.0	6525.5***	6525.5	6.46
Replicate	2	0.20	0.40	0.26	337.2	674.3	0.63	515.1	1030.3	1.02
Environment (Replicate)	2	0.21	0.42	0.28	104.5	1035.2	0.97	220.4	440.8	0.44
Genotype	14	6.31***	88.3	57.9	4110.6***	59437.6	55.5	4360.0***	61039.5	60.5
Genotype*Environment	14	3.82***	53.5	35.1	2976.4***	33089.9	30.9	2285.3***	31944.3	31.6
Error	56	0.12	6.42	-	43.1	14552.8	-	198.60	11121.3	-

-df, MS, SS - degrees of freedom, mean squares, and sum of squares, respectively

-Statistical significance: * = $P \leq 0.05$, ** = $P \leq 0.01$, *** = $P \leq 0.001$

Table 6: Mean squares and sum of squares of yield related traits for Kenyan cassava landraces

Source of Variation	df	Root weight in tonnes/hectare			Number of roots per plant		
		MS	SS	SS (%)	MS	SS	SS (%)
Total	89	-	3033.8	-	-	375.7	-
Environment	1	11.5***	11.5	0.38	28.1***	28.1	7.48
Replicate	2	2.42	4.85	0.16	0.01	0.03	0.01
Environment (Replicate)	2	1.95	3.90	0.13	0.36	0.72	0.19
Genotype	14	133.73***	1872.2	61.7	12.8***	178.7	47.6
Genotype*Environment	14	81.5***	1141.3	37.6	12.0***	168.0	44.7
Error	56	2.36	132.3	-	0.37	20.8	-

-df, MS, SS - degrees of freedom, mean squares, and sum of squares, respectively

-Statistical significance: * = $P \leq 0.05$, ** = $P \leq 0.01$, *** = $P \leq 0.001$

Table 6 contd: Mean squares and sum of squares of yield related traits for Kenyan cassava landraces

Source of Variation	df	Dry matter content (%)			Harvest index		
		MS	SS	SS (%)	MS	SS	SS (%)
Total	89	-	6390.9	-		2.63	
Environment	1	17.0	17.0	0.27	0.63***	0.63	24.0
Replicate	2	151.9*	303.8	4.75	0.00	0.00	0.00
Environment (Replicate)	2	124.6	249.2	3.90	0.01	0.01	0.38
Genotype	14	290.8***	4071.4	63.7	0.07***	1.04	39.5
Genotype*Environment	14	125.0***	1749.6	27.4	0.07***	0.95	36.1
Error	56	41.7	2332.6	-	0.01	0.38	

-df, MS, SS - degrees of freedom, mean squares, and sum of squares, respectively

-Statistical significance: * = $P \leq 0.05$, ** = $P \leq 0.01$, *** = $P \leq 0.001$

Table 7: Mean squares and sum of squares of CBSD symptoms for Tanzanian cassava landraces

Source of Variation	df	Foliar symptoms at 3 MAP			Foliar symptoms at 6 MAP			Foliar symptoms at 9 MAP		
		MS	SS	SS (%)	MS	SS	SS (%)	MS	SS	SS (%)
Total	173	-	90.2	-	-	144.3	-	-	127.3	-
Environment	1	0.03	0.03	0.03	2.74***	2.74	1.89	7.45***	7.45	5.85
Replicate	2	0.02	0.03	0.03	0.22	0.44	0.30	0.43*	0.87	0.68
Block (Environment*Replicate)	44	0.11	5.00	5.54	0.10	4.00	2.77	0.16	6.96	5.47
Genotype	63	1.05***	66.4	73.6	1.42***	89.2	61.8	1.07***	67.3	52.9
Genotype*Environment	63	0.30***	18.8	20.8	0.76***	48.0	33.3	0.71***	44.8	35.2
Error	210	0.08	17.3	-	0.08	16.2	-	0.12	24.5	-

-df, MS, SS - degrees of freedom, mean squares, and sum of squares, respectively

-Statistical significance: * = $P \leq 0.05$, ** = $P \leq 0.01$, *** = $P \leq 0.001$

Table 7 contd: Means squares and sum of squares of CBSD symptoms for Tanzanian cassava landraces

Source of Variation	df	Root necrosis severity			Root necrosis incidence			Usable roots		
		MS	SS	SS (%)	MS	SS	SS (%)	MS	SS	SS (%)
Total	173	-	145.1	-	-	192671.7	-	-	111137.8	-
Environment	1	5.15***	5.20	3.58	21.6	21.6	0.01	627.5***	627.5	0.56
Replicate	2	0.04	0.04	0.03	22.6	45.2	0.02	9.65	19.3	0.02
Block (Environment*Replicate)	44	0.02	1.02	0.70	11.8	495.7	0.26	19.0	834.2	0.75
Genotype	63	1.68***	106.1	73.1	2391.6***	150673.1	78.2	1339.3***	84375.6	75.9
Genotype*Environment	63	0.52***	32.7	22.5	657.7***	41436.1	21.5	401.1***	25271.0	22.7
Error	210	0.03	5.60	-	9.4	1981.1	-	18.2	3817.7	-

-df, MS, SS - degrees of freedom, mean squares, and sum of squares, respectively

-Statistical significance: * = $P \leq 0.05$, ** = $P \leq 0.01$, *** = $P \leq 0.001$

Table 8: Means squares and sums of squares of yield related traits for Tanzanian cassava landraces

Source of Variation	df	Root weight (t/ha)			Number of roots per plant		
		MS	SS	SS (%)	MS	SS	SS (%)
Total	173	-	20294.5	-	-	565.8	-
Environment	1	1936.1***	1936.1	9.54	6.68	6.68	1.18
Replicate	2	3.44	6.88	0.03	1.36	2.71	0.48
Block (Environment*Replicate)	44	7.37	324.5	1.60	0.69	30.4	5.37
Genotype	63	193.1***	12162.0	59.9	5.71***	359.5	63.5
Genotype*Environment	63	92.2***	5808.5	28.6	2.64***	166.5	29.4
Error	210	9.03	1898.2	-	0.65	136.0	-

-df, MS, SS - degrees of freedom, mean squares, and sum of squares, respectively

-Statistical significance: * = $P \leq 0.05$, ** = $P \leq 0.01$, *** = $P \leq 0.001$

Table 8 contd: Means squares and sum of squares of yield related traits for Tanzanian cassava landraces

Source of Variation	df	Dry matter content			Harvest index		
		MS	SS	SS (%)	MS	SS	SS (%)
Total	173	-	9945.1	-		26606.8	
Environment	1	2665.1***	2665.1	26.8	803.9***	803.9	3.02
Replication	2	0.49	0.97	0.01	39.4*	78.8	0.30
Block (Environment*Replicate)	44	7.00	308.2	3.09	11.0	483.2	1.82
Genotype	63	66.9***	4216.9	42.4	350.5***	22084.2	83.0
Genotype*Environment	63	43.7***	2753.9	27.7	50.1***	3156.7	11.7
Error	210	5.23	1098.4	-	11.9	2492.3	

-df, MS, SS - degrees of freedom, mean squares, and sum of squares, respectively

-Statistical significance: * = $P \leq 0.05$, ** = $P \leq 0.01$, *** = $P \leq 0.001$

A large SS for genotype indicated that the landraces were diverse with large differences among the means, contributing to most variations in the traits analysed. The smaller proportion of SS for genotype by environment interaction showed that the differences among the genotypes by environment interaction means were moderately high. Environment had the smallest proportion of SS thus showing that the environment effect on genotypic response was not significant. It is noteworthy that although environment accounted for the smallest SS %, there were a few landraces whose CBSD symptom and root traits expression were significantly affected by the environment factor.

3.3.6 Seasonal influences on traits

Environmental factors can affect CBSD symptoms expression and cassava yield traits including root weight, root number, dry matter content and harvest in a cropping season. In the Kenyan cassava landraces experiment, there was no significant difference in the rainfall patterns and temperature regime of growing seasons 2014/2015 and 2015/2016 (Figure 5). However, the landraces had significantly more severe foliar symptoms root necrosis, and root necrosis incidence in 2015/2016 (Table 1). Significantly lower root weight, number of roots per plant, dry matter content, and harvest index were also recorded in 2015/2016. Good environmental conditions especially abundant rainfall coupled with elevated temperatures promote transient growth in cassava, a period of active growth where plants do not show CBSD symptoms besides producing high yields. However, CBSD symptoms may become more severe under unfavourable environmental conditions. In 2015/2016, hailstorm damage affected the plants at 2 and 6 MAP which led to defoliation and ensuing stunted growth. New leaves grew from the damaged plants, but they developed severe CBSD symptoms. The combination of damaged plants and CBSV infection may have resulted in the expression of severe CBSD symptoms and low root weight, number of roots per plant, dry matter content and harvest index recorded in 2015/2016.

There was no significant difference in the rainfall patterns and temperature regime of growing seasons 2014 and 2015 in the Tanzanian site and environment may have not influenced CBSD foliar and root symptoms exression where higher means were observed in trial one in 2014 (Figure 6). However, a slight seasonal effect was recorded in root weight and dry matter content. There was a higher mean root weight in 2014 (18.7 t/ha) compared to 2015 (14.0 t/ha). In contrast, the mean dry matter content was 27.1% in 2014 and 32.4% in 2015 (Table 1). A high amount of rainfall recorded in November (132.2 mm) and December

(102.9 mm) in 2014 (Figure 6), may have influenced higher root weight, but a lower dry matter content.

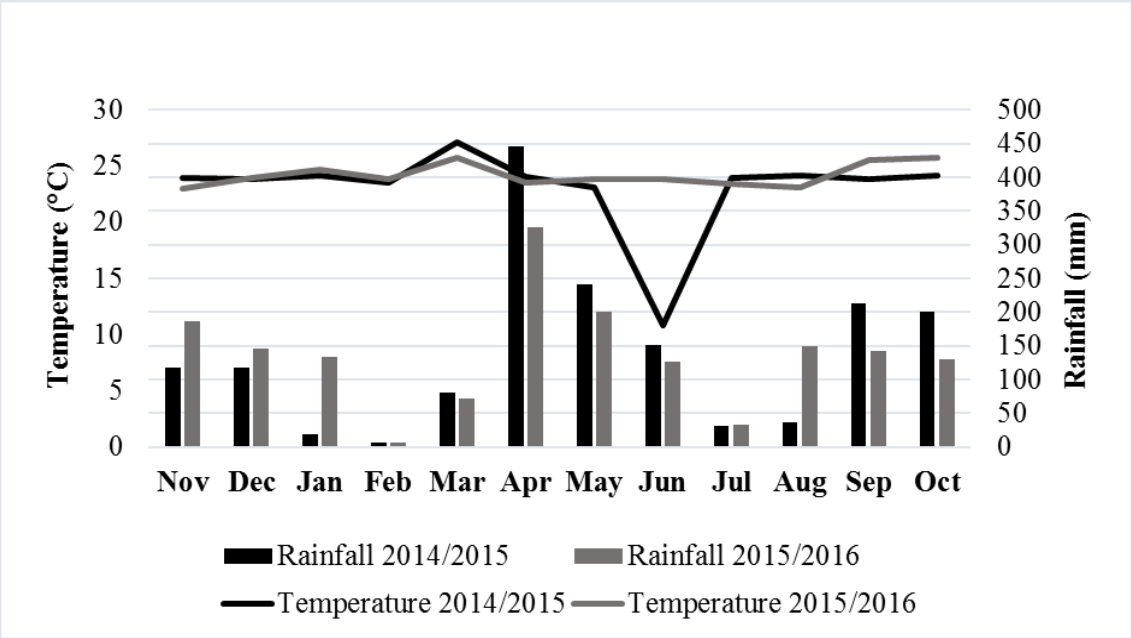


Figure 5: Mean rainfall and temperature for 2014/2015 and 2015/2016 growing seasons in Alupe, Busia, Kenya.

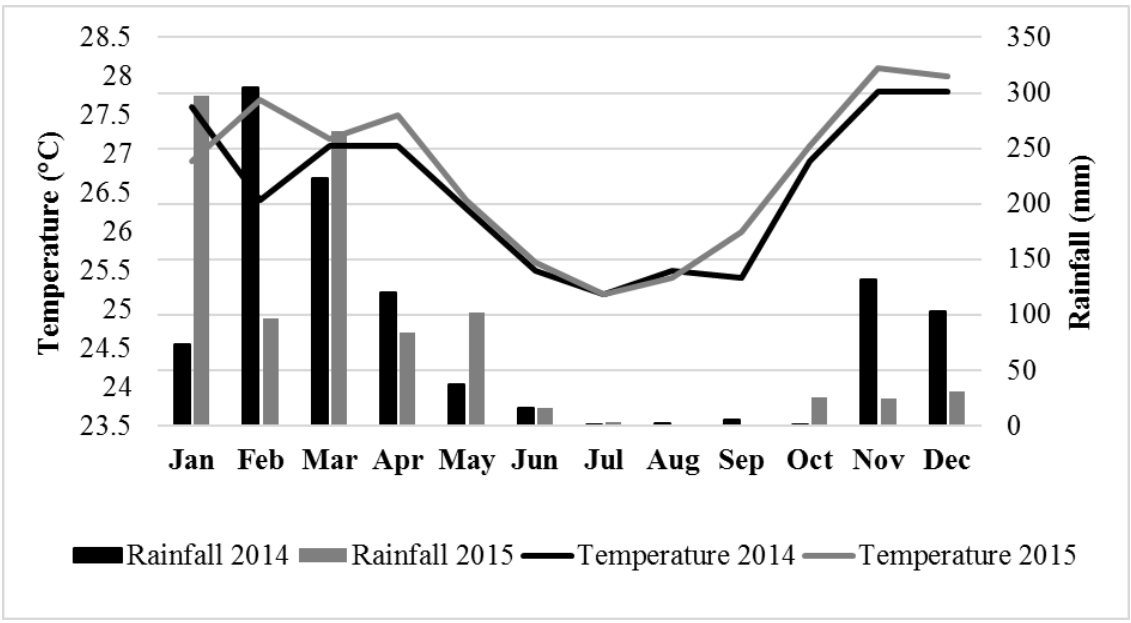


Figure 6: Mean rainfall and temperature for 2014 and 2015 growing seasons in Naliendele, Mtwara, Tanzania.

3.3.7 Disease reaction categories for landraces and varieties screened for CBSD resistance

Characterization of landraces as resistant, tolerant, or susceptible was based on root necrosis since this is the most damaging component of CBSD disease syndrome. Resistant varieties expressed mild or no foliar and root necrosis symptoms while tolerant ones expressed severe foliar symptoms but with minimal or no root necrosis symptoms. Susceptible landraces on the other hand had severe root necrosis symptoms with or without observable foliar symptoms. In the Kenyan trial, no landrace was categorized as resistant as most of them were tolerant including: Migyera, Weite, Merry-go-round, Nyakasamuel, and Manchoberi (Table 9). Although they had low root weights of ≤10 t/ha, they registered high dry matter content ranging from 31 – 41%. Other landraces and improved varieties including: Agric I, Amakuria, Matuja, Nyaboda, MM96/2480, MM96/4466, MM96/9308, and MM98/3567 were susceptible and had severe root necrosis symptoms combined with either moderate or severe foliar symptoms.

In the Tanzania trial, landraces were categorised as resistant tolerant and susceptible. Twenty eight landraces were categorised as resistant including: Azoa, Binamuli, Binti Juma, Binti Pindi, Chimaje, Hamad Rashid, Kiwinda, Makame, Mfaransa, Mkwanyule, Mnacho, Mwendo wa Loya, Namkola, Nkutiao, and Sakada. Among the resistant landraces, Bangi, Benny, Chipanda, Katewanya, Limbanga, Mombasa, Musa Said, Mweda, Ndanda, Salanga, Simanyu, Supa B, and Supa Jangwa registered high root weights ranging from (17 - 35 t/ha) and high dry matter content ranging from (29 - 38%) (Table 10). Twenty seven landraces were categorised as tolerant including: Hingawali, Kalinda, Kibangameno, Kifuu cha Nazi, Kikwada, Kitumbua, Likonde, Mzigo wa Mwizi, Nalilekuchumba, Victory, Chidubwa, Mbuyu, Nachinyaya, Nakuchima, and Nyankagile. Among the tolerant landraces, Binti Ally, Mreteta, Likonde II, Liumbukwa, Vicent, Mdimbe, Nanjenjeha, Namanjongonda, Nanjeja, Ntara, Nyoka, Supa registered higher root weight ranging from 14 - 26 t/ha and higher dry matter content ranging from 25 – 37%. Other landraces including: Cosmas, Kigoma Red, Kikombe, Mnondodya, Ntonto, Sheria, and Sumu ya Panya were susceptible.

Table 9: Disease reaction categories of landraces and varieties screened for CBSD resistance in Kenya

CBSD foliar symptoms	Root necrosis	Root necrosis incidence (%)	Disease reaction category	Landrace/variety
1.0 – 3.0	1.1 – 1.8	3.2 – 30.0	Tolerant	**Migyera, Weite, Merry-go-round, Nyakasamuel, MH95/0183, Manchoberi, Obaro dak** *Root weight = ≤10 t/ha; Root no./plant = 3.1 – 5.5; Dry matter content = 31 – 41%; Harvest index = 0.38 – 0.65*
1.5 – 3.2	2.2 – 5.0	40.6 – 100.0	Susceptible	Agric I, Amakuria, Matuja, Nyaboda, MM96/2480, MM96/4466, MM96/9308, MM98/3567

Note: Landraces in bold had the indicated root weight, root no./plant, dry matter content and harvest within the various disease reaction categories

Table 10: Disease reaction categories of landraces and varieties screened for CBSD resistance in Tanzania

CBSD foliar symptoms	Root necrosis	Root necrosis incidence (%)	Disease reaction category	Landrace/variety
1.0 – 2.0	1.0 – 1.4	0.0 – 10.0	Resistant	Azoa, **Bangi, Benny,** Binamuli, Binti Juma, Binti Pindi, Chimaje, **Chipanda,** Hamad Rashid, **Katewanya,** Kiwinda, **Limbanga,** Makame, Mfaransa, Mkwanyule, Mnacho, **Mombasa, Musa Said, Mweda,** Mwendo wa Loya, Namkola, **Ndanda,** Nkutiao, Sakada, **Salanga, Simanyu, Supa B, Supa Jangwa** *Root weight = 17 - 35 t/ha; No. of roots./plant = 4.3 – 7.0; Dry matter content = 29 – 38%; Harvest index = 0.41 – 0.54*
1.0 – 3.0	1.0 – 2.1	0.0 – 35.2	Tolerant	Kiroba, **Binti Ally,** Hingawali, Kalinda, **Mreteta,** Kibangameno, Kifuu cha Nazi, Kikwada, Kitumbua, Likonde, **Likonde II, Liumbukwa, Vicent, Mdimbe,** Mzigo wa Mwizi, **Nanjenjeha,** Nalilekuchumba, **Namanjongonda, Nanjeja,** Victory, Chidubwa, Mbuyu, Nachinyaya, Nakuchima, **Ntara,** Nyankagile, **Nyoka, Supa**. *Root weight = 14 - 26 t/ha; No of roots/plant = 3.4 – 6.2; Dry matter content = 25 – 37%; Harvest index = 0.36 – 0.51*
1.0 – 2.0	2.0 – 3.4	42.7 – 99.2	Susceptible	Albert, Cosmas, Kigoma Red, Kikombe, Mnondodya, Ntonto, Sheria, Sumu ya Panya

Note: Landraces in bold had the indicated root weight, root no./plant, dry matter content and harvest within the various disease reaction categories

3.4 Discussion

Varied responses to CBSD were recorded among the tested varieties and landraces in field experiments for the two seasons. Many terminologies have been used inconsistently to describe the response of cassava to CBSD and in general of plants to virus infections (Cooper & Jones, 1983). Among them, resistance, tolerance, and susceptibility are most common. In the case of resistant plants, infection by viruses can occur but multiplication and movement are restricted, and the disease symptoms are generally localized or absent (Kang *et al.,* 2005; Cooper & Jones, 1983). These are the characteristics exhibited by 'Namikonga' which perpetually shows no or low symptoms severity for many years, and hence considered resistant. The term tolerance describes a host that can be infected by a virus and in which it can replicate and cause symptoms without significantly diminishing the growth or yield of the plant (Cooper & Jones, 1983). An example is Kiroba, the tolerant control in this study, which had foliar symptoms severity score of up to 2, but had no visible root symptoms, and thus 100% usable roots. Susceptibility on the other hand describes a host plant in which virus spread and multiplication is high, and the development of severe symptoms both on leaves and roots is evident (Maruthi *et al.,* 2014a). In this study 'Albert' was the susceptible control; it expressed both leaf and root symptoms, resulting in reduced usable roots. Using these criteria, the cassava landraces were classified into the resistant, tolerant and susceptible categories.

No Kenyan cassava landrace was classified as resistant since most of them readily expressed foliar and root symptoms. Tolerant landraces with similar reaction as the tolerant control MH95/0183 included: Manchoberi, Weite and Merry go round. They readily expressed foliar symptoms but had minimal root necrosis and high quantities of usable roots (≥ 95.0%). Migyera, Nyakasamuel, and Obaro dak were also be regarded as tolerant although they had slightly higher symptom severity compared to MH95/0183 and slightly lower quantities of root weight ranging from (77.8 – 80.3%). Susceptible landraces and improved varieties including: Nyaboda, MM98/3567, MM96/4466, Matuja, and Amakuria had high root necrosis incidence ranging from 45.8 – 79.5% and low quantity of usable roots ranging from 41.2 – 67.0%. The highly susceptible variety Agric I had 100% root necrosis incidence and no usable roots.

In Tanzania, the resistant landraces had minimal foliar and root symptoms with ≥98.0% usable roots. These included Chimaje, Chipanda, Limbanga, Mfaransa, Mkwanyule, Mweda, Mwendo wa Loya, Ndanda, Nkutiao, Sakada, Supa B, and Supa Jangwa. Landraces

categorised as tolerant included Chidubwa, Kikwada, Mbuyu, Mreteta, Musa Said, Nachinyaya, Nanjenjeha, Nyankagile, Nyoka, and Vicent. These readily developed foliar symptoms but had delayed or absent root symptoms like Kiroba (Hillocks & Jennings, 2003). Other landraces which were also be considered tolerant but had slightly higher foliar and root symptoms compared to Kiroba include Binty Ally, Kalinda, Kibangameno, Kifuu cha Nazi, Kitumbua, Likonde II, Mdimbe, Namanjongonda, Nalilekuchumba, Nanjeja, and Victory. The susceptible landraces included Cosmas, Kigoma Red, Mnondodya, Ntonto, Sheria, and Sumu ya Panya. These landraces had disease reactions comparable to the susceptible control Albert, which had moderate foliar symptom severity, but high root symptoms severity and only about half the roots were usable. The low foliar symptom severity seen in Albert showed that it is not the most susceptible variety and has some levels of tolerance to the disease for foliar symptoms, but not to root necrosis, which is only discovered at harvesting. This is could be the greatest source of food insecurity to cassava farmers growing CBSD-susceptible varieties as the extent of the damage can only be visible at harvest.

In Kenya, the environment (growing season) and genotype contributed to major variations observed in foliar symptom severity at 3, 6, and 9 MAP. A combination of hailstorm damage and CBSV infection may have caused the expression of more severe foliar and root symptoms and the resultant low root weight, number of roots per plant, dry matter content and harvest index observed in growing season 2015/2016. According to Jennings (1957), CBSD root symptoms become more severe under unfavourable environmental conditions. Higher incidences and severity of symptoms have been reported at higher altitudes with cooler temperatures, during low night temperatures and moisture stress (Rwegasira, 2009; Jennings, 1957; Nichols, 1950). Ironically, both stem and root symptoms may disappear or be reduced if conditions become favourable for the growth and development of the plants (Jennings, 1957). In Tanzania, genotype and genotype by season interactions contributed to major variations recorded for all traits tested. They accounted for the largest SS recorded with very highly significant means squares. The seasons accounted for the smallest SS except for a few landraces. The results showed that although the traits examined were mostly under genetic control, the environment in a cropping season influenced the trait expression of a few genotypes. This suggests that CBSD resistance is a quantitative trait as the environment can influence its expression (Kulembeka *et al*., 2012; Pariyo *et al*., 2013; Zacarias & Labuschagne, 2010). Comparable results have been reported on genotype and genotype by environment effects on CBSD symptom expression, root

weight and number of roots per plant (Pariyo *et al.*, 2015; Tumuhimbise *et al.*, 2014; Boakye *et al.*, 2013). CBSD symptoms are usually variable and irregular and depend on many factors including plant age, genetic make-up of a variety, environmental conditions (i.e. altitude, temperature, rainfall quantity) and the virus species (Mohammed *et al.*, 2012; Patil & Fauquet, 2014; Hillocks & Jennings, 2003). In this study, a generally active plant growth with concurrent reduction in disease severity was obseverd in a gowing season with favourable environmental conditions for plant gowth.

The moderate positive correlation ($r>0.50$) between leaf symptoms, and both root necrosis and incidence showed that the presence of leaf symptoms does not always indicate the presence of root necrosis as observed in Merry-go-round, Manchoberi, Weite, Kikwada, Kiroba, Kiwinda, Mfaransa and Vincent, which readily expressed leaf symptoms but with low or no root necrosis. MM96/4466, MM98/3567, Kigoma Red, Cosmas and Ntonto on the other hand had both high leaf symptoms and root necrosis. These results reveal a <50% association between foliar symptoms and root necrosis reported earlier (Abaca *et al.*, 2012; Hillocks *et al.*, 1996). However, a high positive correlation ($r>0.90$) was observed between root necrosis severity and incidence, meaning that varieties with high root necrosis severity also had high root necrosis incidences and consequently greater reductions in usable roots for example Kigoma Red, Mnondodya, and Ntonto. CBSD symptoms both on leaves and roots can also affect key agronomic traits such as root weight and dry matter content (Rwegasira, 2009). There was a significant negative correlation ($P \leq 0.001$, $r = -0.14$) between total root weight and root necrosis incidences indicating that a high root necrosis severity can lead to severely reduced root weight. Some roots with high root necrosis severity may also be constricted or deformed, making them difficult to process, further resulting in reduced root weight (Tomlinson *et al.*, 2017). A significant negative correlation between CBSD symptoms severity (on leaves and roots) and both dry matter content and harvest index shows that the disease impacts on crop profitability. Related results have been reported (Abaca *et al*., 2012; Aigbe & Remison, 2010).

In addition to disease resistance or tolerance, some cassava landraces had desirable root traits. In the Kenya trial, most of the landraces categorised as tolerant including: Weite, Merry-go-round, Nyakasamuel and Manchoberi had low root weight of ≤10.0 t/ha but high dry matter content ranging from 31 – 41%. Dry matter of cassava usually varies from one variety to another and can range between 17% and 47% with the majority lying between 20% and 40% (Braima *et al.*, 2000); values above 30% are considered high. Most of landraces

were collected from Migori County and a survey on the occurrence and estimated losses caused by cassava viruses in the stated county showed that although the local landraces were low yielding, farmers preffered growing them because they had high dry matter content (Masinde *et al.,* 2016). Improved varieties were introduced with the main aim of targeting CMD resistance and not CBSD (GoK, 2006). With the new problem of CBSD, some of these new improved varieties severely succumb to it including Agric I, MM96/4466 and MM98/3567 hence the heavy losses incurred. Local landraces such as Manchoberi and Weite seem to be tolerant with low root necrosis but with low quantitative yield probably due to CMD infection. This brings into focus breeding for resistance to both CBSD and CMD to minimize losses.

In Tanzania, landraces including: Benny, Katewanya, Limbanga, Mombasa, Musa Said, Mweda, Simanyu, Supa B and Supa Jangwa were not only resistant to CBSD, but also had high root weights (≤ 20.0 t/ha) and dry matter content (≤ 30.0 %). There were landraces categorised as tolerant and had high yield (≤ 20.0 t/ha) and dry matter content (≤ 30.0) including: Mreteta, Nyoka, Vicent, and Mdimbe. The tolerant control Kiroba had a mean yield of 22.4 t/ha. Kiroba is, however, reported to be a high yielding variety with a potential production of 40.5 t/ha (Kundy *et al.,* 2014). The low yields recorded for Kiroba in this study could be due to the lowly fertility of the sandy soils at NARI. The other landraces which had root weights comparable Kiroba could have the potential for higher yields with improved soil fertility. The resistant high yielding landraces can be promoted directly for farmer cultivation in disease affected regions. Some other landraces such as Ntonto and Sumu ya Panya were susceptible to CBSD but had desirable root qualities including high yield potential and can be included in cassava breeding programs for CBSD improvement.

The most effective and realistic way of reducing cassava losses due to CBSD is by deploying resistant and tolerant varieties. Cassava landraces identified to be resistant or tolerant in this study already have desirable root traits including high yields and dry matter content and are preferred by farmers. These can be multiplied and used for direct cultivation or in breeding to minimizing the impact of CBSD on affected communities. The tolerant landraces with farmer preffered traits could be taken through virus cleaning to not only increase productivity but also to provide farmers with clean planting materials and minimize the spread of CBSD. Landraces with high yields, dry matter content and harvest index but susceptible to CBSD can be exploited for their superior agronomic characteristics.

3.5 Conclusion

ANOVA revealed significant difference among the landraces in the traits evaluated including CBSD foliar symptoms, root necrosis, root necrosis incidence, usable roots, root weight, root number per plant dry matter content and harvest index. Although the expression of traits was mostly governed by genotype, genotype by environment interaction and environment influenced the expression of traits of some landraces. Disease traits were mostly negatively correlated with yield related traits and a high negative correlation ($P \leq 0.001$, $r > 0.88$) was recorded between root necrosis and usable roots and between root necrosis incidence and usable roots. The Kenya trial identified CBSD tolerant landraces including: Weite, Merry-go-round, Nyakasamuel, and Manchoberi which had low root weights of ≤ 10.0 t/ha but high dry matter content ranging from 31.0 – 41.0%. They had low root weight probably due to CMD infections and this emphasizes the need for deployment of varieties resistant to both CBSD and CMD. The Tanzanian trial identified both resistant and tolerant landraces. Resistant landraces with high root weight (≤ 20.0 t/ha) and high dry matter content (≤ 30.0 %) included Benny, Katewanya, Limbanga, Mombasa, Musa Said, Mweda, Simanyu, Supa B and Supa Jangwa. These could be used directly for cultivation and in cassava breeding programs for transfer of resistance to farmer preffered varieties. Tolerant high yielding landraces were also identified including: Mreteta, Mdimbe, Nyoka, and Vicent and could be taken through virus cleaning so that farmers can have access to clean planting materials for these particular landraces.

3.6 Recommendations

These findings are based on CBSD data generated from two sets of germplasm in the two countries conducted in two planting seasons in two CBSD hot spot areas. Further CBSD and other yield related traits evaluations need to be carried out with the two sets of landraces and varieties combined and replicated in diverse CBSD hot spot sites for more than two seasons to bring out the actual genotype by environment interactions due to the disease. Screening in diverse environments will also enable more genotype and environment interactions. In addition, they can similarly be screened in CMD hotspot areas to confirm if they are also suitable genetic stocks that combine both CMD and CBSD resistance.

CHAPTER FOUR

DEVELOPMENT AND SCREENING OF F1 POPULATIONS FOR RESISTANCE TO CASSAVA BROWN STREAK DISEASE

4.0 Abstract

Cassava mosaic disease (CMD) and cassava brown streak disease (CBSD) are two important biotic constraints for cassava (*Manihot esculenta* Crantz) production in Eastern and Southern Africa. CMD causes general decline in yield in affected plants of susceptible cassava varieties but CBSD causes rotting of edible roots. Deployment of cassava varieties with dual resistance to both diseases is the only sustainable way to control the devastating impact of both diseases. In this study, crosses were carried out between CBSD and CMD resistant parents to develop dually resistant progenies. Two crosses were carried out: 1) Pwani × AR37-80 and 2) Namikonga × AR37-80. Thirty six (36) progenies from each population were screened for CBSD resistance in two growing seasons at a CBSD hotspot area in TARI Naliendele, Tanzania using a partially balanced lattice design with two replicates. Data was collected on CBSD foliar symptoms at 3, 6 and 9 MAP; root necrosis, root necrosis incidence, usable roots and yield related traits. ANOVA revealed significant differences among the progenies in addition to genotype and genotype by environment interaction accounting for the larger variations recorded in CBSD symptoms and root traits evaluated. Disease traits were mostly negatively correlated with yield related traits and a high negative correlation ($P \leq 0.001$, $r > 0.78$) was recorded between root necrosis and usable roots and between root necrosis incidence and usable roots. In the Pwani × AR37-80 F1 population, tolerant progenies identified included: PAR024, PAR057, PAR064, & PAR192 and they had the highest root weight (25.4 – 33.2 t/ha) and moderate dry matter content ranging from 21.0 – 22.9%. In Namikonga × AR37-80 population, resistant progenies identified included: NAMAR050, NAMAR130, NAMAR371, NAMAR402, & NAMARX12 and had the highest root weights ranging from (15.2 – 27.8 t/ha) and moderate dry matter content ranging from (24.1 – 29.4 %) while tolerant progenies NAMAR116 and NAMAR441 and had root weight ranging from 18.2 – 19.5 t/ha and moderate to high dry matter content ranging from 22.4 – 35.8%. Progenies could be suitable genetic stock that combine disease resistance and high yield in one background.

