GENETIC ANALYSIS OF HOST PLANT RESISTANCE TO THE CASSAVA MOSAIC DISEASE

By

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Abstract

The genetic control of resistance to the cassava mosaic disease (CMD) in some African landraces, their relationship with the widely used resistant genetic stock, clone 58308, and molecular markers associated with resistance to CMD in the landraces were evaluated in this study.

The F_1 progenies of 54 cassava crosses and their parents (clone 58308, six improved clones and 15 African landraces) were evaluated in two genetic experiments in three environments in Nigeria. Genetic analysis revealed that additive gene effect was more important in the genetics of resistance to CMD among the landraces, while both additive and non-additive gene effecsts were important for clone 58308.

The segregating F_1 crosses exhibited varying levels of resistance and susceptibility to CMD suggesting polygenic inheritance. Resistant phenotypes were detected in the crosses involving susceptible parents which suggests that resistance to CMD in the clones studied is due to recessive genes and susceptible phenotypes in crosses involving resistant parents suggested that the resistance genes are non allelic. Positive transgressive segregants were also detected in some crosses. The number of effective factors for resistance to CMD ranged from two to seven and was contributed by both parents in a cross. Significant differences in the mean distribution of F_1 progeny disease severity scores further revealed allelic differences between three improved clones and some landraces, and that expression of resistance is influenced by the nature of the female parent.

Bulk segregant analysis (BSA) showed that an SSR marker SSR30-180 was associated with CMD resistance in a cross between the susceptible clone TMSI30555 and the resistant landrace TME7. Linkage analysis revealed that SSR30-

180, which was donated by TME7, was 14.9 cM from a putative CMD resistance locus, CMDRL. Marker-trait association detected by regression analysis further showed that markers, SSR30-180, E-ACC/M-CTC-225, SSR119-A and SSR119 accounted for 57.41%, 22.5%, 32.24% and 37.83% of the total phenotypic variation respectively, for resistance to CMD in the mapping population. The results further showed differences between TME7 and six out of the 15 resistant clones used as parents and checks in the genetic experiments with respect to SSR30-180, suggesting that different alleles are involved in resistance to CMD. Two susceptible clones TME31 and TME117 however, had the marker, which suggests that SSR30-180 is not tightly linked to resistance, thus there is a need to saturate the map with more markers.

These findings are useful in the selection of cassava parental clones for resistance breeding.

Declaration

We declare that this work was carried out by Ms. Yvonne Lokko, of the University of Ghana, Ghana, in collaboration with the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria under our supervision.

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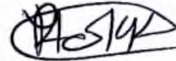

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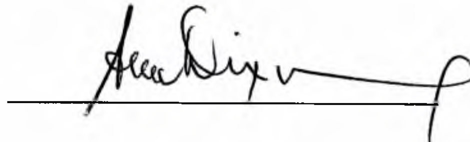

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Dedication

This thesis is dedicated to my father Dr. R.B. Lokko, my brothers and sister, and the loving memory of my mother, Mrs. Mercy Lokko.

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Chapter One

Introduction

The cassava mosaic virus disease (CMD) is considered the most devastating disease of cassava in Africa (Jennings, 1977; Geddes, 1990). It is caused by the *cassava mosaic geminiviruses*, transmitted by the whitefly (*Bemisia tabaci* Genn) and spread through propagation of infected vegetative propagules. The cassava mosaic disease causes severe yield losses ranging from 20 to95%, and the effect of the disease is most severe when plants are infected at the early crop stage (Terry and Hahn, 1980; Otim-Nape *et al.,* 1994a; Thresh *et al.,* 1994a). It is estimated that total crop yield losses due to CMD in Africa is about $2 billion per annum (IITA, 1997).

The most effective means of controlling CMD is by cultivating resistant genotypes (Thresh *et al.,* 1994b). In tackling the early disease challenges in Africa, resistance to CMD was obtained from a cross between cassava and its relative *Manihot glaziovii* Muller von Argau (Nichols, 1947). Progenies from the third backcross generation were evaluated in different African countries and clone 58308, which had edible storage roots and resistance to the disease, was selected in Nigeria. Clone 58308 has been the main source of resistance used by breeders for several decades and has resulted in the selection of several improved cultivated cassava genotypes of the Tropical Manihot Selection (TMS) series, with resistance to CMD (IITA, 1973; Hahn *et al.,* 1989). Some of the genotypes which are widely cultivated in Africa include TMS I30001, TMS I30572, TMS I4(2)1425, TMS I60142 and TMS I90257.

Despite the progress made in resistance breeding, the disease is still a threat to cassava production. Recent disease epidemics in East Africa, caused by a recombinant strain of two of the cassava begomoviruses (Zhou *et al.*, 1997) resulted in losses of 2.2 billion tons losses in storage root yield that amounted to US $440

million (Thresh *et al.,* 1997). Furthermore, exotic germplasm from South America are generally not resistant to CMD and succumb to the disease under field conditions in Africa (Porto *et al.,* 1994). Thus, much of the germplasm introduced from the crop's centre of origin, to broaden the genetic base in Africa has been lost (Jennings and Hershey, 1985). There is therefore a need to increase the levels of resistance within the cassava genepool (Thresh *et al.,* 1994a). To ensure that durable resistance is maintained within the African cassava germplasm, additional sources of resistance with a wider genetic base will be required to diversify resistance to the disease. This would allow the accumulation of desirable gene combinations that would be difficult for the pathogens to circumvent in the long term.

Cassava is open pollinated and in the field hybridises with itself and related wild species creating greater variability for different traits. Silvestre and Arraudeaus (1983), noted that African farmers sometimes take cuttings from spontaneous seedlings for their subsequent planting (cited in Lefevre and Charrier, 1993). These new genotypes, referred to over time as landraces, provide a wealth of new genes for desirable traits and form part of the crop's genetic resource (Gulick *et al.,* 1983; Hershey, 1987). Some of these African landraces, the Tropical *Manihot Esculenta* series (TME), have been identified to be resistant to CMD (Raji, 1995) and could serve as new sources of resistance. However, selection of parents to be incorporated in a breeding programme cannot be based on their performance alone. Inheritance of traits depends on the type of gene action that may be involved and the extent of diversity among the parents. Understanding the mode of gene action and inheritance in the various sources of resistance is important in determining the appropriate breeding strategy to adopt.

To efficiently utilise new sources of resistance to CMD in breeding programmes and to diversify resistance when transferred into elite material, it is essential to compare the various resistant genotypes with each other to determine if the loci for resistance

are similar, and how their effects complement each other to enhance resistance. Establishing genetic relationships between the different sources of resistance would facilitate the choice of parents for developing new resistant cultivars. In addition, by combining different genes that relate to different sources of resistance, epistatic interaction may be identified such that higher levels of resistance can be developed to protect the crop.

Resistance to CMD derived from clone 58308 has been reported to be polygenic, recessive and inherited largely in an additive manner (Hahn and Howland, 1972). Polygenic inheritance was also reported for some of the African landraces (Lokko *et al.*, 1998). Hahn *et al.* (1980a), deduced from this that resistance to the disease must be attributed to the combined action of a number of loci which are linked on a chromosome or a set of chromosomes. Population improvement and recurrent selection was therefore recommended in breeding for resistance to the disease (Hahn *et al.*, 1979). Interestingly, a major dominant gene responsible for resistance in an African landrace TME3 (known as 2nd Agric in Nigeria) has been reported (IITA, 2000; Akano *et al.*, 2002). Further progress in understanding CMD resistance could be made by identifying the individual gene or genes affecting resistance and determining their position on the chromosome map. These resistance genes could then be transfered to susceptible clones using conventional crossing coupled with marker assisted selection (MAS) and by genetic transformation.

The expression of CMD in different cassava genotypes is known to be influenced by genotype x environment interaction (GXE) on the trait (Fargette *et al.*, 1994). Significant GXE effect on a trait reduces the reliability of the phenotype as an indicator of the genotype (Kang, 1998). To obtain more precise genetic information on resistance to CMD, experiments are conducted in different environments (i.e. locations x years). This would demonstrate the GXE effect on the expression of the trait.

The detection of multiple genes for virus resistance using segregation analysis alone is not efficient because of the differences due to genotype by environment interaction (McMullen and Louie, 1989). Molecular markers which are not affected by environmental conditions and are insensitive to gene interactions allow geneticists and to locate and follow the numerous interacting genes that determine a complex trait as well as tagging those controlled by single genes (Botstein, et al., 1980). The two main strategies used to identify molecular markers associated with traits of interest are genetic linkage mapping and bulk segregant analysis (Tanksley *et al.,* 1989; Michelmore *et al.,* 1991; Giovannoni *et al.,* 1991).

Genetic linkage mapping as a tool for localising and isolating both simple and complex traits such as resistance to CMD can provide a more direct method for selecting desirable genes via their linkage to easily detectable molecular markers (Tanksley *et al.,* 1989). Once the trait is identified and mapped, MAS could be used to verify its introduction into new genotypes developed in a breeding programme. MAS can reduce breeding population sizes, continuous recurrent testing, and the time required in developing a superior line by selecting the desired genotype early in the breeding programme.

Bulk segregant analysis (BSA) is a rapid method for identification of markers in specific regions of the genome (Michelmore *et al.,* 1991; Giovannoni *et al.,* 1991). The technique has the potential of detecting unique regions in the genome associated with the trait of interest. DNA samples are pooled from individuals with extreme phenotypes for a particular trait, or marker allele(s), and are then screened to determine molecular markers associated with the trait of interest. A marker of interest can then be converted into a sequence characterised amplified region (SCAR) marker, for the rapid screening of the trait in other populations.

A genetic linkage map of cassava has been constructed at the Centro Internacional de Agricultura Tropical (CIAT) in Colombia (Fregene *et al.*, 1997). The fully saturated map would allow cassava breeders and geneticists to identify quantitative trait loci (QTLs) and clone the genes associated with resistance to CMD. Another map that is being developed at the International Institute of Tropical Agriculture (IITA) in Nigeria, to identify genes for resistance to CMD, could be used together with the CIAT map in developing a consensus map of cassava. A consensus genetic linkage map would be of tremendous importance to cassava research in that it would allow researchers to (i) identify informative markers to use in experiments, (ii) identify QTL of economic traits, (iii) generate a genomic database of cassava and (iv) could also be used for comparative mapping with other species. To facilitate saturating the maps with molecular markers associated with CMD resistance, more segregating populations need to be developed and evaluated in different environments to accumulate quantitative data on resistance to CMD. Bulk segregant analysis (BSA) and linkage analysis could then be used to place the markers on the maps.

The objectives of this study were therefore to:

i) evaluate the relative importance of general and specific combining ability, estimate heterosis and heritability, and determine maternal effects for resistance to CMD;

ii) evaluate the genetic relationships among the various sources of resistance to CMD;

iii) identify molecular markers linked to genes for resistance to CMD.

Chapter Two

Literature Review

2.1 Importance of Cassava and production constraints

The cassava crop is a perennial shrub belonging to the *Euphorbiaceae* family. It was introduced into Africa and Asia from South America by Portuguese travellers in the fifteenth century (Jennings and Hershey, 1985; Allem, 1994).