***Key words*:** Cassava, CBSD resistance, F1 population, Yield related traits

4.1 Introduction

CBSD is caused by two RNA viruses belonging to the genus *Ipomovirus* in the family *Potyviridae*: *Cassava brown streak virus* (CBSV) and *Ugandan cassava brown streak virus* (UCBSV) (Winter *et al.,* 2010; Legg *et al.,* 2011; Vanderschuren *et al.,* 2012; Ndunguru *et al.,* 2015), which are together called cassava brown streak ipomoviruses (CBSIs) (Maruthi *et al.,* 2017). CBSD above-ground symptoms include leaf chlorosis along the secondary and tertiary veins, and elongated necrotic lesions on stems (Nichols, 1950; Hillocks and Jennings, 2003; Tomlinson *et al.,* 2017). The major economic damage arises from the necrotic rotting of cassava roots which reduces nutritional and industrial quality and renders the roots unpalatable and marketable (Hillocks and Jennings, 2003; Winter *et al.,* 2010). In Southern Coastal Tanzania, for example, yield losses of between 70 - 100% have been reported in susceptible cultivars (Hillocks *et al.,* 2001).

CMD is caused by 11 Cassava mosaic begomoviruses (CMBs) of the family Geminiviridae (Legg *et al.,* 2011 & 2015). Among the CMB species, *African cassava mosaic virus* (ACMV), *East African cassava mosaic virus* (EACMV) and *East African cassava mosaic virus* – Uganda variant (EACMV-Ug) are the most prevalent in East Africa (Legg *et al.,* 2015). CMD affected plants show yellow to a pale green chlorotic mosaic pattern on leaves, leaf distortion, stunted growth, and reduced root yield. According to Owor *et al.* (2004), CMD reduced the number of tuberous roots and the root yield by 68% and 50%, respectively in a local Ugandan cultivar, Ebwanateraka, with infected plants giving no root yield in severe infections. Losses up to 100% have been reported in highly susceptible varieties (Thresh *et al.,* 1994; Tembo *et al.,* 2017) or in mixed infections of CMD and CBSD (Fondong *et al.,* 2000; Pita *et al.,* 2001). CMD symptoms severity depends on strains/species of the virus, sensitivity of the cassava variety, plant age and environmental factors, such as soil fertility and soil moisture (Hillocks & Thresh, 2000).

Efforts to control CBSD and CMD were initiated in the early 1930s at the East African Cassava Research Institute at Amani in northeastern Tanzania (Jennings, 1976 & 2003; Nichols, 1950). Due to a lack of resistance in cassava, breeders resorted to introgression of disease resistance through interspecific crosses with wild *Manihot* species (Nichols, 1950). The breeding work successfully developed several hybrids including 46106/27, which showed high levels of field resistance to CBSD (Hillocks and Jennings, 2003; Jennings, 2003). Many of these hybrids dissipated into local farming systems. It has been shown that one hybrid 46106/27, known as Amani in Tanzania, is closely related to, but not identical to,

a Tanzanian local cultivar Namikonga (Kulembeka, 2010; Pariyo *et al.*, 2013). Namikonga is, therefore, suspected to be an interspecific hybrid from the Amani program that was subsequently adopted by the farming communities and given a local name. At present, Namikonga still expresses field resistance to CBSD and is used as one of the best sources of CBSD resistance in conventional breeding programs (Jennings, 2003; Kaweesi *et al.,* 2014; Maruthi *et al.,* 2014a). More recently, breeders have been exploiting other natural sources of CBSD resistance (Kawuki *et al.,* 2016) and more recently cassava varieties immune to CBSD have been found (Sheat *et al.,* 2019). Genetic engineering has generated immunity to CBSVs in the model cassava cultivar 60444 (Vanderschuren *et al.,* 2012). A diallel analysis conducted by Kulembeka *et al.* (2012) found that CBSD resistance in Namikonga was due to two or more genes with additive effects.

Currently, deployed resistance against CMD in Africa is of two types: i) quantitative resistance derived from *Manihot glaziovii* and ii) qualitative resistance conferred by a single resistance gene(s). Two known sources of CMD resistance are recognized, one largely influenced by a single dominant gene known as CMD2 discovered in a Nigerian landrace TME3 (Akano *et al.,* 2002; Rabbi *et al.,* 2014), and a more quantitative source of CMD resistance called CMD1, derived from an Amani interspecific cross, TMS 30572 (now TMS-I30572) (Fregene *et al.,* 2000; Mohan *et al.,* 2013). A third putative source of resistance, known as CMD3, has also been described (Okogbenin *et al.,* 2012). CBSD and CMD combined can cause estimated annual losses in excess of US$3 billion (Thresh *et al.,* 1997; Hillocks and Maruthi, 2015) and adversely affect food security in the entire region (Patil *et al.,* 2015). While CMD is of economic importance across Sub-Saharan Africa (SSA), CBSD remains localized in East and Southern Africa (ESA), although there is a high risk of the disease spreading to West Africa unless contained (Legg *et al.,* 2011).

Dual infections of CMD and CBSD are common in farmer's fields and they are a serious threat to cassava production and food security in SSA. Deployment of cassava varieties with dual resistance to both diseases is the only sustainable way to control the devastating impact of both diseases (Mohammed *et al.,* 2015). More recently, breeding has been focussing on dual resistance to both CMD and CBSD and elite cassava varieties have been released. Crossing the cassava variety 'Namikonga' (CBSD resistant but CMD susceptible) with 'AR42-4' (CBSD susceptible but CMD resistant) developed a new cassava hybrid 'Pwani' which is resistant to CMD but tolerant to CBSD with no or delayed root necrosis (Tumwegamire *et al.,* 2018). Additionaly, 'Pwani' is a tolerant sweet variety with yield

potential of 51.0 tonnes/ha (Table 11). ‘Dodoma’, also a sweet variety with a yield potential of 30.3 tonnes/ha developed by crossing (AR11-12♀ × Namikonga♂); and ‘Mkumba’ and ‘Makutupora’ (IITA, 2012). The known resistant variety, ‘Namikonga’, has poor root qualities (low harvest index), but its general combining ability for CBSD resistance (Kulembeka *et al.,* 2012) makes it a suitable progenitor, hence many elite varieties have been developed by crossing susceptible varieties with it. Apart from AR42-2, AR37-80, and other lines were introduced from the International Centre for Tropical Agriculture (CIAT) in Colombia to Tanzania to improve levels of dry matter content, CMD, and cassava green mite (CGM) resistance in local germplasm (Blair *et al.,* 2007; Okogbenin *et al.,* 2012). The AR37-80 was developed through marker assisted selection (MAS), being positively selected for markers for the CMD2 resistance locus and for CGM resistance. It is resistant to CMD and CGM but susceptible to CBSD (Blair *et al.,* 2007; Okogbenin *et al.,* 2012). The large-scale adaption of dual-resistant varieties, however, is yet to be achieved in the worst affected countries of eastern and southern Africa. The aim of this study was to develop F1 populations by crossing CBSD and CMD resistant parents and screen the F1 progenies for CBSD resistance.

Table 11: Pedigree of cassava varieties used for generating F1 crosses

Variety	Pedigree	Country of Origin	Response to CBSD	Response to CMD	Notes	References
Namikonga	Thirdback cross from inter-specific hybrid (46106/27) from *Manihot glaziovii* onto *Manihot esculenta* (Amani breeding program)	Tanzania	R	S	Late root bulking. Parent of mapping population from which QTLs for CBSD resistance have been derived. There is genetic variation within the variety	Hillocks & Jennings, (2003); Jennings, (1960)
Pwani	Cross between Namikonga × AR 42-4.	Tanzania	T	S	A sweet variety grown in Eastern, Southern and Western zones of Tanzania with yield potential of 23-51 t/ha. Used as parent in mapping population to generate QTLs for CBSD resistance	IITA (2012)
AR37-80	A CIAT cross between a CMD resistant line (C33) from IITA and CW259 - 42	CIAT, Colombia	S	R	Susceptible parent of mapping population.	Okogbenin *et al.*, (2007)

R – Resistant, S – Susceptible, T - Tolerant

4.2 Materials and methods

4.2.1 Genetic crosses

A crossing block consisting of three parents Namikonga (CBSD resistant), Pwani (CBSD tolerant) and AR37-80 (CBSD susceptible) was established at Tanzania Agricultural Research Institute (TARI)-Naliendele in Tanzania in the 2012 growing season. Crossing was done with the resistant/tolerant parents used as females and susceptible parents as pollen donors. The crosses were (1) Namikonga♀ × AR37-80♂ and (2) Pwani♀ × AR37-80♂. Genetic crosses were performed by hand pollination according to IITA (1990) and Kawano (1980). Flowering takes place any time of the year depending on the weather and age of plants but the main flowering period in the area is between June and September and it is during this time that the highest number of fruits can be obtained. Change in colour of the anthers from green to yellow determined pollen maturity and collection was done in the mornings. Mature unopened female flowers were bagged with white muslin bags to prevent honey bees or other insects from pollinating them when they opened. Pollination was performed in the afternoons by rubbing anthers on the stigmas of opened female flowers. After pollination, the muslin bags were replaced to prevent unwanted environmental pollination. Pollination of mature unopened female flowers was assured through emasculation by removal of the perianth. The pollinated flowers were labelled to show the cross combinations. After fifty-six (56) days, mosquito netting bags replaced the muslin bags to allow air and light into the developing fruit. It was essential to enclose the fruit in these bags because mature fruits dehisce explosively, scattering the seeds. Mature seeds were harvested 70 - 90 days after pollination. The average number of seeds obtained in Namikonga × AR37-80 and Pwani × AR37-80 F1 populations were 79 and 67, respectively.

4.2.2 Seed germination and seedling establishment

Due to challenges in cassava seed germination, all the seeds from the two F1 families were sown to ensure a proper sample size. Seeds were germinated and grown in seed trays holding sterilised forest soil in a screen house at TARI - Naliendele. Since temperatures up to 35°C are needed for cassava seed germination (Ellis *et al.,* 1982), the seed trays were placed in the screen house to ensure maintenance of the right temperature. Forty days after sowing, (March 2013), the seedlings were transported and transplanted in the field at TARI-Makutupora in Dodoma, Tanzania. TARI-Makutupora station is good for seed multiplication because it is a disease-free site. Seedlings and mature stakes (about 25 cm long) from each of

the four parents were also planted in single rows at spacing of 1.0 m x 1.0 m. No fertiliser was applied and at 10 MAP (January 2014), the plants were harvested and about four to five cuttings from each of the F1 progenies were obtained. Based on the availability of enough vegetative cuttings, 36 progenies from each F1 family were selected for CBSD field resistance screening.

4.2.3 CBSD screening of F1 progenies

Screening of CBSD infections in the field was conducted in the 2014/2015 and 2015/2016 cropping seasons at TARI-Naliendele, a hot spot for CBSD infection. Naliendele lies on the coastal belt of the Indian Ocean at 10° 22' 20"S, 40° 10' 34"E and 111 m above sea level. The site receives rainfall from December-May with scattered showers in August-October (TMA, 2009). The soils are very deep, well drained, weak structured, dark reddish brown loamy sand topsoil over a reddish brown moderately structured sandy loam to sandy clay loam subsoil (Mugogo & Njapuka, 2007).

4.2.4 Field layout

A partially balanced lattice design with two replicates was used for this study. Three cassava cuttings (about 25 cm long with 4 to 5 nodes and viable buds) from each F1 progeny in both families and parents were planted at spacing of 1.0 m x 1.0 m. To increase CBSD inoculum pressure, cuttings from a known susceptible and infected variety, Albert, were also planted after every ten rows of the test progenies to act as disease spreaders. In addition, infected Albert cuttings were planted in the the first and last rows. This configuration ensured that every plant is exposed to similar high inoculum pressure and no plant escapes infection. Neither fertiliser nor irrigation were applied, and the field was rain fed throughout the growing period. The field was also kept weed free throughput the growing period.

4.2.5 Data collection

Data collection was done as described in section 3.2.4. Foliar severity at 3, 6 and 9 MAP was scored according to Hillocks *et al*. (1996) while root severity was scored according to Gondwe *et al*. (2002). Data was also collected on root necrosis, root necrosis incidence and percentage of resultant usable roots. In addition, data was collected on other yield reated traits including: root weight in tonnes per hectare, number of roots per plant, dry matter content (Kawano, 1987) and harvest index (Hühn, 1990).

4.2.6 Data analysis

Means were obtained by taking the average of the three replications for CBSD leaf symptoms' severity at 3; 6 and 9 MAP, root necrosis, root necrosis incidence, usable roots (%), number of roots per plant, root weight in tonnes per hectare, root dry matter content and harvest index. The means were subjected to combined ANOVA that was carried out for the two cropping seasons and treatment means separated using Dunnett's method to compare each progeny against a control (Pwani for the Pwani × AR37-80 F1 population) and (Namikonga for the Namikonga × AR37-80 F1 population), at 95% confidence level. Spearman's correlation analysis was carried out to determine the correlation between CBSD symptoms, root weight, number of root per plant, dry matter content, and harvest index as described in section. Progenies were classified into three disease reaction categories based on foliar symptoms, root necrosis and root necrosis incidence as described in section 3.2.5.

AR37-80

NAMIKONGA

PWANI

Figure 7: AR37-80, Namikonga, and Pwani leaf and root CBSD symptoms

4.3 Results

4.3.1 CBSD foliar symptoms

CBSD mean foliar severity increased throughout the growing seasons for Pwani × AR37-80 F1 population. The mean foliar severity was 2.2 (on a scale of 1 to 5) at 3 MAP, 2.6 at 6 MAP, and 2.6 at 9 MAP (Table 12). At 3 MAP, Pwani had a foliar severity of 2.0 and it was not significantly ($P \leq 0.05$) different from other progenies apart from PAR074 which had a significantly lower foliar severity of 1.3. Contrastingly, PARA1, PAR093, PAR060, PAR047, and PAR037 had significantly ($P \leq 0.05$) higher foliar severity ranging from 2.8 – 3.1 (Table 12). At 6 MAP, Pwani's foliar severity increased to 2.1 and it was not significantly different from other genotypes apart from PAR024, PAR030, PAR047, and PAR060, which had significantly higher foliar severities ranging from 2.9 – 3.2. At 9 MAP, Pwani had a foliar severity of 2.0 which was significantly lower than that of AR37-80 (2.7). Likewise, PAR043, PAR093, PARA1, and PAR192 have significantly ($P \leq 0.05$) higher foliar severity ranging from 2.7 – 3.2. Although Pwani had milder symptoms in comparison to AR37-80, the two parents did not have significant difference in foliar symptom expression. This could be attributed to the ability of both varieties to express foliar symptoms.

Comparable results were recorded for Namikonga ×AR37-80 population where the CBSD mean foliar severity increased throughout the growing seasons. The mean foliar severity was 1.6, 1.7 and 1.8 at 3, 6, and 9 MAP, respectively (Table 13). At 3 MAP, Namikonga had foliar severity of 1.5 which was not significantly ($P \leq 0.05$) different from other progenies. NAMAR097, NAMAR200, NAMAR370, NAMAR480, NAMAR601, and NAMARX37 had a significantly lower foliar severity of 1.0. On the other hand, NAMAR055, NAMAR540, NAMAR519, and NAMAR156B had significantly ($P \leq 0.05$) high foliar severity ranging from 2.1 – 2.5. At 6 MAP, the foliar severity for Namikonga was mild at 1.5 and it was significantly lower than that of AR37-80 (2.5). Foliar symptoms expression of NAMAR013, NAMAR103, and NAMAR412 was analogous to AR37-80 as they had significantly higher foliar severity ranging from 2.1 – 3.0. NAMARX37 and NAMAR240, however, had significantly ($P \leq 0.05$) low foliar severity of 1.0. At 9 MAP, Namikonga's foliar severity was still low at 1.5 and was not significantly different from the other genotypes apart from NAMAR492, NAMAR055, NAMAR516, and NAMAR412 which had foliar severity ranging from 2.3 – 3.3. In comparison to AR37-80, Namikonga had mild foliar symptoms and this is possibly due to its ability to suppress symptom expression unlike AR37-80 which readily expresses symptoms.

Table 12: Means of CBSD symptoms and yield related traits for Pwani × AR37-80 F1 population

Genotype	Foliar 3	Foliar 6	Foliar 9	Root N	Root NI (%)	Usable R (%)	Root W	Root No.	Dry M (%)	H Index
PWANI	**2.0**	**2.1**	**2.0**	**1.0**	**0.0**	**100.0**	**30.1**	**7.2**	**27.7**	**0.46**
AR37-80	2.0	2.6	2.7*	2.7***	63.6***	46.8***	4.3***	3.1***	31.5	0.36
PAR008	1.6	1.8	2.2	1.2	13.1*	99.2	5.5***	5.2	25.9	0.46
PAR013	2.2	2.5	2.7*	1.3	25.5***	99.8	9.6***	3.8***	33.4	0.38
PAR014	1.8	2.5	2.9***	1.6*	45.7***	94.8	3.8***	3.1***	31.4	0.40
PAR018	2.4	2.6	2.6	1.3	23.4***	89.6*	9.9***	6.4	24.7	0.39
PAR023	1.4	1.7	2.0	1.3	13.3**	95.8	11.2***	6.3	22.1	0.44
PAR024	2.5	2.9*	2.9***	1.7**	27.0***	70.4***	25.4	6.1	22.0	0.43
PAR026	2.2	2.5	2.2	2.2***	72.5***	85.0***	22.9	4.4**	22.9	0.48
PAR028	2.0	2.7	2.7*	1.0	0.0ns	100.0	4.8***	3.8***	22.0	0.60***
PAR030	2.2	3.1***	3.1***	1.5	23.6***	79.7***	9.2***	3.3***	31.2	0.55
PAR033	1.6	2.3	2.3	1.7**	27.0***	76.2***	5.2***	4.7*	29.1	0.34**
PAR037	3.1***	2.7	3.0***	1.2	13.9***	99.8	5.1***	5.8	24.5	0.41
PAR040	1.8	2.5	2.2	2.1***	63.2***	66.2***	26.7	5.7	22.6	0.54
PAR043	2.3	2.7	3.2***	3.1***	83.3***	48.2***	6.7***	3.5***	25.2	0.42
PAR047	3.0**	3.2***	3.2***	1.6*	24.9***	85.6***	16.6***	7.6	23.0	0.43
PAR057	2.7	2.6	2.9***	1.0	0.0ns	100.0	32.6	8.2	21.0*	0.52
PAR058	2.1	2.4	2.4	2.1***	60.3***	66.7***	5.0***	4.7*	32.5	0.31***
PAR060	2.8*	3.0**	3.1***	1.3	20.4***	94.1	11.9***	4.9*	23.7	0.50

Table 12 contd: Means of CBSD symptoms and yield related traits for Pwani × AR37-80 F1 population

Genotype	Foliar 3	Foliar 6	Foliar 9	Root N	Root NI (%)	Usable R (%)	Root W	Root No.	Dry M (%)	H Index
PAR063	1.8	2.5	2.3	1.0	0.0^{ns}	100.0	8.6***	2.0***	24.4	0.36
PAR064	2.3	2.4	2.4	1.2	12.6*	99.2	29.3	5.5	21.5	0.54
PAR071	2.2	2.6	2.6	1.4^{ns}	25.0***	99.8^{ns}	3.5***	2.6***	25.3	0.51
PAR074	1.3*	2.2	2.3	1.7*	43.6***	94.9^{ns}	4.9***	2.6***	22.5	0.54
PAR076	2.6	2.7	3.0***	3.6***	96.7***	12.4***	5.8***	2.2***	22.1	0.40
PAR080	2.1	3.0**	2.6	1.3	16.6***	92.3	3.0***	5.1*	20.2*	0.51
PAR083	1.7	2.3	2.2	1.3	13.1*	94.4	14.9***	9.1	25.6	0.46
PAR084	2.1	1.9	2.2	1.9***	38.8***	66.7***	12.6***	4.9*	30.0	0.46
PAR093	3.0**	3.1***	3.0***	2.2***	71.2***	87.2***	2.9***	2.8***	32.3	0.37
PAR110	2.0	3.0**	3.0***	1.2	26.1***	73.8***	11.6***	3.8***	20.1*	0.46
PAR111	1.8	3.0**	2.7*	2.1***	42.9***	58.2***	14.6***	4.5**	22.6	0.45
PAR124	2.3	2.5	2.6	2.0***	44.5***	76.0***	21.0***	5.6	23.7	0.61***
PAR135	2.4	2.4	2.5	1.9***	59.7***	84.9***	13.1***	6.4	20.8*	0.50
PAR136	1.8	2.7	2.7*	2.1***	47.1***	72.6***	6.7***	5.3	21.7*	0.40
PAR140	2.0	3.0**	3.1***	2.2***	64.8***	55.5***	15.3***	6.6	24.2	0.40
PAR192	1.8	3.1***	2.7*	1.1	9.4	97.4	33.2	11.1***	22.9	0.42
PARA1	2.9**	2.8	2.8***	1.4	49.2***	69.1***	7.2***	6.0	25.9	0.44

Table 12 contd: Means of CBSD symptoms and yield related traits for Pwani × AR37-80 F1 population

Genotype	Foliar 3	Foliar 6	Foliar 9	Root N	Root NI (%)	Usable R (%)	Root W	Root No.	Dry M (%)	H Index
Mean	2.2	2.6	2.6	1.7	35.0	81.6	12.6	5.1	25.0	0.45
Mean Y1	2.0	2.8	2.9	1.9	48.4	76.3	14.1	5.4	19.5	0.46
Mean Y2	2.3	2.4	2.4	1.4	21.6	86.8	10.9	4.8	30.6	0.44
Dunnett	0.7	0.8	0.7	0.6	10.2	10.4	7.9	2.2	5.7	0.11
CV	13.3	11.6	10.1	14.1	12.9	5.1	16.6	16.5	11.0	8.5

-Means separation done by dunnett test (PWANI is the control)

-Statistical significance: * = $P \leq 0.05$, ** = $P \leq 0.01$, *** = $P \leq 0.001$

-Mean (2014) and (2015) are means for cropping season one and two respectively

-CV - coefficient of variation

-Dunnet significant difference value

-Foliar 3, 6, 9 – foliar symptoms at 3, 6, 9 months after planting; Root N – Root necrosis; Root NI (%) – Root necrosis incidence; Useable R (%) – Useable roots; Root W – Root weight (t/ha); Root No. – Number of roots per plant; Dry M (%) – Dry matter content; H Index – Harvest index

Table 13: Means of CBSD symptoms and yield related traits for Namikonga × AR37-80 F1 popuation

Genotype	Foliar 3	Foliar 6	Foliar 9	Root N	Root NI (%)	Usable R (%)	Root W	Root No.	Dry M (%)	H Index
NAMIKONGA	**1.5**	**1.5**	**1.5**	**1.0**	**0.0**	**100.0**	**19.1**	**6.0**	**46.8**	**0.36**
AR37-80	2.4**	2.5***	2.7***	2.6***	74.5***	59.2***	7.0***	4.9	30.9***	0.51*
NAMAR013	1.4	2.1*	1.8	1.7	24.5**	75.1***	8.5***	4.8	34.8*	0.50*
NAMAR050	1.7	1.9	1.7	1.1	2.9	98.8	27.1*	8.9*	29.4***	0.58***
NAMAR055	2.1*	2.3***	2.5***	2.8***	84.5***	32.1***	16.0	4.9	32.5*	0.35
NAMAR091	1.7	2.0	2.5***	1.4	45.3***	100.0	31.1***	4.3	30.3***	0.46
NAMAR097	1.0*	1.7	1.5	3.1***	77.4***	29.3***	23.5	5.0	28.9***	0.52***
NAMAR103	1.5	2.3***	2.3**	1.0	0.0	100.0	13.8	5.2	25.5***	0.44
NAMAR110	1.5	1.5	1.6	1.0	0.0	100.0	12.0	7.0	25.8***	0.50*
NAMAR116	1.7	1.7	1.8	1.4	31.9***	98.3	18.2	3.8	35.8	0.37
NAMAR130	1.5	1.0	1.3	1.0	0.0	100.0	16.3	6.4	24.1***	0.55***
NAMAR156B	2.3**	1.9	1.6	3.5***	89.3***	25.8***	17.3	6.5	31.2***	0.47
NAMAR200	1.0*	1.1	1.1	1.0	0.0	100.0	11.1*	7.1	34.2**	0.41
NAMAR284	1.8	2.4***	2.5***	1.8*	66.2***	76.1***	16.8	7.5	28.7***	0.42
NAMAR321	2.1*	2.3***	2.3**	3.5***	98.4***	22.5***	14.0	4.8	26.0***	0.26
NAMAR334	1.3	1.0	1.3	1.1	15.0	95.7	6.6***	3.9	26.5***	0.38
NAMAR370	1.0*	1.6	1.3	2.0***	50.5***	75.2***	28.9***	3.7	32.9**	0.36
NAMAR371	1.1	1.7	1.4	1.0	4.4	99.6	27.8**	8.0	28.6***	0.50*
NAMAR402	1.8	1.0	1.6	1.0	0.0	100.0	15.2	4.3	26.7***	0.45

Table 13 contd: Means of CBSD symptoms and yield related traits for Namikonga × AR37-80 F1 population

Genotype	Foliar 3	Foliar 6	Foliar 9	Root N	Root NI (%)	Usable R (%)	Root W	Root No.	Dry M (%)	H Index
NAMAR409	1.1	1.1	1.0	1.6	28.5***	90.1	7.7***	4.8	34.8*	0.46
NAMAR435	2.0	2.3***	2.4**	2.6***	69.8***	44.7***	17.6	5.6	22.5***	0.41
NAMAR441	1.2	1.3	1.1	1.8*	29.0***	72.9***	19.5	2.2***	22.4***	0.45
NAMAR444	1.5	2.0	1.9	1.0	7.3	100.0	7.5***	6.3ns	34.8*	0.45
NAMAR475	1.5	2.7***	2.5***	2.2***	53.3***	76.1***	6.2***	2.7**	28.0***	0.43
NAMAR479	1.1	1.0	1.0	1.1	1.5	100.0	14.5	4.7	27.5***	0.41
NAMAR480	1.0*	1.5	1.3	2.1***	48.9***	55.1***	5.5***	2.7**	28.9***	0.20**
NAMAR492	2.5***	2.1*	2.3*	2.4***	56.4***	49.8***	7.2***	4.5	32.8*	0.39
NAMAR510	1.5	1.1	1.2	1.0	0.6	98.0	5.4***	5.3	26.7***	0.53***
NAMAR516	2.0	2.0	2.7***	1.3	18.5	86.9***	11.4*	8.1	33.4**	0.34
NAMAR519	2.5***	2.5***	2.7***	1.8*	42.0***	57.3***	13.2	3.7	33.1***	0.40
NAMAR540	2.1*	2.1*	2.0	2.9***	98.9***	34.4***	2.4***	2.7**	30.6***	0.37
NAMAR549	1.5	1.4	1.5	2.6***	87.6***	39.3***	22.3	6.4	36.7	0.33
NAMAR601	1.0*	1.9	2.0	1.6	22.6**	63.7***	11.6*	4.0	38.1	0.27
NAMAR661	2.0	1.7	2.0	1.8**	51.4***	73.9***	5.3***	3.4*	24.8***	0.39
NAMARX12	2.0	1.4	1.9	1.1	4.2	98.9	22.5	5.4	27.5***	0.39
NAMARX37	1.0*	1.0*	1.6	1.6	32.7***	78.9***	10.0***	4.8	31.7***	0.39

Table 13 contd: Means of CBSD symptoms and yield related traits for Namikonga × AR37-80 F1 population

Genotype	Foliar 3	Foliar 6	Foliar 9	Root N	Root NI (%)	Usable R (%)	Root W	Root No.	Dry M (%)	H Index
Mean	1.6	1.7	1.8	1.8	36.8	75.3	14.5	5.1	30.3	0.42
Mean Y1	1.5	1.9	2.0	1.9	42.3	66.7	16.7	5.7	26.0	0.46
Mean Y2	1.7	1.5	1.6	1.6	31.2	75.1	12.2	4.6	34.6	0.37
Dunnett	0.6	0.6	0.7	0.8	19.1	11.5	7.3	2.4	11.3	0.13
CV	12.9	13.5	15.0	19.3	18.5	14.1	17.5	19.4	13.3	11.8

-Means separation done by dunnett test (NAMIKONGA is the control)

-Statistical significance: * = $P \leq 0.05$, ** = $P \leq 0.01$, *** = $P \leq 0.001$

-Mean (2014) and (2015) are means for cropping season one and two respectively

-CV - coefficient of variation

-Dunnet significant difference value

-Foliar 3, 6, 9 – foliar symptoms at 3, 6, 9 months after planting; Root N – Root necrosis; Root NI (%) – Root necrosis incidence; Useable R (%) – Usable roots; Root W – Root weight (t/ha); Root No. – Number of roots per plant; Dry M (%) – Dry matter content; H Index – Harvest index

4.3.2 CBSD root symptoms

The mean root necrosis severity was 2.1 for the Pwani × AR37-80 F1 population (Table 12). Pwani had the least root necrosis with a severity score of 1.0 and was not significantly different from most of the progenies, apart from a few, including PAR014, PAR026, PAR043, and PAR026 which had significantly higher ($P \leq 0.05$) root necrosis severities ranging from 1.6 – 3.6. Comparable results were recorded for root necrosis incidence where Pwani did not register any statistical root necrosis incidence (0.0%) (Table 12). Other progenies including parent AR37-80 had significantly ($P \leq 0.05$) higher root necrosis incidences ranging from 12.6 – 96.7%. Pwani had low mean root necrosis severity and a resultant high percentage of usable roots (up to 100.0%) and was not significantly different from progenies including: PAR008, PAR013, PAR014, PAR023, PAR028, PAR037, PAR057, and PAR063 (Table 12). Significantly low quantities were not only recorded in AR37-80 also in progenies including: PAR043, PAR058, PAR 0.76, and PAR 140 ranging from 12.4 – 66.7%.

Similar results were recorded for Namikonga × AR37-80 F1 population with a mean root necrosis severity of 1.8 (Table 13). Namikonga had the least root necrosis with a severity score of (1.0). Progenies NAMAR055, NAMAR097, NAMAR056, and NAMAR475 including the susceptible parent AR37-80 had significantly higher ($P \leq 0.05$) root necrosis severity ranging from 2.1– 3.5. Comparable results were recorded for root necrosis incidence with Namikonga, NAMAR103, NAMAR130, NAMAR200, NAMAR402, and NAMAR479 having no root necrosis incidence (Table 13). Contrastingly, significantly ($P \leq 0.05$) higher root necrosis incidences were recorded in AR37-80 (74.5%), NAMAR055 (84.5%), NAMAR 097 (77.4%), NAMAR156B (89.3%) and NAMAR321 (98.4%). Since Namikonga, NAMAR050, NAMAR103, NAMAR130, NAMAR200, NAMAR402, and NAMAR479 had minimal or no root necrosis, they also had high percentages of usable roots (99.0 – 100.0%).

4.3.3 Cassava yield related traits

The mean root weight for the Pwani × AR37-80 F1 population was 12.6 t/ha (Table 12). The highest mean root weight was recorded in Pwani, PAR024, PAR026, PAR040, PAR057, PAR064, and PAR192, ranging from 22.9 – 33.2 t/ha. AR37-80 had a significantly ($P \leq 0.05$) lower mean root weight of 4.3 t/ha. Most of the progenies had intermediate root weight between those of the parents ranging from 5.1 – 16.6 t/ha. The mean root number per plant was 5.1 (Table 12). The highest mean root number per plant was recorded in PAR192

(11.1), PAR083 (9.1), PAR057 (8.2), PAR047 (8.6), PAR047 (7.6) and Pwani (7.2). On the other hand, AR37-80, PAR080, PAR093, PAR076, PAR071, PAR063, and PAR014 had significantly ($P \leq 0.05$) lower mean root number per plant ranging from 2.0 – 5.1. Most of the progenies had intermediate mean root number per plant between those of their parents and ranged from 3.1 - 6.6.

The mean dry matter content was 25.0% (Table 12). Both Pwani and AR37-80 had high dry matter content of 27.7% and 31.5%, respectively. Most of the progenies had mean dry matter contents that were not significantly different from both parents and ranged from 21.5 - 33.4%. Progenies PAR057, PAR080, PAR110, PAR135, and PAR136 had significantly lower mean dry matter content ranging from 20.1 – 21.7%. The mean harvest index was 0.45 (Table 12). Pwani had a high mean harvest index of 0.46 which did not differ significantly ($P \leq 0.05$) with that of AR37-80 at 0.36. The highest significant mean harvest indices were recorded in PAR124 (0.61) and PAR028 (0.60). Harvest indices for the other progenies ranged from 0.36 – 0.55. AR37-80 is a South American variety and its poor adaptation to East African environments could be the cause of its poor root traits in this study. Pwani on the other hand is a high yielding CBSD tolerant Tanzanian local variety.

Namikonga × AR37-80 F1 population had comparable results where a mean root weight of 13.3 t/ha was recorded (Table 13). Namikonga itself had a high root weight of 19.4 t/ha. The highest mean root weights were recorded in NAMAR050 (28.2 t/ha), NAMAR370 (29.0 t/ha), and NAMAR371 (27.8 t/ha). Progenies including: NAMAR055, NAMAR097, NAMAR321, and NAMAR441 which had mean root weights ranging from 14.0 – 24.6 t/ha while AR37-80, NAMARX37, NAMAR444, and NAMAR540 had significantly ($P \leq 0.05$) lower mean root weights ranging from 2.8 – 13.0 t/ha. The mean root number per plant was 5.0 (Table 13). Namikonga had a mean root number per plant of (5.8) and it was not significantly different from other progenies including AR37-80, NAMAR055, NAMAR200, NAMAR130, and NAMAR510 whose root mean number per plant ranged from 4.2 -7.4. On the contrary, NAMAR240, NAMAR370, NAMAR475 and NAMAR601 had significantly ($P \leq 0.05$) lower mean root number per plant ranging from 2.4 – 3.6, while NAMAR050 (8.9), NAMAR371 (8.0), and NAMAR561 (8.1) had significantly higher mean root number per plant. Namikonga had the highest mean dry matter content of 47.5% when compared to AR37-80 at 31.2%. The other progenies had mean dry matter content comparable to AR37-80 ranging from 22.9 – 34.7%. The mean harvest index was 0.42 with Namikonga registering 0.35 (Table 13). Significantly higher harvest indices were recorded in NAMAR013,

NAMAR050, NAMAR130, NAMAR110, NAMAR371, and NAMAR510 ranging between 0.50 and 0.58. NAMAR480, however, had a significantly lower harvest index of 0.21.

4.3.4 Correlation analysis

Correlation analysis quantified the association between different parameters tested in both populations. Accordingly, a significant positive correlation was observed between foliar symptom severity at 3 MAP and 6 MAP for Pwani ×AR37-80 ($P \leq 0.01$, $r = 0.52$) and Namikonga × AR37-80 ($P \leq 0.01$, $r = 0.47$) (Table 14 and 15). Similar results were observed on analysis between foliar symptoms severity at 3 MAP and 9 MAP for Pwani × AR37-80 ($P \leq 0.01$, $r = 0.67$) and Namikonga × AR37-80 ($P \leq 0.01$, $r = 0.60$). Additionaly, a high positive correlation was observed betweeen foliar symptoms severity at 6 MAP and 9 MAP for Pwani ×AR37-80 ($P \leq 0.01$, $r = 0.81$) and Namikonga × AR37-80 ($P \leq 0.01$, $r = 0.89$) The results showed that foliar symptoms observed as early as 3 MAP persisted throughout the whole season up to 9 MAP.

In the Pwani × AR37-80 population, there was no significant correlation between foliar symptoms at 3 MAP and root necrosis; foliar symptoms at 6 MAP and root necrosis; and foliar symptoms at 9 MAP and root necrosis (Table 14). The expression of foliar and root symptoms varied, with some progenies having high foliar severity but no root necrosis, for example, Pwani, PAR028 and PAR057. On the other hand, AR37-80, PAR026, PAR043 and PAR076 expressed high CBSD severity on both roots and leaves. Although Pwani × AR37-80 population depicted no significant correlation between foliar and root symptoms, Namikonga × AR37-80 population depicted a significant positive correlation between foliar symptoms at 3 MAP and root necrosis ($P \leq 0.05$, $r = 0.38$), between foliar symptoms at 6 MAP and root necrosis ($P \leq 0.01$, $r = 0.49$) and between foliar symptoms at 9 MAP and root necrosis ($P \leq 0.05$, $r = 0.33$) (Table 15). Equivalent results were observed for correlations between foliar symptoms and root necrosis incidence where there was no correlation between foliar symptoms and root necrosis incidence in Pwani × AR37-80 population, while there was significant positive correlation between foliar symptoms and root necrosis incidence in the Namikonga × AR37-80 population (Table 14 and 15). Even so, there was a remarkably high positive correlation between root necrosis and root necrosis incidence: ($P \leq 0.01$, $r = 0.93$) and ($P \leq 0.01$, $r = 0.98$) for Pwani × AR37-80 and Namikonga × AR37-80, respectively (Table 14 and 15). High root necrosis and root necrosis incidence resulted in reduced quantitiess of usable roots as depicted in both populations where high negative correlation was recorded between root necrosis and usable roots, and between root necrosis incidence

and usable roots of ($P \leq 0.01$, $r = -0.78$) and ($P \leq 0.01$, $r = -0.96$) for Pwani × AR37-80 and Namikonga × AR37-80, respectively (Table 14 and 15).

Although there was a negative correlation between CBSD symptoms and root weight, it was nonetheless not significant in both populations (Table 14 and 15). A non-significant negative correlation was recorded between foliar symptoms and root number per plant in both populations. Contrastingly, a significant negative correlation was recorded between root number per plant and root necrosis, and between root number per plant and root necrosis incidence ($P \geq -0.35$) in both populations. This showed that high root necrosis and high root necrosis incidence resulted in reduced root number per plant. Comparable to root weight, there was a non-significant negative correlation between CBSD symptoms and dry matter content and between CBSD symptoms and harvest index. It is noteworthy that a non-significant positive correlation was observed between foliar symptoms at 6 MAP and harvest index and between foliar symptoms at 9 MAP and harvest index in Namikonga × AR37-80 F1 population (Table 15). Progenies NAMAR013, NAMAR261, and NAMAR412 including parent AR37-80 had severe foliar symptoms, which may have contributed to a reduction in shoot weight and consequently a higher root weight to shoot weight ratio (harvest index). Additionally, a significant positive correlation was recorded between dry matter content and harvest index ($P \leq 0.05$, $r \geq 038$) in both populations (Table 14 and 15).

Table 14: Correlation coefficient values for CBSD symptoms and yield related traits for Pwani × AR37-80 F1 population

	Foliar 3	Foliar 6	Foliar 9	Root N	Root NI	Usable R	Root W	Root No.	Dry M	H Index
Foliar 3	1	0.52**	0.67**	0.15	0.21	-0.18	0.02	0.03	0.05	-0.09
Foliar 6		1	0.81**	0.17	0.15	-0.22	-0.02	-0.03	-0.14	-0.06
Foliar 9			1	0.32	0.28	-0.41*	-0.14	-0.05	-0.06	-0.14
Root N				1	0.93**	-0.89**	-0.21	-0.35*	0.14	-0.25
Root RI					1	-0.78**	-0.25	-0.39*	0.19	-0.24
Usable R						1	0.16	0.27	-0.07	0.25
Root W							1	0.66**	-0.33*	0.32
Root No.								1	-0.31	0.08
Dry M									1	0.49**
H Index										1

-Spearman's correlation used

-Statistical significance: * = $P \leq 0.05$, ** = $P \leq 0.01$, *** = $P \leq 0.001$

-Foliar 3, 6, 9 – foliar symptoms at 3, 6, 9 months after planting; Root N – Root necrosis; Root NI (%) – Root necrosis incidence; Useable R (%) – Usable roots; Root W – Root weight (t/ha); Root No. – Number of roots per plant; Dry M (%) – Dry matter content; H Index – Harvest index

Table 15: Correlation coefficient values for CBSD symptoms and yield related traits for Namikonga × AR37-80 F1 population

	Foliar 3	Foliar 6	Foliar 9	Root N	Root NI	Usable R	Root W	Root No.	Dry M	H Index
Foliar 3	1	0.47**	0.60**	0.38*	0.40*	-0.40*	-0.18	-0.02	-0.15	-0.08
Foliar 6		1	0.89**	0.49**	0.50**	-0.44**	-0.17	-0.12	-0.07	0.06
Foliar 9			1	0.33*	0.38*	-0.32	-0.27	-0.07	-0.14	0.01
Root N				1	0.98**	-0.96**	-0.15	-0.36*	-0.08	-0.22
Root NI					1	-0.95**	-0.17	-0.39*	-0.05	-0.27
Usable R						1	0.14	0.40*	0.04	0.34*
Root W							1	0.46**	-0.04	0.21
Root No.								1	0.06	0.39*
Dry M									1	0.38*
H Index										1

-Spearman's correlation used

-Statistical significance: * = $P \leq 0.05$, ** = $P \leq 0.01$, *** = $P \leq 0.001$

-Foliar 3, 6, 9 – foliar symptoms at 3, 6, 9 months after planting; Root N – Root necrosis; Root NI (%) – Root necrosis incidence; Usable R (%) – Usable roots; Root W – Root weight (t/ha); Root No. – Root number per plant; Dry M (%) – Dry matter content; H Index – Harvest index

4.3.5 Mean squares and sum of squares for evaluated traits

In Pwani × AR37-80 population, the highest sum of squares (SS) weas due to genotype by environment interaction (G×E) (42.3 – 45.4%) and genotype (33.6 – 45.7%) while environment (3.07 – 17.5%) had the least for foliar symptoms at 3, 6, and 9 MAP (Table 16). Simillarly in Namikonga × AR37-80 population, the highest SS was due to genotype (46.2 – 49.8%) followed closely by G×E interaction (44.2 – 46.8%) and environment (2.44 – 6.81%) having the least (Table 18). The findings showed that both genetic make up and G×E interaction played an equal role in influencing foliar symptoms expression of the progenies. Although SS due to environment was the least for both populations, mean squares were significant ($P \leq 0\ 001$) indicating that the expression of foliar symptoms of a few progenies was significantly affected by environmental factors. In both population, SS due to genotype was the highest (55.3 – 63.7%) followed by G×E interaction (28.0 – 39.9%) and finally environment (1.79 – 17.9%) for root necrosis, root necrosis incidence and usable roots (Table 16 and 18). Generally, more severe CBSD symptoms were observed in growing season one.

In Pwani × AR37-80 population, SS due to genotype was the highest ranging from (79.0 – 82.3%) followed by G×E interaction (13.8 – 17.2%) and environment (2.02 – 3.56%) for root weight and root number per plant (Table 17). In dry matter content, SS due to environment (53.3%) was the highest followed by genotype (22.5%) and G×E interaction (21.9%) (Table 17). Harvest index on the other hand had the highest SS due to G×E interaction (52.5%) followed by genotype (44.7%) and environment (1.21%) (Table 18). The findings showed that variations in root weight and root number per plant observed among the progenies was largely due to genetic make up while environment and G×E interaction significantly influenced dry matter content and harvest index, respectively.

In Namikonga × AR37-80 population, root weight had the highest SS due to genotype (81.7%) while G×E interaction and environment had the least with 8.24% and 8.20%, respectively (Table 19). Number of roots per plant had the highest SS due to genotype (49.1%), followed closely by G×E interaction (41.2%) and environment had the least with (6.72%). Dry matter content and harvest index had the highest SS due to genotype (38.9 – 53.0%) followed by environment (20.0 – 38.9%) and G×E interaction (14.7 – 24.4%). The findings showed that although genotype of progenies largely influenced yield related traits, G×E interaction and environment also played a role in the variations observed particularly for

number of roots per plant, dry matter content and harvest index as their effects were highly significant ($P \leq 0\ 001$) (Table 19) resulting in the variation between the two environments.

4.3.6 Environmental influences on traits tested

Temperature and rainfall can influence CBSD symptom expression as well as yield related traits including: root weight, number of roots per plant, dry matter content and harvest index. There was no significant difference in the rainfall patterns and temperature regime of growing seasons 2014 and 2015 and environment may have not influenced CBSD foliar and root symptoms exression where higher means were observed in growing season 2014 (Figure 6). Generally, more severe leaf symptoms were seen in season one in comparison to season two in both populations (Table 12 and 13).

Environmental conditions influenced root weight, number of roots per, dry matter and harvest index. The Pwani × AR37-80 population had mean root weight of 14.1 and 10.9 while the Namikonga × AR37-80 population registered 15.5 and 11.2 t/ha in seasons one and two, respectively (Table 12 and 13). The mean harvest index for Pwani × AR37-80 was 0.46 and 0.44 in seasons one and two, respectively, while that of Namikonga × AR37-80 was 0.46 and 0.37, respectively (Table 12 and 13). The higher root weight and harvestable portions in season one could be attributed to higher quantitiess of rainfall at harvesting in December (102.9 mm) in comparison to 30.5 mm in season two (Figure 6). Rainfall quantities may also have affected dry matter content since a lower mean dry matter content was recorded in season one for Pwani × AR37-80 (19.5%) and Namikonga × AR37-80 (30.6%) compared to season two where the Pwani × AR37-80 and Namikonga × AR37-80 populations had 25.9% and 34.8%, respectively (Table 12 and 13).

Table 16: Mean squares and sums of squares for CBSD symptoms for Pwani × AR37-80 F1 population

Source of Variation	df	Foliar symptoms at 3 MAP			Foliar symptoms at 6 MAP			Foliar symptoms at 9 MAP		
		MS	SS	SS (%)	MS	SS	SS (%)	MS	SS	SS (%)
Total	93	-	53.8	-	-	42.8	-	-	44.4	-
Environment	1	1.65***	1.65	3.07	4.95***	4.95	11.6	7.75***	7.75	17.5
Replicate	1	1.25***	1.25	2.32	1.02***	1.02	2.38	0.61**	0.61	1.37
Block (Environment *Replicate)	21	0.09	1.91	3.55	0.09	1.86	4.34	0.11	2.31	5.20
Genotype	35	0.70***	24.6	45.7	0.46***	15.9	37.2	0.43***	14.9	33.6
Genotype*Environment	35	0.70***	24.4	45.4	0.55***	19.1	44.6	0.54***	18.8	42.3
Error	50	0.08	4.06	-	0.09	4.48	-	0.07	3.48	-

-df, MS, SS - degrees of freedom, mean squares, and sum of squares, respectively

-Statistical significance: * = $P \leq 0.05$, ** = $P \leq 0.01$, *** = $P \leq 0.001$

Table 16 contd: Mean squares and sums of squares of CBSD symptoms for Pwani × AR37-80 F1 population

Source of Variation	df	Root necrosis severity			Root necrosis incidence			Usable roots		
		MS	SS	SS (%)	MS	SS	SS (%)	MS	SS	SS (%)
Total	93	-	79.5	-	-	145520.0	-	-	78195.0	-
Environment	1	9.71***	9.71	12.2	25974.7***	25974.7	17.9	3957.5***	3957.5	5.06
Replicate	1	0.23*	0.23	0.29	7.93	7.93	0.01	45.0	45.0	0.06
Block (Environment*Replicate)	21	0.07	1.25	1.57	11.5	241.1	0.17	23.0	482.1	0.62
Genotype	35	1.32***	46.0	57.9	2297.6***	80415.3	55.3	1334.7***	46713.5	59.7
Genotype*Environment	35	0.64***	22.3	28.0	1110.9***	38880.9	26.7	771.3***	26996.9	34.5
Error	50	0.06	32.8	-	20.3	1016.6	-	16.9	847.1	-

-df, MS, SS - degrees of freedom, mean squares, and sum of squares, respectively

-Statistical significance: * = $P \leq 0.05$, ** = $P \leq 0.01$, *** = $P \leq 0.001$

Table 17: Mean squares and sums of squares of yield related traits for Pwani × AR37-80 F1 population

Source of Variation	df	Root weight (t/ha)			Number of roots per plant		
		MS	SS	SS (%)	MS	SS	SS (%)
Total	93	-	12210.0	-	-	569.1	-
Environment	1	435.1***	435.1	3.56	11.5***	11.5	2.02
Replicate	1	0.01	0.01	0.00	1.40	1.40	0.25
Block (Environment*Replicate)	21	2.09	43.8	0.36	0.41	8.68	1.52
Genotype	35	286.9***	10043.1	82.3	12.8***	449.5	79.0
Genotype*Environment	35	48.2***	1688.0	13.8	2.80***	98.0	17.2
Error	50	4.36	217.9	-	0.71	35.4	-

-df, MS, SS - degrees of freedom, mean squares, and sum of squares, respectively

-Statistical significance: * = $P \leq 0.05$, ** = $P \leq 0.01$, *** = $P \leq 0.001$

Table 17 contd: Mean squares and sums of squares of yield related traits for Pwani × AR37-80 F1 population

Source of Variation	df	Dry matter content			Harvest index		
		MS	SS	SS (%)	MS	SS	SS (%)
Total	93	-	8285.4	-		13333.9	
Environment	1	4418.9***	4418.9	53.3	161.1***	161.1	1.21
Replicate	1	4.80	4.80	0.06	27.1	27.1	0.20
Block (Environment*Replicate)	21	8.70	182.7	2.21	8.99	188.9	1.41
Genotype	35	53.2***	1863.6	22.5	167.4***	5959.1	44.7
Genotype*Environment	35	51.9***	1815.4	21.9	199.9***	6997.7	52.5
Error	50	7.58	379.0	-	14.3	712.8	-

-df, MS, SS - degrees of freedom, mean squares, and sum of squares, respectively

-Statistical significance: * = $P \leq 0.05$, ** = $P \leq 0.01$, *** = $P \leq 0.001$

Table 18: Mean squares and sums of squares of CBSD symptoms for Namikonga × AR37-80 F1 population

Source of Variation	df	Foliar symptoms at 3 MAP			Foliar symptoms at 6 MAP			Foliar symptoms at 9 MAP		
		MS	SS	SS (%)	MS	SS	SS (%)	MS	SS	SS (%)
Total	93	-	55.8	-	-	80.5	-	-	83.8	-
Environment	1	1.36***	1.36	2.44	5.48***	5.48	6.81	5.56***	5.56	6.64
Replicate	1	0.00	0.00	0.00	0.04	0.04	0.05	0.18	0.18	0.21
Block (Environment *Replicate)	21	0.03	0.57	1.02	0.05	1.08	1.34	0.07	1.54	1.83
Genotype	35	0.80***	27.8	49.8	1.06***	37.2	46.2	1.13***	39.5	47.1
Genotype*Environment	35	0.75***	26.1	46.8	1.05***	36.7	45.6	1.06***	37.0	44.2
Error	50	0.04	2.09	-	0.06	2.76	-	0.08	3.74	-

-df, MS, SS - degrees of freedom, mean squares, and sum of squares, respectively

-Statistical significance: nonsignificant (ns) = $P > 0.05$, * = $P \leq 0.05$, ** = $P \leq 0.01$, *** = $P \leq 0.001$

Table 18 contd: Mean squares and sums of squares of CBSD symptoms for Namikonga × AR37-80 F1 population

Source of Variation	df	Root necrosis severity			Root necrosis incidence			Usable roots		
		MS	SS	SS (%)	MS	SS	SS (%)	MS	SS	SS (%)
Total	93	-	122.7	-	-	216404.0	-	-	142497.0	-
Environment	1	6.21***	6.21	5.06	6951.4***	6951.4	3.21	2546.0***	2546.0	1.79
Replicate	1	0.12	0.12	0.10	22.8	22.8	0.01	35.1	35.1	0.03
Block (Environment*Replicate)	21	0.12	2.51	2.05	36.9	774.5	0.36	48.3	1015.0	0.71
Genotype	35	2.09***	73.0	59.5	3940.4***	137913.6	63.7	2345.4***	82089.2	57.6
Genotype*Environment	35	1.17***	40.9	33.3	2021.2***	70741.7	33.7	1623.2***	56811.7	39.9
Error	50	0.13	6.51	-	53.9	2697.1	-	31.3	1564.3	-

-df, MS, SS - degrees of freedom, mean squares, and sum of squares, respectively

-Statistical significance: * = $P \leq 0.05$, ** = $P \leq 0.01$, *** = $P \leq 0.001$

Table 19: Mean of squares and sums of squares of yield related traits for Namikonga × AR37-80 F1 population

Source of Variation	df	Root weight (t/ha)			Number of roots per plant		
		MS	SS	SS (%)	MS	SS	SS (%)
Total	93	-	8396.8	-	-	610.2	-
Environment	1	688.2***	688.2	8.20	41.0***	41.0	6.72
Replicate	1	33.8*	33.8	0.40	0.22	0.22	0.04
Block (Environment*Replicate)	21	5.84	122.4	1.46	0.88	18.4	3.02
Genotype	35	196.0***	6860.3	81.7	8.55***	299.3	49.1
Genotype*Environment	35	19.8***	692.1	8.24	7.18***	251.3	41.2
Error	50	5.44	272.2	-	0.90	45.2	-

-df, MS, SS - degrees of freedom, mean squares, and sum of squares, respectively

-Statistical significance: * = $P \leq 0.05$, ** = $P \leq 0.01$, *** = $P \leq 0.001$

Table 19 contd: Means of squares and sums of squares of yield related traits for Namikonga × AR37-80 F1 population

Source of Variation	df	Dry matter content			Harvest index		
		MS	SS	SS (%)	MS	SS	SS (%)
Total	93	-	7265.1	-		16968.2	
Environment	1	2826.7***	2826.7	38.9	3387.2***	3387.2	20.0
Replicate	1	0.04	0.04	0.00	65.9	65.9	0.38
Block (Environment*Replicate)	21	25.9	544.8	7.50	18.0	377.1	2.22
Genotype	35	80.7***	2823.6	38.9	256.9***	8990.5	53.0
Genotype*Environment	35	30.6*	1070.0	14.7	118.5***	4147.5	24.4
Error	50	16.5	823.5	-	24.1	1204.5	-

-df, MS, SS - degrees of freedom, mean squares, and sum of squares, respectively

-Statistical significance: * = $P \leq 0.05$, ** = $P \leq 0.01$, *** = $P \leq 0.001$

3.3.7 Disease reaction categories of F1 progenies screened for CBSD resistance

Characterization of progenies as resistant, tolerant, or susceptible was based on root necrosis since this is the most damaging component of CBSD disease syndrome. Resistant progenies expressed mild or no foliar and root necrosis symptoms while tolerant ones expressed severe foliar symptoms but with minimal or no root necrosis symptoms. Susceptible progenies on the other hand had severe root necrosis symptoms with or without observable foliar symptoms. The Pwani × AR37-80 F1 population had only tolerant and suceptible categories similar to the parents used for crosses. Progenies categorized as tolerant included: PAR008, PAR013, PAR018, PAR023, PAR028, PAR030, PAR033, PAR037, PAR047, PAR060, PAR063, PAR071, PAR080, PAR083, & PAR110. These had low root weights (< 17.0 t/ha) and moderate to high dry matter content ranging from 20.1 – 33.4%. Progenies PAR024, PAR057, PAR064, & PAR192 had the highest root weight (25.4 – 33.2 t/ha) and moderate dry matter content ranging from 21.0 – 22.9% (Table 20). Other progenies including: PAR014, PAR040, PAR043, PAR058, PAR074, PAR076, PAR093, PAR111, PAR124, PAR135, PAR136, PAR140, PARA1 were susceptible and had severe root necrosis symptoms.

The Namikonga × AR37-80 F1 population had resistant, tolerant and suceptible categories. Resistant progenies included NAMAR050, NAMAR130, NAMAR200, NAMAR371, NAMAR402, NAMAR444, NAMAR479, NAMAR510, & NAMARX12. NAMAR050, NAMAR130, NAMAR371, NAMAR402, & NAMARX12 had high root weights ranging from (15.2 – 27.8 t/ha) and moderate dry matter content ranging from (24.1 – 29.4 %) (Table 21). Tolerant progenies included: NAMAR013, NAMAR116, NAMAR334, NAMAR409, NAMAR441, NAMAR601, NAMARX37. Among the tolerant progenies, NAMAR116 and NAMAR441 had root weight ranging from 18.2 – 19.5 t/ha and modearate to high dry matter content ranging from 22.4 – 35.8%. Other progenies including: NAMAR055, NAMAR091, NAMAR097, NAMAR156B, NAMAR284, & NAMAR321 were susceptible.

Table 20: Disease reaction categories of Pwani × AR37-80 F1 progenies

CBSD foliar symptoms	Root necrosis	Root necrosis incidence (%)	Disease reaction category	Landrace/variety
1.4 – 3.2	1.0 – 1.7	0.0 – 27.0	Tolerant	Pwani, PAR008, PAR013, PAR018, PAR023, **PAR024**, PAR026, PAR028, PAR030, PAR033, PAR037, PAR047, **PAR057,** PAR060, PAR063, **PAR064,** PAR071, PAR080, PAR083, PAR110, **PAR192** *Root weight = 25.4 – 33.2 t/ha; Root no./plant = 6.1 – 11.1; Dry matter content = 21.0 – 22.9%; Harvest index = 0.42 – 0.54*
1.8 – 3.2	1.4 – 3.6	42.9 – 96.7	Susceptible	AR37-80, PAR014, PAR040, PAR043, PAR058, PAR074, PAR076, PAR093, PAR111, PAR124, PAR135, PAR136, PAR140, PARA1

Note: Landraces in bold had the indicated root weight, number of root per plant, dry matter content and harvest within the various disease reaction categories

Table 21: Disease reaction categories of Namikonga × AR37-80 F1 progenies

CBSD foliar symptoms	Root necrosis	Root necrosis incidence (%)	Disease reaction category	Landrace/variety
1.0 – 2.0	1.0 – 1.1	0.0 – 7.3	Resistant	Namikonga, **NAMAR050**, **NAMAR130**, NAMAR200, **NAMAR371**, **NAMAR402,** NAMAR444, NAMAR479, NAMAR510, **NAMARX12** *Root weight = 15.2 – 27.8 t/ha; Root no./plant = 4.3 – 8.9; Dry matter content = 24.1 – 29.4%; Harvest index = 0.45 – 0.58*
1.4 – 2.1	1.1– 1.8	15.0 – 32.7	Tolerant	NAMAR013, **NAMAR116,** NAMAR334, NAMAR409, **NAMAR441**, NAMAR601, NAMARX37 *Root weight = 18.2 – 19.5 t/ha; Root no./plant = 2.2 – 3.8; Dry matter content = 22.4 – 35.8%; Harvest index = 0.37 – 0.45*
1.0 – 2.7	1.4 – 3.5	42.0 – 98.9	Susceptible	AR37-80, NAMAR055, NAMAR091, NAMAR097, NAMAR156B, NAMAR284, NAMAR321, NAMAR370, NAMAR435, NAMAR475, NAMAR480, NAMAR492, NAMAR519, NAMAR540, NAMAR 549, NAMAR661

Note: Landraces in bold had the indicated root weight, number of roots per plant, dry matter content and harvest within the various disease reaction categories

4.4 Discussion

Varied responses to CBSD were recorded among F1 populations in the field experiments for two cropping seasons. In the literature, resistance, tolerance and susceptibility have been used interchangeably to describe the response of cassava to CBSD (Cooper & Jones, 1983). Relative to a susceptibility, disease resistance is the reduction of pathogen growth on or in the plant and hence a reduction of disease. Disease tolerance on the other hand describes the exhibition of little disease damage despite substantial pathogen levels (Kang *et al.,* 2005; Cooper & Jones, 1983). Namikonga has been considered resistant to CBSD for many years because it exhibits no or minimal symptoms severity and in this study it had foliar severity scores up to 1.5 with no root necrosis hence had 100% usable roots. The known tolerant variety Pwani had foliar symptom severity score of up to 2.0, but with no visible root symptoms, resulting in 100% usable roots. Susceptible variety AR37-80 expressed high foliar and root severity scores up to 2.7 and accompanying significant reduction in usable roots.

In Pwani × AR37 – 80 F1 population, most of the progenies had high foliar symptoms comparable to both parents. This could be attributed to the susceptible nature of AR37-80 which readily displays both foliar and root symptoms and the tolerant Pwani which expresses leaf symptoms but shows no or delayed root necrosis (IITA, 2012). In the Namikonga × AR37-80 F1 population, there were progenies expressing both leaf and root symptoms ≤2 and were considered resistant while others had leaf symptoms ≥2 with no root necrosis and were considered tolerant. Namikonga had foliar severity up to 1.5 indicating that it could also express disease symptoms under high disease pressure conditions. The Namikonga × AR37-80 cross produced resistant, tolerant and susceptible progenies. A diallel analysis conducted by Kulembeka *et al.* (2012) found that CBSD resistance in Namikonga was due to two or more genes with additive effects. In Additive gene action more than one allele contribute equally to the production of qualitative phenotypes resulting in a variation of phenotypes (Acquuah, 2013). In the Namikonga × AR37-80 F1 population, various phenotypes were observed which led to the categorization of progenies as resistant, tolerant and susceptible. This is corroborated by work from IITA (2012) on the cross between AR11-12 (a CBSD susceptible variety) and Namikonga (a CBSD resistant variety) which produced Pwani, a tolerant variety.

In the Pwani × AR37-80 population, PAR028, PAR057, PAR063 and PAR192 had foliar severity ranging from 1.8 – 3.1, root severity ranging from 1.0 – 1.1, no root necrosis

incidence and 100% usable roots. These can be considered tolerant to CBSD. Contrastingly, PAR043, PAR076 and PAR093 had a foliar severity range of 2.0 – 3.6, root severity range of 2.2 – 3.6, root necrosis incidence range of 63.6 – 96.7%, and usable roots percentage range of 12.4 – 48.2%. These were classified as susceptible to CBSD. In the Namikonga × AR37-80 population, there were F1 progenies with disease reactions comparable to either parent while a few had intermediate disease reactions. NAMAR130, NAMAR200, NAMAR402, NAMAR479, NAMAR510 had a foliar severity range of 1.0 – 1.8, root severity of 1.0, root necrosis incidence range of 0.0 – 1.25%, and usable roots percentage range of 99.3 – 100.0% and were considered resistant. On the other hand, NAMARX12 and NAMAR444 recorded a foliar severity of 2.0, root severity of 1.1, root necrosis incidence range of 3.8 – 6.1, and usable roots percentage range of 98.7 – 100.0%, hence their classification as tolerant. Progenies NAMAR055, NAMAR097, NAMAR56, and NAMAR321 had foliar severity ranging from 1.0 – 2.7, root severity ranging from 2.7 – 3.5, root necrosis incidence ranging from 76.5 – 98.3%, and usable roots percentage ranging from 23.1 – 58.3% and were classified as susceptible.

Pwani had the highest root weight of 30.1 t/ha and this was expected as it is a high yielding variety with a yield potential of 50 t/ha (IITA, 2012). Namikonga had 19.4 t/ha and its lower yield was expected since it has poor root qualities (low harvest index) besides being susceptible to CMD (Masumba *et al.*, 2017; Kaweesi *et al.*, 2014; Kulembeka *et al.*, 2012; Hillocks and Jennings, 2003; Jennings, 1960). AR37-80 on the other hand had very low root weight of 4.3 t/ha and 6.5 t/ha in Pwani × AR37-80 and Namikonga × AR37-80 populations, respectively. AR37-80 is a South American variety and could be poorly adapted hence the low yield observed in both F1 populations (Okogbenin *et al.*, 2007) and some progenies derived from it. F1 progenies in Pwani × AR37 – 80 cross had root weights similar to either parents or intermediate between what the parents had. Similarly, F1 progenies in Namikonga × AR37 – 80 cross had root weights similar to either parents or intermediate between what the parents had. However, a few progenies had significantly higher root weight that both Namikonga and AR37-80 and they included: NAMAR050 (27.1 t/ha), NAMAR091 (31.1 t/ha), NAMAR370 (28.9 t/ha), & NAMAR371 (27.8 t/ha). Their higher yield maybe due to CMD resistance acquired from their CMD resistant parent AR37-80.

CBSD symptoms on leaves and roots can affect key agronomic traits like root weight, root number, dry matter content and harvest index (Aigbe & Remison, 2010). In this study, root weight, dry matter content and harvest index were mostly negatively correlated with

CBSD symptoms. Similar observations have been reported by Aigbe & Remison (2010) and Abaca *et al.* (2012) where the presence of viral disease on cassava plants significantly affects dry matter partitioning in the storage roots. Hillocks *et al*. (2002) also reported that dry matter allocation to cassava leaves and roots occurs between 3 – 6 MAP, a period that coincides with drastic increases in leaf severity hence hampering dry matter content allocation. Number of roots per plant was significantly negatively correlated ($r \geq 0.35$) with both root necrosis and root necrosis incidence showing that high root necrosis and root necrosis incidence caused root rot and as a result reduced number of roots per plant. Negative correlation between root weight and dry matter content showed that an increase in root weight is not necessarily related to increased dry matter content. Environmental conditions such as high rainfall around harvesting time may have resulted in increased root weight contributed largely by increased moisture uptake. There was a highly significant positive correlation between root necrosis and root necrosis incidence ($r \geq 0.93$) in both populations. Both root necrosis and root necrosis incidences were highly negatively correlated with usable roots ($r \geq -0.78$ and resulted in reduced quantities of usable roots.

In Pwani × AR37-80 population, the highest sum of square (SS) were due to genotype by environment interaction (G×E) (42.3 – 45.4%) and genotype (33.6 – 45.7%) while in Namikonga × AR37-80 population, the highest SS was due to genotype (46.2 – 49.8%) followed closely by G×E interaction (44.2 – 46.8%) for CBSD foliar symptoms. This showed that both genotype and G×E interaction contributed almost equally to CBSD foliar symptom expression. SS due to genotype was the highest (55.3 – 63.7%) followed by G×E interaction (28.0 – 39.9%) and finally environment (1.79 – 17.9%) for root necrosis, root necrosis incidence and usable roots in both populations indicating that the variation in the traits was largely due to genotype of progenies although G×E interaction influenced the expression of some progenies. Very high SS due to genotype of 82.3% and 81.7% for Pwani × AR37-80 and Namikonga × AR37-80, respectively were recorded in root weight depicting a highly significant genetic control. On the contrary, SS due environment was either comparable to or higher than SS due to genotype particularly for number of roots per plant, dry matter and harvest index. The mean harvest index for Pwani × AR37-80 was 0.46 and 0.44 in seasons one and two, respectively, while that of Namikonga × AR37-80 was 0.46 and 0.37, respectively. The higher harvestable portions in season one could be attributed to higher quantitiess of rainfall at harvesting in December (102.9 mm) in comparison to 30.5 mm in season two. Rainfall quantities may also have affected dry matter content since a lower mean

dry matter content was recorded in season one for Pwani × AR37-80 (19.5%) and Namikonga × AR37-80 (30.6%) compared to season two where the Pwani × AR37-80 and Namikonga × AR37-80 populations had 25.9% and 34.8%, respectively.