Cassava is cultivated mainly for its storage roots, which are a major staple in Africa, and serves as a foreign exchange earner in many countries (Dorosh, 1988; Cock, 1985b). The storage roots are consumed directly after boiling or processed into various food products (Allem and Hahn, 1991). The storage roots are also processed into starch for industrial purposes, flour for confectionery and animal feed. The peels also serve as fodder for livestock (Bokanga, 1998; Mandal, 1993). The leaves which contain appreciable amounts of vitamins, minerals and proteins are consumed as green vegetables in many parts of Africa particularly in Zaire, Sierra Leone, Liberia, Guinea and Cameroon (Lancaster and Brooks, 1983; West *et al.*, 1988; Bokanga, 1994).

The crop can grow in poor soils on marginal lands where other crops cannot grow and requires minimal fertiliser, pesticides and rainfall (FAO, 2000; Cock and Howeler, 1978). It can be harvested any time from 6 to 24 months after planting, and can be left in the ground as a food reserve for household food security in times of famine, drought and war (FAO, 2000; Best and Henry, 1994; Cock, 1985a).

Global cassava production in 1999 was over 160 million tons with Africa contributing over 50% of the global production cultivated on about 75 million hectares of farmland (Thresh *et al.*, 1994b; FAO, 2000).

Cassava is cultivated in diverse agroecologies in Africa including the coastal belts, the rain forest, the moist and dry savannah, as well as the Sahel regions (Allem and Hahn, 1991; IITA 1998). It has been estimated that in Africa, the annual production of 6.1 t/ha under farmer's conditions, is a shortfall from its potential of 30.5-51 t/ha (Hahn *et al.,* 1989; FAO, 2000). This shortfall in production is attributed to yield losses due to diseases, pest and civil strife in some African countries, which disrupt farming activities (FAO, 1997). The major diseases that affect the crop in Africa are, the cassava mosaic disease (CMD), cassava bacterial blight (CBB), and the cassava anthracnose disease (CAD). The cassava green mite (CGM) is also an important pest of the crop (Théberge *et al.,* 1985; Yaninek *et al.,* 1989). Yield losses in storage root caused by any one of these diseases or pest in isolation is estimated at over 80% (Yaninek *et al.,* 1989; Lozano. 1992; Thresh *et al.,* 1997; Otim-Nape *et al.,* 1994a).

2. 2 Viruses affecting cassava

Cassava is affected by a number of viruses belonging to several genera and families. The viruses of major economic importance are, the *cassava mosaic geminiviruses* (CMG) which are *begomoviruses* belonging to the *geminiviridae*; the *cassava common mosaic virus* (CsCMV) which is a *potexvirus*; the *cassava vein mosaic virus* (CsVMV) belonging to the *caulimoviridae* family; the *cassava frog skin virus* (CsFSV) belongs to the *redoviridae* family (Bock and Harrison, 1985; Fauquet and Fargette, 1990; de Kochko *et al.*, 1998; Calvert *et al.*, 1996). The CsCMV, CsVMV and CsFSV are important in South America, while the *cassava mosaic geminiviruses* are important in India and in Africa (de Kochko *et al.*, 1998; Calvert *et al.*, 1996; Harrison *et al.,* 1997b). The *cassava brown streak virus* (CBSV) belonging to the family *potyviridae*, genus *Ipomovirus* has recently been identified as a major problem in East Africa (Msikita et al., 2000).

2.3 Cassava mosaic disease

The cassava mosaic disease was first reported in East Africa in 1894 (Jennings, 1994). It is now prevalent in many parts of Africa, India and Sri Lanka (Thresh *et al.*, 1994a). The disease is caused by the cassava begomoviruses and transmitted by the whitefly (*Bemisia tabaci* Genn), which feeds on young cassava leaves. The disease is spread through propagation of infected vegetative propagules and infected plants serve as a source of inoculum for further whitefly spread (Storey and Nichols, 1938; Théberge *et al.*, 1985). The cassava mosaic disease has been rated as the most important vector-borne disease of any crop in Africa (Geddes, 1990).

2.3.1 Characteristics of cassava mosaic viruses

The cassava begomoviruses are composed of two circular single-stranded DNA particles belonging to the family *Geminiviridae* (Harrison and Robinson, 1988; Hong *et al.*, 1993). There are five distinct *cassava mosaic geminiviruses* (CMG), which have been identified based on their nucleotide sequences. These include the *African cassava mosaic virus* (ACMV), the *East African cassava mosaic virus* (EACMV), the *Indian cassava mosaic virus* (ICMV), the *South African cassava mosaic virus* (SACMV), and the Uganda variant of the *East African cassava mosaic virus* (EACMV-Ugv) (Zhou *et al.*, 1997; Berrie *et al.*, 1998). ACMV and EACMV have been reported in West Africa, East Africa, and some parts of Southern Africa while the ICMV occurs in India and Sri Lanka (Harrison *et al.*, 1997a; Ogbe *et al.*, 1999; Offei *et al.*, 1999). The Ugandan variant has been reported in only East Africa and SACMV has also been reported in only Southern Africa. Recently, another strain of EACMV was reported in Cameroon (Fondong *et al.*, 2000).

The viruses can be detected and distinguished serologically by their reaction with specific monoclonal antibodies (Mabs) raised against them and by the polymerase chain reaction (PCR) using primers based on the differences in the nucleotide

sequences of their genome (Thomas *et al.,* 1986; Zhou *et al.,* 1997). Nucleotide sequence analysis and serological reactions suggest that Ugv and SACMV are variants of EACMV and ACMV (Zhou *et al.,* 1997; Berrie *et al.,* 1998). Biological differences, such as ease of mechanical transmission into *Nicotiana benthamiana* and optimum temperature for replication, may also be used to distinguish between the viruses (Robinson *et al.*, 1984; Hong *et al.*, 1993).

2.3.2. Effects of CMD on cassava

The distinct symptom of the disease is mosaic (Rossel *et al.,* 1992). The symptoms first appear in young leaves and shoot apices after infection. When the whiteflies feed on the plant, the virus enters and moves down rapidly to the base of the stem. It is then systemically transferred into side branches and shoot apices causing characteristic disease symptoms (Storey and Nichols, 1938; Jos *et al.,* 1984; Rossel *et al.,* 1992). Infected stem cuttings also sprout into infected plants due to the upward translocation of the virus particles.

The internal anatomy of cassava plants and the test plant *Nicotiana benthamiana* infected with cassava mosaic virus, have been shown to include changes in phloem parenchyma and sieve tube cells (Horvat and Verhoyen, 1981; Adejare and Coutts, 1982). Reduced stem girth, plant height and petiole length of infected plants have been attributed to infection (Thankappan and Chacko, 1976). In some cases, the infected plants become stunted (Kaiser and Teemba, 1979). Recent studies have shown that most plants with such symptoms of severe stunting may be due to mixed infection, involving both ACMV and EACMV or ACMV and UgV (Harrison *et al.,* 1997b; Fondong *et al.,* 2000).

Adverse effects of the disease on the physiology of plants have also been studied. These include a reduction in the plant photosynthetic leaf area that affects the plant's metabolism (Vuylsteike, 1981; Nian *et al.,* 1976). Vuylsteike (1981) described the

adverse effects on the leaves and metabolism as an increase in chlorophylase production in the leaves, and an increase in amylase in the storage roots, which adversely affects the accumulation of starch in the storage roots. Thus at harvest, storage root yield, quality and starch content are reduced. The quality and nutrient content of the leaves is also reduced due to the reduction in leaf sizes, mosaic pattern and physiological changes (Alagianagalingan and Ramakrishaman,1970; Nian *et al.,* 1976).

Various authors have shown a positive correlation between symptom severity and yield reduction (Hahn *et al.,* 1980a; Otim-Nape *et al.,* 1994a). Fauquet *et al.,* (1987a) observed that varietal responses to the disease under the same growth conditions also vary. Yield losses sustained in different cassava genotypes are reported to range from 20 to 95% (Thankappan and Chacko, 1976; Terry and Hahn, 1980; Seif, 1982; Fargette *et al.,* 1988). The effect of CMD on the yield of storage roots, however, depends on the variety and age of the plant (Thresh *et al.,* 1994a). Losses are more severe when plants are infected during their early growth stage and plants infected through infected clonal material sustain greater storage root yield losses than those infected by whiteflies (Terry and Hahn, 1980; Fargette and Viê, 1988).

Fargette *et al.,* (1988) estimated yield losses from CMD per annum of up to 30 million tons out of 51 million tons production in are incurred in Côte d'Ivoire. An epidemic of CMD during the 1990s in Uganda caused over 2.2 billion tons storage root losses that amounted to US $440 million (Thresh *et al.,* 1997). It is estimated that total crop yield losses due to CMD in Africa is about $2 billion per annum (IITA, 1997).

2.3.3 Control

Various control methods have been suggested for the control of CMD. Since infected planting material is the primary source of the viruses, most of these methods are

based on phytosanitation, cultural practices and the use of resistant material (Thresh *et al.,* 1998b; Fauquet and Fargette, 1990; Bock, 1994).

Phytosanitation include the use of virus-free planting material derived from thermotherapy, and meristem culture, roguing out diseased plants from within crop stands and removing diseased cassava plants around an intended planting site and exploiting the phenomenon of reversion (Thresh *et al.,* 1998a).

In thermotherapy, the plants are grown under temperatures of 37°C and 39°C for 28 to 105 days, to reduces the virus concentration and incidence of the disease in some genotypes (Kaiser and Teemba, 1979; Kaiser and Louie, 1982). In some genotypes, symptoms reappear when heat treatment is stopped, whiles others can remain healthy for 4 to 5 months after heat treatment (Gibson, 1994).

Virus-free cassava plants are produced through meristem-tip culture (Kaiser and Teemba, 1979; Adejare and Coutts, 1981; Ng *et al.*, 1992). The use of heat therapy before meristem-tip culturing gives a higher number of virus-free plants and also allows the use of larger pieces (0.5 – 0.8 mm) of the meristematic dome to regenerate disease-free plants (Adejare and Coutts, 1981).

Roguing is recommended at the early stage of growth before the infected plants serve as inoculum for further spread (Thresh and Otim-Nape, 1994). This practice, however, is not effective in reducing CMD under high disease pressure and among genotypes that do not have substantial resistance to virus infection (Thresh *et al.,* 1998b; Fargette *et al.,* 1990). Fargette *et al.* (1990) observed that CMD spread between fields was more important than within fields. Thresh *et al.*, 1998b deduced from this that roguing could be effective only if practised by farmers throughout whole localities. In the 1950s in East Africa for instance, roguing as a control measure was effective with strict enforcement by the local authorities (Thresh *et al.*, 1998b; Thresh *et al.,* 1994b).

Reversion whereby the infected cuttings produce disease-free plants in the subsequent generation has also been exploited to select and produce healthy cuttings for CMD epidemiological studies in Côte d'Ivoire (Fargette *et al.,* 1985; Fargette *et al.,* 1988). Fargette and Vié (1995) using this method showed that although the healthy plant may become infected in the cause of the growth period, these plants have less yield loss than plants that were raised from infected material.

The recommended cultural practices to control CMD are targeted at time of planting and stem types. Cuttings obtained from the lower portions of the main stem have been found to sprout into more infected plants than cuttings from the upper portion of the main stem and stem branches (Njock *et al.,* 1994). Ogbe *et al.,* (1996), comparing cuttings from 6 to 8 month-old mother-plants with those from 10 to 14 month-old mother-plants, observed more infected plants in the former. It has, therefore, been recommended that cuttings from the upper portion of mature plants be used for planting to control the disease.