The varied sum of squares showed that although the considered traits were mostly under genetic control, environmental factors significantly influenced the trait expression of a few genotypes. Boakye *et al*. (2013); Pariyo *et al.* (2015) and Tumuhimbise *et al.* (2014) reported similar observations Pariyo *et al.* (2015) reported a high genetic control in evaluated traits as well as significance in mean squares for genotype, environment, and genotype by environment interaction in CBSD root necrosis and necrosis incidence when 19 cassava genotypes were evaluated in three locations. Likewise, Boakye *et al*. (2013) and Tumuhimbise *et al*. (2014) reported a high genetic control and high significance in mean squares of genotype, environment, and genotype by environment interaction in yield related traits.

A study by Nichols (1950) showed that symptom expression and resistance to the virus depended on environmental conditions with severely diseased plants dying at higher altitudes with cooler temperatures. High incidence and severity of symptoms have also been reported during low night temperatures but both stem and root symptoms may disappear or be reduced if conditions become more favourable to plant growth (Jennings, 1957). In this study, there was no significant difference in the rainfall patterns and temperature regime of growing seasons 2014 and 2015 and environment may have not influenced CBSD foliar and root symptoms exression where higher means were observed in growing season 2014. CBSD symptoms are usually variable and irregular and depend on many factors including plant age, cultivar (genotype), environmental conditions (i.e. altitude, temperature, rainfall quantity) and virus species (Patil & Fauquet, 2014; Mohammed *et al.*, 2012; Hillocks & Jennings, 2003).

Although it is challenging to get a genotype with a combination of both CBSD resistance and good root and harvestable qualities (Jennings, 2003), this studies managed to identify some progenies which were minimally affected by CBSD and also had good root qualities. In Pwani × AR37-80 population, tolerant progenies PAR024, PAR057, PAR064, & PAR192 had the highest root weight (25.4 – 33.2 t/ha) and moderate dry matter content ranging from 21.0 – 22.9%. In Namikonga × AR37-80 population, resistant progenies NAMAR050, NAMAR130, NAMAR371, NAMAR402, & NAMARX12 had high root weights ranging from (15.2 – 27.8 t/ha) and moderate dry matter content ranging from (24.1 – 29.4 %) while tolerant progenies NAMAR116 and NAMAR441 had root weight ranging

from 18.2 – 19.5 t/ha and moderate to high dry matter content ranging from 22.4 – 35.8%. Results in this study showed that there were F1 progenies with low CBSD symptoms expression that could be used as reliable sources of CBSD resistance/tolerance.

4.5 Conclusion

ANOVA revealed significant difference among the progenies in the traits evaluated including CBSD foliar symptoms, root necrosis, root necrosis incidence, usable roots, root weight, root number per plant dry matter content and harvest index. Sum of squares (SS) showed that CBSD foliar symptoms expression was equally influenced by both genotype and genotype by environment interaction. SS due to genotype was the highest (55.3 – 82.7%) for root necrosis, root necrosis incidence, usable roots and root weight in both populations indicating that the variation in the traits was largely due to genotype of progenies. Finally, SS due environment was either comparable to or higher than SS due to genotype particularly for number of roots per plant, dry matter and harvest index. Disease traits were mostly negatively correlated with yield related traits and a high negative correlation ($P \leq 0.001$, $r > 0.78$) was recorded between root necrosis and usable roots and between root necrosis incidence and usable roots. In the Pwani × AR37-80 F1 population, tolerant progenies identified included: PAR024, PAR057, PAR064, & PAR192 and they had the highest root weight (25.4 – 33.2 t/ha) and moderate dry matter content ranging from 21.0 – 22.9%. In Namikonga × AR37-80 population, resistant progenies identified included: NAMAR050, NAMAR130, NAMAR371, NAMAR402, & NAMARX12 and had the highest root weights ranging from (15.2 – 27.8 t/ha) and moderate dry matter content ranging from (24.1 – 29.4 %) while tolerant progenies NAMAR116 and NAMAR441 and had root weight ranging from 18.2 – 19.5 t/ha and moderate to high dry matter content ranging from 22.4 – 35.8%. The findings indicate that these can be used in future breeding programmes to generate cassava varieties with farmer preferred traits.

4.6 Recommendations

These findings are based on CBSD data generated from two planting seasons in a CBSD hot spot area. Further CBSD and other yield related traits evaluation could be carried out in diverse CBSD hot spot sites combined with diverse seasons to confirm the genotypic reactions recorded in this study. In addition, F1 progenies can similarly be screened in CMD hotspot areas to substantiate if CMD resistance was introgressed into these progenies from

the CMD resistant parent AR37-80. There were resistant progenies but poor yield related traits. In view of the difficulties associated with conventional breeding, apart from backcrossing to restore and enhance yield and quality of the tuberous roots produced, genetic engineering would offer potential for the rapid transfer of resistance genes to the traditional cultivars.

CHAPTER FIVE

ANALYSIS OF SYMPTOMS EXPRESSION AND VIRUS ACCUMULATION IN CBSV GRAFT INOCULATED CASSAVA VARIETIES

5.0 Abstract

Cassava brown streak disease (CBSD) is a viral disease and an important biotic constaint to cassava production. The most feasible and sustainable control of CBSD is through deployment of resistant varieties that restrict virus multiplication and symptom development, thus limiting the spread and impact of the virus. Conventional screening for CBSD resistance in the field may have 'escapes' where test varieties are not inoculated. This study therefore aimed at characterization of cassava varieties through graft inoculation and monitoring virus accumulation and symptoms expression with time, with the aim of idenfiying varieties that restict virus accumulation and symptoms development. Graft inoculation was carried out on 12 varities with cassava brown streak virus (CBSV) and foliar symptoms severities were recorded as well as virus accumulation monitored by quantitative real time PCR at 3, 4, 8, 12, 16, 20, and 24 weeks after grafting. Virus accumulation and symptoms expression on roots was also recorded at 32 weeks when the roots were harvested. The correlation between change in foliar symptoms severity with time and change in foliar virus quantity with time was studied and the varieties showed different plant defence mechanisms. Although Kaleso (r = 0.89) and Nase 1 (r = 0.58) had a positive correlation, they nonetheless had comparable restricted virus accumulation and CBSD symptom development, portraying their resistance to CBSD. On the contrary, Kibandameno (r = 0.94), Ebwanateraka (r = 0.99), CHO5/2003 (r = 0.95) and LM1/2008/363 (r = 0.92) had high positive correlation, but with significantly higher virus quantities and severe foliar symptoms when compared to Kaleso and Nase 1. This portrayed their susceptibility to CBSD. Colicanana (r = -0.85), MECU 72 (r = -0.86), Mkuranga 1 (r = -0.45), and Sauti (r = -0.68) had a negative correlation as they accumulated high virus quantities but developed minimal symptoms or vice versa. Apart from Kaleso, Nase 1 can be a good progenitor in breeding for CBSD resistance. This information is beneficial for implementing knowledgeable breeding strategies with the aim of minimizing CBSV dissemination and achieving durable CBSD resistance.

***Key words*:** Cassava, CBSV resistance, Graft inoculation, Virus quantification, qRT-PCR.

5.1 Introduction

CBSD has been wide spread in the East African Coastal areas since its first description in northern Tanzania in 1936 (Tomlinson *et al*., 2017). It is an important disease caused by viruses: Ugandan cassava brown streak virus (UCBSV) and cassava brown streak virus (CBSV). These two viruses belong to the genus *Ipomovirus* in the family *Potyviridae* and they have (+) ss RNA genomes (Legg *et al.,* 2011; Ndunguru *et al.,* 2015; Vanderschuren *et al.,* 2012; Winter *et al.,* 2010). CBSV and UCBSV produce similar disease symptoms although the latter causes milder symptoms and lower pathogenicity. CBSD causes economic losses resulting from damage to the aboveground parts including leaf chlorosis, leaf necrosis, stem lesions/die back and stunted growth on infected plants (Hillocks & Jennings, 2003; Winter *et al.,* 2010). Damage to below ground parts include root constrictions and dry corky necrotic rot on starchy tissues which renders roots unpalatable and unmarketable (Hillocks & Jennings, 2003; Winter *et al.,* 2010).

The CBSD symptoms are usually variable and irregular and depend on many factors including plant age, cultivar (genotype), environmental conditions (i.e. altitude, temperature, rainfall quantity) and virus species (Patil & Fauquet, 2014; Mohammed *et al.,* 2012; Hillocks & Jennings, 2003). Virus infected plants normally display different plant defense mechanisms including resistance which is the ability of the host to restrict or hinder pathogen invasion, development or multiplication. Tolerance on the other hand refers to plants that show minimal disease damage despite substantial pathogen levels and susceptibility is the sum-total of qualities that make a plant fit for pathogen establishment and multiplication (Miller *et al.,* 2005).

Control strategies for CBSD have been focussing on host plant resistance especially with Amani hybrid genotypes i.e. Kaleso (Namikonga) which has been a consistent resistant parent in many breeding programmes. Kulembeka *et al.* (2012) reported that Namikonga had the highest general combining ability for CBSD resistance. Recently, breeding efforts have led to the identification of several cassava varieties with CBSD resistance/tolerance including: Kiroba and Pwani. These varieties readily express leaf symptoms but with no, mild or delayed root symptoms (Kaweesi *et al.,* 2014; Mohammed *et al.,* 2012). They give some relief against the disease but nonetheless sustain and promote the spread of the virus. Therefore, there is urgent need of CBSD resistant farmer preferred varieties that do not replicate/propagate the virus to minimise the impact of the disease on affected populations.

It is worth noting that CBSD resistance breeding programs have been aiming at producing varieties with minimal root necrosis. Foliar symptoms expression has been considered acceptable provided that root symptoms are absent or mild. Previous studies have shown that plants that develop severe foliar symptoms also tend to accumulate high virus quantities and consequently promote the dissemination of the virus (Kaweesi *et al.,* 2014; Maruthi *et al.,* 2014a). This suggests that breeding perspectives in the future should therefore focus on not only minimizing both foliar and root symptoms but also virus quantities. Detection and quantification of viral RNA by reverse transcription-PCR (RT-PCR) and quantitative real time PCR (qRT-PCR) have been popular due to their high sensitivity, reliable specificity, and their ability to measure viral titre precisely (Adams *et al.,* 2013). These procedures can make an immediate impact in the identification of CBSD resistance.

The aim of this study was to characterize CBSD resistance of selected cassava varieties by quantifying virus accumulation and evaluating the relationship between relative virus quantity and symptom severity on leaves and roots.

5.2 Materials and methods

5.2.1 CBSV establishment

Twelve varieties were tested including Kaleso a known resistant variety (Table 22). Stem cutting were obtained from disease free cassava and potted in a mixture of compost soil and peat moss at a ratio of 1:1. The plants were maintained at a relative constant environment at 28 ± 5°C and 50 - 60% relative humidity (RH) in a quarantine glasshouse at the Natural Resources Institute-UK. The severe CBSV isolate collected from Nampula, Mozambique (CBSV-[MZ:Nam1-1:07]) was used to inoculate the cassava varieties (Mohammed *et al.,* 2012; Patil *et al.,* 2010). The isolate had been reared in a very susceptible cassava variety ‘Naliendele’ through graft inoculation and maintained at a relative constant environment at 28 ± 5°C and 50 - 60% RH which was good for symptom development. Stem cuttings were obtained from CBSV infected variety Naliendele and potted. The infected cuttings were used as scions during graft inoculation.

Table 22: Pedigree of cassava varieties tested

Common name	Pedigree	Country of origin
LM1/2008/363	Clone (yet to be released)	Kenya
Kibandameno (TME 1786)	IITA landrace accession	Kenya
Kaleso	Known as Namikonga in Tanzania. Third backcross from inter-specific hybrid (46106/27) from *M. glaziovii* from Amani breeding program (Resistant control)	Kenya
Sauti (CH92/077)	IITA landrace accession	Malawi
CHO5/203	Clone (yet to be released)	Malawi
Kalawe (CH02/0066)	Locally bred/improved variety	Malawi
Colicanana	Improved Cassava variety developed by IITA	Mozambique
Mkuranga1 (KBH 2002/066)	Cassava variety tolerant to CMD and developed by IITA from clone 96/1613 HS	Tanzania
Kipusa (KBH 2006/026)	Cassava variety tolerant to CMD and developed by IITA	Tanzania
Ebwanateraka	Local landrace	Uganda
Nase 1 (TMS 60142)	Cassava line developed by IITA, from clone 58308 of Amani breeding programme (Hahn *et al.,* 1989)	Uganda
Mecu 72	Introduction from CIAT which is also resistant to whiteflies (Bellotti & Arias, 2001)	Colombia

5.2.2 Graft inoculation

Graft inoculation was carried out when both clean and infected plants were three months old. Five clean plants per variety were graft inoculated with scions (10 cm in length) from infected var. Naliendele expressing clear CBSD symptoms, while two control plants from each variety were grafted with scions from healthy plants. To prevent the excessive loss of moisture and drying of scions, they were enclosed in plastic bags with a few punch holes. Moisture was maintained within the plastic bags till the scions were established; approximately two weeks after graft inoculation. The protective plastic bags were there after

removed and plants kept in the glasshouse for symptoms observation. Plants were maintained in a relatively constant environment at 28 ± 5°C and 50 - 60% RH for symptom development.

5.2.3 CBSD symptoms scoring

CBSD foliar symptoms were recorded and photos taken for each variety at 2, 3, 4, 8, 12, 16, 20 and 24 weeks after grafting. The severity of the foliar symptoms was rated on a scale of 1 to 5 according to Hillocks *et al*. (1996) as described in section 3.2.4. Prior to harvesting of roots, the plants were subjected to water stress from 24 – 32 weeks after grafting to enhance root bulking. At 32 weeks after grafting, roots were harvested. Unfortunately, most of the varieties did not develop tuberous roots and only had fibrous roots. However, Ebwanateraka, Colicanana, and Kipusa developed tuberous roots and were observed for CBSD root symptoms by cutting the roots at 1 cm interval and photos taken. Both foliar and root symptoms were photographed using camera (Nikon D5000).

5.2.4 Sampling for virus detection and quantification in cassava

Sampling was done according to Abarshi *et al*. (2010) and leaves and root samples were collected from the ten varieties grafted. Data was not collected for Kipusa and Kalawe to avoid variability in experimental results as both had only one surviving plant. Leaf samples were collected at 2, 3, 4, 8, 12, 16, 20 and 24 weeks after grafting from leaves (third or fourth leaf from top). 100 mg of diseased fresh plant leaf tissue were punched into eppendorf tubes, frozen rapidly in liquid nitrogen and stored at -80 °C prior to CBSV testing. Roots were harvested at 32 weeks after grafting and were stored at -80°C prior to CBSV testing.

5.2.5 Nucleic acid isolation and CBSV amplification

Nucleic acids were extracted from leaves following the CTAB protocol described by Lodhi *et al*. (1994) and optimized for cassava viruses by Maruthi *et al.* (2002). The CTAB extraction buffer contained (2% (w/v) CTAB, 1.4 M NaCl, 1% (v/v) 2-mercaptoethanol, 20 mM EDTA, and 100 mM Tris-HCl, pH 8.0). The buffer was preheated to 60°C and 2-mercaptoethanol added fresh before use. CTAB buffer (1 ml) was added to each 2 ml Eppendorf tube containing approximately 100 mg of leaf tissue sample and stainless steel beads. The leaf tissues were finely crushed using Tissue-Lyser II - Qiagen at 30 Hz per minute for 10 minutes. Seven hundred and fifty microlitres of the sample was poured into a 1.5 ml eppendorf tube before heating the samples at 60°C for 30 minutes. An equal volume

(750 µl) of phenol:chloroform:isoamylalcohol (25:24:1) was added to the samples and centrifuged at 13000 revolutions per minute (rpm) for 10 minutes. Five hundred microliters of supernatant was transferred into new eppendorf tubes and 500 µl of cold (-20°C) isopropanol added to precipitate DNA. The samples were incubated at -20°C for at least 1 hour followed by centrifuging at 13000 rpm (4°C) for 10 minutes. The supernatant was discarded and pellet washed in 500 µl of 70% ethanol by vortexing then centrifuging for 5 minutes at 13000 rpm. The ethanol was poured out and DNA pellet vacuum-dried for 5 minutes. The dried pellet was suspended in 100 µl of 1X TE buffer and stored at -20°C.

Nucleic acids were also extracted from approximately 100 mg of root tissue pooled from different roots per plant using the same protocol, although the phenol:chloroform:isoamylalcohol step was done twice for extra purity and removing any remaining traces of proteins and lipids (Abarshi *et al*., 2010). In addition, NaCl concentration was increased to 3.0 M for CTAB root extraction buffer to purify nucleic acids from salts. The quality of nucleic acids extracted were assessed using Nanodrop 2000 (Thermo scientific) and the ones that presented 260/280 and 260/230 purity indices equal to or greater than 2.0 and were selected for cDNA synthesis. cDNA synthesis was carried out using QuantiTect reverse transcription kit (Qiagen) following the manufacturer's instructions. The amount of RNA used in each cDNA synthesis reaction was 1µg as recommended by Moreno *et al*. (2011).

The nucleic acids were assessed using Nanodrop 2000 and the ones that presented 260/280 and 260/230 purity indices equal to or greater than 2.0 and were selected for cDNA synthesis which was carried out using QuantiTect reverse transcription kit – Qiagen following the manufacturer's instructions. CBSVs-specific reverse (CBSV R4) and forward (CBSV F3) primers and Iowa black FQ probe with fluorescent dye JOE were used for virus amplification (Otti *et al.,* 2016; Abarshi *et al.,* 2012 and 2010) (Table 24). Previously identified reference gene PP2A and BHQ1 probe with fluorescent dye JOE were used as internal controls for data normalization (Otti *et al.,* 2016; Moreno *et al.,* 2011) (Table 24). A typical qPCR reaction mixture contained Invitrogen 2 × qPCR master mix (10µl), CBSV/PP2A forward and reverse primers (2 µl), Iowa black FQ probe/BHQ1 probe (0.2µl), cDNA (1 µl) and water (4.8 µl) adding up to a total volume of 20 µl. The mixture was dispensed into qPCR plates which were then sealed using adhesive seals to provide protection against evaporation. Thermal cycling conditions used in qPCR were as follows: (50°C, 2 minutes), (94°C, 2 minutes), (94°C, 15 seconds, 40 cycles), (54°C, 20 seconds, 40 cycles)

and (60°C, 30 seconds). The qRT-PCR reactions were performed with a Thermal Cycler Real Time system.

CBSVs-specific reverse (CBSV R4) & forward (CBSV F3) primers and Iowa black FQ probe with fluorescent dye JOE were used for virus amplification (Table 23). Previously identified reference gene PP2A and BHQ1 probe with fluorescent dye JOE were used as internal controls for data normalization (Table 1). PP2A was used as an internal control for normalization of gene expression data, since it is a host reference gene with stable expression patterns in cassava (Liu *et al.,* 2012; Moreno *et al.,* 2011). A typical qPCR reaction mixture contained Invitrogen 2 × qPCR master mix (10 µl), CBSV/PP2A forward and reverse primers (2 µl), Iowa black FQ probe/BHQ1 probe (0.2 µl), cDNA (1 µl) and water (4.8 µl) adding up to a total volume of 20 µl. The mixture was dispensed into Biorad qPCR plates which were then sealed using micro seal 'B' adhesive seals (Bio-Rad, USA) to provide protection against evaporation. Thermal cycling conditions used in qPCR were as follows: (50°C, 2 minutes), (94°C, 2 minutes), (94°C, 15 seconds, 40 cycles), (54°C, 20 seconds, 40 cycles) and (60°C, 30 seconds). The RT-qPCR reactions were performed using the CFX Connect™ Real-Time PCR Detection System (Bio-Rad, USA).

5.2.6 Data analysis

Amplification data was analyzed using the CFX maestro sofware. Relative quantifications were performed based on the quantification cycle (Cq) method described by Livak & Schmittgen (2001). Cq is the cycle at which the fluorescence of a sample crosses a threshold line or first increases above baseline fluorescence. The fold change (relative quantity) in virus n(target gene) relative to the reference gene (PP2A) at various time points was determined by the formula given as:

$$2^{\wedge -\Delta\Delta Cq} = 2^{\wedge -(\Delta Cq\ sample\ -\ \Delta Cq\ control)} \qquad (5.1)$$

where: $2^{\wedge -\Delta\Delta Cq}$ = Relative quantity,
ΔCq sample = [(Cq $_{CBSV}$) - (Cq $_{PP2A}$)] sample
ΔCq control = [(Cq $_{CBSV}$) - (Cq $_{PP2A}$)] positive control

The Cq values for samples were derived from the graft inoculated/infected plants. While Cq values for positive control was derived from susceptible variety

Kibandameno.Virus quantities were normalized to the concentration of the virus detected in Kibandadameno at the 2 weeks time point.

Foliar severity and relative virus quantity data at different time points were subjected to analysis of variance (ANOVA) using R statistical software's generalized linear model with quasi-binomial errors and a logitlink. On the other hand, quasi-poisson errors and a logitlink was used for ANOVA of count parameters (foliar and root virus quantities) (Kabacoff, 2011). Means were separated using Dunnett's method to compare each variety against resistant control (Kaleso), at 95% confidence level. Change in foliar symptoms severity with time and change in foliar virus quantity with time for each plant in each variety was calculated using the formula below:

$$Slope(m) = \frac{\Delta y}{\Delta x} \quad where: \Delta y = change\ in\ y,\ \Delta x = change\ in\ x \tag{5.2}$$

Correlations were there after calculated using the slope values for each plant in each variety. It is worth noting that varieties Kalawe and Kipusa had one surviving plant each therefore data collected from them was not analysed. However, photos were taken to show their CBSD symptoms severity.

Table 23: Primers used for detecting CBSVs in qRT-PCR

Virus / target gene	EMBL/Gene Bank accession number	Primer/probe	Sequence (5'-3')	Quantity used (µ mol l^{-1})	Reference
CBSV	FN434436	CBSV F3	GGARCCRATGTAYAAATTTGC	2.0	(Abarshi *et al.*, 2012)
		CBSV R4	GCWGCTTTTATYACAAAMGC	2.0	(Abarshi *et al.*, 2012)
		Probe	JOE-TTCCAGCCA/ZEN/AGCAATWYTG ATGTATCAGAATAGTGTGA-Iowa black FQ	0.2	(Otti *et al*., 2016)
PP2A	CK650945	PP2AF	TGCAAGGCTCACACTTTCATC	2.0	(Moreno *et al*., 2011)
		PP2AR	CTGAGCGTAAAGCAGGGAAG	2.0	(Moreno *et al*., 2011)
		Probe	JOE-CTTTCTGTTGTGCCCCCACCATGC-BHQ1	0.2	(Otti *et al.*, 2016)

5.3 Results

5.3.1 CBSD symptoms

Graft inoculation was successful (Figure 8) with the most sensitive varieties (Kibandameno and CHO5/2003) showing symptoms with a mean (1.3) at 2 weeks after grafting (Table 24). Symptoms detection commenced at 4 weeks after inoculation for the other varieties and was observed till 24 weeks. The resistant control Kaleso, maintained mild foliar severity throughout the experiment with lowest mean score (1.0) at 2 weeks and a maximum (2.5) at 24 weeks (Table 24, Figure 9). A similar trend was observed in Nase 1, which had a minimum score (1.0) at 2 weeks and a maximum (2.8) at 20 weeks. Varieties LM1/2008/363, Kibandameno, Mecu 72, Ebwanateraka, Sauti and Colicanana had significantly ($P \leq 0.01$) higher foliar severity than Kaleso that ranged from 1.5 – 4.8, between 3 – 20 weeks after grafting. Ebwanateraka, LM1/2008/363 and CHO5/203 had a steady increase of symptoms with the highest mean severity (4.0), recorded at 24 weeks (Table 24). The observation of foliar symptoms in Mkuranga 1 commenced at 12 weeks after graft inoculation and a maximum foliar severity (3.0) observed between 16 – 24 weeks. CBSD can severely affect plant foliage as was observed in Colicanana and Sauti. These varieties developed severe foliar symptoms and die-back (Figure 9), but they tended to recover and regenerate new shoots. The development of new shoots resulted in lower foliar severity at certain time points for example Colicanana at 12 weeks and Sauti at 24 weeks.

Figure 8: Successful grafts. A - Ebwanateraka, B – Nase 1, C – Mecu 72, D – Kibandameno, and E – Well established graft union.

Ebwanateraka, Kipusa, and Colicanana developed tuberous roots and photos were taken to show CBSD symptoms severity. Ebwanateraka showed brownish streaks on both the

pith and rind while Kipusa had moderate root necrosis (Figure 10). Colicanana on the other hand depicted severe root damage as it had extensive dry corky necrotic rot (Figure 10).

5.3.2 Foliar and root virus quantities

Foliar virus was detected early at 2 weeks in varieties Kibandameno and CHO5/203 (Table 25). Kaleso and Nase 1 maintained the lowest foliar virus quantities throughout the experiment with a maximum of 2622.3 and 3249.6 folds, respectively, at 24 weeks. Further, at 24 weeks, significantly ($P \leq 0.05$) high foliar virus quantities ranging from 15260 – 16270 folds were recorded in Ebwanateraka, Sauti, CHO5/203, LM1/2008/363, MECU 72, Mkuranga 1, and Kibandameno (Table 25). Although Colicanana had severe foliar severity, it had low foliar virus quantities comparable to Kaleso and Nase 1. It mostly had necrotic leaves with low virus quantities which increased when the plant recovered and developed new shoots. Similarly, significantly high root virus quantities ranging from 10054 – 59759.0 folds were recorded in Kibandameno, LM1/2008/363, Sauti, CHO5/203, Ebwanateraka, and Mecu 72. While Colicanana had the highest root virus quantity of 951968.6 folds, Mkuranga 1, Nase 1 and Kaleso registered the lowest root virus quantities of 4836.3, 3249.6, and 2622.3 folds respectively.

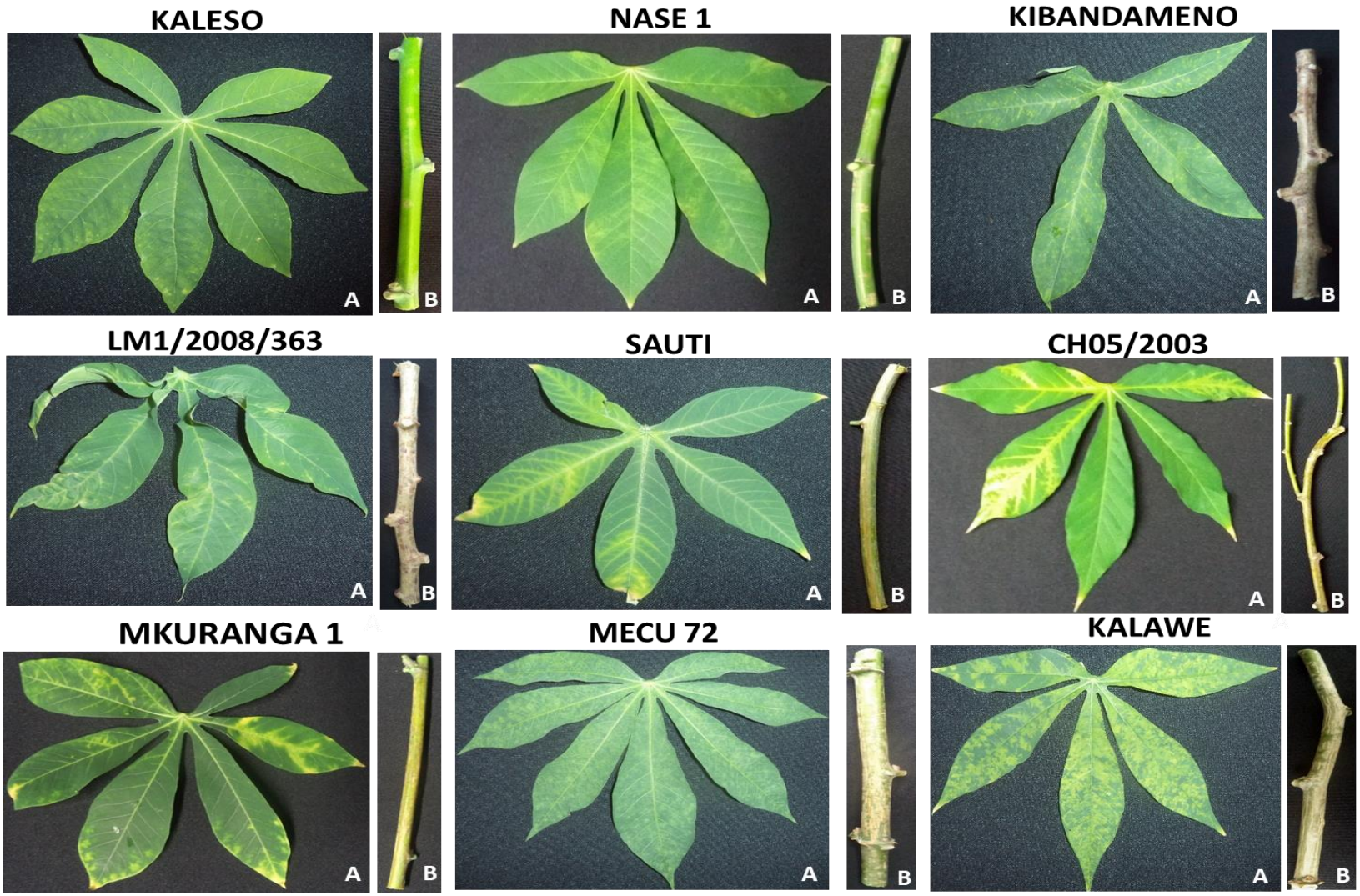


Figure 9: Depiction of leaf and stem symptoms on varieties without tuberous roots; A – leaf symptoms, B – stem symptoms.

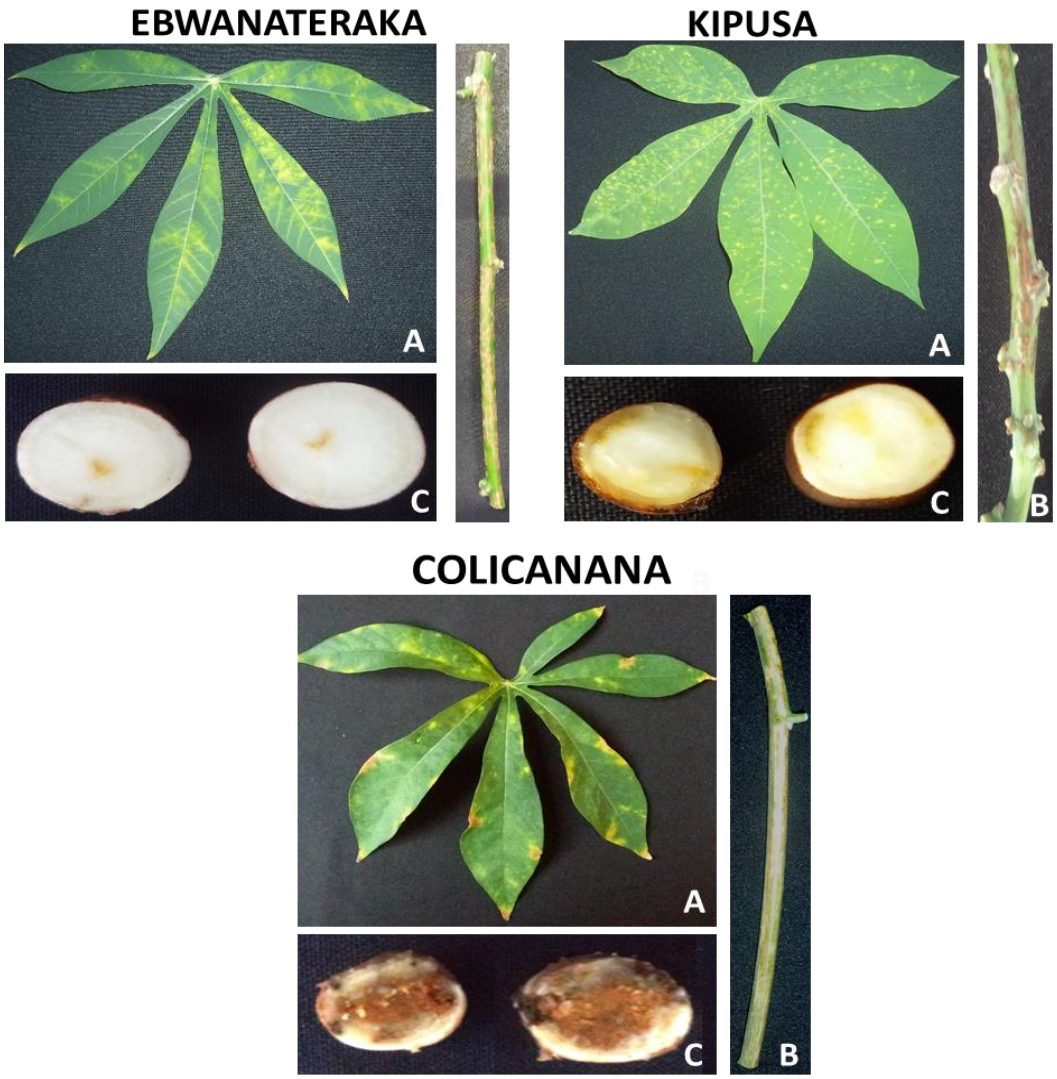
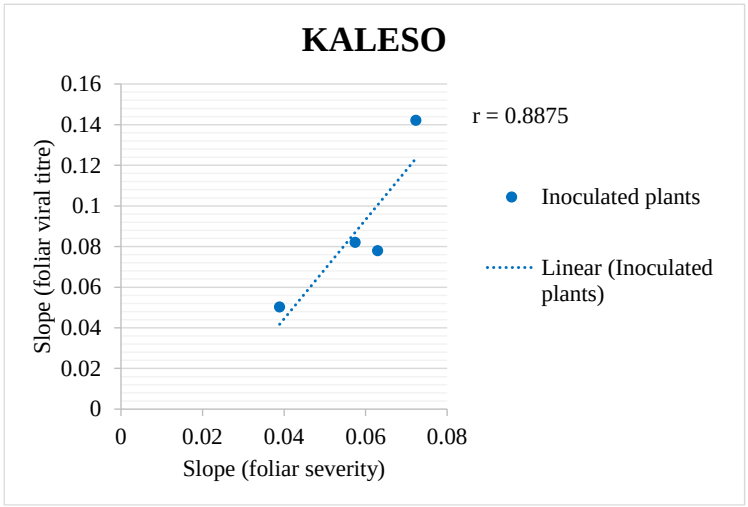


Figure 10: Depiction of leaf stem, and root symptoms on varieties that developed tuberous roots; A – leaf symptoms, B – stem symptoms, C – root symptoms

5.3.3 Disease reactions based on change in foliar symptoms and change in foliar virus quantity with time

Correlation between change in foliar symptoms/change in foliar virus quantity (x) with time (y) revealed different trends of disease reaction. Correlation was calculated to indicate the proportion of the variance in the dependent variable (y) that is predictable from the independent variable (x). High positive correlation was observed in Kaleso (r= 0.8875) (Figure 10), implying that 89% of the variability in foliar severity can be accounted for by the foliar virus quantity. In this variety, foliar virus quantity and symptoms increased concurrently but were generally maintained at low levels. A moderate positive correlation (r = 0.5812) was observed in Nase 1 (Figure 11). This variety maintained low foliar severity but slightly higher virus quantities than Kaleso. Additionally, although foliar virus quantities were either increasing or decreasing between 12 to 20 weeks, the foliar severity nonetheless remained constant at 2.0 for Nase 1. A high positive correlation was also observed in Kibandameno (r = 0.9348), Ebwanateraka (r = 0.9993), LM1/2008/363 (R^2 = 0.9193) and CHO5/203 (R^2 = 0.5919) (Figure 12). The foliar severity and virus quantities for these varieties increased simultaneously throughout the experiment and were significantly higher compared to Kaleso and Nase1. Some varieties (Mecu 72 (r = 0.8637), Sauti (r = 0.6843), Colicanana (r = 0.8479) and Mkuranga 1 (0.4455) had negative correlations indicating that high foliar severity does not always result in high foliar virus quantities (Figure 13). Mecu 72 showed increased foliar severity with reduced foliar virus quantity and vice versa at different time points. Sauti and Colicanana had high foliar severity with no/little detectable virus quantities between 4 and 12 weeks after inoculation (Table 24 and 25). High foliar severity seemed an impediment to virus accumulation in Sauti and Colicanana, as increased virus quantities were only observed when the plants recovered and new shoots with minimal severity emerged. Additionally, variety Mkuranga 1, for example, had detectable virus quantities from 3 – 8 weeks even though it was not showing any foliar symptoms. The foliar severity and virus quantities however increased concurrently from 16 to 24 weeks.



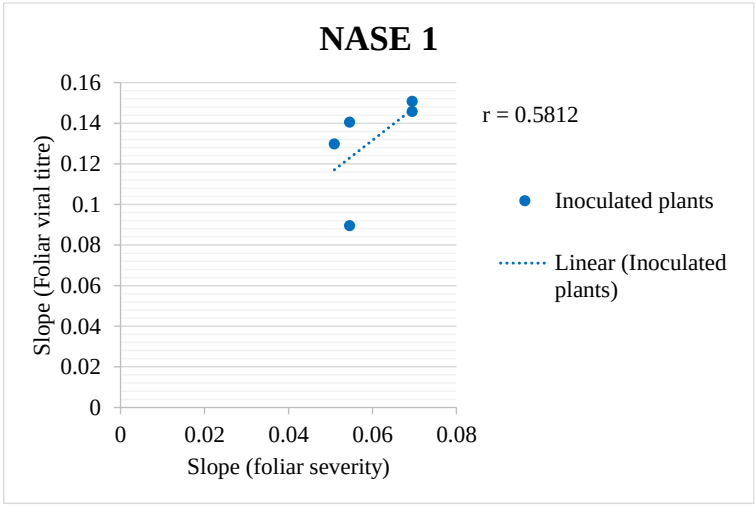
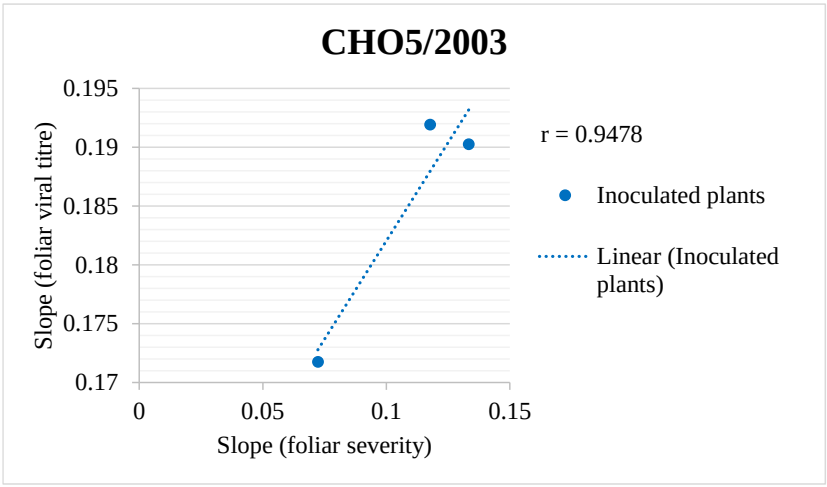
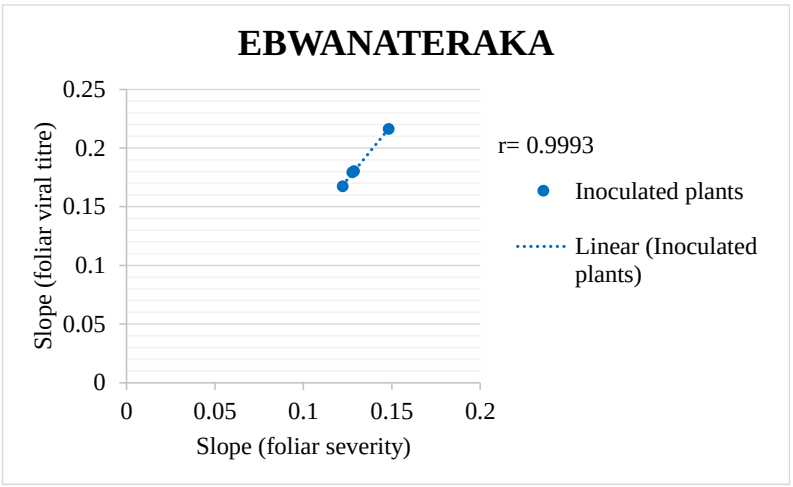
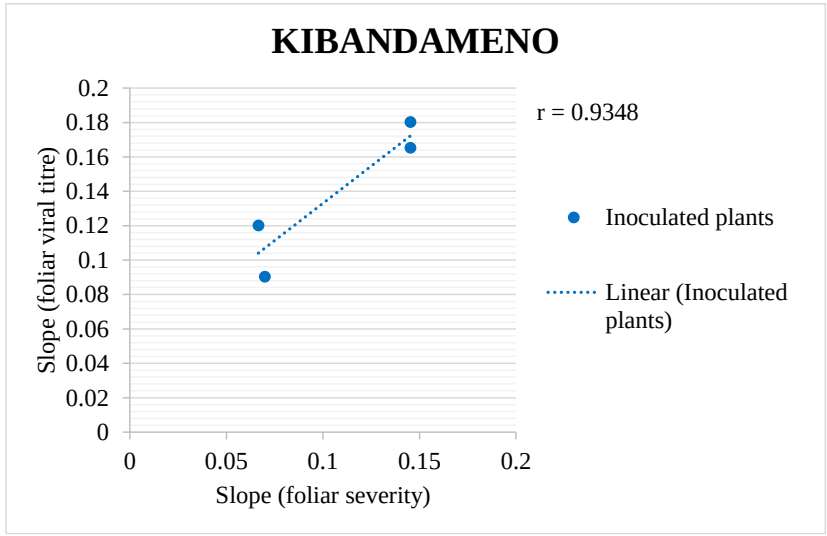


Figure 11: Varieties showing positive correlation with low foliar severity and foliar viral relative quantity







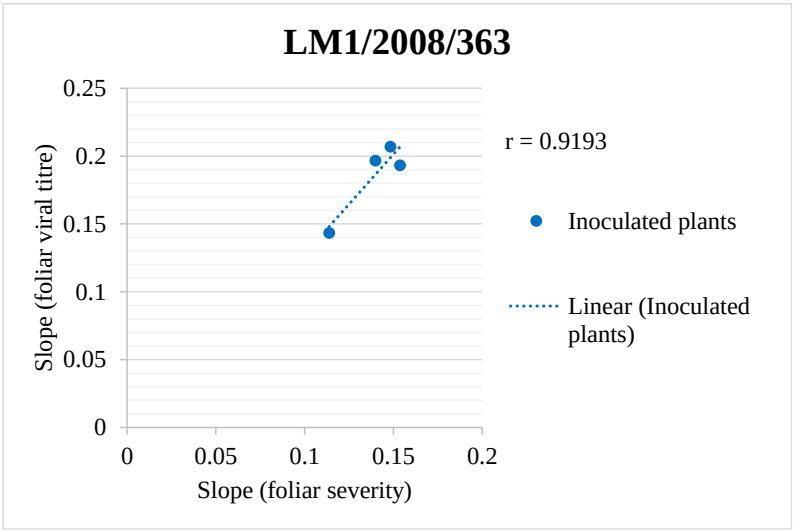
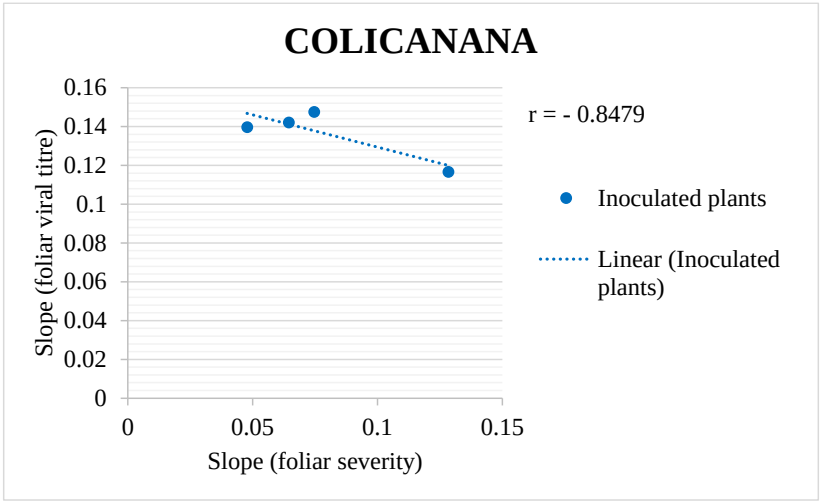
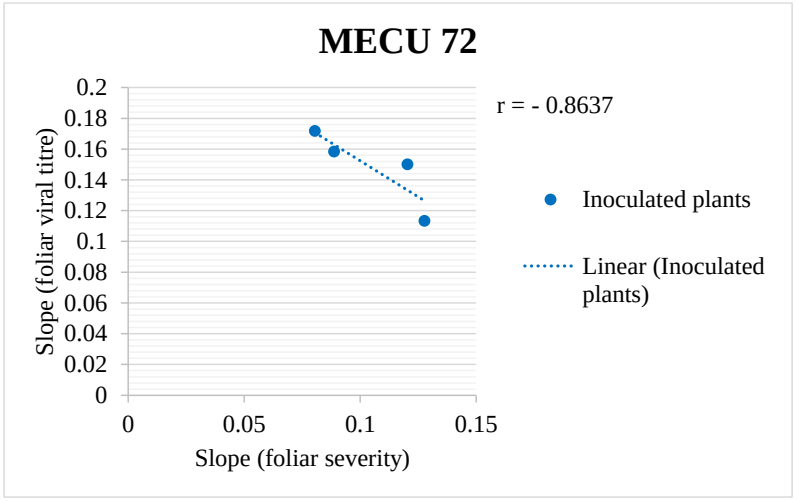
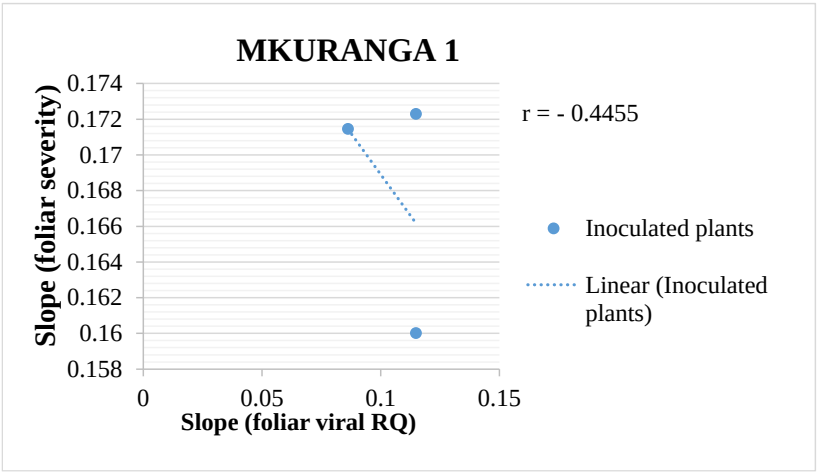


Figure 12: Varieties showing positive correlation with high foliar severity and foliar viral relative quantity







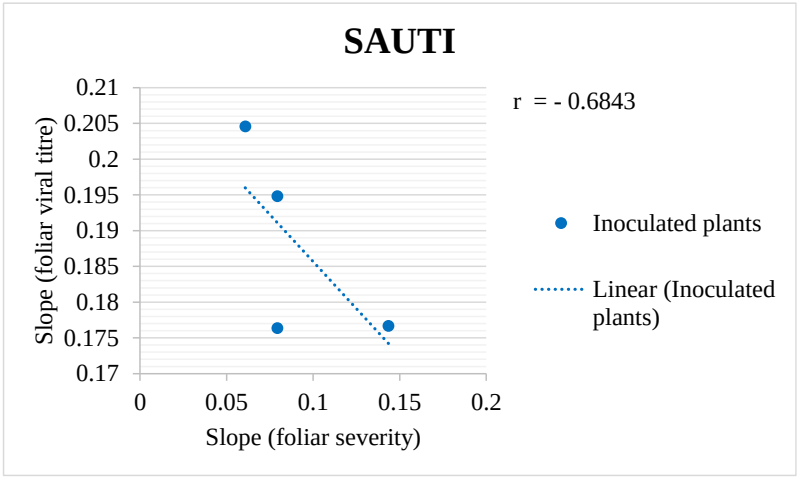


Figure 13: Varieties showing negative correlation between foliar severity and foliar viral relative quantity

Table 24: Means of foliar symptoms across time for different varieties

Variety	Foliar symptoms							
	Time in weeks after graft inoculation							
	2	3	4	8	12	16	20	24
Kaleso	**1.0±0.0**	**1.0±0.0**	**1.3±0.2**	**1.3±0.2**	**1.8±0.3**	**1.8±0.2**	**1.8±0.3**	**2.5±0.2**
LM1/2008/363	1.0±0.0	1.0±0.0	1.3±0.2	1.8±0.5	2.5±0.3	2.8±0.3	3.5±0.3*	4.0±0.1
Kibandameno	1.3±0.0	1.5±0.1*	1.8±0.4	2.0±0.4	3.8±0.3*	3.8±0.3**	3.8±0.3**	3.0±0.2
Mecu 72	1.0±0.0	1.0±0.0	1.6±0.3	3.2±0.4*	3.0±0.3	2.8±0.3	3.8±0.3**	3.3±0.2
Ebwanateraka	1.0±0.0	1.0±0.0	1.7±0.4	2.7±0.6	3.0±0.4	2.3±0.3	4.0±0.3***	4.0±0.2
Sauti	1.0±0.0	1.0±0.0	4.2±0.3***	4.2±0.4***	4.2±0.2***	2.0±0.2	4.8±0.2***	3.0±0.2
Nase 1	1.0±0.0	1.0±0.0	1.4±0.3	2.0±0.4	2.0±0.3	2.0±0.2	2.8±0.3	2.0±0.1
Kipusa	-	-	-	-	-	-	-	-
Mkuranga 1	1.0±0.0	1.0±0.0	1.0±0.0	1.0±0.0	2.3±0.4	3.0±0.4	3.0±0.5	3.0±0.2
CHO5/203	1.3±0.1	1.3±0.1	1.3±0.6	1.7±0.4	1.7±0.3	2.0±0.3	3.0±0.4	4.0±0.2
Kalawe	-	-	-	-	-	-	-	-
Colicanana	1.0±0.0	1.0±0.0^{ns}	4.0±0.4***	4.8±0.2***	3.0±0.4^{ns}	4.3±0.2***	3.7±0.4***	3.3±0.2
Mean (μ)	1.1±0.0	1.1±0.0	2.0±0.3	2.6±0.3	2.8±0.3	2.6±0.3	3.4±0.3	3.1±0.2

-μ - population mean, ± - standard error

-Means separation done by dunnett test (Kaleso is the control)

-Statistical significance: nonsignificant * = $P \leq 0.05$, ** = $P \leq 0.01$, *** = $P \leq 0.001$

Table 25: Means and standard errors of foliar virus relative quantities from 2 to 16 weeks after graft inoculation

Variety	Foliar virus quantities					
	Time in weeks after graft inoculation					
	2	3	4	8	12	16
Kaleso	**0.00±0.0**	**0.00±0.0**	**0.78±0.1**	**352.3±18.6**	**457.0±45.3**	**40.3±5.4**
LM1/2008/363	0.00±0.0	0.00±0.0	0.13±0.03	4505.2±353.1***	4681.9±284.9**	9842.1±448.7***
Kibandameno	0.30±0.03	256.2±23.9***	161.0±25.7***	6397.6±684.7***	5825.6±293.9***	2030.2±47.4***
Mecu 72	0.00±0.0	0.00±0.0	83.7±6.4	1969.2±126.2***	11405.0±800.2***	3145.6±261.3***
Ebwanateraka	0.00±0.0	0.00±0.0	172.2±22.1***	3723.1±497.5***	2785.9±345.5	1673.4±232.9***
Sauti	0.00±0.0	0.00±0.0	0.00±0.0	0.00±0.0	0.00±0.0	423.7±48.7
Nase 1	0.00±0.0	0.00±0.0	1554.5±86.1***	15.1±3.0	3727.3±197.6*	596.5±63.7
Kipusa	-	-	-	-	-	-
Mkuranga 1	0.00±0.0	67.7±3.2***	70.5±2.5	3750.0±398.1***	835.8±80.6ns	787.5±108.2
CHO5/203	0.17±0.01	141.3±1.2***	129.6±6.0**	134.1±30.9	32741.0±2944.8***	3513.5±189.0***
Kalawe	-	-	-	-	-	-
Colicanana	0.00±0.0	0.00±0.0	0.00±0.0	48.0±3.0	3273.7±323.7	713.3±100.3
Mean (μ)	0.02±0.01	24.5±10.6	170.3±81.1	1905.9±408.2	6354.7±1444.9	2340.5±488.9

-μ - population mean, ± standard error,

-Means separation done by dunnett test (Kaleso is the control)

-Statistical significance: * = $P \leq 0.05$, ** = $P \leq 0.01$, *** = $P \leq 0.001$

Table 25 contd: Means and standard errors of foliar virus quantities at 20 and 24 weeks after graft inoculation and root virus relative quantities

Variety	Foliar and root virus quantities		
	Time in weeks after graft inoculation		
	20	24	Root virus quantities
Kaleso	**1068.8±46.9**	**8398.9±478.0**	**2622.3±174.2**
LM1/2008/363	22265.0±1667.3***	34603.0±1582.9***	54029.0±1217.1***
Kibandameno	8071.5±343.1*	15260.0±385.3*	59759.0±541.6***
Mecu 72	6054.7±297.4	28914.0±1242.0***	10054.0±324.9*
Ebwanateraka	30779.0±1305.5***	162704.1±34114***	18924.0±1184.1**
Sauti	32352.0±2443.0***	81892.0±1573.2***	25114.0±1263.5***
Nase 1	2258.7±194.9	10844.0±551.2	3249.6±127.8
Kipusa	-	-	-
Mkuranga 1	26450.0±1104.3***	17213.0±303.7***	4836.3±277.6
CHO5/203	90923.±5502.9***	16081.0±781.4**	24401.0±2315.7***
Kalawe	-	-	-
Colicanana	4915.7±894.0	2511.7±270.5	951968.6±26387.0***
Mean (μ)	20690.0±4111.1	36764.0±7780.4	36764.0±7780.4

-μ - population mean, ± standard error,

-Means separation done by dunnett test (Kaleso is the control)

-Statistical significance: * = $P \leq 0.05$, ** = $P \leq 0.01$, *** = $P \leq 0.001$

5.4 Discussion

Host plant resistance has been widely exploited in controlling CBSD and recently there has been interest in promoting varieties that inhibit virus multiplication and symptoms expression. Several studies under glass house and field conditions revealed restriction of virus multiplication and symptom expression in the known resistant variety Kaleso (Kaweesi *et al.*, 2014; Maruthi *et al.*, 2014a; Miller *et al.*, 2005). The susceptible variety Albert expressed severe shoot and root symptoms coupled with high virus quantities while tolerant varieties like Kiroba depicted intermediate virus quantities and disease symptoms. In this study, resistance was observed in the resistant control Kaleso by maintaining low virus quantities both on leaves and roots and showing mild foliar symptoms. Similar findings have been reported by Kaweesi *et al*. (2014) and Maruthi *et al*. (2014a), who observed minimal disease damage coupled with low viral loads. Disease reaction for Nase 1 proved that foliar virus load is not always highly positively correlated to foliar symptoms severity as this variety could harbor slightly higher foliar viral load than Kaleso but with mild foliar symptoms. Even so, Nase 1 had low root virus quantities comparable to Kaleso. Similar results were reported for this variety by Kaweesi *et al*. (2014) and it was categorized as a tolerant variety with restricted symptom development.

Plants that tend to show foliar symptoms and have detectable virus quantities early after graft inoculation tend to accumulate high virus quantities as was observed for Kibandameno and CHO5/203. Comparable results were reported by Maruthi *et al*. (2014a) where Albert and Kiroba a susceptible and tolerant variety, respectively showed early symptoms and detectable virus quantities unlike Kaleso. Although LM1/2008/363 and Ebwanateraka did not show early foliar symptoms and detectable virus quantities, they nonetheless accumulated high foliar and root virus quantities comparable to Kibandameno and CHO5/203. A study by Pariyo *et al*. (2015) categorized Kibandameno as susceptible, since it had high foliar and root severity. Variety Mkuranga 1 had detectable virus quantities without symptoms till 12 weeks after graft inoculation. This has an implication on spreading of CBSD through planting materials since a plant may not have symptoms but still have virus. Varieties Colicanana, Sauti, and Mecu 72 had severely affected foliage with the development of leaf chlorosis, necrosis, stem lesions and stunted growth. Most of the leaves in these varieties were necrotic rendering them unsuitable for virus multiplication (Hull, 2014) and consequently low detectable virus quantities. They also tended to recover and

develop new shoots. The new shoots had lower foliar severity and thus increased detectable virus quantities, as they were suitable for virus multiplication (Hull, 2014).

Different plant defense mechanisms were observed in this study. Restricted virus accumulation and symptom development was observed in Kaleso. Although it had a high positive correlation between change in foliar symptoms with time and change in foliar virus quantity with time, foliar symptom severity and virus quantities remained significantly low portraying its resistance to CBSD. The other mechanism observed was restricted symptom development with moderate virus quantities in tolerant varieties such as Nase 1. Varieties Kibandameno, Ebwanateraka, LM1/2008/363, and Mecu 72 too had high positive correlation between change in foliar symptoms with time and change in foliar virus quantity with time but they had significantly higher virus quantities and developed severe foliar and root symptoms when compared to Kaleso and Nase 1. Mkuranga 1 portrayed the mechanism where a variety accumulates high virus quantities but without visible foliar symptoms. This empasizes the risk of spreading the virus through asymptomatic plants. Expression of severe foliar symptoms coupled with high virus quantities is a trait portrayed by susceptible plants as they are not able to restrict the growth and development of a pathogen. Root symptoms maybe positively correlated with root virus quantities as Kibandameno had low moderate root necrosis severity coupled with moderate root virus quantities while Colicanana had the most severe root necrosis coupled with the highest root virus quantity. This is an indicator that susceptible plants with severe root necrosis will also have high virus quantities when compared to resistant plants with low root necrosis severity and virus quantities.

Variety Kaleso has been consistently resistant to CBSD for many years and has the highest general combining ability for disease resistance (Kulembeka *et al.,* 2012; Nichols, 1947). The findings in this study confirmed the resistance status Kaleso, as it had minimal CBSD symptoms severity coupled with low viral load. Nase 1 that has previously been categorized as tolerant (Kaweesi *et al.,* 2014) was comparable to Kaleso, though it had slightly higher foliar viral load. Both Kaleso and Nase 1 can be good parents for CBSD resistance breeding. Field evaluations by Adiga *et al*. (2016) showed that varieties Kibandameno, CHO5/203, Sauti, Colicanana, and Mkuranga 1 had low foliar severity ranging from 1.0 – 1.7. In this study however, high foliar severity ranging from 3.0 – 4.8, in addition to high foliar and root viral quantities were recorded for these varieties. Breeding for CBSD resistance has fundamentally focused on reducing root necrosis and the expression of shoot symptoms is considered acceptable provided that root symptoms are absent or mild.

However, yield losses due to foliar symptoms have been reported which could surpass root losses due to necrosis (Ndyetabula, 2016). Moreover, plants that develop severe foliar symptoms could also be harboring high virus quantities, and as a result promote the dissemination of the virus. This advocates for future breeding perspectives to focus on reducing both root and shoot symptom expression and virus quantities.

5.5 Conclusion

Evaluating plant response to CBSD by qRT-PCR is important in confirming the resistance or susceptibility status of a variety. This enables the elimination of varieties that may not express symptoms under field screening but may still be susceptible. Kaleso was classified as resistant due to its ability to restrict both symptoms expression and virus accumulation. Nase 1 on the other hand was classified as moderately resistant as it had restricted symptoms development but with slightly higher foliar viral load when compared to Kaleso. Mkuranga 1 was categorised as tolerant. It had severe foliar symptoms and high foliar viral load but low root viral load that was not significantly different from that of Kaleso and Nase 1. Colicanana, Kibandameno, Ebwanateraka and LM1/2008/363 were categorised as susceptible as they had severe foliar symptom development and accumulation of high virus quantities on both leaves and roots. Both Kaleso and Nase 1 can be good parents for CBSD resistance breeding. To achieve durable CBSD resistance, it is important that breeders understand symptom expression and virus quantities of different genotypes to enable them to make noble choices of parents for crossing. This information will be useful for breeders implementing informed breeding strategies with the aim of reducing the spread of CBSV.

5.5 Recommendations

This study revealed a multifaceted situation with regards to cassava plants defense mechanisms against CBSD. There is need for further research to fully understand the base of the different defense mechanisms. Additionally, the varieties can be grown in the field where the varieties can develop tuberous roots for root necrosis severity and virus accumulation evaluations.

CHAPTER SIX

ELIMINATION OF CASSAVA BROWN STREAK VIRUS USING IN VITRO NODAL BUD CULTURE TECHNIQUE

6.0 Abstract

Cassava brown streak disease (CBSD) is an economically important disease caused by the cassava brown streak ipomoviruses (CBSIs). As with other plant viruses, the use of clean planting materials can limit its spread and impact. This study evaluated the effects of chemotherapy and thermotherapy, variety, and regeneration media on virus cleaning and plantlet survival. CBSV infected cuttings of three varieties: Tz 130, Muzege and Nachinyaya that were established and maintained in a glasshouse at the Natural Resources Institute, UK were used as test plants. Cassava leaves were sampled from of each variety, virus indexing done by qRT-PCR and CBSV positive plants used for culture initiation. The cultures were subjected to five treatments, viz: control (no treatment), thermotherapy (35 °C), thermotherapy + sub culturing into regeneration media, chemotherapy (ribavirin 25 mg/L) + thermotherapy (35 °C), and chemotherapy + thermotherapy + sub culturing. The best performing variety with regards to virus cleaning and survival was Tz 130. When plantlets for Tz 130 were subjected to chemotherapy + thermotherapy, they did not survive but survival improved by up to 30% when they were sub cultured into regeneration media and 100% of the plantlets were viruse free. Plantlets subjected to thermotherapy had survival of 30% due to poor root development but this improved to 77% when they were sub cultured into regeneration media and 91.3% of the plantlets were virus free. When Muzege and Nachinya were sub-cultured into regeneration media after thermotherapy, survival improved ranging from (40.0 – 56.7 %) but success in virus cleaning was low (0 – 5.9%). Likewise, when Muzege and Nachinyaya were subcultured into regeneration media after combining chemotherapy and thermotherapy, survival improved to (30.0 – 37.0 %) but success in virus cleaning was still low (11. – 18.2 %). It is concluded that Tz 130 may has a stronger CBSV resistance mechanism that acts synergistically with virus cleaning methods in achieving virus elimination. Thermotherapy combined with node bud culture and regeneration can be used to produce virus free cassava. Likewise chemotherapy + thermotherapy combined with node bud culture and regeneration can be used to produce virus free cassava although mass propagation would be required because survival rates are low.

Key words: Cassava, CBSV, Virus elimination, Node-bud culture, *In Vitro* techniques

6.1 Introduction

Cassava brown streak disease (CBSD) is a major contributor to yield losses in cassava and like other virus diseases of vegetatively propagated crops; phyto-sanitary practices can play a key role in limiting the impact and spread of CBSD (Legg *et al.*, 2017; Hillocks & Jennings, 2003). In view of the cryptic symptoms of CBSD, where symptoms are typically mild and primarily confined to lower leaves, it can be difficult to distinguish between healthy and infected plants. This has the consequence that infected planting material readily propagate cassava brown streak viruses (CBSVs). Additionally, the semi-persistent transmission of these viruses means that the whitefly vector retains them for relatively short periods of time, limiting the distance over which they can be carried (Jeremiah, 2014). CBSD therefore appears to be spread by vectors over short distances but readily carried over longer distances through transport of planting materials.

Unlike bacterial and fungal diseases, viral diseases have no effective chemical control (Lebot, 2019). The supply of virus-free planting materials is therefore important for sustainable crop production and is a prerequisite for the international exchange of germplasm to avoid risks of introducing diseases to uninfected areas. Besides mass propagation of high quality planting materials, biotechnological techniques are also important in producing virus free material. Techniques such as *in vitro* thermo and /or chemotherapies offer a viable option for virus elimination and regeneration of virus-free plantlets (Mwangangi *et al.*, 2014; Wasswa *et al.*, 2010; Zapata *et al*., 1995; Mellor & Stace-Smith, 1970). Community phytosanitation and use of virus free planting materials has the potential to deliver area-wide and sustained reductions of CBSD occurrence. Legg *et al.* (2017) reported that the use of virus free planting materials resulted in reduced CBSD inoculum pressure, reduced CBSD incidences from >90% to < 3% in newly introduced improved varieties and high yield (two to four-fold) in previously cultivated local varieties. This provides significant productivity gains for growers who specifically have preferences for particular varieties that are unfortunately susceptible to CBSD.

Apical meristems are often used for production of virus free plantlets because they are generally either free or carry very low virus titres (Zapata *et al.*, 1995). The explanations for the escape of the meristem by virus invasion are (i) some viruses move readily in a plant body through the vascular system which is absent in meristem, (ii) high metabolic activity in the actively diving meristem cell does not allow virus replication due to competition, and (iii) a high endogenous auxin level in shoot apices may also inhibit virus multiplication (Razdan,

2003). Apart from meristem tip culture, node bud culture has also been used for virus elimination in cassava (Maruthi *et al.*, 2014b).