Another strategy to control CMD is to avoid planting when the whitefly populations are high. In the rainforest and transition forest agroecologies of Nigeria, for example, the major peak of whitefly population is between April and June. This is followed by a low population of the insect between July and August and a minor peak during September to December (Leuschner, 1978). Fargette *et al.* (1994) demonstrated that plants are generally more susceptible to secondary infection by whiteflies during the first 8-12 weeks. Terry and Hahn (1980), also showed that CMD infection at two months greatly reduced yield of storage roots. The results of Terry and Hahn (1980) also showed that losses due to late infections were lower in plants derived from clean planting material than plants from infected cuttings. Fauquet *et al.* (1988) suggested the use of low CMD incidence or low pressure sites to produce virus free planting material for distribution and as experimental material.

Plant resistance is the most important means to control virus diseases and the resultant crop losses due to them (Fraser, 1990). Resistant cassava genotypes tend to have lower CMD spread with mild to moderate symptoms (Jennings, 1960; Otim-Nape *et al.*, 1998) and the resistant genotypes also have higher storage root yield when compared with the susceptible genotypes (Terry and Hahn, 1980). Resistant varieties were used to control the spread of CMD (Cours-Darne, 1968) in Madagascar in the 1920s. More recently in the 1990s improved resistant cassava varieties were used when there was an outbreak of UgV in Uganda (Otim-Nape *et al.*, 1994b).

2.4 Host plant resistance to the cassava mosaic disease (CMD)

Breeding for resistance to CMD started in the 1920s in East Africa and Madagascar (Jennings, 1994). Initially several cassava varieties were screened and the resistant ones released to farmers to stop the spread of the disease (Cours-Darne, 1968). There was, however, the need for higher levels of resistance. Story and Nichols (1938), developed an interspecific cross between *M. esculenta* and *M. glaziovii* Muller von Argau, which resulted in hybrids which had non-tuberous root and showed mild and transient symptoms. After three backcrosses to cassava, they were able to restore root quality and retain resistance to CMD (Nichols, 1947). Intercrosses between the third backcross selections produced a number of promising clones with good quality roots and resistance to virus was enhanced (Jennings, 1994). Open pollinated seeds from these clones were distributed to cassava breeders in Africa including Nigeria where clone 58308 was selected (Hahn *et al.*, 1989; Jennings, 1994). The clone 58308 has poor root yield but has maintained resistance to CMD. It has high parental value for CMD and has been used as the main source of resistance in IITA's breeding programme resulting in several improved cultivars of the Tropical Manihot Selection (TMS) series, with resistance to CMD (IITA, 1973; Hahn *et al.*, 1989).

Resistance from this source is known to be polygenic, recessive, and is additively inherited with a heritability of over 60% (Hahn *et al.,* 1980a; Hahn *et al.,* 1989). Therefore, population improvement and recurrent selection are recommended in breeding for resistance to the disease (Hahn *et al.,* 1989). This would accumulate desirable gene combinations that would be difficult for the pathogens to circumvent in the long term.

Mutation breeding, somaclonal variation and genetic engineering have been suggested as additional tools in breeding for resistance to the cassava mosaic virus disease (Ahiabu *et al.,* 1995; Ahloowalia, 1995; Maluszynski *et al.,* 1995; Fauquet and Beachy, 1991). These methods, however, require skills in mutant induction and selection, generating somaclones and transgenics, and an efficient regeneration system to avoid chimeras and undesirable mutants.

Although no genes conferring resistance to CMD have been cloned, transformation protocols for cassava have been established (Schöpke *et al.,* 1997). The coat protein sequences of the virus have been characterised (Hong *et al.,* 1993) and could be used to generate gene constructs for transformation. The defective infective (DI) DNA of the ACMV-B particle has been shown to induce amelioration of symptoms in transgenic *N. benthamiana* plants and the gene was passed on when the transgenics were used as a source of inoculum (Stanley *et al.,* 1990). This could also be adapted in cassava transformation, for resistance to CMD.

2.4.1 Mechanism of resistance

The mechanism of resistance to CMD is not fully understood and no immunity to CMD has been found within the *Manihot* species. Resistance to CMD is either by resistance to virus infection or resistance to the vector, *B. tabaci* (Rossel *et al.,* 1992). Cassava genotypes which are resistant to virus infection show mild symptoms, which may be restricted to some shoots and have higher storage root

yield when compared to the susceptible genotypes (Jennings, 1976; Otim-Nape *et al.*, 1998; Fargette *et al.*, 1996; Terry and Hahn, 1980). They recover from symptoms with age (Njock *et al.*, 1996) and contain low virus concentrations (Fargette *et al.*, 1996). The ability of resistant genotypes to localise or restrict the movement of the virus has been referred to as non-systemicity (Thresh *et al.*, 1998b; Njock, 1994). Infected cuttings of resistant genotypes are also able to sprout into healthy plants in subsequent generations. This phenomenon is known as reversion (Fauquet *et al.*, 1987b).

It has been observed that the extent of reversion is higher when plants are infected by the whitefly earlier in its growth cycle rather when infection is due to existing virus concentration in the mother cuttings used in planting or when infection by whitefly is delayed later in the plant's growth cycle or (Thresh *et al.*, 1998b). Simulation models have postulated that virus infection of resistant genotypes could be maintained at an equilibrium below 50% by the combined effect of reversion and the use of virus-free planting material (Fargette *et al.*, 1994; Fargette and Vié, 1995).

Resistance to the whitefly vector has also been established. Fauquet *et al.* (1987b) observed that there was a clearly pronounced resistance to the vector. Fargette *et al.* (1996) also reported that resistant and susceptible cassava genotypes display a wide range of responses to whitefly population. Legg (1994), also observed variation in the suitability of some cassava genotypes, as hosts *of B. tabaci*. In contrast, Hahn *et al.* (1980b) observed similar numbers of whiteflies on resistant and susceptible genotypes and inferred that resistance to the vector was unlikely.

Resistance to CMD is assessed by a number of components of resistance. These include field incidence (percentage of infected plants); vector resistance (number of adult whiteflies per plant); inoculation resistance, virus resistance (virus content estimated by ELISA), virus diffusion resistance (development of symptoms with time),

symptom intensity and non-systemicity incidence of young growing tips or shoot tips (Fargette *et al.,* 1996; Changa, 1992). Fargette *et al.* (1996) demonstrated a significant correlation between symptom severity and ACMV titre among resistant genotypes in two separate trials. Resistance to virus multiplication and movement were the major responses to ACMV detected among resistant cassava landraces and improved cultivars (Ogbe, 2001).

2.5. Genetics of cassava

Cassava is a highly heterozygous species (Kawano, 1978; Lefevre and Charrier, 1993). When selfed, it exhibits inbreeding depression but with outcrossing it results in wide segregation of most traits (Kawano, 1978). The F_1 is, therefore, genetically equivalent to an F_2 with respect to loci common to parents (Magoon and Krishnan, 1977). It is propagated using stem cuttings to reproduce the genotype since propagation via seeds results in trait losses in the genotype due to the highly heterozygous nature (Kawano, 1980). Apomixis whereby seeds are produced without undergoing the sexual process has also been reported (Grattapagalia *et al.,* 1996). Cultivated cassava genotypes generally have erratic flowering (Bonierbale *et al.,* 1997a). Development of crosses for genetic studies has to some extent been difficult because of some levels of sterility, low seed set per pollination, and seed sterility that exist in cassava (Kawano, 1980; Hahn *et al.,* 1979). Nevertheless, some genetic studies and intraspecific and interspecific crosses have been made for genetic studies and genotype improvement. Some of the well documented genetic studies in cassava include studies on cytogenetics of cassava and its wild relatives (Bai, 1987; Fregene, 1996), genome analysis and diversity studies (Awoleye *et al.,* 1994; Grattapagalia *et al.,* 1996; Lefevre and Charrier, 1993), hybridisation (Haysom *et al.,* 1994; Asiedu *et al.,* 1992; Magoon 1967; IITA, 1988; Bai *et al.,* 1993; Wanyera, 1993) and the mode of inheritance of some morphological traits (Hahn *et al.,* 1989; Bryne, 1987; Rajendran, 1989; Amma *et al.,* 1995).

2.5.1 Genome analysis

Cassava generally has a diploid genome (2n=36). However, some authors have described it as being an allotetraploid. Since the breeding behaviour of allotetraploids is similar to that of diploids (Wricke and Weber, 1986), this is not certain. Although most cassava genotypes studied are diploid, spontaneous polyploids such as triploids (3*x*) and tetraploids (4 *x*) of some genotypes have been reported (IITA, 1980; Hahn *et al.*, 1989). The nucleic acid content of diploid cassava is 1.67 picogram per nucleus, that is, 772 mega base pairs in the haploid genome (Awoleye *et al.*, 1994). Lefevre and Charrier (1993) confirmed the heterozygous nature of the cassava genome and its relatives in their study on isozymes diversity among *Manihot* germplasm. Their studies also confirmed the diploid genome based on the inheritance of the isozyme markers. Similar studies on the inheritance of molecular markers also indicated a diploid genome (Fregene *et al.*, 1994).

2.5.2 Cytogenetics

Fregene (1996), reviewed the work of several authors who postulated that cassava is a segmental allotetraploid with basic chromosome number x =9. Jos and Nair (1979), however, observed regular 18 bivalents during meiosis in several cassava genotypes the chromosomes suggesting a diploid genome with $2n=2x=36$ chromosomes. They also detected the presence of pachytene abnormalities such as deletions, duplications, inversion and inregular pairing, in cassava clones that were partially pollen-sterile and concluded that this was due to hybridisation (Jos and Nair, 1979). Normal pairing at meiosis were observed in different independent studies of interspecific F_1 crosses, between cassava and *M. glaziovii* and between cassava and arborescent cassava, which confirmed that interspecfic hybridisation occurs within the genus (Magoon *et al.*, 1969; IITA, 1988; Bai, 1982; Wanyera, 1993).

2.5.3 Hybridisation and genetic diversity

There is no genetic barrier to hybridisation within the genus *Manihot* (Haysom *et al.,* 1994; Asiedu *et al.,* 1992; Magoon, 1967; Bai, 1982; Wanyera, 1993). Spontaneous hybrids between cassava and other *Manihot* species have been reported to occur naturally in Africa and in Brazil (Nassar, 1994).

In earlier hybridisation studies in the late 1930s, Doughty suggested that tree cassava is a natural hybrid between cassava and *M. glaziovii* (Fregene, 1996). Doughty's suggestion was later confirmed by other workers who observed normal pairing at meiosis in an F_1 between cassava and *M. glaziovii* and in an F_1 between cassava and arborescent cassava (Magoon, 1967; IITA, 1988; Bai, 1982; Wanyera, 1993). Resistance to the cassava mosaic and the cassava bacterial blight diseases were derived from an interspecific cross between cassava and *M. glaziovii* (Nichols, 1947; Jennings, 1977; Hahn *et al.,*1989).

The extent of genetic diversity in the crop may be attributed to the occasional use of seeds in propagation since cassava would combine easily with itself and wild relatives. Silvestre and Arraudeaus (1983), noted that African farmers sometimes take the cuttings from spontaneous seedlings for their subsequent planting (cited in Lefevre and Charrier, 1993). These new genotypes, referred to as the landraces provide a wealth of allelic genes for some traits and form part of the crops genetic resource which could be included in breeding programmes for the improvement of the crop (Gulick *et al.,* 1983; Hershey, 1987). Some of these African landraces, collected in West Africa have been identified to be resistant to CMD (Raji, 1995; Mignouna and Dixon, 1997).