Plant thermotherapy refers to achieving a cellular environment which is progressively less adequate for virus vitality (Mink *et al.*, 1998). During heat treatment, the elevated temperature is unsuitable for virus replication which lowers virus titre and enhances elimination of viruses from infected plants (Walkey, 1976). The success of virus elimination depends on the plant virus species, the host (cultivar), and whether the plant has single or mixed infection (Allam, 2000). Elevated temperatures in vitro have been effective in eliminating several viruses known to infect vegetatively propagated crops (Allam, 2000). The absence of mosaic symptoms on the leaves of rooted cassava plantlets and effects after subjecting diseased donor cassava explants to heat treatment for at least 30 days at 35° - 38°C has been reported by (Adejare & Coutts, 1981; Chellappan *et al.*, 2005). Wasswa *et al*. (2010) also reported a CBSV elimination efficiency of 40% with a 49% survival at 36°C for 4 weeks.

Chemotherapy is centered on base analogues, with the presumption that such molecules could inhibit the synthesis of the virus'nucleic acid (Panattoni *et al.,* 2013). Investigations have provided valuable contributions with respect to antiviral chemotherapy performance in clinical medicine (Panattoni *et al.,* 2013). The potential similarities between animal and plant hosts' metabolic pathways present in both, has been the starting point for experimentations on phyto-viruses (Panattoni *et al.,* 2013). In this regard, the discovery of ribavirin presented a defining moment in research (Sidwell *et al.,* 1972). Ribavirin compound is a guanosine analogue with broad-spectrum activity against animal viruses and appears to be active against plant virus replication (Sidwell *et al.,* 1972). The efficiency of ribavirin in the elimination of plant viruses is documented in some crops including: onion, potato, *Citrus spp*, garlic, sugarcane, grapes and apples (Panattoni *et al.,* 2013; Nascimiento *et al.,* 2003; Fletcher *et al.,* 1998) and is dependent on the concentration used, the host plant and type of infected tissue (Paunovic *et al.,* 2007).

Improved virus elimination can also be achieved if chemotherapy is combined with thermotherapy (Luciana *et al.,* 2007). Joint effects of thermotherapy at 37°C and ribavirin applied to *in vitro* plants was highly efficient in eliminating potato virus Y resulting in 83.3% of virus free potato plants (Nascimiento *et al.,* 2003). Further reports support the use of thermotherapy together with the addition of antiviral agents into the growth medium as the best treatments for virus elimination in potato (Fletcher *et al.,* 1998; Griffiths *et al.,* 1990).

The success of virus elimination also depends on the plant virus species and whether the plant is single or mixed infected (Allam, 2000). Plantlet regeneration can also be a challenge after thermo and/or chemotherapy treatments and survival is usually very low. Thermotherapy on successful single node cuttings has been successfully used for virus elimination in cassava (Maruthi *et al.,* 2014b). Mwangangi *et al.* (2014) also reported successful virus elimination of up to 84% and plant regeneration when cassava meristem tips were subjected to thermotherapy. Unfortunately, meristem tips that were subjected to a combination of chemotherapy and thermotherapy did not survive. The aim of this study was to optimize in vitro techniques for virus elimination, plantlet regeneration after treatment and testing the effects of treatments on different cassava varieties.

6.2 Materials and methods

6.2.1 Cassava plant virus indexing

Three cassava varieties Muzege, Nachinyaya and TZ 130 that were previously graft inoculated with CBSV were tested for presence of viruses through quantitative reverse transcriptase-polymerase chain reaction (qRT-PCR). Leaf samples were collected from top, middle and bottom sections of the plant and nucleic acid extracted using the Cetyl Trimethyl Ammonium Bromide (CTAB) method as described in section 5.2.5 Total nucleic acid assessment, cDNA synthesis and amplifications were done as described in sections 5.2.5 and relative quantification of virus was performed based on quantification (Cq) method as described in section 5.2.6.

6.2.2 Experimental design

A completely randomized design with three replicates was used for this study. The treatment were developed according to Maruthi *et al.,* (2014b) with a few mordifications. They included: thermotherapy at 35°C for two weeks; chemotherapy (ribavirin 25 mg/L or 0.10 mM) + thermotherapy at 35°C for two weeks; thermotherapy at 35°C for two weeks + sub culturing to regeneration media; chemotherapy (ribavirin 25 mg/L or 0.10 mM) + thermotherapy at 35°C for two weeks + sub culturing to regeneration media and a control of plantlets grown at 28°C without treatment.

6.2.3 Treatment media

Murashige and Skoog (MS) basal medium was used to prepare one litre medium as follows. MS medium (2.2 g) (Sigma M5519, Germany), 20 g of sucrose ($C_{12}H_{22}O_{11}$) (Sigma, Germany) and 950 ml of de-ionized water were added to a 2 L capacity beaker and mixed well using a magnetic stirrer. Two millilitres of Plant Preservative Mixture (PPM 0.2 - 0.25%) (Plant Cell Technology, UK) was added into the solution. PPM is an antibiotic mixture which helps kill any disease-causing microorganisms that may have survived the initial sterilization procedures. pH was then adjusted to 5.7 - 5.8 using 1N HCl and/or 1M NAOH solutions before making up the solution to 1 litre. Two grams of phyagel (Sigma, Germany) was added to the solution and thoroughly mixed. The medium (8ml/tube) was dispensed into clean borosilicate glass tubes (100 x 25 mm) (Timstar, UK) and capped with autoclavable plastic caps (Timstar, UK). The tubes were wrapped in two layers of greaseproof paper and aluminium foil and autoclaved in Boxer autoclave (Boxer, UK) at 121°C for 15 minutes. The package was dried in a hot box oven (Gallenkamp, UK) for 4 hours at 60 °C and allowed to cool to room temperature before use.

6.2.4 Regeneration media

One litre of regeneration media was prepared according to Mapayi *et al*. (2013) with a few modifications. Murashige and Skoog basal Medium (4.43 g) (Sigma M5519, Germany), 100 mg of myo-inositol ($C_6H_{12}O_6$) (Fischer Scientific, UK), 30 g sucrose ($C_{12}H_{22}O_{11}$) (Sigma, Germany) and 950 ml de-ionized water were added to a 2 L capacity beaker and mixed well using a magnetic stirrer. Growth regulators were added: 1-Naphthaleneacetic acid (NAA~0.01mg/L) and 6-Benzylaminopurine (BAP~0.05mg/L). Two millilitres of Plant Preservative Mixture (PPM) (0.2 – 0.25%) (Plant Cell Technology, UK) was also added and the pH adjusted to 5.7. De-ionized water was added to make up the solution to 1 litre before adding 2 g phytagel and dispensing into tubes. The tubes were autoclaved as described earlier.

6.2.5 Sterilization and initiation of explants

The top green parts of cassava plants having at least five nodes were cut from CBSV positive cassava plants using sterile blades. At most five stems were transferred into 500 ml capacity glass bottles and washed three times using sterile distilled water then immersed in 70% ethanol for 3 - 5 seconds. Ethanol was removed and 250 ml sterilizing solution

containing 10% sodium hypochlorite and 2 drops of tween 20 added to each glass bottle. The bottles were shaken on a flask shaker SF1 (Bibby scientific, UK) at 400 oscillations/min for 25 minutes then rinsed four times with sterile distilled water. Under a laminar flow hood, node buds (2 - 3 mm) were excised then cultured in the prepared treatment media.

6.2.6 Treatments

Thermotherapy

Node buds were excised and cultured on treatment media supplemented with 0.2 mg/L 1-Naphthaleneacetic acid (NAA). Thermotherapy was done in a precision incubator (LEEC, UK) set at 35°C, 60% R.H and 14:10 light: dark hours for two weeks. The plantlets were thereafter transferred to a growth room at 28°C, 60% R.H and 14:10 dark:light hours for 8 weeks before potting into soil.

Thermotherapy + Subculturing

Node buds were excised and cultured on treatment media with thermotherapy for two weeks. The grown plantlets (at most 2 nodes) were sub-cultured into regeneration media after the two-week thermotherapy treatment. These plantlets were then transferred to a growth room at 28°C, 60% R.H and 14:10 dark:light hours for 8 weeks before potting into soil.

Chemotherapy + Thermotherapy

Node buds were excised and cultured on treatment media supplemented with 0.2 mg/L 1-Naphthaleneacetic acid (NAA) and ribavirin (25mg/L). Thermotherapy was done for two weeks before transferring to a growth room at 28°C, 60% R.H and 14:10 dark:light hours for 8 weeks before potting into soil.

Chemotherapy + Thermotherapy + Subculturing

Node buds were excised and cultured on treatment media supplemented with ribavirin (25mg/L). Thermotherapy was done for two weeks and the grown plantlets (3 nodes) sub-cultured in regeneration media after two weeks of the chemotherapy and thermotherapy treatment before transferring to a growth room at 28°C, 60% R.H and 14:10 dark:light hours for 8 weeks before potting into soil.

Control (No treatment)

Node buds were excised and cultured on treatment media supplemented with 0.2 mg/L 1-Naphthaleneacetic acid (NAA). The plantlets were then transferred to a growth room at 28°C, 60% R.H and 14:10 dark:light hours for 8 weeks before potting into soil.

6.2.7 Potting

Plantlets were potted into a mixture of compost soil (John Innes No. 2) and peat moss (Jiffy substrates) at a ratio of 1:1 (Figure 14). The plants were left to grow for 3 months before scoring for the presence or absence of CBSD symptoms and testing for the presence of virus by qRT-PCR as described in section 5.2.5 and 5.2.6.

Figure 14: Acclimatization of deflasked plantlets using a humidity dome. A) Newly deflasked plantlets; B) Plantlets covered in a humidity dome; C) Fully acclimatized plantlets at 4 weeks.

6.2.8 Data collection and analysis

Data was collected on the number of leaves, nodes, and roots at 2, 4, 6 and 8 weeks after culturing. Generalised linear model (fixed effects model) was used to estimate the effect of fixed effects that is time, variety, treatment, and treatment by variety by time interaction on growth parameters: leaves, nodes and roots. The generalised linear model is as follows:

$$Yijkl = \mu + Ti + Vj + Gk + Ti*Vj*Gk + eijkl$$

where

Yijkl = observation,

μ – overall mean,

Ti – effect of i^{th} treatment,

Vj – effect of j^{th} variety,

Gk - effect of k^{th} time,

Ti*Vj*Gk – interaction of treatment by variety by time.

ANOVA was done using R function lm and means separated by Tukey's HSD test in R package agricolae (Mendiburu, 2020). The function xyplot in R package lattice (Sarkar, 2008) was used to make scatter plots to show relationship between growth parameters and time

6.3 Results

6.3.1 CBSV presence in parent materials used in tissue culture

All the three varieties used for virus elimination tested positive for CBSV and had visible foliar symptoms (Figure 15). The housekeeping gene (PP2A) and virus (CBSV) had higher expression with early quantification cycles of <30 (Figure 15) depicting the presence of virus. Relative quantification revealed prsence of virus ranging from 0.1 and 41.4 (Table 26).

6.3.2 Effect of chemotherapy and thermotherapy treatment with time on growth parameters

The effect of the different treatments with time on growth parameters was observed using correlation values. The growth parameters analysed included: leaves, nodes, and roots. Chemotherapy combined with thermotherapy treatment had a negative effect on growth parameters with significant correlation coefficients ranging from r = -0.3 - 0.2 in Tz 130, Nachinyaya, and Muzege (Figure 16, 17, 18). Although callus tissues were observed at 2 weeks after treatment, most of the plantlets had leaf chlorosis (Figure 20). This treatment hampered growth as most of the plantlets had either apical necrosis or were dead at 8 weeks.

Growth improved when plantlets subjected to chemotherapy combined with thermotherapy treatment were subcultured into regeneration media. The correlation coefficient values for this treatment ranged from r = 0.1 - 0.4 for all the three varieties (Figure 16, 17, 18). At 2 weeks after plantlets were subjected to this treatment and subsequently subcultured into regeneration media, they had developed shoots (Figure 21). The plantlets continued growing and at 8 weeks, they had pronounced rooting, as well as greater shoot elongation and increased number of leaves.

Similar to chemotherapy combined with thermotherapy treatment, thermotherapy treatment had negative effect on growth since it had low positive correlation coefficient values ranging from r = 0.0 - 0.3 (Figure 16, 17, 18). At 2 weeks after plantlets were

subjected to this treatment, there was callus formation in addition to shoot development. Most plantlets however had leaf chlorosis and were dead at 8 weeks (Figure 22). There was minimal plant gowth in this treatment and improvement was observed only when plantlets were subcultured into regeneration media. Thermotherapy and subsequent subculturing had improved effect on growth, as depicted by the positive correlation coeffiecient values ranging from r = 0.2 - 0.5. Plantlets subjected to this treatment had better growth with vigorous rooting, shooting, and stem elongation (Figure 23).

Control treatment had the highest positive correlation coefficient values ranging from r = 0. 4 - 0.6 (Figure 16, 17, 18). This treatment resulted in enhanced plantlet growth with visible roots at 2 weeks after culturing. Vigorous gowth continued throughout the culturing period and at 8 weeks, the plantlets had pronounced shoot proliferation, elongation and rooting when compared to plantlets in other treatments (Figure 24).

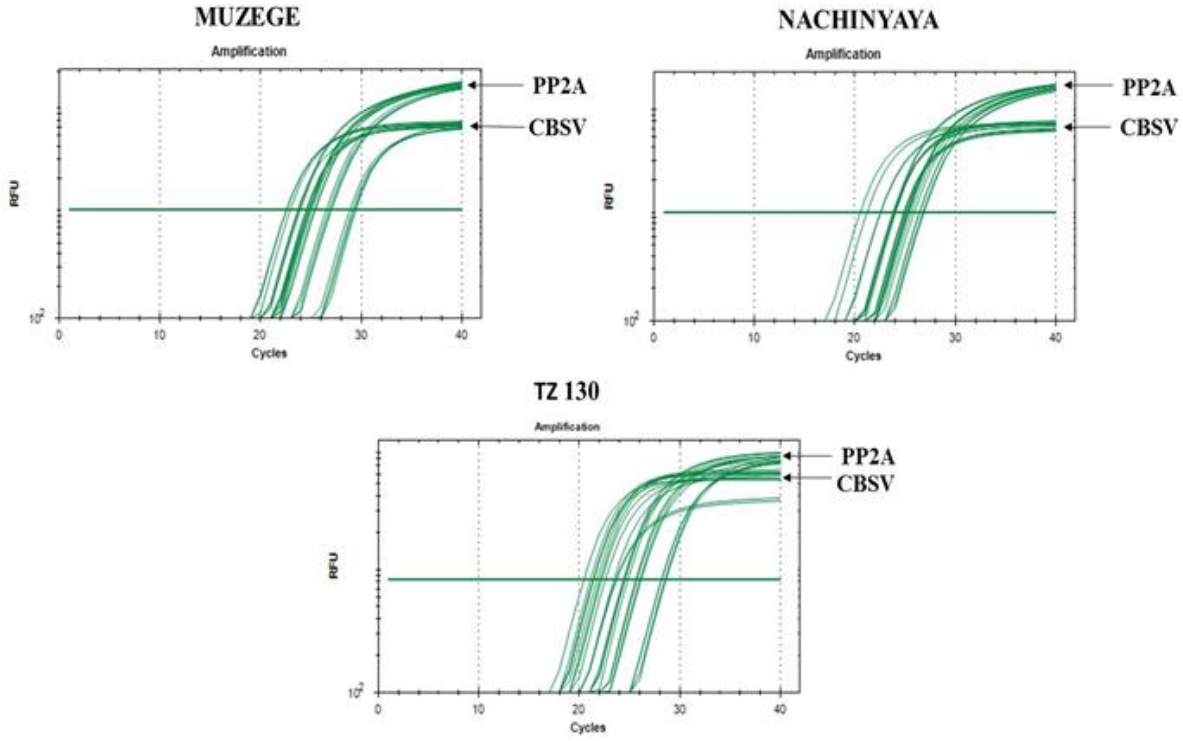


Figure 15: Amplification of housekeeping gene (PP2A) and CBSV in Muzege, Nachinyaya, and TZ 130.

Table 26: Quantification cycles and relative virus quantities in parent materials used for virus cleaning

Parent plant tested for CBSV presence	Quantification cycle		Relative virus quantity ($2^{\wedge-\Delta\Delta Cq}$)
	CBSV	PP2A	
Muzege (1)	23.3	27.0	41.4
Muzege (2)	29.2	26.6	0.2
Muzege (3)	24.7	24.9	1.2
Muzege (4)	24.5	24.8	1.2
Muzege (5)	29.1	25.3	0.1
Nachinyaya (1)	23.9	25.2	2.5
Nachinyaya (2)	24.0	25.3	2.5
Nachinyaya (3)	24.9	26.4	2.8
Nachinyaya (4)	20.8	25.7	29.9
Nachinyaya (5)	25.0	26.8	3.5
TZ 130 (1)	20.7	24.4	13.0
TZ 130 (2)	21.3	24.6	9.9
TZ 130 (3)	21.7	25.8	17.2
TZ 130 (4)	22.0	25.9	14.9
TZ 130 (5)	23.6	28.5	29.9
Positive control	26.8	25.2	-

Note: Relative virus quantities (>0.0) indicate presence of virus

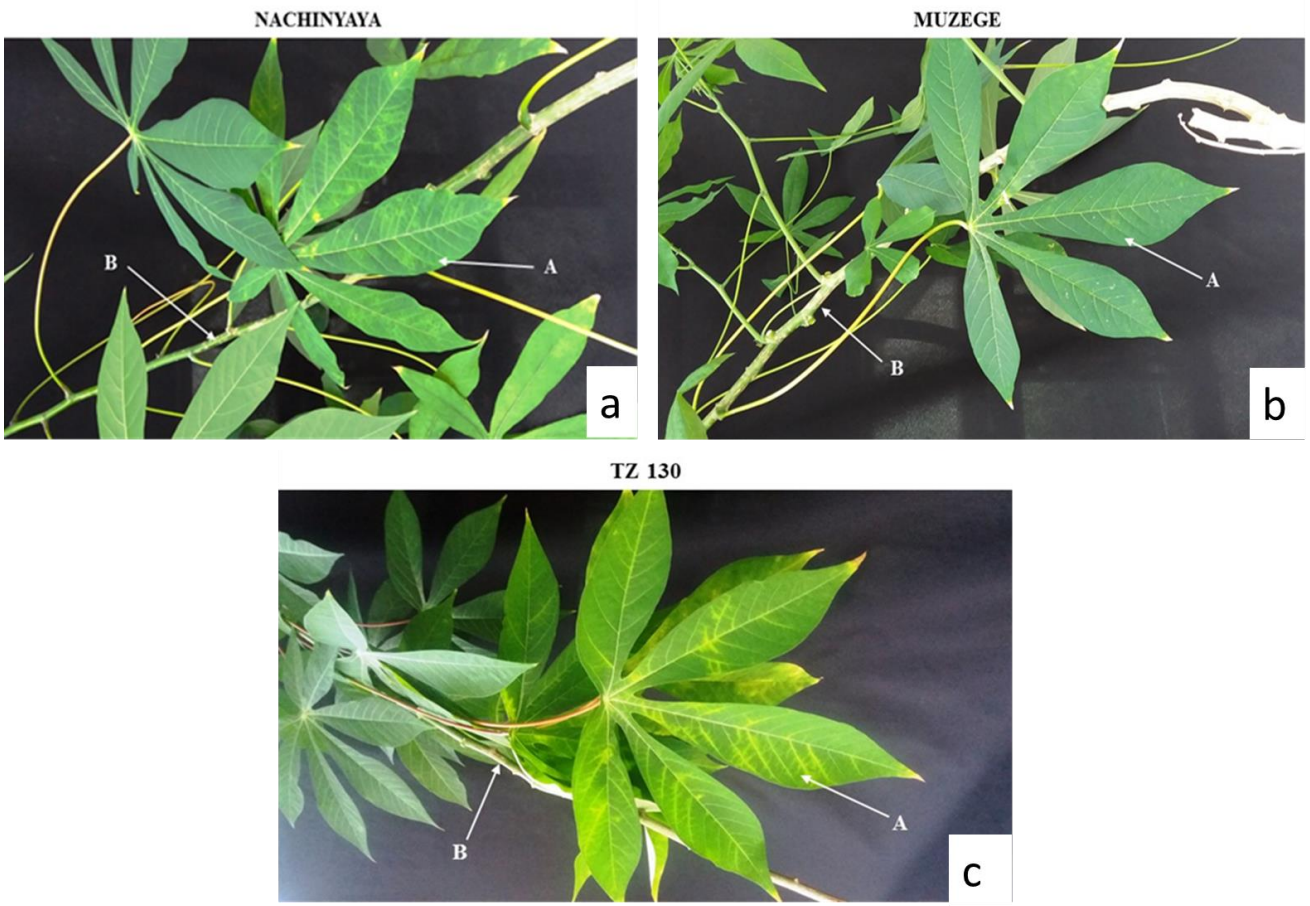


Figure 16: CBSD symptom expression on 3 varieties. **a)** Nachinyaya (A – visible leaf symptoms, B – stem lesions); **b)** Muzege (A – none visible leaf symptoms, B – stem lesions) and **c)** Tz 130. A – conspicuous leaf symptoms, B – stem lesions

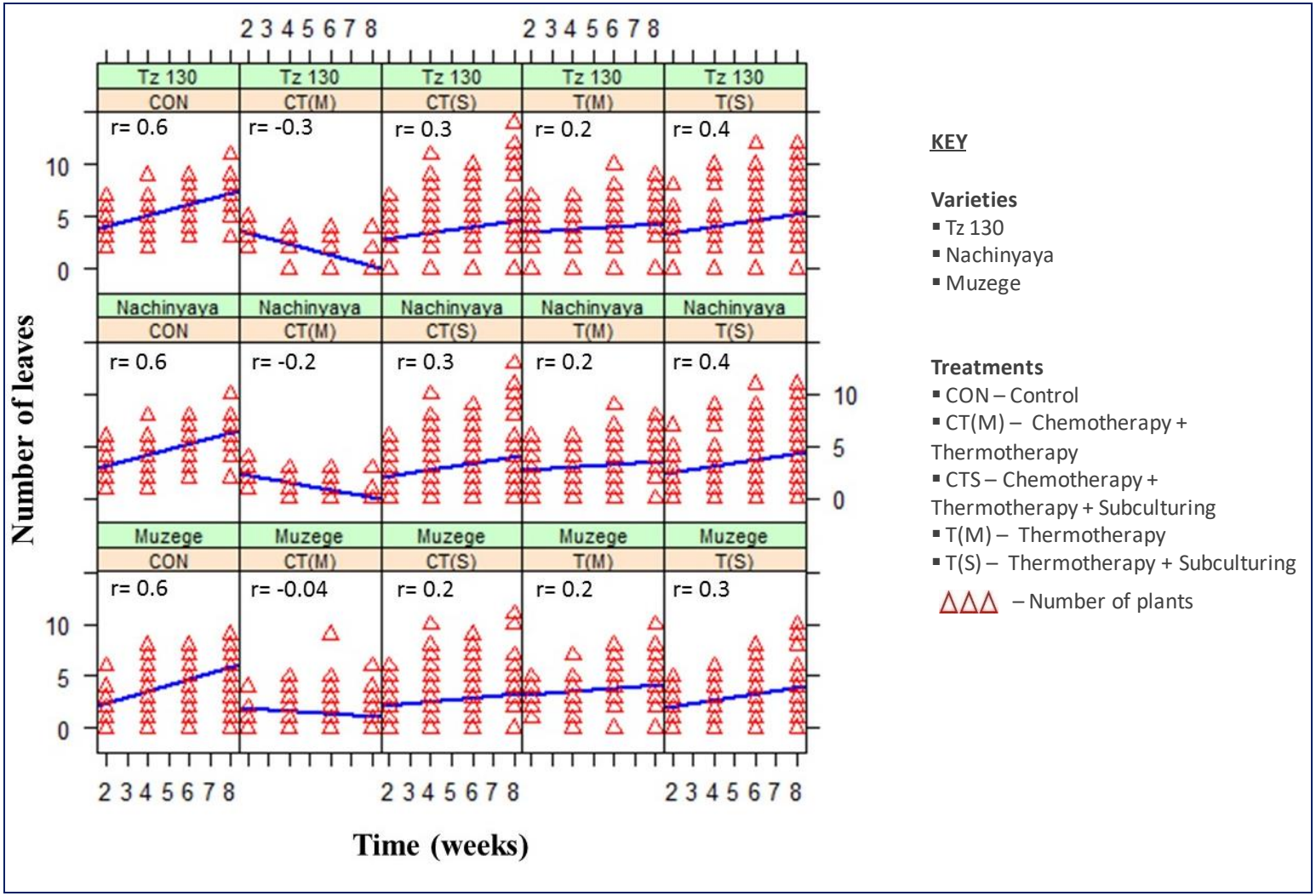


Figure 17: Trend of leaves development in cassava varieties subjected to different treatments

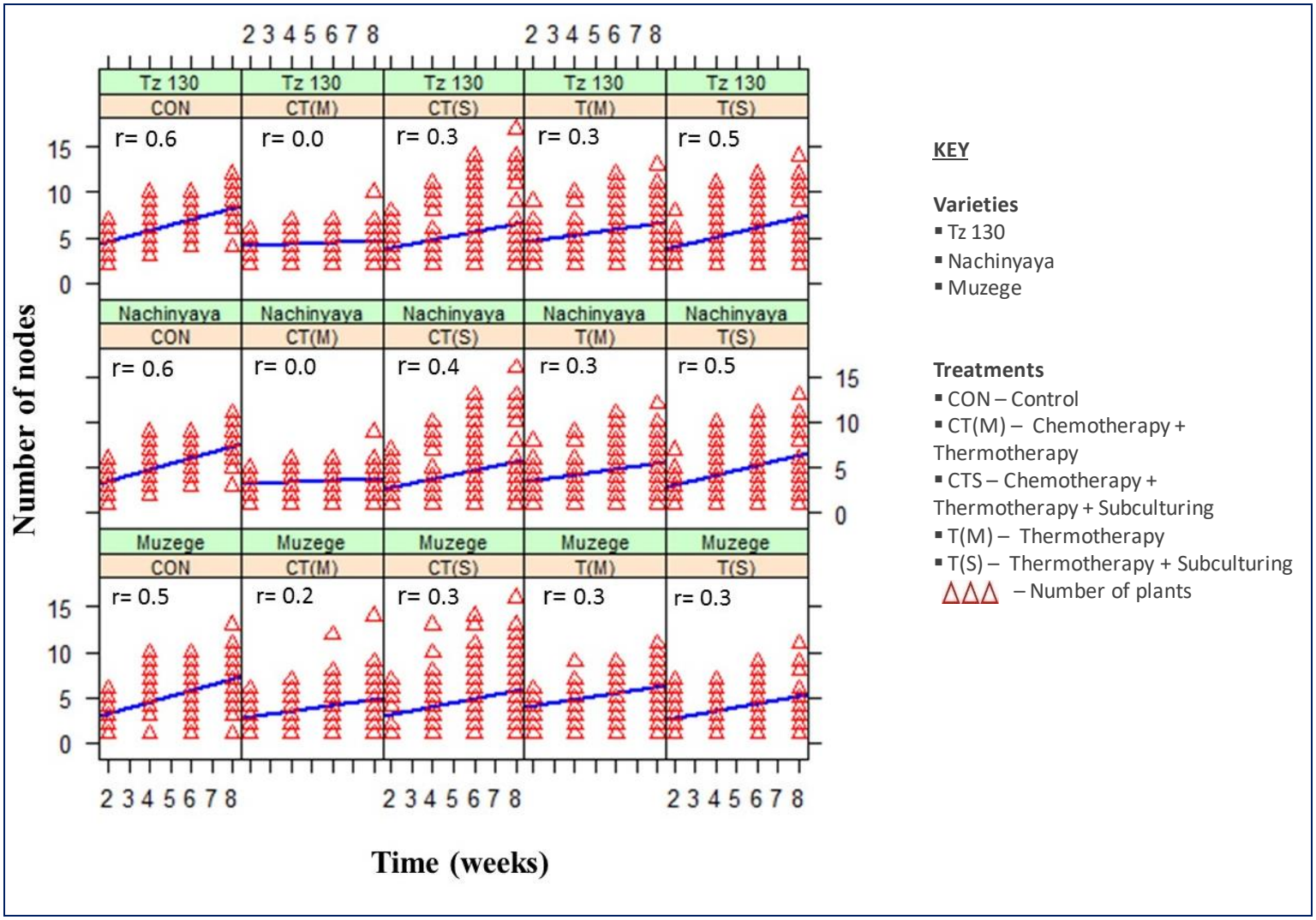


Figure 18: Trend of nodes development in cassava varieties subjected to different treatments

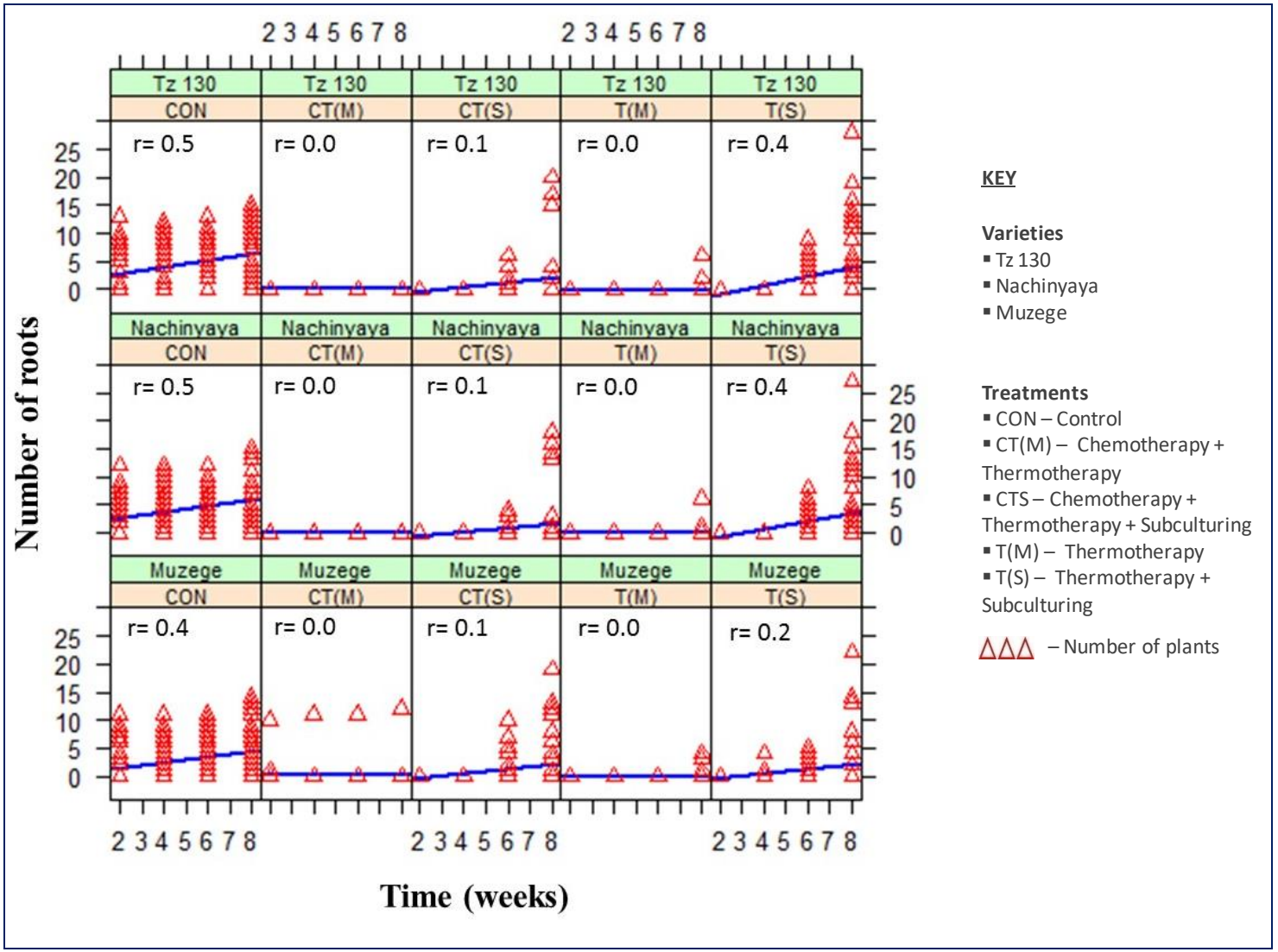


Figure 19: Trend of roots development in cassava varieties subjected to different treatments

6.3.3 Significance of fixed effects on the number of leaves, nodes and roots at 8 weeks after treatment

The fixed effects in this study included variety, treatment, and time and data was analysed to evaluate their influence on the development of leaves, nodes, and roots. There was a highly significant difference ($P < 0.001$) in the mean number of leaves and nodes developed among the varieties (Table 27). Tz 130 had a significantly higher mean number of leaves of 3.9 in comparison to both Nachinyaya and Muzege which had a mean of 3.1 (Table 28). Comparable results were recorded for the mean number of nodes where Tz 130 had a higher mean number of 5.5 while both Nachinyaya and Muzege had a lower mean of 4.5. Although there was no significant difference in the mean root number, Tz 130 had a wider root number range (0 – 28) in comparison to Nachinyaya (0 – 27) and Muzege (0 – 22).

The different treatments resulted in a highly significant difference ($P \leq 0.001$) in the development of leaves, nodes, and roots (Table 27). Plantlets subjected to control treatment had the best growth as depicted by the highest mean number of leaves (4.8), nodes (5.7) and, roots (3.9) recorded (Table 28). There was no significant difference in growth in treatments thermotherapy, thermotherapy combined with subculturing into regeneration media, and chemotherapy combined with thermotherapy and subculturing into regeneration media. However, the mean number of leaves and nodes of plantlets subjected to these treatments were significantly higher than in chemotherapy combined with thermotherapy. The mean number of roots in treatment control (3.9) was significantly higher than in thermotherapy combined with subculturing into regeneration media (1.3). Further the mean number of roots was low (0.8) when plantlets were subjected to chemotherapy combined with thermotherapy and subculturing treatment. Both chemotherapy and chemotherapy combined with thermotherapy treatments had the least mean number of roots of 0.1.

Like variety and treatment, time had significant ($P <0.001$) influence on the growth of leaves, nodes, and roots (Table 27). The mean number of leaves developed was highest at 8 weeks (3.9) and was not significantly different from 4 and 6 weeks. The lowest mean number of leaves (2.7) was recorded at 2 weeks after treatment (Table 28). Likewise, the mean number of nodes was significantly higher at 8 weeks (6.0) when compared to 6 weeks (5.3), 4 weeks (4.5) and 2 weeks (3.5). The mean number of roots was also significantly higher at 8 weeks (2.7) compared to the other time points. The eighth week time point had the highest number of leaves, nodes, and roots, all of which are important for plant survival when

transferred to soil. Treatment by variety by time interaction had no significant effect on the development of leaves, nodes, and roots.

Table 27: Anova table for fixed effects on growth parameters

Parameter	Response	df	SS	MS	F value	Pr(>F)
Leaves	Variety	2	316.1	158.0	31.6	3.039e-14***
	Treatment	4	2462.6	615.7	123.2	<2.2e-16***
	Time	3	370.9	123.6	24.7	1.034e-15***
	variety × treatment	8	106.2	13.3	2.66	0.006701**
	variety × time	6	16.6	2.77	0.55	0.766988ns
	treatment × time	12	917.2	76.4	15.3	<2.2e-16***
	variety × treatment × time	24	71.9	3.00	0.60	0.936805ns
	residuals	2003	10012.7	5.00	-	-
Nodes	Variety	2	445.5	222.8	36.2	3.596e-16***
	Treatment	4	703.8	176.0	28.6	<2.2e-16***
	Time	3	1842.4	614.2	99.8	<2.2e-16***
	variety × treatment	8	108.7	13.6	2.21	0.02432*
	variety × time	6	2.8	0.47	0.08	0.99829
	treatment × time	12	284.0	23.7	3.85	7.661e-06***
	variety × treatment × time	24	41.2	1.72	0.28	0.99980
	residuals	2003	12325.5	6.15	-	-
Roots	Variety	2	41.1	20.5	2.85	0.0583907
	Treatment	4	4317.0	1079.2	149.6	<2.2e-16***
	Time	3	1401.4	467.2	64.7	<2.2e-16***
	variety × treatment	8	221.2	27.7	3.83	0.0001751***
	variety × time	6	15.1	2.52	0.35	0.9104771
	treatment × time	12	869.4	72.5	10.0	<2.2e-16***
	variety × treatment × time	24	67.2	2.80	0.39	0.9967549
	residuals	2003	14455.2	7.22	-	-

-df, MS, SS - degrees of freedom, mean squares and sum of squares respectively

-Statistical significance: * = $P \leq 0.05$, ** = $P \leq 0.01$, *** = $P \leq 0.001$

Table 28: Effect of fixed effects on growth of leaves, nodes and roots

Fixed effects		Leaves	Min	Max	Nodes	Min	Max	Roots	Min	Max
Varieties	Tz 130	3.9±2.5a	0	14	5.5±2.8a	2	17	1.5±3.5a	0	28
	Nachinyaya	3.1±2.5b	0	13	4.5±2.7b	1	16	1.3±3.2a	0	27
	Muzege	3.1±2.8 b	0	11	4.5±2.7b	1	16	1.1±2.9a	0	22
Treatment	CON	4.8±2.1a	0	11	5.7±2.4a	1	13	3.9±4.1a	0	15
	TS	3.7±2.8b	0	12	4.8±3.0b	1	14	1.3±3.6b	0	28
	TM	3.6±2.3b	0	10	5.1±2.6b	1	13	0.1±0.5d	0	6
	CTS	3.2±3.0b	0	14	4.6±3.5b	1	17	0.8±2.8c	0	20
	CTM	1.5±1.5c	0	9	3.9±1.7c	1	14	0.1±1.1d	0	3
Time (wks)	2	2.7±1.6a	0	8	3.5±1.9a	1	9	0.5±1.9a	0	13
	4	3.4±2.2b	0	11	4.5±2.3b	1	13	0.7±2.4ab	0	12
	6	3.6±2.8bc	0	12	5.3±2.8c	1	14	1.2±2.7c	0	13
	8	3.9±3.5c	0	14	6.0±3.4d	1	17	2.7±4.7d	0	28

Means separation by Tukey's HSD test. a, b, c letter codes denoting significance at $P \leq 0.05$. Treatments include: CON – Control, CT – Chemotherapy + Thermotherapy, CTS – Chemotherapy + Thermotherapy + Sub culturing, T – Thermotherapy, TS – Thermotherapy + Sub culturing

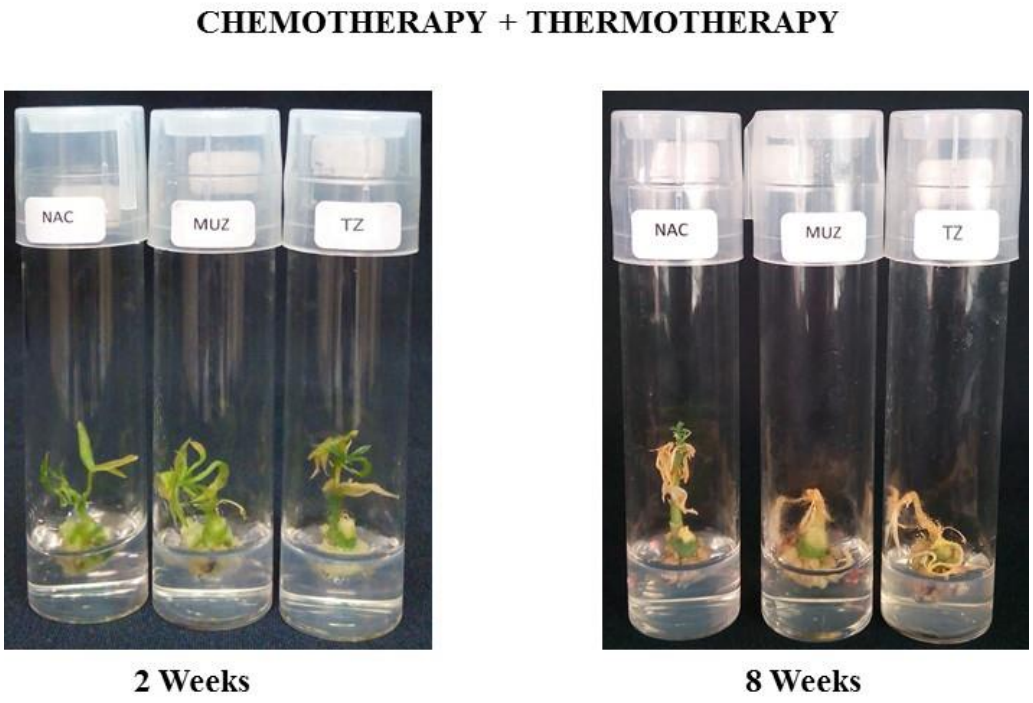


Figure 20: Growth of plantlets at 2 and 8 weeks after the chemotherapy + thermotherapy treatment

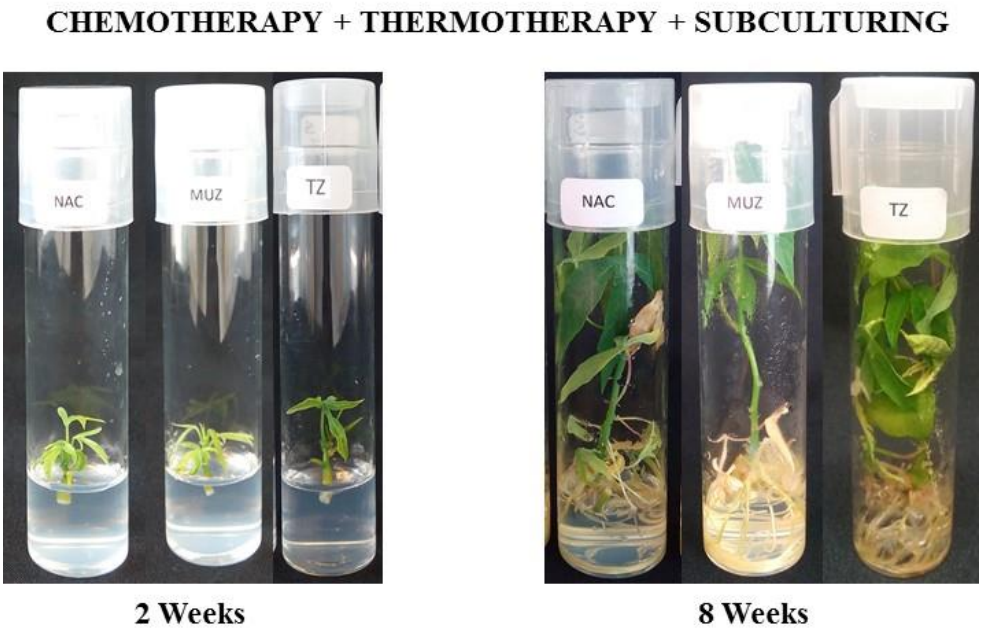


Figure 21: Growth of plantlets at 2 and 8 weeks after the chemotherapy + thermotherapy + subculturing treatment

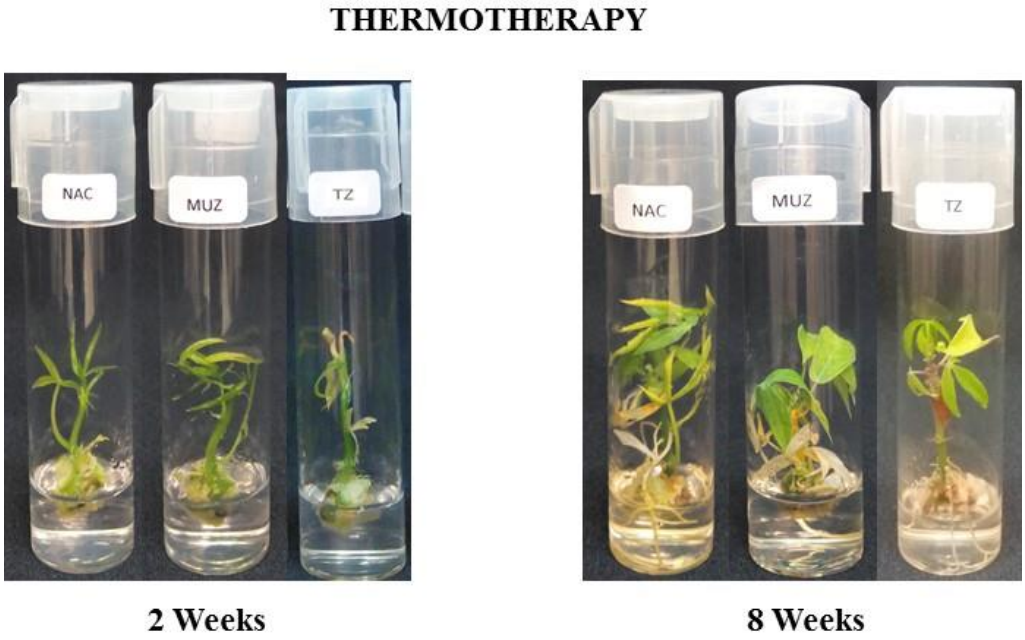


Figure 22: Growth of plantlets at 2 and 8 weeks after the thermotherapy treatment

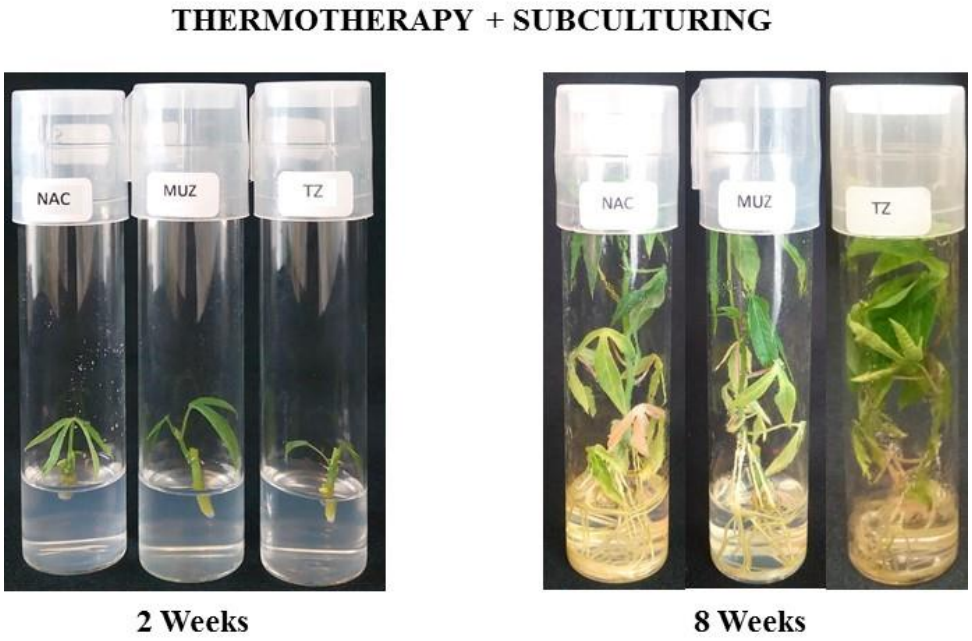


Figure 23: Growth of plantlets at 2 and 8 weeks after the thermotherapy + subculturing treatment

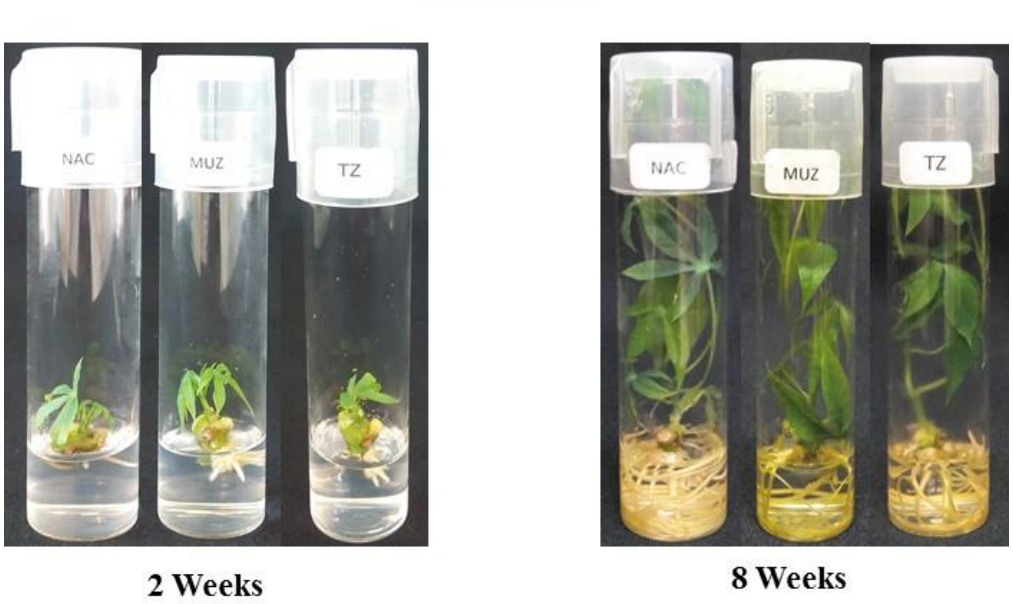


Figure 24: Growth of plantlets at 2 and 8 weeks in the control

6.3.5 Survival of CBSV negative plants after treatment

Variety Tz 130 had the highest survival in all treatments except in chemotherapy combined with thermotherapy, where all plantlets in all the varieties died (Table 29). In control, Tz 130 had 100.0% survival, Nachinyaya (90.0%) and Muzege (86.7%). All the plants in control however tested positive for CBSV. In thermotherapy treatment, low survival was observed; Tz 130 (30.0%), Nachinyaya (10.0%) and Muzege (16.7%). Additionaly, Tz 130 had 66.7% virus free plants in comparison to Nachinyaya and Muzege which had no virus free plants. Thermotherapy combined with subculturing into regeneration media significantly improved plantlet survival. Accordingly, Tz 130 had 76.7% survival and 91.3% virus free plants compared to Nachinyaya which had 56.7% survival and 5.9% virus free plants. Muzege had the lowest survival of 40.0% with no virus free plants. Chemotherapy combined with thermotherapy treatment had no surviving plants. However, survival improved when plantlets were subcultured into regeneration media. Muzege had 30.0% survival with 11.1% virus free plants while Nachinyaya had 36.7% survival with 18.2% virus free plants. Tz 130 on the other hand had 100% virus free plants with a 30.0% survival.

Table 29: Survival and CBSV negative plants in different varieties exposed to different treatments

Treatments	Varieties	No. of plantlets cultured	No. of surviving plantlets	Survival (%)	CBSV negative plantlets	CBSV negative plantlets (%)
CON	Muzege	30	26	86.7	0	0.0
	Nachinyaya	30	27	90.0	0	0.0
	Tz 130	30	30	100.0	0	0.0
TS	Muzege	30	12	40.0	0	0.0
	Nachinyaya	30	17	56.7	1	5.9
	Tz 130	30	23	76.7	21	91.3
T	Muzege	30	5	16.7	0	0.0
	Nachinyaya	30	3	10.0	0	0.0
	Tz 130	30	9	30.0	6	66.7
CTS	Muzege	30	9	30.0	1	11.1
	Nachinyaya	30	11	36.7	2	18.2
	Tz 130	30	9	30.0	9	100.0
CT	Muzege	30	0	0.0	-	-
	Nachinyaya	30	0	0.0	-	-
	Tz 130	30	0	0.0	-	-

Treatments include: CON – Control, CT – Chemotherapy + Thermotherapy, CTS – Chemotherapy + Thermotherapy + Sub culturing, T – Thermotherapy, TS – Thermotherapy + Sub culturing

6.4 Discussion

Several *in vitro* techniques were studied for the ability to regenerate CBSV free plants. Chemotherapy and thermotherapy treatments have been used oftenly to produce virus free plants, because the methods are not cumbersome and time consuming. Challenges however, have been experienced in getting surviving plants after treatments. This study evaluated the effect of different treatments including chemotherapy combined with thermotherapy, chemotherapy combined with thermotherapy followed by sub culturing into regeneration media, thermotherapy, and thermotherapy combined with sub culturing into regeneration media on growth parameters. Effects of treatments on survival and virus cleaning in different varieties were also tested.

Plant thermotherapy involves providing an environment unsuitable for cellular biological activity and consequently inadequate for virus replication (Mink *et al.,* 1998). Ribavirin, an anti-viral drug used for chemotherapy is an analogue of guanosine and is effective in hindering the duplication of viral genetic material (Carter & Saunders, 2007). Thermotherapy treatment involves keeping plants at temperatures of 35 - 54°C, considering the plant's physiological tolerance limits, for a suitable time (Panattoni *et al.,* 2013). In this study, a ribavirin concentration of 25 mg/l combined with thermotherapy at 35°C for two weeks resulted in very minimal plants growth, dead terminal growing points and no surviving plants at the end of culture time. This was also reported by Mwangangi *et al*. (2014) who observed high plant mortality when chemotherapy was combined with thermotherapy. Ribavirin at active doses is normally phytotoxic and can cause an increase in culture time and death (Kidulile *et al.,* 2018; Singh, 2015). Survival however improved to 30 – 36.7% when plants subjected to this treatment were transferred into regeneration media. Plants subjected to thermotherapy treatment had good leaf and node development but very poor root growth resulting in low survival (10 – 30%). Heat treatment could have negatively affected the plants resulting into poor root growth by 8 weeks after culturing. Plantlets subjected to thermotherapy treatment may need more culture time to develop roots that will improve on their survival when transferred to soil. Survival significantly improved in this treatment when plants were transferred to regeneration media and it ranged from 40 – 77%.

Murashige and Skoog (MS) media used for tissue culture has macro elements, microelements, vitamins and organics which are important in culture growth and development (Trigiano & Gray, 2010). In this study, the control plants had 100% survival even with half strength MS media supplemented with 20 g/l sucrose and no growth

hormones. Maruthi *et al*. (2014b) also reported high survival rates when cassava nodes are cultured in half strength MS media without growth hormones. Plants subjected to chemotherapy combined with thermotherapy treatment followed by transfer to regeneration media had slight improvement with regards to survival which ranged from 30 – 37% among the varieties. Chemotherapy combined with thermotherapy treatment was highly phytotoxic, but regeneration media led to survival of a few plants, some of which were virus free. Virus cleaning success ranged from 11 – 100% among the different varieties in this treatment.

Regeneration media used had full salt strength and was supplemented with growth hormones (NAA and BAP), myo-inositol and 30 g/l sucrose. The hormone NAA is an auxin which influences cell enlargement, bud formation and root initiation while BAP, a cytokinin, influences bud formation. Myo-inositol is an energy source playing a major role in cell division resulting to cell growth (Abobkar & Ahmed, 2012) while sucrose acts as a morphogenic trigger in the formation of auxiliary buds and branching of adventitious roots (Vinterhalter & Vinterhalter, 1997). This media therefore greatly contributed to the development of leaves, stems, and roots and as a result improved plant survival.

Respectively, the survival and virus cleaning successes in various treatments were 76.7% and 91.3% for Tz 130, 56.7% and 5.9% for Nachinyaya, and 40.0% and 0.0% for Muzege in the thermotherapy and subculturing into regeneration media treatment. In the thermotherapy treatment, the survival and virus cleaning successes respectively were 30.0% and 66.7% for Tz 130, 10.0% and 0% for Nachinyaya, and 16.7% and 0% for Muzege. In the chemotherapy combined with thermotherapy and subculturing into regeneration media treatment, the survival and virus cleaning success respectively were 30.0% and 100.0% for Tz 130, 36.7% and 18.2% for Nachinyaya, and 30.0% and 11.1% for Muzege. Tz 130 was the outstanding variety in success, concurring with Allam (2000) observation that that success of virus elimination is dependent on many factors including: the plant virus species, the host (cultivar), and whether the plant is single or mixed infected.

Earlier studies by Kaweesi *et al.* (2014) have shown that Tz 130 has tolerance to CBSD with low mean foliar incidence (17%), foliar severity (1.17) coupled with low root necrosis incidence and severity. Nachinyaya on the other hand is also tolerant but with higher foliar incidence (42.8%), foliar severity (2.4), root incidence (36.9%) and root severity (2.4) (Ferguson *et al.,* 2015; Rwegasira & Chrissie, 2012). CBSD tolerance varies with some varieties expressing more symptoms (Kaweesi *et al.,* 2014). It is noteworthy that since Tz

130 can restrict CBSD symptoms more that Nanchinyaya, it may have a stronger resistance mechanism which acts synergistically with virus cleaning methods to produce virus free plants. Muzege and Nachinyaya had the least success even when chemotherapy and thermotherapy treatments were combined, and this led to conclusion that success in virus elimination and regeneration is variety dependent. Maruthi *et al*. (2014b) noted that not all varieties can be cleaned in one cycle and the more susceptible varieties can take 2 - 3 cycles of intense therapy. Plants with unsuccessful virus cleaning could be taken through a second virus cleaning cycle where the duration of treatment exposure is increased to maximize on the chances of getting surviving virus free plants. Meristem tip culture could also improve on virus cleaning in these varieties.

6.5 Conclusion

In this study, node-bud culture combined with thermotherapy (35°C for two weeks) and subculturing in regeneration media was the most effective method as it achieve the highest success in virus elimination (91.3%) and post treatment plantlets survival (77.7%). Node-bud culture combined with thermotherapy, chemotherapy, and subculturing in regeneration also achieved a high success in virus elimination (100.0%) but with poor post treatment plantlets survival (30.0%). It is noteworthy that successful virus elimination proved to be variety dependent, as Tz 130 had the highest success when compared to Nachinyaya and Muzege. These virus elimination technique can be used to produce CBSV free planting materials that will reduce the incidence and spread of the virus.

6.6 Recommendations

Several factors have been found to influence elimination of viruses in plants such as effect of thermotherapy and chemotherapy, duration of treatment, plant genotype and biological nature of the virus. There is therefore a need for more studies on the effects of these factors on virus cleaning. Studies should also be carried out on the effects of different cleaning methods on elimination of the Ugandan cassava brown streak virus (UCBSV) strain in infected cassava and the effect of treatment duration on virus cleaning and survival of plants. When plants are exposed to antiviral chemicals, there is a possibility of mutations hence this should also be investigated in virus cleaned plants.

CHAPTER SEVEN

GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

7.1 General Discussion

Cassava (*Manihot esculenta* Crantz) is the third most important source of calories in the tropics and the eighth most important food crop in the world after sugarcane, maize, rice, wheat, potatoes, soybeans, and vegetables in terms of global annual production (FAOSTAT, 2018). Despite its importance, and its average yield in Africa remains low at is 9.0 t/ha, which is far below the genetic potential of 90 t/ha (Lebot, 2019; FAOSTAT, 2018). Cassava brown streak disease (CBSD) caused by cassava brown streak viruses is one of the constraints affecting cassava production. CBSD causes losses in production through reduced growth as well as spoilage of harvested roots due to necrotic rot (Nichols, 1950). Quantitative assessments of yield losses have demonstrated that losses of up to 70% occur and can be higher in highly susceptible varieties (Hillocks *et al.,* 2001). Apart from whiteflies, surveys have revealed that the transportation of infected materials to areas in which CBSD was previously absent has enabled the disease to spread from independent hotspots (Legg *et al.,* 2011). This is because farmers exchange cassava stems used for vegetative planting material locally and over long distances. Deployment of CBSD resistant cassava varieties and planting virus free cassava are the only sustainable ways of controlling the devastating impact of the disease.

Varied responses are normally observed when field screening is done with the aim of identifying CBSD resistant varieties. Many terminologies have been used inconsistently to describe the response of cassava to CBSD and in general of plants to virus infections (Cooper & Jones, 1983). Among them, resistance, tolerance, and susceptibility are most common. In the case of resistant plants, infection by viruses can occur but multiplication and movement are restricted, and the disease symptoms are generally localized or absent (Kang *et al.,* 2005; Cooper & Jones, 1983). The term tolerance describes a host that can be infected by a virus and in which it can replicate and cause symptoms without significantly diminishing the growth or yield of the plant (Cooper & Jones, 1983). With regards to CBSD, tolerant varieties express moderate to severe foliar symptoms but minimal or no visible root symptoms thus have 100% utilisable roots. Susceptibility on the other hand describes a host plant in which virus spreads and multiplication is high, and the development of severe symptoms both on leaves and roots is evident (Maruthi *et al.,* 2014a). Using these criteria, cassava landraces were classified into the resistant, tolerant and susceptible categories.

This study identified twenty eight (28) resistant landraces and they expressed minimal foliar and root symptoms just like the known resistant variety 'Namikonga' (Masumba *et al.*, 2017, Kaweesi *et al.*, 2014; Kulembeka *et al.*, 2012). Growing these resistant landraces would increase cassava productivity as they have minimal root damage. Apart from resistant landraces, twenty seven (27) tolerant landraces were also identified and they expressed moderate to severe foliar but with minimal or no root necrosis (Hillocks *et al.*, 2011). It is noteworthy that some of the landraces identified as resistant or tolerant had good yield related traits including: high root weight, root number per plant and dry matter content. F1 progenies were developed by crossing cassava brown streak disease with cassava mosaic disease resistant parents. Two populations developed were Namikonga × AR37-80 and Pwani × AR37-80. The populations were screened for CBSD resistance and it led to the identification of resistant and tolerant progenies some of which some had high yield. In both landraces and the F1 populations, sum of squares (SS) due to genotype was the highest for most of the traits evaluated including: CBSD foliar symptoms at 3, 6, and 9 MAP, root necrosis, root necrosis incidence, usable roots, root weight, number of roots per plant, and harvest index. The findings indicated that the traits are heritable and thus can be selected in breeding programmes. CBSD symptoms were mostly negatively correlated with yield related trait. A high positive correlation was recorded between root necrosis severity and root necrosis incidence indicate that the data collected for incidence can be sufficient and recommended, because scoring for incidence is quicker and less subjective (absence or presence) than scoring for severity on a wide scale (1–5) especially in multi-location trials.

Grafting assured inoculation of cassava varieties which led to their characterisation based on foliar symptoms development and virus accumulation with time. Apart from Kaleso, Nase 1 was identified as a good CBSD progenitor since it had restricted virus accumulation and symptom development. *In vitro* chemotherapy and thermotherapy techniques were evaluated with the aim of producing virus free cassava. Chemotherapy (25 mg/l) combined with thermotherapy (35° C) treatment had no surviving plants while thermotherapy treatment had few surviving plants ranging from (10 – 30%). Ribavirin at active doses is normally phytotoxic and can cause an increase in culture time and death (Kidulile *et al.*, 2018; Singh, 2015). Survival however improved when plantlets exposed to both treatments were sub-cultured into a full salt strength Murashige and Skoog regeneration media, supplemented with growth hormones (NAA and BAP), myo-inositol and 30 g/l sucrose. Success in virus

cleaning seemed variety dependent and the highest survival of 77% and virus cleaning success of 100% was observed in variety Tz 130.

7.2 Conclusions

Screening for CBSD resistance in Kenya identified CBSD tolerant landraces including: Weite, Merry-go-round, Nyakasamuel, and Manchoberi which had low root weights of ≤ 10.0 t/ha but high dry matter content ranging from 31.0 – 41.0%. They had low root weight probably due to CMD infections and this emphasizes the need for deployment of varieties resistant to both CBSD and CMD. The Tanzanian trial identified both resistant and tolerant landraces. Resistant landraces with high root weight (≤ 20.0 t/ha) and high dry matter content (≤ 30.0 %) included Benny, Katewanya, Limbanga, Mombasa, Musa Said, Mweda, Simanyu, Supa B and Supa Jangwa. These could be used directly for cultivation and in cassava breeding programs for transfer of resistance to farmer preffered varieties. Tolerant high yielding landraces were also identified including: Mreteta, Mdimbe, Nyoka, and Vicent and could be taken through virus cleaning so that farmers can have access to clean planting materials for these particular landraces.

F1 populations were developed and screened for CBSD resistance. In the Pwani × AR37-80 F1 population, tolerant progenies identified included: PAR024, PAR057, PAR064, & PAR192 and they had the highest root weight (25.4 – 33.2 t/ha) and moderate dry matter content ranging from 21.0 – 22.9%. In Namikonga × AR37-80 population, resistant progenies identified included: NAMAR050, NAMAR130, NAMAR371, NAMAR402, & NAMARX12 and had the highest root weights ranging from (15.2 – 27.8 t/ha) and moderate dry matter content ranging from (24.1 – 29.4 %) while tolerant progenies NAMAR116 and NAMAR441 and had root weight ranging from 18.2 – 19.5 t/ha and moderate to high dry matter content ranging from 22.4 – 35.8%. The findings indicate that they can be used in future breeding programmes to generate cassava varieties with farmer preferred traits.

Analysis of symptoms expression and virus accumulation in CBSV graft inoculated plants is important in confirming the resistance or susceptibility status of a variety and enables the elimination of varieties that may not express symptoms under field screening but may still be susceptible. This is important especially when choosing CBSD resistant progenitors for breeding CBSD resistant farmer preferred varieties. Kaleso was classified as resistant due to its ability to restrict both symptoms expression and virus accumulation. Different plant defense mechanisms were observed in this study including restricted

symptoms expression coupled with minimal virus accumulation of viruses in resistant varieties and severe symptom expression coupled with high virus accumulation in susceptible varieties. Apart from Kaleso, Nase 1 was identified as a good progenitor since it restricted symptom expression albeit having slightly higher virus quantities than Kaleso. This information will be useful for breeders implementing informed breeding strategies with the aim of reducing the spread of CBSV.