The incorporation of the African landraces into the cassava breeding programme at IITA was hindered for several years due to the fact that many of them exhibit shy flowering or no flowering at all. It was discovered in 1990 that these landraces

flowered at Ubiaja, in Edo State, Nigeria, and this has encouraged the development of several crosses for genetic improvement and for genetic studies (IITA, 1990).

2.6 Quantitative genetics in cassava breeding

2.6.1 Mating designs

Most traits studied in cassava are polygenic (Byrne, 1987; Rajendran, 1989; Amma *et al.,* 1995; Hahn *et al.,* 1989). Variation in polygenic traits is attributed to quantitative trait loci, QTLs. Quantitave traits in plants and animals are studied using a variety of genetic models and designs. The analysis of mating designs allows genetic analysis to be carried out after one generation and provides tests of the adequacy of the model (Mather and Jinks, 1982).

The diallel and the North Carolina design II (NCD II) mating designs provide genetic interpretations including combining abilities and the inheritance of quantitative traits (Kang, 1994). The concept of general and specific combining ability was first defined by Sprague and Tatum, (1942). Knowledge on the relative importance of general combining ability (GCA) and specific combining ability (SCA) which represent two major modes of gene action for quantitative traits is essential in formulating an efficient breeding strategy. GCA of a line refers to the average value of the line based on its performance when crossed with other lines. It is due largely to additive gene effect. SCA is the deviation of a cross from the average GCA of the parent lines and is due to non-additive gene effects (Sprague and Tatum, 1942; Falconer and Mackay, 1997).

The theory of the diallel was first developed by Jinks and Hayman (1953). Hayman (1954a; b) gave some assumptions which should be satisfied before a meaningful interpretation of the analysis is done. These assumptions are that:

i, the parents are homozygous,

ii, there is normal diploid segregation,

iii, there are no maternal effects,

iv, genes are independently distributed among the parents, and

v, there is no allelic interaction or no epistasis.

These assumptions, have been shown to be unrealistic (Kempthorne, 1956; Matzinger and Kempthorne, 1956; Gilbert, 1973; Feyt, 1976). Kempthorne (1956) considered that the assumption concerning the independent distribution of genes in the parents is not reliable because it implies that the presence or absence of an allele at a particular locus is statistically independent of the presence or absence of an allele at any other locus. Failure of the assumption is a result of linkage of genes. Gilbert (1973) suggested that because of the biochemical pathways involved in the expression of traits, the assumption of no epistasis cannot be justified. Feyt (1976) showed that genes at n loci cannot be independent unless a minmum of 2^n parents are used in a diallel cross. In other words, if four gene loci condition a trait, these loci cannot be independent unless 2^4=16 parents are used in a cross (Kang, 1994). This further shows that this assumption is unrealistic. Matzinger and Kempthorne (1956) considered varying degrees of inbreeding as acceptable to complete homozygosity of the parents.

The North Carolina design II (NCD II) mating scheme is a cross-classification design that was first proposed by Comstock and Robinson (1948). It differs from the diallel in that different sets of parents are used as males and females. Thus, it accommodates more parents in determining combining abilities than a diallel and provides the same sort of genetic information (Hallauer and Miranda, 1988). The main effects of males and females are equivalent to GCA and the female x male interaction is equivalent to SCA.

Both the diallel and NCD II mating designs have been used to obtain genetic information on morphological and agronomical traits of importance in cassava (Hahn *et al.,* 1989; Rajendran, 1989; Amma *et al.,* 1995). Rajendran (1989) reported additive gene action for storage root yield and non-additive gene action for the yield components (harvest index, storage root number and storage root weight). Amma *et al.,* (1995) reported that root quality traits, starch content, dry matter and cyanide (HCN) content are predominantly non-additive. Low HCN is also recessive (Kawano, 1978; Amma *et al.* 1995).

Resistance to CMD derived from clone 58308 has been reported to be polygenic, recessive and inherited largely in an additive manner (Hahn *et al.,* 1980a, 1989; IITA 1973; Msabaha, 1983). Recently, the presence of a major dominant resistant gene in the African landrace TME3 (known locally in Nigeria as "2nd agric") designated as *CMD2* was reported (Akano *et al.,* 2002; IITA, 2000).

CBB, which was also derived from the 58308 source of resistance, is inherited largely in an additive manner. However, some degree of non-additive effect is also involved (Hahn *et al.,* 1980a; 1989; Umemura and Kawano, 1983; Jennings, 1977).

2.6.2 Heterosis

Heterosis is defined as the amount by which the mean of an F_1 exceeds its better parent or midparent (Kang, 1994). Heterosis occurs when directional dominance exists, or there is allelic interaction or overdominance at some or all alleles and when the parents differ in gene frequency. However, the genetic basis of heterosis is not quite clear (Kang, 1994; Falconer and Mackay, 1997).

Evidence from maize and other crops suggest that the genetic basis of heterosis is due to partial or complete dominance (Hallauer and Miranda, 1988; Brummer, 1999).

Overdominance arising from dominant alleles in repulsion at linked loci and epistasis, may also play a role in heterosis expression (Xiao *et al.*, 1995; Yu *et al.*, 1998).

The condition that differences in allelic frequencies should exist for heterosis to be expressed, suggests that crosses between diverse populations would result in heterosis. However, highly divergent populations or certain cases of multiple allele populations may prevent heterosis expression (Moll *et al.*, 1962; Bonierbale *et al.*, 1993). The amount of heterosis exhibited in crosses between local and exotic cultivars of a crop is an important criterion in selecting cultivars to be used in a cross (Kang, 1994). To maximise the potential of heterosis of a trait in a breeding programme, crosses among the various populations, lines or clones need to be evaluated. If the progenies express heterosis the two parents are accorded to different heterotic groups (Hallauer and Miranda, 1988; Brummer, 1999).

The extent of heterosis for most traits in cassava has not been studied extensively. Amma *et al.* (1995) detected positive and significant high parent heterosis for dry matter content and starch in a diallel cross involving inbred lines. They also detected negative and significant heterosis for HCN. Heterosis for resistance to CMD was observed in crosses involving some improved clones and resistant African landraces (Lokko *et al.*, 1998).

2.6.3. Number of genes and complementarity among genes affecting quantitative traits

In Mendelian genetics, analysis of the number of genes is determined by observing the segregation ratios. In quantitative genetics, however, effective factors are estimated to determine if a few or many genes condition the quantitative trait. This is because quantitative genetics is based on the infinitesimal model where a large number of loci (polygenes) all with small effects affect the trait (Lynch and Walsh,

1998). Environmental effects usually modify these multiple genetic factors. The most important assumption for estimating effective factors is additivity of gene action.

Molecular markers or biometrical methods can be used to estimate effective factors for quantitative traits (Lynch and Walsh, 1998; Zeng *et al.,* 1990). Most of the biometrical estimating techniques do not estimate the exact number of genes but rather the number of effective factors (Wright, 1968; Lande, 1981; Cockerham, 1986; Zeng *et al.,* 1990; Zeng 1992). This is because the number of genes affecting quantitative traits do not have the same effect and the total response of a trait depends primarily on the number of loci, which affect it (Falconer and Mackay 1997).

The biometrical methods for estimating effective factors are based on the method which was first developed by Castle (1921) and Wright (1968). Using the Castle–Wright formula, the number of effective factors in theory cannot exceed the number of independent segregating chromosome segments, which is the haploid number. Since there are usually 1 or 2 recombination events in eukaryotes, the maximum number of effective factors can be 2 to 3 times the haploid chromosome number. However, each segment can contain many genes (Lynch and Walsh, 1998).

The number of effective factors affecting resistance to CMD has not previously been reported. Hahn *et al.* (1980a) inferred from the polygenic nature of resistance to CMD that resistance must be attributed to the combined action of the number of loci which are linked on a chromosome or a set of chromosome of a genome or genomes. They, however, suggested that since cassava is genetically heterozygous and probably an allotetraploid, a study on the genetic mechanism of resistance to CMD is complicated and difficult.

The main purpose of testing gene complementarity is to examine allelic relationships between different sources of a trait in order to determine whether the genes are allelic among themselves. Progenies between parents varying for a quantitative trait

would exhibit phenotypes ranging between those of the parents. The nature of the progeny's distribution is determined by the number of genes which account for the differences between the parents and environment (Lynch and Walsh, 1998). Gene complementarity among sources of resistance to disease and pests has been reported in oats, soybean and sorghum (Fox *et al.,* 1997; Wang *et al.,* 1998; Dixon *et al.,* 1991).

2.6.4. Heritability

Heritability is defined as the percentage of total phenotypic variability for a trait that is due to the genes, and their interaction with other genes (Kang, 1994). If the environmental effect on the trait were negligible, then heritability would be 100%. Heritability is an indication of ease with which a trait can be transferred to the progeny. The value is not a constant and is defined for a particular trait in a population at a particular time. Variation in the estimate may, therefore, occur from population to population or within a population from time to time due mainly to changes in the additive genetic variance. Two forms of heritability are useful in breeding; heritability in the broad sense which is the ratio of total genetic variation to phenotypic variation, and in the narrow sense which is the fraction of the phenotypic variation that is due to additive genetic variation (Falconer and Mackay, 1997).

From a seven-parent diallel cross, heritability of 60% was estimated for resistance to CMD (Hahn and Howland 1972; IITA, 1973). In related studies, selected clones from 10,000 F_1 crosses made by crossing 58308 with three other cassava clones, gave narrow sense heritability of 35% and 30% for resistance to CMD estimated by parent offspring regression for two separate readings (Hahn *et al.,* 1980a). Broad sense heritability of 48% and 68% for resistance to CMD estimated from variance components of half sib families from unreplicated trials in two planting seasons of 952 clones were also reported. Similar high heritability estimates were obtained for

CBB from these data sets (Hahn *et al.,* 1980b). Based on these results, resistance to CMD and CBB is reported to be highly heritable (Hahn *et al.,* 1989).

Amma et al. (1995) reported high heritability (76.81%) for low HCN, based on narrow sense heritability, which was similar to an earlier report on the heritability of low HCN by Kawano (1978). Dixon et al.,(1994), on the other hand, reported that heritability for low HCN can vary from 0 to 50% depending on the composition of genotypes evaluated, number of years, locations, and seasons. Iglesias and Hershey (1991) observed that starch and dry matter content were highly heritable traits based on broad sense heritability, Amma *et al.,* (1995), however, reported low heritability (3.25% for dry matter and 7.58% for starch content) based on narrow sense heritability.

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2.6.5 Genetic correlation studies

The phenotypic values of different traits are often found to be correlated. This may be due to the fact that genes, which control the expression of a quantitative trait, are likely to influence the expression of others. Genetic correlation or covariance between traits can be mainly attributed to pleiotropy, which is the influence of a single gene or locus on more than one trait, or gene linkage, which is a cause of transient correlation (Falconer and Mackay, 1997).

Resistance to CMD and CBB is reported to be genetically correlated (IITA, 1973; Hahn 1978; Hahn *et al.,* 1980b). Hahn *et al.* (1980b) obtained phenotypic (r^2=0.423) and genotypic (r^2=0.899) correlations between resistance to CMD and CBB in half-sib families and genetic correlation (r^2=0.689) in 925 clones. They attributed the correlation to genetic linkage, quoting the observation by Magoon *et al.* (1969) of random transmission of parental chromosomes in *Manihot* interspecific hybrids in support. Using canonical analyses, Jennings (1977) observed that the joint resistance was inconsistent in a population of both *M. glaziovii* and cassava

genotypes from the 58308 stock. Jennings proposed the alternative hypothesis of pleiotropic effect (Jennings, 1977; Jennings and Hershey, 1985).

This reported linkage between resistance to CBB and CMD could have positive implications in breeding for resistance to the two diseases of cassava in that selection for one would lead to progress in the other.

2.7 Biometrical analysis

2.7.1 Analysis of mixed models in genetic studies

For most genetic experiments, genetic material is evaluated over a set of years and locations or environments, to obtain precise genetic information. In such analysis, the genetic materials are considered fixed and the years and locations or environmental effects considered random (McIntosh, 1983; Zhang and Kang 1997). For random effects models, inferences are based on variances while in a fixed effects model, the inferences are based on means (Littell *et al.,* 1996; Kang, 1994). Mixed model analyses, involving both fixed and random effects, allow the proper analysis of experimental data involving both random and fixed effects (Littell *et al.,* 1996). The analysis of variance (ANOVA), the general linear models (GLM) and the variance components analysis (VARCOMP) which are statistical procedures available with the statistical analysis system (SAS), handle random, fixed and mixed model analyses with specifications.

The GLM and ANOVA procedures in SAS use the method of least squares to fit general linear models. ANOVA handles balanced data only, while GLM handles unbalanced data and also allows the estimation of least square means. Least square means are the expected values of class means that would be expected for a balanced design of the class variable with all the covariates at their true mean (SAS, 1999). Least square means are thus suitable for estimating genetic effects such as combining abilities and heterosis in a mating experiment. It has been argued that

most analyses involving fixed effects models may indeed be mixed models and that the conventional least squares estimates in the analysis of variance may not lead to the 'best' estimates and may not be as informative as it should be (SAS, 1999). Henderson mixed model procedure of best linear unbiased predictors (BLUPs) are estimated in PROC MIXED procedure in SAS (SAS, 1997; Littell *et al.,* 1996).

The VARCOMP procedure in SAS computes estimates of the variance components in a general linear model. It is designed to handle models that have random effects. The minimum variance/norm quadratic unbiased estimate (MIVQUE0) method produces estimates which are locally the best quadratic unbiased estimates, given that the true ratio of each component to the residual error component is zero (SAS, 1997; SAS, 1999). The technique is similar to the type I methods which equates mean squares involving the random effects to its expected mean square, but the random effects in MIVQUE0 are adjusted for the fixed effects.

With fixed genetic effects in the analysis of data from either a diallel or a NCD II experiment, it is not appropriate to draw inferences about hypothetical populations (Ross *et al.,* 1983). However, with qualifications, important quantitative information such as the ratio of additive variance to total genetic variance can be drawn from such studies that may aid in the improvement of the crop for the trait (Ross *et al.,* 1983; Pedersen *et al.,* 1983; Rooney *et al.,* 1997). The ratio of additive variance to total genetic variance in the population gives an indication of the relative importance of GCA in predicting progeny performance. The closer this ratio is to 1, the greater the chances of predicting progeny performance based on GCA (Baker, 1978; Kang, 1994). If the genetic model assumed is a fixed effects model, the analogous mean square components of the fixed effects can be estimated to compute the ratios (Baker, 1978; Wricke and Weber, 1986; Ross *et al.,* 1983; Kang 1994). The MIVQUE0 procedure is, therefore, suitable for estimating the analogous mean square components.

2.7.2. The diallel analysis

A number of genetic models and statistical procedures exist for analysing and interpreting the results of diallel crosses. Depending on the objectives and availability of materials for an experiment, reciprocal crosses could be included in a diallel analysis. The methods used for the analysis of a diallel cross include;

i, Hayman's method of analysis of variance for the genetic parameters 'a', 'bs', 'c' and 'd' (Hayman, 1954a),

ii, Combining ability analysis which partitions combining ability into general combining ability (GCA) and specific combining ability (SCA) (Griffing, 1956b; Kempthorne and Curnow, 1961; Pooni *et al.,* 1984; Singh and Chaudhary, 1985).

iii, Gardener and Eberhart's analysis II which estimates the effects v_i, h_{ij}, h and s_{ij}' variety and heterosis effects (Gardener and Eberhart, 1966).

The genetic parameter 'a' of Hayman's method refers to additive gene effect and is equivalent to GCA, while the 'bs' which refers to dominance gene effect is equivalent to SCA. The paramter 'c' refers to maternal effects and 'd' refers to reciprocal effects of the differences due to 'c'. The SCA is further partitioned into 'b_1' (directional dominance which compares the mean of the F_1 and the mid parent value), 'b_2' (directional dominance which quantifies gene distribution over arrays) and 'b_3' (discrepancy in reciprocals due to dominance).

Using the Hayman analysis of variance, significance of the b effects allows further analysis of the data using the graphical analysis procedure, *Wr/Vr* graphs (Mather and Jinks, 1982). Here, Wr is the covariance between the parents and Vr is the array variances, an array being the crosses in which a particular parent is involved (Singh and Chaudhary, 1985). In the absence of non-allelic interaction and with independent

distribution of genes among parents, Wr is related to Vr by a regression slope. The distance between the origin and the intercept of the regression line on the curve provides a measure of the degree of dominance. The order of the array points along the regression lines gives an indication of dominant and recessive genes among the parents (Singh and Chaudhary, 1985).

The theories underlying the analysis of diallel crosses in relation to combining ability were later explained by Griffing (1956a; b). Griffing (1956a) described four methods of the diallel crosses depending on the material used. Method (1) includes selfed parents, F_1s and reciprocals. Method (2) has the selfed parents and F_1s only. Method (3) involves the F_1s and reciprocals, and method (4) involves the F_1s only. In Griffing's approaches to the analysis of diallel crosses, variance components due to general and specific combining ability are estimated which are then translated into genetic components such as additive and dominance variance. The analysis may be based on fixed effects model I or random effects model II. The procedure for the analysis of variance of the two methods is the same except for the expectation of mean squares (Griffing 1956b; Pooni *et al.,* 1984; Singh and Chaudhary, 1985). The first part of the analysis consist of testing the null hypothesis, that there are genotypic differences among F_1 crosses, parents and the reciprocals. Significant differences among the genotypes are a prerequisite for the combining ability analysis (Singh and Chaudhary, 1985).

The average heterosis 'h' in the Gardener and Eberhart analysis II is equivalent to the parent versus cross orthogonal contrast in a traditional analysis of variance and the specfic heterosis 's_{ij}' is equivalent to SCA (Hallauer and Miranda,1988; Pooni *et al.,* 1984). The Gardener and Eberhart analysis II also gives two estimates of the parent, the variety heterosis, 'h_{ij}' and the variety mean square 'v_i' which includes information of the performance of the varieties themselves and the varieties within crosses. These two estimates are not equivalent to either the parent mean square in

a traditional analysis of variance or GCA of Griffing's methods (Hallauer and Miranda,1988; Pooni *et al.,* 1984). In the analysis of variance of genetic data, an orthogonal contrast between the parents and crosses sums of squares is used as an estimate of average heterosis in a mating experiment (Hallauer and Miranda, 1988).

The various methods of analyses of diallels were evaluated by Arunachalam (1976) who concluded that the various assumptions made for the Hayman, (1954a) and the Gardener and Eberhart (1966) methods were generally not fulfilled in practical situations with experimental material. He concluded that for testing parents and F_1 materials, the combining ability analysis gives the best estimates of the gene action in parent and/or hybrid combinations.

2.7.3 The number of effective factors

The most widely used method for estimating the number of effective factors, is the Castle–Wright formula (Castle, 1921; Wright, 1968), which utilises information on the phenotypic means and variances of the parent lines and subsequent generations (F_1, F_2, B_1, B_2 etc)

The Castle–Wright formula is based on the assumptions that,

i. all segregating loci contribute equally to the trait,

ii. no linkage exists among loci affecting the trait,

iii. no dominance, or the degree of direction of dominance of plus factors is similar for all loci,

iv. all plus factors are contributed by one parent and all minus factors by the other,

v. no epistasis occurs among alleles at contributing loci, and

vi. environmental and genotypic variances are independent and combine additively to give total variability.

Since Castle (1921) and Wright (1968) developed their formula for inbred lines, a number of models have been proposed for the estimation of effective factors in genetically variable populations which are mainly modifications of the model given by Castle and Wright (Lawrence and Frey, 1976; Lande, 1981; Cockerham, 1986; Zeng *et al.,* 1990; Zeng, 1992; Fenster and Ritland, 1994).

Fenster and Ritland (1994), suggested that since the degree of effective dominance can change among segregating generations due to epistasis, assumption iii) would not be valid and proposed a formula for estimating effective factors which incorporated dominance based on the segregating generations.

Lawrence and Frey (1976) also pointed out that if assumption iv) for the Castle-Wright formula is not met, then the numerator would be small and the number of factors would be underestimated. The range of the F_1 segregates instead of the parental range was suggested as the numerator in the formula for estimating effective factors. They further argued that the range of the segregating population was a better estimate than the parental range if the parents did not represent genotypic extremes. The range of the segregating population is also appropriate when there is non-additive variance involved in the inheritance of the trait (Dixon *et al.,* 1991; Lawrence and Frey, 1976; Fenster and Ritland, 1994).

Lande (1981) also gave a modification of the Castle-Wright formula for use with genetically variable populations. Cockerham (1986) and Zeng (1992) separately proposed methods for correcting sampling error. Cockerham proposed using the experimental variance as a correction factor for the squared difference between the mean of the two parents, while Zeng (1992) proposed using least squares to estimate the genetic variance and a modified estimator.

Multize and Barker (1985), showed that the estimated number of effective factors increased as heritability and the probability of type I error increased. There was, however, a downward bias of the estimated number of effective factors when heritability decreased, the probability of type I error lowered and when there was an increase in the type II error.

Zeng *et al.* (1990), observed that the Wright method is of little value in estimating the number of loci affecting a quantitative trait since linkage would prevent the estimation from exceeding the number of chromosomes. The sample variance may prevent one from concluding that a large number of loci are involved. They, therefore, proposed that the alternative is to estimate the number of genes using molecular markers.

2.8 Molecular marker technology and its application in cassava breeding and genetics

Molecular markers, which include biochemical (isozymes and storage proteins) and DNA markers, exist in every genotype. They occur in large numbers and their expression is independent of phenotypic value and as such are powerful tools of genetic research (Soller and Beckmann, 1983; Beckmann and Soller, 1986). They allow geneticists and plant breeders to locate and follow the numerous interacting genes that determine a complex trait as well as tagging those controlled by single genes. Molecular markers offer many advantages over conventional phenotypic markers. They are i) developmentally stable, ii) detectable in all tissues, iii) unaffected by environmental conditions, and iv) virtually insensitive to epistatic or pleiotropic effects. They provide a choice of codominant markers which can be identified in heterozygotes or dominant markers which are identified as present or absent subclasses (Botstein *et al.,* 1980; Williams *et al.,* 1990).

Molecular markers have made an immense contribution to cassava breeding and genetics. The areas covered include, the development of genetic maps (Fregene *et*

al., 1997), the assessment of genetic diversity (Bonierbale *et al.*, 1997b; Lefevre and Charrier, 1993; Beeching *et al.*, 1993; Mignouna and Dixon 1997; Sanchez *et al.*, 1999; Fregene *et al.*, 2000), taxonomical studies (Second *et al.*, 1997), understanding the phylogenetic relationships in the genus (Calvalho *et al.*, 1993; Roa *et al.*, 1997; Oslen and Schaal, 1999; Roa *et al.*, 2000), confirmation of ploidy (Lefevre and Charrier, 1993; Fregene *et al.*, 1994) and cultivar identification (Ocampo *et al.*, 1993; Wanyera, 1993; Laminski *et al.*, 1997).