Success in virus cleaning and post treatment survival was dependent on variety as Tz 130 had the highest success when compared to Nachinyaya and Muzege. Thermotherapy treatment at 35°C for two weeks and subculturing in regeneration media was the most effective method as it achieve the highest success in virus elimination (91.3%) and post treatment plantlets survival (77.7%). Thermotherapy combined with chemotherapy and subculturing in regeneration media also achieved a high success in virus elimination (100.0%) but with poor post treatment plantlets survival (30.0%). Node-bud culture combined with thermotherapy (35°C) and subculturing into regeneration media can produce virus free plants. Node-bud culture combined with chemotherapy, thermotherapy, and sub-culturing into regeneration media can produce virus free plants although mass propagation will be required because survival rates are low. These virus elimination technique can be used to produce CBSV free planting materials that will reduce the incidence and spread of the virus.

7.3 Recommendations

i) The findings of screening landraces for CBSD resistance are based on data generated from two sets of germplasm in Kenya and Tanzania, conducted in two planting seasons in two CBSD hot spot areas. Further CBSD and other yield related traits evaluations need to be carried out with the two sets of landraces and varieties combined and replicated in diverse CBSD hot spot sites for more than two seasons to bring out the actual genotype by environment interactions due to the disease. Screening in diverse environments will also enable more genotype and environment interactions. In addition, they can similarly be screened in CMD hotspot areas to confirm if they are also suitable genetic stocks that combine both CMD and CBSD resistance.

ii) The findings of screening F1 populations for CBSD resistance are based on CBSD data generated from two planting seasons in one CBSD hot spot area. Further CBSD

and other yield related traits evaluation could be carried out in diverse CBSD hot spot sites combined with diverse seasons to confirm the genotypic reactions recorded in this study. In addition, F1 progenies can similarly be screened in CMD hotspot areas to substantiate if CMD resistance was introgressed into these progenies from the CMD resistant parent AR37-80. There were resistant progenies but poor yield related traits. In view of the difficulties associated with conventional breeding, apart from backcrossing to restore and enhance yield and quality of the tuberous roots produced, genetic engineering would offer potential for the rapid transfer of resistance genes to the traditional cultivars.

iii) The study on analysis of symptoms expression and virus accumulation in CBSV graft inoculated cassava varieties revealed a multifaceted situation with regards to cassava plants defense mechanisms against CBSD. There is need for further research to fully understand the base of the different defense mechanisms. Additionally, the varieties can be grown in the field where the varieties can develop tuberous roots for root necrosis severity and virus accumulation evaluations.

iv) Several factors have been found to influence elimination of viruses in plants such as effect of thermotherapy and chemotherapy, duration of treatment, plant genotype and biological nature of the virus. There is therefore a need for more studies on the effects of these factors on virus cleaning. Studies should also be carried out on the effects of different cleaning methods on elimination of the Ugandan cassava brown streak virus (UCBSV) strain in infected cassava and the effect of treatment duration on virus cleaning and survival of plants. When plants are exposed to antiviral chemicals, there is a possibility of mutations hence this should also be investigated in virus cleaned plants.

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APPENDICES

Appendix 1: ANOVA tables for Kenyan cassava genotypes

Cassava brown streak disease leaf symptoms at 3 MAP					
Source	**DF**	**Sum of squares**	**Mean square**	**F value**	**Pr>F**
Environment	1	8.5254	8.5254	65.6	<.0001
Replicate	2	0.0802	0.0401	0.31	0.7357
Environment (Replicate)	2	0.0002	0.0001	0.00	0.9991
Genotype	14	20.616	1.4726	11.3	<.0001
Environment*Genotype	14	3.8696	0.2764	2.13	0.0238
Model	33	33.092	1.0028	7.71	<.0001
Error	56	7.2796	0.1299		
Corrected total	89	40.371	0.4536		

Cassava brown streak disease leaf symptoms at 6 MAP					
Source	**DF**	**Sum of squares**	**Mean square**	**F value**	**Pr>F**
Environment	1	23.819	23.819	188.2	<.0001
Replicate	2	0.1429	0.0714	0.56	0.5718
Environment (Replicate)	2	0.1042	0.0521	0.41	0.6644
Genotype	14	13.990	0.9993	7.90	<.0001
Environment*Genotype	14	10.230	0.7307	5.77	<.0001
Model	33	48.285	1.4632	11.6	<.0001
Error	56	7.0862	0.1265		
Corrected total	89	55.371	0.6221		

Cassava brown streak disease leaf symptoms at 9 MAP					
Source	**DF**	**Sum of squares**	**Mean square**	**F value**	**Pr>F**
Environment	1	80.656	80.656	399.7	<.0001
Replicate	2	0.4276	0.2138	1.06	0.3535
Environment (Replicate)	2	0.2987	0.1493	0.74	0.4817
Genotype	14	33.410	2.3864	11.8	<.0001
Environment*Genotype	14	31.634	2.2596	11.2	<.0001
Model	33	146.43	4.4372	22.0	<.0001
Error	56	11.304	0.2018		
Corrected total	89	157.73	1.7723		

Root necrosis at harvesting					
Source	**DF**	**Sum of squares**	**Mean square**	**F value**	**Pr>F**
Environment	1	10.000	10.000	87.3	<.0001
Replicate	2	0.4007	0.2003	1.75	0.1833
Environment (Replicate)	2	0.4247	0.2123	1.85	0.1661
Genotype	14	88.274	6.3053	55.1	<.0001
Environment*Genotype	14	53.530	3.8236	33.4	<.0001
Model	33	152.63	4.6251	40.4	<.0001
Error	56	6.4147	0.1146		
Corrected total	89	159.04	1.7870		

Root necrosis incidence (%) at harvesting					
Source	**DF**	**Sum of squares**	**Mean square**	**F value**	**Pr>F**
Environment	1	12854.6	12854.6	49.5	<.0001
Replicate	2	674.26	337.13	1.30	0.2814
Environment (Replicate)	2	1035.2	517.62	1.99	0.1460
Genotype	14	59437.6	4245.5	16.3	<.0001
Environment*Genotype	14	33089.9	2363.6	9.10	<.0001
Model	33	107091.0	3245.2	12.5	<.0001
Error	56	14552.8	259.9		
Corrected total	89	121644.3	1366.8		

Usable roots at harvesting (%) at harvesting					
Source	**DF**	**Sum of squares**	**Mean square**	**F value**	**Pr>F**
Environment	1	6625.5	6625.5	33.4	<.0001
Replicate	2	1030.3	515.13	2.59	0.0837
Environment (Replicate)	2	440.81	220.41	1.11	0.3368
Genotype	14	61039.5	4360.0	22.0	<.0001
Environment*Genotype	14	31944.3	2285.3	11.5	<.0001
Model	33	101130.3	3064.6	15.4	<.0001
Error	56	11121.3	198.60		
Corrected total	89	112251.7	1261.3		

Root weight (tonnes/hectare)					
Source	**DF**	**Sum of squares**	**Mean square**	**F value**	**Pr>F**
Environment	1	11.520	11.520	4.88	0.0313
Replicate	2	4.848	2.424	1.03	0.3649
Environment (Replicate)	2	3.9016	1.9508	0.83	0.4430
Genotype	14	1872.2	133.73	56.6	<.0001
Environment*Genotype	14	1141.3	81.518	34.52	<.0001
Model	33	3033.7	91.930	38.9	<.0001
Error	56	132.25	2.3616		
Corrected total	89	3165.9	35.572		

Root number per plant					
Source	**DF**	**Sum of squares**	**Mean square**	**F value**	**Pr>F**
Environment	1	28.112	28.112	75.5	<.0001
Replicate	2	0.0260	0.0130	0.03	0.9657
Environment (Replicate)	2	0.7202	0.3601	0.97	0.3862
Genotype	14	178.69	12.764	34.3	<.0001
Environment*Genotype	14	167.97	11.998	32.2	<.0001
Model	33	375.52	11.379	30.6	<.0001
Error	56	20.840	0.3722		
Corrected total	89	396.36	4.4535		

Dry matter content (%)					
Source	**DF**	**Sum of squares**	**Mean square**	**F value**	**Pr>F**
Environment	1	16.987	16.987	0.41	0.5257
Replicate	2	303.79	151.90	3.65	0.0325
Environment (Replicate)	2	249.19	124.59	2.99	0.0583
Genotype	14	4071.4	290.81	6.98	<.0001
Environment*Genotype	14	1749.6	124.97	3.00	0.0018
Model	33	6390.9	193.67	4.65	<.0001
Error	56	2332.6	41.654		
Corrected total	89	8723.6	98.017		

Harvest index					
Source	**DF**	**Sum of squares**	**Mean square**	**F value**	**Pr>F**
Environment	1	0.6250	0.6250	90.7	<.0001
Replicate	2	0.0016	0.0008	0.11	0.8934
Environment (Replicate)	2	0.0127	0.0063	0.92	0.4047
Genotype	14	1.0382	0.0742	10.8	<.0001
Environment*Genotype	14	0.9533	0.0681	9.88	<.0001
Model	33	2.6308	0.0797	411.6	<.0001
Error	56	0.3858	0.0069		
Corrected total	89	3.0166	0.0339		

Appendix 2: ANOVA tables for Tanzanian cassava local landraces

Cassava brown streak disease leaf symptoms at 3 MAP					
Source	**DF**	**Sum of squares**	**Mean square**	**F value**	**Pr>F**
Environment	1	0.0272	0.0272	0.33	0.5661
Replicate	2	0.0318	0.0159	0.19	0.8245
Blocks (Environment*Replicate)	44	4.9739	0.1130	1.37	0.0737
Genotype	63	66.408	1.0541	12.8	<.0001
Environment*Genotype	63	18.786	0.2982	3.62	<.0001
Model	173	102.36	0.5917	7.19	<.0001
Error	210	17.278	0.0823		
Corrected total	383	119.63	0.3124		

Cassava brown streak disease leaf symptoms at 6 MAP					
Source	**DF**	**Sum of squares**	**Mean square**	**F value**	**Pr>F**
Environment	1	2.7354	2.7354	35.4	<.0001
Replicate	2	0.4352	0.2176	2.82	0.0621
Blocks (Environment*Replicate)	44	3.9584	0.0955	1.24	0.1617
Genotype	63	89.238	1.4165	18.3	<.0001
Environment*Genotype	63	47.996	0.7618	9.86	<.0001
Model	173	161.15	0.9315	12.1	<.0001
Error	210	16.229	0.0773		
Corrected total	383	177.38	0.4631		

Cassava brown streak disease leaf symptoms at 9 MAP					
Source	**DF**	**Sum of squares**	**Mean square**	**F value**	**Pr>F**
Environment	1	7.4510	7.4510	63.9	<.0001
Replicate	2	0.8676	0.4338	3.72	0.0259
Blocks (Environment*Replicate)	44	6.956	0.1581	1.36	0.0826
Genotype	63	67.249	1.0674	9.15	<.0001
Environment*Genotype	63	44.782	0.7108	6.10	<.0001
Model	173	142.96	0.8264	7.09	<.0001
Error	210	24.490	0.1166		
Corrected total	383	167.45	0.4347		

Root necrosis at harvesting					
Source	**DF**	**Sum of squares**	**Mean square**	**F value**	**Pr>F**
Environment	1	5.1523	5.1523	193.4	<.0001
Replicate	2	0.0399	0.0200	0.75	0.4742
Blocks (Environment*Replicate)	44	1.0179	0.0231	0.87	0.7055
Genotype	63	106.05	1.6833	63.2	<.0001
Environment*Genotype	63	32.699	0.5191	19.5	<.0001
Model	173	164.03	0.9481	35.6	<.0001
Error	210	5.5950	0.0266		
Corrected total	383	169.62	0.4429		

Root necrosis incidence at harvesting (%)					
Source	**DF**	**Sum of squares**	**Mean square**	**F value**	**Pr>F**
Environment	1	21.603	21.603	2.29	0.1317
Replicate	2	45.178	22.589	2.39	0.0937
Blocks (Environment*Replicate)	44	495.70	11.266	1.19	0.2059
Genotype	63	150673.1	2391.6	25435	<.0001
Environment*Genotype	63	41436.1	657.72	69.7	<.0001
Model	173	219954.9	1271.4	134.8	<.0001
Error	210	1981.1	9.4336		
Corrected total	383	221936.0	579.47		

Usable roots at harvesting (%)					
Source	**DF**	**Sum of squares**	**Mean square**	**F value**	**Pr>F**
Environment	1	627.53	627.53	34.5	<.0001
Replicate	2	19.306	9.653	0.53	0.5888
Blocks (Environment*Replicate)	44	834.20	18.959	1.04	0.4088
Genotype	63	84375.6	1339.3	73.7	<.0001
Environment*Genotype	63	25271.0	401.13	22.1	<.0001
Model	173	125153.8	723.43	39.8	<.0001
Error	210	3817.7	18.179		
Corrected total	383	128971.4	336.74		

Root weight (tonnes/hectare)					
Source	**DF**	**Sum of squares**	**Mean square**	**F value**	**Pr>F**
Environment	1	1936.1	1936.1	214.2	<.0001
Replicate	2	6.8755	3.4377	0.38	0.6841
Blocks (Environment*Replicate)	44	324.45	7.3737	0.82	0.7869
Genotype	63	12162.0	193.05	21.4	<.0001
Environment*Genotype	63	5808.5	92.199	10.2	<.0001
Model	173	22401.0	129.49	14.3	<.0001
Error	210	1898.2	9.0389		
Corrected total	383	24299.1	63.444		

Root number per plant					
Source	**DF**	**Sum of squares**	**Mean square**	**F value**	**Pr>F**
Environment	1	6.6782	6.6782	10.3	0.0015
Replicate	2	2.710	1.355	2.09	0.1259
Blocks (Environment*Replicate)	44	30.369	0.6902	1.07	0.3728
Genotype	63	359.45	5.7056	8.81	<.0001
Environment*Genotype	63	166.51	2.6430	4.08	<.0001
Model	173	616.03	3.5609	5.50	<.0001
Error	210	135.98	0.6475		
Corrected total	383	752.02	1.9635		

Dry matter content (%)					
Source	**DF**	**Sum of squares**	**Mean square**	**F value**	**Pr>F**
Environment	1	2665.1	2665.1	509.5	<.0001
Replicate	2	0.9712	0.4856	0.09	0.9114
Blocks (Environment*Replicate)	44	308.17	7.0039	1.34	0.0914
Genotype	63	4216.9	66.935	12.8	<.0001
Environment*Genotype	63	2753.9	43.712	8.36	<.0001
Model	173	10556.9	61.023	11.7	<.0001
Error	210	1098.4	5.2305		
Corrected total	383	11655.4	30.432		

Harvest index					
Source	**DF**	**Sum of squares**	**Mean square**	**F value**	**Pr>F**
Environment	1	803.91	803.91	67.7	<.0001
Replicate	2	78.751	39.376	3.32	0.0381
Blocks (Environment*Replicate)	44	483.15	10.981	0.93	0.6089
Genotype	63	22084.2	350.54	29.5	<.0001
Environment*Genotype	63	3156.7	50.107	4.22	<.0001
Model	173	29587.8	170.98	14.4	<.0001
Error	210	2492.3	11.868		
Corrected total	383	32071.0	83.736		

Appendix 3: ANOVA tables for the Pwani × AR37-80 F_1 population

Cassava brown streak disease leaf symptoms at 3 MAP					
Source	**DF**	**Sum of squares**	**Mean square**	**F value**	**Pr>F**
Environment	1	1.6469	1.6469	20.3	<.0001
Replicate	1	1.2469	1.2469	15.4	0.0003
Blocks (Environment*Replicate)	21	1.9108	0.0910	1.12	0.3600
Genotype	35	24.562	0.7018	8.64	<.0001
Environment*Genotype	35	24.436	0.6982	8.59	<.0001
Model	93	64.598	0.6946	8.55	<.0001
Error	50	4.0622	0.0812		
Corrected total	143	68.660	0.4801		

Cassava brown streak disease leaf symptoms at 6 MAP					
Source	**DF**	**Sum of squares**	**Mean square**	**F value**	**Pr>F**
Environment	1	4.9506	4.9506	55.3	<.0001
Replicate	1	1.0167	1.0167	11.4	0.0015
Blocks (Environment*Replicate)	21	1.8631	0.0887	0.99	0.4894
Genotype	35	15.935	0.4553	5.09	<.0001
Environment*Genotype	35	19.098	0.5457	6.10	<.0001
Model	93	43.758	0.4705	5.26	<.0001
Error	50	4.4751	0.0895		
Corrected total	143	48.233	0.3373		

Cassava brown streak disease leaf symptoms at 9 MAP					
Source	**DF**	**Sum of squares**	**Mean square**	**F value**	**Pr>F**
Environment	1	7.7469	7.7469	111.3	<.0001
Replicate	1	0.6136	0.6136	8.82	0.0046
Blocks (Environment*Replicate)	21	2.3069	0.1099	1.58	0.0940
Genotype	35	14.886	0.4253	6.11	<.0001
Environment*Genotype	35	18.768	0.5362	7.71	<.0001
Model	93	48.620	0.5230	7.51	<.0001
Error	50	3.479	0.0696		
Corrected total	143	52.100	0.3643		

Root necrosis at harvesting					
Source	**DF**	**Sum of squares**	**Mean square**	**F value**	**Pr>F**
Environment	1	9.7136	9.7136	174.3	<.0001
Replicate	1	0.2336	0.2336	4.19	0.0459
Blocks (Environment*Replicate)	21	1.2492	0.0595	1.07	0.4105
Genotype	35	46.046	1.3156	23.6	<.0001
Environment*Genotype	35	22.257	0.6359	11.4	<.0001
Model	93	87.910	0.9453	17.0	<.0001
Error	50	32.787	0.0557		
Corrected total	143	90.698	0.6342		

Root necrosis incidence at harvesting (%)					
Source	**DF**	**Sum of squares**	**Mean square**	**F value**	**Pr>F**
Environment	1	25974.7	25974.7	1277.6	<.0001
Replicate	1	7.9336	7.9336	0.39	0.5350
Blocks (Environment*Replicate)	21	241.12	11.482	0.56	0.9232
Genotype	35	80415.3	2297.6	113.0	<.0001
Environment*Genotype	35	38880.9	1110.9	54.6	<.0001
Model	93	163111.1	1753.9	86.3	<.0001
Error	50	1061.6	20.331		
Corrected total	143	164127.7	1147.8		

Usable roots at harvesting (%)					
Source	**DF**	**Sum of squares**	**Mean square**	**F value**	**Pr>F**
Environment	1	3957.5	3957.5	233.6	<.0001
Replicate	1	45.002	45.002	2.66	0.1094
Blocks (Environment*Replicate)	21	482.08	22.956	1.35	0.1879
Genotype	35	46713.5	1334.7	78.8	<.0001
Environment*Genotype	35	26996.9	771.34	45.5	<.0001
Model	93	86891.0	934.31	55.2	<.0001
Error	50	847.13	16.943		
Corrected total	143	87738.1	613.55		

Root weight (tonnes/hectare)					
Source	**DF**	**Sum of squares**	**Mean square**	**F value**	**Pr>F**
Environment	1	435.07	435.07	99.9	<.0001
Replicate	1	0.0117	0.0117	0.00	0.9588
Blocks (Environment*Replicate)	21	43.798	2.0856	0.48	0.9662
Genotype	35	10043.1	286.94	65.9	<.0001
Environment*Genotype	35	1688.0	48.228	11.1	<.0001
Model	93	14369.8	154.51	35.5	<.0001
Error	50	217.86	4.3571		
Corrected total	143	14587.6	102.01		

Root number per plant					
Source	**DF**	**Sum of squares**	**Mean square**	**F value**	**Pr>F**
Environment	1	11.447	11.447	16.2	0.0002
Replicate	1	1.4003	1.4003	1.98	0.1658
Blocks (Environment*Replicate)	21	8.6783	0.4133	0.58	0.9106
Genotype	35	449.49	12.843	18.1	<.0001
Environment*Genotype	35	97.999	2.7999	3.96	<.0001
Model	93	662.45	7.1231	10.1	<.0001
Error	50	35.391	0.7078		
Corrected total	143	697.84	4.8799		

Dry matter content (%)					
Source	**DF**	**Sum of squares**	**Mean square**	**F value**	**Pr>F**
Environment	1	4418.9	4418.9	583.0	<.0001
Replicate	1	4.8034	4.8034	0.63	0.4298
Blocks (Environment*Replicate)	21	182.66	8.6982	1.15	0.3355
Genotype	35	1863.6	53.244	7.02	<.0001
Environment*Genotype	35	1815.4	51.868	6.84	<.0001
Model	93	8463.2	91.002	12.0	<.0001
Error	50	379.01	7.5802		
Corrected total	143	8842.2	61.834		

Harvest index (%)					
Source	**DF**	**Sum of squares**	**Mean square**	**F value**	**Pr>F**
Environment	1	161.08	161.08	11.3	0.0015
Replicate	1	27.127	27.127	1.90	0.1739
Blocks (Environment*Replicate)	21	188.85	8.9931	0.63	0.8752
Genotype	35	5859.1	167.40	11.7	<.0001
Environment*Genotype	35	6997.7	199.93	14.0	<.0001
Model	93	14667.9	157.72	11.1	<.0001
Error	50	712.77	14.255		
Corrected total	143	15380.1	107.55		

Appendix 4: ANOVA tables for the Namikonga × AR37-80 F_1 population

Cassava brown streak disease leaf symptoms at 3 MAP					
Source	**DF**	**Sum of squares**	**Mean square**	**F value**	**Pr>F**
Environment	1	1.3611	1.3611	32.6	<.0001
Replicate	1	0.0025	0.0025	0.06	0.8078
Blocks (Environment*Replicate)	21	0.5681	0.0271	0.65	0.8613
Genotype	35	27.811	0.7946	19.0	<.0001
Environment*Genotype	35	26.066	0.7448	17.8	<.0001
Model	93	63.551	0.6833	16.4	<.0001
Error	50	2.0894	0.0418		
Corrected total	143	65.640	0.4590		

Cassava brown streak disease leaf symptoms at 6 MAP					
Source	**DF**	**Sum of squares**	**Mean square**	**F value**	**Pr>F**
Environment	1	5.4834	5.4834	99.5	<.0001
Replicate	1	0.0367	0.0367	0.67	0.4181
Blocks (Environment*Replicate)	21	1.0826	0.0516	0.94	0.5514
Genotype	35	37.213	1.0632	19.3	<.0001
Environment*Genotype	35	36.649	1.0471	19.3	<.0001
Model	93	99.254	1.0672	19.4	<.0001
Error	50	2.7557	0.0551		
Corrected total	143	102.01	0.7134		

Cassava brown streak disease leaf symptoms at 9 MAP					
Source	**DF**	**Sum of squares**	**Mean square**	**F value**	**Pr>F**
Environment	1	5.5617	5.5617	74.4	<.0001
Replicate	1	0.1806	0.1806	2.42	0.1264
Blocks (Environment*Replicate)	21	1.5370	0.0732	0.98	0.5026
Genotype	35	39.476	1.1279	15.1	<.0001
Environment*Genotype	35	36.982	1.0566	14.1	<.0001
Model	93	102.37	1.1008	14.7	<.0001
Error	50	3.7374	0.0747		
Corrected total	143	106.11	0.7420		

Root necrosis at harvesting					
Source	**DF**	**Sum of squares**	**Mean square**	**F value**	**Pr>F**
Environment	1	6.2084	6.2084	47.7	<.0001
Replicate	1	0.1167	0.1167	0.90	0.3481
Blocks (Environment*Replicate)	21	2.5131	0.1197	0.92	0.5690
Genotype	35	72.993	2.0855	16.0	<.0001
Environment*Genotype	35	40.890	1.1683	8.98	<.0001
Model	93	133.44	1.4349	11.0	<.0001
Error	50	6.5051	0.1301		
Corrected total	143	139.95	0.9787		

Root necrosis incidence at harvesting (%)					
Source	**DF**	**Sum of squares**	**Mean square**	**F value**	**Pr>F**
Environment	1	6951.4	6951.4	128.9	<.0001
Replicate	1	22.801	22.801	0.42	0.5186
Blocks (Environment*Replicate)	21	774.46	36.879	0.68	0.8284
Genotype	35	137913.6	3940.4	73.1	<.0001
Environment*Genotype	35	70741.7	2021.2	37.5	<.0001
Model	93	234911.7	2525.9	46.8	<.0001
Error	50	2697.1	53.942		
Corrected total	143	237608.8	1661.6		

Usable roots at harvesting (%)					
Source	**DF**	**Sum of squares**	**Mean square**	**F value**	**Pr>F**
Environment	1	2546.04	2546.04	81.4	<.0001
Replicate	1	35.106	35.106	1.12	0.2946
Blocks (Environment*Replicate)	21	1015.0	48.331	1.54	0.1047
Genotype	35	82089.2	2345.4	75.0	<.0001
Environment*Genotype	35	56811.7	1623.2	51.9	<.0001
Model	93	156137.0	1678.9	53.7	<.0001
Error	50	1564.3	31.286		
Corrected total	143	157701.2	1102.8		

Root weight (tonnes/hectare)					
Source	**DF**	**Sum of squares**	**Mean square**	**F value**	**Pr>F**
Environment	1	688.19	688.19	126.4	<.0001
Replicate	1	33.834	33.834	6.21	0.0160
Blocks (Environment*Replicate)	21	122.36	5.8369	1.07	0.4073
Genotype	35	6860.3	196.01	36.0	<.0001
Environment*Genotype	35	692.10	19.774	3.63	<.0001
Model	93	9618.2	103.42	19.0	<.0001
Error	50	272.20	5.4440		
Corrected total	143	9890.4	69.164		

Root number per plant					
Source	**DF**	**Sum of squares**	**Mean square**	**F value**	**Pr>F**
Environment	1	40.960	40.960	45.4	<.0001
Replicate	1	0.2177	0.2177	0.24	0.6255
Blocks (Environment*Replicate)	21	18.373	0.8749	0.97	0.5139
Genotype	35	299.28	8.5508	9.47	<.0001
Environment*Genotype	35	251.29	7.1798	7.95	<.0001
Model	93	709.35	7.6274	8.45	<.0001
Error	50	45.149	0.9030		
Corrected total	143	754.50	5.2762		

Dry matter content (%)					
Source	**DF**	**Sum of squares**	**Mean square**	**F value**	**Pr>F**
Environment	1	2826.7	2826.7	171.6	<.0001
Replicate	1	0.04	0.04	0.00	0.9609
Blocks (Environment*Replicate)	21	544.83	25.944	1.58	0.0950
Genotype	35	2823.6	80.673	4.90	<.0001
Environment*Genotype	35	1070.0	30.570	1.86	0.0221
Model	93	8151.0	87.645	5.32	<.0001
Error	50	823.51	16.470		
Corrected total	143	8974.5	62.759		

Harvest index (%)					
Source	**DF**	**Sum of squares**	**Mean square**	**F value**	**Pr>F**
Environment	1	3387.2	3387.2	140.6	<.0001
Replicate	1	65.880	65.880	2.74	0.1044
Blocks (Environment*Replicate)	21	377.06	17.995	0.75	0.7661
Genotype	35	8990.5	256.87	10.7	<.0001
Environment*Genotype	35	4147.5	118.50	4.92	<.0001
Model	93	19696.0	211.76	8.79	<.0001
Error	50	1204.4	24.088		
Corrected total	143	209000.4	1461.5		

Appendix 5: Author's own publications

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Occurrence and estimated losses caused by cassava viruses in Migori County, Kenya

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A farm survey was conducted in Kuria East and Suna West sub-counties to determine the incidence, severity and estimated losses of cassava brown streak disease (CBSD) and cassava mosaic disease (CMD) on cassava crops in farmers' fields. The results showed that cassava is the second most important staple crop after maize in Migori County. CMD incidence ranged from 0.0 to 56.7%) in Kuria East and 10.0 to 55.0% in Suna West. CBSD incidences were much higher at 5.0 to 74.0% in Kuria East and 10.0 to 77.5% in Suna West. Both CMD and CBSD had an effect on yield reduction and total root loss ranged from 10.7 to 47.2% in Kuria East and 11.5 to 33.2 in Suna West. The percent mean total root loss in Kuria East was 25.9%; equivalent to 1299.6 US dollars/ha while in Suna East was 24.7%; equivalent to 1259.5 US dollars/ha. The best performing variety with regards to low CBSD and CMD incidence, low root losses and high yield were TMS 30572 and MH95/0183. The findings of this study are expected to provide impetus for the development and promotion of new high yielding, locally adapted and resistant cassava varieties.

Key words: Cassava, CBSD, incidence, root necrosis, yield loss.

INTRODUCTION

Cassava (*Manihot esculenta* Crantz) is a staple food for more than 800 million people world-wide (Lebot, 2009). It was initially adopted as a popular famine reserve crop but in recent times has emerged to be a profitable cash crop of industrial significance in the world economy (Larsson et al., 2013; Tonukari et al., 2015). In Kenya, the crop is grown for both food and income on approximately 72,482 ha with an annual output of 1.1 million tonnes (FAOSTAT, 2013). Western Kenya, where Migori County is located, accounts for 60% of total cassava production in Kenya. In Migori County, cassava is a staple food crop occupying about 8800 ha with mean yields of 6 and 12

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Genotype by environment interactions in identifying cassava (*Manihot esculenta* Crantz) resistant to cassava brown streak disease

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ABSTRACT

Cassava landraces were evaluated for resistance to cassava brown streak disease (CBSD) for two cropping seasons at a disease hotspot area in Naliendele, Tanzania. Based on reactions to CBSD, several landraces including Chimaje, Mfaransa and Supa B were considered to be resistant to the disease while Kikwada, Mbuyu, and Nyoka were tolerant. ANOVA revealed that the largest sum of squares (SS) (41.9–86.7%) was attributed to the genotype of the cassava landraces, while a smaller proportion of SS (8.1–38.2%) was due to genotype by environment interactions for all traits tested, which included disease symptoms, root weight, number of roots per plant and dry matter content. Environment accounted for the smallest effect (0.01–26.3%), however, the mean squares was nonetheless significant for a few genotypes, which indicated that their disease expression was indeed influenced by the environment. Increased CBSD severity was associated with low temperatures and rainfall. Increased rainfall towards harvesting led to higher root weight but lower dry matter content in the first cropping season. Correlation analysis showed that the presence of CBSD symptoms reduces the amount of usable roots, total root weight, and root dry matter content. Many resistant/tolerant landraces also had high root weight and dry matter content, and they can be used by farmers to reduce CBSD losses. The landraces described here form novel sources of CBSD resistance that can be used for breeding disease-resistant cassava varieties with superior agronomic characteristics.

1. Introduction

3 Cassava brown streak disease (CBSD) is arguably the most dangerous threat to cassava (*Manihot esculenta* Crantz), which is Africa's most important food security crop. The disease causes losses to cassava root production and quality. *Cassava brown streak virus* (CBSV) (Monger et al., 2001) and *Ugandan cassava brown streak virus* (UCBSV) belonging to the genus *Ipomovirus*, famly *Potyviridae* cause CBSD (Mbazibwa et al., 2009). Both viruses, together called cassava brown streak ipomoviruses (CBSIs), have a positive-sense single stranded RNA genome (Winter et al., 2010; Ndunguru et al., 2015). CBSD symptoms include foliar chlorosis and necrosis, brown streaks on stems, constrictions and dry corcky necrotic rot of roots and stunted plant growth (Hillocks and Jennings, 2003; Winter et al., 2010; Vanderschuren et al., 2012). CBSIs are mainly spread by the propagation of infected cassava cuttings by farmers, but also transmitted in a semi-persistent manner by the insect vector whiteflies, *Bemicia tabaci* (Gennadius) (Maruthi et al., 2005, 2017; Mware et al., 2009).

Early reports on CBSD distribution identified the disease to be mostly restricted to the East African coast and the shores of Lake Malawi (Nichols, 1950). For many years, it was believed that the disease does not spread at altitudes 1000 m above sea level (Nichols, 1950; Hillocks et al., 1999). However, in recent years, outbreaks of CBSD have been reported at mid altitude levels (1200–1500 m above sea levels) in Uganda, western Kenya and Tanzania, Mozambique, Rwanda, Burundi, and in isolated parts of the Democratic Republic of Congo (Mahungu et al., 2003; Alicai et al., 2007; Jeremiah and Legg, 2008; Osogo et al., 2014). CBSD is currently estimated to cause annual economic losses in excess of US$ 726 million (Maruthi M N, unpublished data). A definitive cause for the sudden upsurge in CBSD incidences is yet to be identified, however, the introduction of the virus to mid-altitude areas and the presence of high whitefly populations are considered to be the key drivers of new CBSD outbreaks (Legg et al., 2011, 2014).

CBSD symptom expression and resistance to the virus depends on the type of the variety (resistant or susceptible), growing conditions

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Appendix 6: Research authorisation

UNIVERSITY of GREENWICH 125 YEARS Established 1894
NRI | Natural Resources Institute

TO WHOM IT MAY CONCERN

12 July 2020

Dear Sir/ madam,

This is to confirm that Ms Emily Masinde visited the Natural Resources Institute (NRI) of the University of Greenwich in the UK for one year from March 2015 to March 2016 as part of her PhD programme. She was permitted to study in the UK as part of the project LimitCBSD – 'Limiting the impact of cassava brown streak disease on cassava value chain, smallholders and women farmers in eastern Africa' funded by the African Union commission. Emily's studies were funded by LimitCBSD. The project was led by me from NRI in which the Egerton University was one of the partners. Emily made excellent progress and successfully completed all her research objectives while in the UK, which contributed towards her PhD studies.

If you have further queries on this matter, please do not hesitate to contact me.

Yours sincerely,

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