2.8.1 Isozyme markers

Isoenzymes or isozymes of a gene product refer to any two distinguishable proteins that catalyse the biochemical reaction of the gene. Their usefulness as molecular markers depends on the rarity of the allozyme variant (group of isozymes that catalyses the same gene product), how tightly linked they are to a gene and how difficult a direct screening method is. The main limitation of isozyme markers is that only few gene products can be revealed. Nevertheless, isozymes have been used in cassava breeding and genetics (Wanyera, 1993; Lefevre and Charrier, 1993; Ocampo *et al.*, 1993; Laminski *et al.*, 1997).

Wanyera, (1993) demonstrated the usefulness of isoenzymes in confirming true hybrids in a cross between *M. glaziovii* and *M. esculenta.* Lefevre and Charrier, (1993) detected genetic diversity among several cassava clones with isozyme markers. The study also confirmed that cassava is a true diploid based on the inheritance of the markers. Ocampo *et al.* (1993) used the esterase isozyme to fingerprint the cassava germplasm collection held at the CIAT. This isozyme was also used in fingerprinting South African elite cultivars (Laminski *et al.*, 1997). Fregene *et al.* (1997) placed three Isozyme markers on the cassava genetic linkage map developed at CIAT. Isozymes markers were also used to develop a procedure for identifying varieties of cassava (Hussain *et al.*, 1987; Ramirez *et al.*, 1987).

2.8.2 DNA markers

A DNA marker is basically a small region of DNA showing sequence polymorphism in different individuals within a species (Liu, 1997). The markers are based on the enormous variation or polymorphism in DNA sequences of organisms. DNA markers eliminate the limitations in genome investigations using morphological and isozyme markers, such as gene expression and environmental interaction, heritability, and low map resolution (Vogel *et al.,* 1996). DNA-based marker systems can also be used for indirect selection of tagged loci affecting qualitative or quantitative traits and to monitor loci during introgression or selection programmes, thus reducing the number of backcross generations (Baird *et al.,* 1996). Many DNA fingerprinting techniques have been developed to generate markers and are generally based on one of two strategies; classical, hybridisation-based fingerprinting and PCR-based fingerprinting.

Classical, hybridisation-based fingerprinting which was first developed for use in human genetics and is now used as a general method of genetic analysis involves the cutting of genomic DNA with restriction enzymes followed by electrophoretic separation of the DNA fragments. The restriction fragment length polymorphism (RFLP) generated is then detected with probes targeted to specific regions of the genome, after southern blot transfer to a nylon membrane (Soller and Beckman, 1983).

PCR-based fingerprinting involves the amplification of particular DNA sequences using specific or arbitrary primers and a thermostable polymerase. Amplification products are separated by electrophoresis and detected by staining with ethidium bromide on agarose gels, silver on polyacrylamide gels or the use of radioactive or fluorescent labelled primers in the amplification reaction (Ehrlich *et al.*, 1989). Techniques in this category include random amplified polymorphic DNA (RAPD) (Williams *et al.*, 1990), DNA amplification fingerprinting (DAF) (Caetano-Anollés *et*

al., 1991), arbitrarily primed-PCR (AP-PCR) (Welsh and McClelland, 1990) microsatellites (Hamada *et al.,* 1982) and amplified fragment length polymorphism (AFLP) (Vos *et al.,* 1995).

DNA markers vary in their level of polymorphism and the amount of information generated. The most informative DNA markers are characterised by high polymorphism information coefficient (PIC), indicating relatively large number of alleles with similar frequencies in each locus (Botstein *et al.,* 1980). Polymorphism may also be due to single site alterations due to mutations, which abolish or create a restriction site, insertions, deletions, or inversions between two restriction sites. In this case, the level of polymorphism is low. Polymorphism may also be due to variable number of tandem repeats (VNTRs) resulting in a marker system with high levels of polymorphism and can detect variation between closely related relatives. Markers that detect single site alterations include RFLPs, RAPD, and AFLPs, while minisatellites and microsatellite markers detect VNTRs.

2.8.3 Restriction Fragment Length Polymorphism (RFLP)

RFLP is a powerful tool in genetic mapping. RFLP markers are cloned DNA segments that can be used to reveal base pair changes or rearrangements in homologous DNA sequences (Beckman and Soller, 1986). They are codominant markers and scoring is simple because of the low copy number. However, the use of RFLP markers requires that many probes are used which requires a complementary DNA (cDNA) or genomic library of the species already exist.

The use of RFLP markers has led to the rapid construction of linkage maps in many plant species and the placement of genes that control qualitative traits on these maps. Single locus disease resistance genes were the first category of genes to be mapped extensively using RFLPs. This was partly because most characterised disease resistance genes are controlled by alleles at a single locus and most are

dominant genes that are easy to assay. RFLPs have also been used to study and identify markers that are tightly linked to genes controlling quantitative traits (Tanksley, 1993; Young, 1996). In RFLP mapping, regions of interest are detected based on their map positions by analying flanking markers when the defined intervals are small.

RFLP markers have contributed to DNA marker technology in cassava. Angel *et al.* (1993) initiated work on a detailed genetic map of cassava for tagging agronomically important traits and to clone cassava genes. Currently the map has 239 RFLP markers (Fregene *et al.,* 2001). Beeching *et al.,* (1993), assessed the genetic diversity of several accessions of cassava with RFLPs. Recently, Oslen and Schaal (1999) demonstrated the geographic origin of cassava from its relative, *M. esculenta* subspecies *flabellifolia* based on sequence variation of the single copy nuclear gene glyceraldehyde 3-phosphate dehydrogenase (*G3pdh*).

Despite the potentials of RFLP markers in breeding, there are limitations. It is laborious and intensive and does not generally offer the opportunity to target tight linkages to specific loci because of the specific probe used. In addition, RFLPs cannot detect all the differences that exist between nucleotides, only those that occur at restriction sites. The value of the marker comprising an RFLP map depends on the degree to which such markers can be used in a population other than the mapping population. This is a function of the degree of genotypic diversity in the primary gene pools (i.e the mapping population) but also depends on the usefulness of the probes used.

Combinations of RFLP and RAPD markers are also commonly used in the constructions of linkage maps for many plant species (Fregene *et al.*, 1994; Zhang *et al.,* 1996). Calvalho *et al.* (1993) used RFLP of mtDNA and RAPDDs to identify phylogenetic relationships in the *Manihot* genus.

2.8.4 Random Amplified Polymorphic DNA (RAPD)

RAPD primers usually have 10 base pair sequences that are used to amplify unknown regions of a genome. The short sequences of the primers make possible a multitude of primer binding sites throughout the genome. Efficient amplification of DNA fragments may occur when two primer binding sites occur in close proximity (Burrow and Blake, 1998). RAPD markers are dominant since the polymorphism is detected as a failure of one allele to amplify due to mutations in the primer-binding site.

RAPD markers are the most extensively used markers in cassava biotechnology, especially in determination of phylogenetic relationships and genetic diversity in *Manihot* species (Marmey *et al.,* 1994; Laminski *et al.,* 1997; Schaal *et al.,* 1997). They are simple, rapid, and only small amounts of DNA are required. Gomez *et al.* (1996) subjected 328 RAPD markers to linkage analysis in cassava. Out of this, 30 were placed on the cassava linkage map at CIAT (Fregene *et al.,* 1997). Currently, the map has 80 RAPD markers (Fregene *et al.,* 2001). Marmey *et al.* (1994) demonstrated genetic diversity among African cassava accessions using RAPD markers. Mignouna and Dixon (1997) observed that several African landraces with varying levels of resistance to CMD were genetically different from the genetic stock 58308 based on RAPD analysis.

DAFs and AP-PCR are basically variations of the RAPD technique. DAF differ from RAPDs, in that the primers are made up of short (sequences 5-8 nucleotides long) and the fragments are separated on polyacrylamide gels to give better resolution of the DNA fragments. DAFs and AP-PCRs have not been reported in cassava.

2.8.5 Variable number of tandem repeats (VNTR)

The two classes of VNTR markers are the minisatellite with repeat units from about 10-45 base pairs and microsatellite or simple sequence repeats (SSR) with repeat

units of 2-4 nucleotides (Jeffrey *et al.,* 1985). Eukaryotic genomes contain extended repetitive elements, many of which are mobile such as the retrotransposons which are flanked by long terminal direct repeats (LTRs) (Rhode, 1996). The presence or absence of flanking LTRs which occur in high copy numbers in the genome have been characterised in a number of plant species and have been the basis to establish a PCR-based marker system known as the inverse sequence-tagged repeats (ISTR).

2.8.6 Microsatellite or simple sequence repeats (SSRs)

Microsatellites or simple sequence repeats (SSR)s are well distributed within the genome of eukayotes (Morgante and Olivieri, 1993). They are flanked by unique sequences that serve as templates for specific primers to amplify the SSR allele via the polymerase chain reaction. Microsatellite polymorphism which can be detected by autoradiography, fluorescence labelling, silver staining and ethidium bromide staining have been used to complement both the RFLP and PCR based markers.

SSRs have a high level of allelic diversity as a result of the variable number of repeat units within their structure, and this makes them valuable as genetic markers (Hamada *et al.,* 1982; Morgante and Olivieri, 1993). They are often multiallelic, can be multiplexed, automated for high throughput genotyping and convenient to exchange between laboratories (Powell *et al.,* 1996b; Chen *et al.,* 1997). They are especially suited for distinguishing between closely related genotypes. Although the procedure for obtaining microsatellites is laborious and expensive, their conversion to PCR markers allows the screening of large numbers of alleles for defined loci. The markers are codominant and are, therefore, a suitable option for mapping and molecular characterisation in cassava (Agyare-Tabbi *et al.,* 1997; Chavarriaga-Aguirre *et al.,* 1998).

Chavarriaga-Aguirre *et al.*, (1998) isolated and characterised 14 highly heterozygous GA rich microsatellite DNA in cassava. A total of 521 accessions from the cassava core collection at CIAT were successfully screened with four SSR loci developed into PCR-based markers, to determine genetic diversity (Chavarriaga-Aguirre *et al.*, 1999). Cassava microsatellites were also used to assess genetic diversity among cassava accessions and assess genetic relationships between cassava and its wild relatives (Roa *et al.*, 2000). Recently, Mba *et al.* (2001), developed and characterised 172 new SSR markers and placed 36 of these on the cassava linkage map.

2.8.7 Amplified fragment length polymorphism (AFLP)

The amplified fragment length polymorphism (AFLP) technology, which is based on a combination of restriction digestion and PCR, was developed by Vos *et al.* (1995). It involves the selective amplification by PCR of a subset of restriction fragments of agenomic sample. DNA is digested with two restriction enzymes, a frequent cutter and a rare cutter. Double-stranded DNA adapters are then ligated to the ends of the DNA fragments to generate template DNA for amplification. Thus, the sequence of the adapters and the adjacent restriction site serve as primer binding sites for subsequent amplification of the restriction fragments by PCR. Selective nucleotides extending into the restriction fragments are added to the 3′ ends of the PCR primers such that only subsets of the restriction fragments are recognised. Only restriction fragments in which the nucleotides flanking the restriction site match the selective nucleotides will be amplified. The subset of amplified fragments are then analysed by denaturing polyacrylamide gel electrophoresis to generate the fingerprint.

The frequent cutter generates fragments with the optimal size range for separation on denaturing gels and the number of fragments to be amplified is reduced by using the rare cutter, since only the rare cutter/frequent cutter fragments are amplified (Vos *et al.*, 1995). In most AFLP studies, genomic DNA is digested with the enzymes

EcoR1/Mse1 that is suitable for species with AT (adenine and thiamine) rich genomes (Vos *et al.*, 1995; Janessen *et al.*, 1996). Other enzyme combinations such as *Pst1/Mse1*, *HindII/Mse1* are useful for genomes with 40-50% GC (guanine and cytocine) content, while *Apa1/Taq1* are used for GC rich genomes (Wong *et al.*, 1999).

AFLP markers have high multiplex ratios since several genomic fragments are analysed in on assay and require no prior sequence data (Rusell *et al.*, 1997; Powell *et al.*, 1996a). They are generally dominant markers based on the presence or absence of a particular product. However, with appropriate software, such as Keygene (Wageningen, the Netherlands) they can be scored as codominant markers

The main advantage of AFLP technology over other marker systems is that variability can be assessed at a large number of independent loci and data can be obtained quickly and reproducibly (Sanchez *et al.*, 1999). AFLP markers have been extensively used in cassava molecular marker technology (Bonierbale *et al.*, 1997b; Second *et al.*, 1997; Roa *et al.*, 1997; Sanchez *et al.*, 1999; Fregene *et al.*, 2000). Fregene *et al.* (2000) were able to detect a considerable number of duplicates in some African landraces with AFLP markers. They also detected a unique AFLP fragment among the African landraces, which was associated with branching pattern. Sanchez *et al.* (1999) established genetic similarities among cassava accessions resistant to the CBB disease. AFLP markers were also used to demonstrate genetic relationships among different cassava accessions and other *manihot* species (Bonierbale *et al.*, 1997b; Second *et al.*, 1997; Roa *et al.*, 1997).

2.9 Determination of molecular markers associated with traits of interest

The strategies available to facilitate detection of molecular markers associated with specific traits of interest include bulk segregant analysis (Michelmore *et al.*, 1991; Giovannoni *et al.*, 1991), linkage mapping (Tanksley *et al.*, 1989) and QTL analysis

(Dudley, 1993; Tanksley, 1993). For markers associated with disease resistance genes, degenerate primers based on isolated candidate resistant genes can be used to screen for resistance gene analogs (Kanazin *et al.*, 1996; Collins *et al.*, 1998).

2.9.1 Bulk segregant analysis

Bulk segregant analysis (BSA) is a rapid method for identification of markers in specific regions of the genome (Michelmore *et al.*, 1991; Giovannoni *et al.*, 1991). The method is especially useful when there are no other markers available within the region of interest. It involves comparing two pooled DNA samples of individuals from a segregating population. The two DNA pools contrast for a trait (e.g. resistant and susceptible) or a marker allele in a previously mapped population are analysed to identify markers distinguishing them. The individuals in each bulk are identical for the trait or gene of interest but are arbitrary for other traits. So the genomic region is studied against a randomised genetic background of unlinked loci. Markers that are polymorphic between the pools will be genetically linked to the loci determining the trait used to make up the pools.

Giovannoni *et al.* (1991) demonstrated the use of DNA pooling to saturate specific chromosomal regions with additional markers, based on DNA pools from an existing tomato mapping population. Kiehne and Neale (1998) adapted this strategy in the outcrossing species loblolly pine, and identified nine new RAPD markers linked to a chromosomal interval containing the previously mapped QTL for wood specific gravity. Reamon-Buttner *et al.* (1998) detected nine AFLP makers, tightly linked to the sex locus of the dioecious plant *Asparagus officinalis* L, which is important to plant breeding in developing sex-specific PCR primers.

Bulk segregant analysis has also been explored in tagging molecular markers linked to major disease and pest resistance genes (Michelmore *et al.*, 1991; Haley *et al.*, 1993; Miklas *et al.*, 1993; Garcia *et al.*, 1996; Seo *et al.*, 1997; Fritz *et al.*, 1999;

Correa *et al.*, 2000). Michelmore *et al.*, (1991), were the first to report three RAPD markers linked to major disease resistance genes using contrasting DNA bulks composed of F_2 individuals of known genotype. Akano *et al.* (2002) recently used BSA to identify a SSR marker linked to the CMD resistance gene, *CMD2.* Molecular markers linked to two major rust resistance genes in common bean, were detected using introgression and bulk segregant analysis (Miklas *et al.*, 1993; Haley *et al.*, 1993). The bean studies, however, revealed that a major limiting factor in the application of dominant markers linked in coupling with dominant alleles or in repulsion with recessive resistant alleles is the amplification of the DNA fragments of equal sizes in susceptible germplasm (Miklas *et al.*, 1996). Sequencing results of a RAPD marker, which distinguished between resistance and susceptibility to the Russian wheat aphid (Fritz *et al.*, 1999) revealed a deletion in the susceptible parent relative to the resistant parent. Markers of interest are therefore, usually developed into sequence characterised amplified regions (SCARs) which are codominant markers for resistance screening (Garcia *et al.*, 1996; Seo *et al.*, 1997; Fritz *et al.*, 1999; Correa *et al.*, 2000).

2.9.2 Linkage mapping

Linkage maps are based on recombination frequencies. The percentage of a segregating progeny that are recombinants for a pair of linked loci is the recombination frequency. The recombination frequency gives an estimate of the distance between two loci in a chromosome, on the assumption that the probability of crossover is proportional to the distance between loci.

Construction of genetic linkage maps requires that the most appropriate mapping population(s) is available. Pairwise recombination frequencies of these populations are calculated from genetic data such as DNA marker scores, linkage groups are established and map distances estimated, and then map order is determined (Staub

et al., 1996). Different marker systems may be included in the analysis of the population. Computer packages such as Linkage 1 (Suiter *et al.*, 1983), GMendel (Holloway and Knapp, 1994), Mapmaker (Lander and Botstein, 1986; Lander *et al.*, 1987), MapManager (Manly and Cudmore, 1996) and JoinMap (Stam, 1993) have been developed to aid in the analysis of genetic data for map construction. These programmes use data obtained from segregating populations to estimate recombination frequencies that are then used to determine the linear arrangement of genetic markers by minimising recombination events.

Genetic linkage maps can provide a more direct method for selecting desirable genes via their linkage to easily detectable molecular markers (Tanksley *et al.*, 1989). Once a trait is identified and mapped, marker-assisted selection (MAS) could be used in early identification of the trait. This would reduce, breeding population sizes, continuous recurrent testing and the time required in developing a superior line. The cassava genetic linkage map developed at CIAT (Fregene *et al.*, 1997), currently has five known genes including two CMD resistance genes *CMD1* and *CMD2* placed on it and a number of quantitative trait loci (QTL) associated with some linkage groups (Fregene *et al.*, 2001).

2.9.3 Quantitative trait loci (QTL)

Molecular markers are being utilised in studying quantitative traits loci (QTLs) that affect traits and have been suggested as tools to enhance the efficiency of selecting quantitative traits in breeding programmes (Dudley, 1993; Young, 1996). The theory of QTL mapping was first described in 1923 by Sax and later elaborated by Thoday in 1961 who suggested that if the segregation is simply inherited, monogenes could be used to detect linked QTLs (Thoday, 1961). Young (1996) suggested that defined sequences of DNA, in modern day QTL mapping act as the linked monogenic markers used to detect QTLs.

Mapping QTLs with molecular markers require the analysis of large segregating populations (Tanksley, 1993). A population derived from distinctively different parents (e.g. resistant and susceptible) is analysed with the marker to test for linkage of markers to a QTL. This is based on its position on the map from flanking markers. The population is then classified into homozygous or heterozygous for the marker of the parents and compared statistically with phenotypic values to determine linkage of the molecular marker with the QTL. One of the first published reports of QTL mapping with DNA markers involved fruit size, pH and soluble solids in tomato (Paterson *et al.,* 1988).

QTL mapping for quantitative disease resistance has been reported in a number of plant species (Young, 1996). Wang *et al.* 1994, mapped QTLs affecting resistance to the rice blast fungus in a population consisting of recombinant inbred lines. QTL's associated with resistance to potato late blight was studied in a F_1 cross between two highly heterozygous parents where the phenotypic disease responses were compared with DNA marker data (Leonards-Schippers *et al.,* 1994). The results indicated that resistance to the pathogen is a cumulative phenotype resulting from a small number of race-specific and race-nonspecific resistance loci. Other QTL mapping studies on quantitative disease resistance include resistance to leaf spot fungus of maize, resistance to the soybean cyst nematode, and tomato bacterial wilt (Bubeck *et al.,* 1993; Danesh *et al.,* 1994; Concibido *et al.,* 1994). Recently, QTLs associated with dry matter yield and dry matter percentage have been linked to linkage group D of the cassava framework map (Fregene *et al.,* 2001).

A number of computer software have been developed for QTL analysis, these include MAPMARKER/QTL, QTLSTAT, LINKAGE, MAPQTL, QGENE, Map Manager QT and PGRI (Liu, 1998).

2.9.4 Screening for resistance gene analogs

Genes conferring resistance to several diseases caused by a wide range of pathogens including viruses, fungi, bacteria and nematode have been isolated using map-based cloning or insertional mutation (Michelmore, 1995; Staskawicz *et al.*, 1995). Most of the cloned resistance genes have been found to have common structural motifs known as leucin rich repeats (LRR) which are actually the specificity determinants (Thomas *et al.*, 1997; Wang *et al.*, 1998). Leucin rich repeats containing resistant genes can be grouped into the transmembrane R proteins which lack nucleotide binding sites (NBS) and the cytoplasmically located R proteins with NBS (Barker *et al.*, 1997; Ori *et al.*, 1997).

The presence of these conserved sequences among R genes has provided an opportunity for isolating new resistant genes or resistant gene analogs (RGAs) based on PCR methods by designing degenerate primers based on the conserved sequences (Kanazin *et al.*, 1996; Collins *et al.*, 1998; Gentzbittel *et al.*, 1998).

The cloning and analysis of these resistant gene analogs has opened new avenues of research on plant disease and produced markers tightly linked to resistance genes. Gentzbittel *et al.*, (1998) isolated RGAs in sunflower using NBS and protein kinase conserved motiffs. They further mapped three of the clones to linkage group 1 where downy mildew resistance genes are located. RGA were also mapped to four downy mildew R genes clusters in lettuce (Shen *et al.*, 1998).

2.9.5 Sequence tagged sites (STS)

A recent addition to the tools for fine physical mapping of genes of interest is sequence tagged sites (STS). An STS is a short DNA sequence generally, between 100-500 bp that is easily recognisable and occurs only once in a chromosome or genome studied (Brown, 1999). STS are derived in the genome from expressed sequence tags (EST), which are obtained from cDNA clones; simple, sequence

length polymorphism (SSLP), or from random genomic sequences. To date, six ESTs have been placed on the cassava genetic map (Fregene *et al.,* 2001). Single nucleotide polymorphism (SNPs) are another group of site specific markers, which are the most common type of DNA sequence variation and occur every 100 to 300 base pairs (Lohmann *et al.,* 2000). Due to their high frequencies, the chances are high that disease resistance or susceptibility would be caused by or closely associated with specific SNPs.

2.9.6 Type of population

The choice of an appropriate mapping population depends on the type of marker systems employed (Tanksley *et al.,* 1988). Generally F_2s and backcross progenies are used in the construction of linkage maps, QTL analysis, and BSA for homozygous species. In a heterozygous species, the segregating F_1 is used (Liu, 1998; Pillay and Kenny, 1996; Fregene *et al.,* 1997). However, this should be an informative cross between two divergent parents that are heterozygous for the markers (Falconer and Mackay, 1997). The cassava genetic map at CIAT is based on an F_1 population of two geographically divergent parents. The female parent TMS I30572, having resistance from clone 58308, while the South American male parent has no resistance to CMD (Fregene et al., 1997).

Maximum genetic information is obtained from a completely classified F_2 or segregating F_1 population using a codominant marker system. Information from a dominant marker system can be equivalent to a completely classified F_2 population if progeny tests (i.e. F_3 or F_2, BC) are used to identify heterozygous F_2 individuals (Staub *et al.,* 1996). Progeny testing is required when the phenotypes do not consistently reflect genotype or where trait expression is controlled by QTLs.

Recombinant inbred lines (RIL) are suitable for QTL mapping in that they consist of many distinct lines each a mosaic of different homozygous segments from the

original parent. Dominant markers also supply as much information as codominant markers in RIL (i.e. an array of genetically related lines; usually F_8 or more), doubled haploid, or backcross populations in coupling phase because in these populations, all loci are homozygous, or nearly so (Burr and Burr, 1991;Liu, 1997).

Other types of population used in identification of markers tightly linked to disease resistance genes are the near-isogenic lines (NILs) (Muehlbauer *et al.,* 1988; Young *et al.,* 1988). A donor-parent (DP) carrying the gene of interest is repeatedly backcrossed to a recurrent parent (RP), selection of the desired gene and recovery of the RP phenotype is continued until the newly developed line is theoretically nearly isogenic with the RP, except for the chromosome segment containing the target gene. Linkage between a molecular marker and the target gene can be assessed by determining the marker genotype of the RP, its various NIL derivatives, and their corresponding DPs (Melchinger, 1990). Although the development of NILs through back11crossing for economically important genes is costly and time consuming (Kelly, 1995), the use of such NILs has permitted the successful tagging of three disease resistance genes in common bean (Haley *et al.,* 1994; Miklas *et al.,* 1996).

Inter-gene-pool crossing technique was used successfully to tag two rust resistance genes in common bean (Haley *et al.,* 1993; Miklas *et al.,* 1993) and in tomato, interspecific populations have been applied in mapping of some disease resistance genes (Paterson *et al.,* 1990).

Chapter Three

Materials and Methods

The study was carried out in the experimental fields and at the Cellular and Molecular Technology Laboratories (CMTL) of the International Institute of Tropical Agriculture (IITA) in Ibadan, Nigeria.

3.1 Analysis of variance and combining ability analyses

3.1.1 Genetic Material

Diverse cassava genotypes with varying levels of resistance and susceptibility to CMD, including the resistant genetic stock 58308, six improved clones and 15 African landraces and the progenies from their F_1 crosses were used in the study. The cassava genotypes, TMS I30572 (moderately resistant), TMS 91/02324 (resistant to CMD), a resistant landrace TME1 (Antiota) and a susceptible landrace TME117 (Isunikankiyan) were included as checks. The parental and check genotypes are described in Table 3.1.

3.1.2 Experimental design

Seeds of the F_1 crosses, parental and check genotypes were provided by the cassava breeding unit at IITA for the study. The seeds were obtained from two mating designs. One was a 4X4 diallel mating scheme in all possible combinations including selfs and reciprocals. It involved the resistant genetic stock 58308 and three improved clones, TMS I30001, TMS I30572 (resistant to CMD), and TMS I30555 (susceptible to CMD). The other mating design was a 3X18 North Carolina design II (NCD II) with TMS I30001, TMS I30572, and TMS I30555 as the female parents, three resistant improved clones and the 15 landraces were the male parents.

Table 3.1 Description of cassava clones used as parents in 4X4 diallel and 3X18 North Carolina Design II matings and checks

Clone	Pedigree information, local name and origin	Genetic Experiment	
		Diallel	Design II
TMS I30001	Pedigree information lost	Parent 1	Female 1
TMS I30555	58308 X Oyarugba dudu	Parent 2	Female 2
TMS I30572*	58308 XBranca de Santa Caterina (OP)[+]	Parent 3	Female 3
TMS I60142	KR685 OP		Male 1
TMS I90257	58308 X Oyarugba dudu		Male 2
TMS I4(2)1425	58308 X Oyarugba fufun		Male 3
58308	*M. esculenta X M. glaziovii*	Parent 4	
91/02324*	TME1 OP		
TME1*	Antiota (Ondo, Ondo State, Nigeria)		Male 4
TME2	Odungbo (Opeji, Ogun State, Nigeria)		Male 5
TME4	Atu (Iwo, Kwara State, Nigeria)		Male 6
TME5	Bagiwawa (New Busa, Niger State, Nigeria)		Male 7
TME6	Lapai-1 (Lapai, Niger State, Nigeria)		Male 8
TME7	Oko-Iyawo(New Lapai, Niger State, Nigeria)		Male 9
TME8	Amala (Ireuekpen, Edo State, Nigeria)		Male 10
TME9	Olekanga (Ogbomosho, Oyo State, Nigeria)		Male 11
TME10	Orente (Ogbomosho, Oyo State, Nigeria)		Male 12
TME11	Igueeba (Warri, Delta State, Nigeria)-		Male 13
TME12	Tokunbo (Ibadan, Oyo State, Nigeria)		Male 14
TME14	Abbey Ife (Abbey-Ife, Osun State, Nigeria)		Male 15
TME31	Bakince-Iri (Bahago, Sokoto State, Nigeria)		Male 16
TME41	Danbusa (Kanji, Niger State, Nigeria)		Male 17
TME117*	Isunikankiyan (Ibadan, Oyo State, Nigeria)		Male 18

*Clone used as check
[+]OP=open pollinated

In 1997, the seedlings from these crosses were established in a seedling nursery in Mokwa, Niger State, Nigeria, to produce woody cuttings for the experiments. Mokwa is in the Southern Guinea Savanna with a ferric luvisol soil type. It is generally a low disease pressure site for CMD.

The genetic material was then established in two experiments at Mokwa and Ibadan (Oyo State, Nigeria) during the 1998 growing season and in Ibadan during the 1999 growing season. Ibadan is in the forest-savanna transition with a ferric luvisol soil type. The physical and chemical properties of soil and rainfall pattern of the two locations during the two growing seasons are described in Appendix I. In Mokwa, the two experiments together with their parents, and three checks (TMS I30572, TME1, and TME117) were evaluated, while in Ibadan, the two experiments, their parents and four checks (91/02324, TMS I30572, TME1, and TME117) were evaluated.

The design for each experiment was a randomised complete block with two replications. Plants were established using 20 cm long cuttings. Plants were spaced at 0.5 m apart in rows (ridges 30 cm high and 10 m long) which were 1 m apart giving a plant population of 20,000 plants per hectare. Due to differential seed set of the parents, the number of progenies varied for the different crosses. The number of progenies obtained from the seedling nursery ranged from 16 to 300 in the diallel experiment and 52 to 934 in the NCD II experiment. To ensure the survival of each F_1 genotype, two cuttings per genotype were planted in each replicate. The second cutting was removed at 6 weeks after planting (WAP) when the plants were established. Twenty cuttings of each parental or check genotype were planted in each replicate with the same spacing as the crosses. The experiments were evaluated for CMD under rain-fed conditions. No fertiliser or herbicide was applied during the course of the experiments. Hand weeding was done when necessary.

3.1.3 Assessment of CMD

Individual plants in each F_1 cross and 10 plants each of the parents and checks were assessed for their reaction to CMD under natural infection by whiteflies. Assessment was based on their phenotypic expression of severity of symptoms using the standard five point scoring scale system for CMD (IITA, 1990). Disease severity scores of the whole plants and shoot tips (from the shoot apex to the first fully expanded leaf) were assessed at 6, 12 and 20 WAP in 1998 and at 6 and 12 WAP in 1999. Table 3.2 describes the five point scoring scale and an illustration of the disease severity scores on cassava leaves is presented in Plate 3. 1.

3.1.4 Data analyses

Data collected from the three environments where the experiments were conducted were analysed using the statistical analysis system (SAS) package (SAS, 1999). An environment was defined as the particular location and year that the experiments were conducted. Thus, the three environments were Mokwa 1998, Ibadan 1998, and Ibadan 1999.

The weighted means for incidence and disease severity of CMD in shoot apices and whole plants of each of the checks, parents, and crosses at the different scoring dates were determined from the data collected and used for the genetic analysis. Incidence was determined as the percentage of diseased plants or percentage of plants with diseased shoot tips. The weighted average disease severity score of a parent, check, or F_1 cross was defined as the summation of the product of the frequency count and the value of the disease severity class, divided by the total number of plants evaluated, and was determined for each replicate. If the weighted average disease severity score of a parent, check, or F_1 progeny cross was less than or equal to 2, it was classified as resistant, otherwise it was classified as susceptible.

Table 3.2 Standard five point scale scoring systems for CMD (IITA, 1990)

Disease Score	Symptom	Status
1	No obvious symptoms.	Highly resistant (HR)
2	Mild chlorotic patterns or mild leaf distortion at the base	Resistant (R)
3	Strong mosaic on the entire leaf, distortions of leaves	Moderately susceptible (MS)
4	Severe mosaic distortion, reductions of leave lamina with about 2/3 of the leaves affected	Susceptible (S)
5	Severe mosaic, severe distortion of leaves, stunting of entire plant and about 4/5 of leaves affected	Highly susceptible (HS)

Plate 3.1 Cassava leaves, with varying levels of cassava mosaic disease severity
CMD 1, No obvious symptoms.
CMD 2, Mild chlorotic pattern, mild leaf distortion at the base.
CMD 3, Mosaic on the entire leaf, distortion of leaf
CMD 4, Severe mosaic distortion, reduction of leaf lamina.
CMD 5, Severe mosaic, severe distortion of leaf and severe reduction of leaf lamina.

A simple phenotypic correlation analysis performed on the average CMD incidence and disease severity scores of all genotypes in the two experiments showed a consistent significant linear relationship between incidence and severity at the different scoring dates across environments and in the individual environments (Appendix II). Genetic analyses of the F_1 crosses were based on CMD severity at 12 WAP and the assessment of genetic relationships among the parents was based on all the CMD severity and incidence responses.

The analyses of variance for each experiment were performed on the individual environments and a combined analysis of variance was performed with data from the three environments. The analyses were based on mixed models with the genotypes considered fixed; replicate, environment, and genotype by environment interaction (GXE) considered as random effects.

Appropriate F-tests were performed according to the expectations of the means squares, based on the procedures described by McIntosh (1983) for mixed model analysis. In the individual environment analyses, the genotypic components were tested with the pooled error. For the combined analyses, the environment was tested with the replicates nested within environments, Env(Rep) mean square, and the genotypic components were tested with their respective genotype by environment (GXE) interactions. The GXE effects were tested with the pooled error.

3.1.4.1 3X18 North Carolina Design II (NCD II) analysis

The general linear model (GLM) procedure in SAS which uses the method of least squares to fit general linear models was used for the analyses of variance (SAS, 1999). Mean squares were calculated from Type III sums of squares. Genotypes were partitioned into the variation due to test genotypes (parents and crosses) and checks. Test genotypes were then partitioned into parents and crosses. The parents were further partitioned into female, male, resistant and susceptible parents and one

degree of freedom orthogonal contrasts, female versus male parents and resistant versus susceptible parents were made to test for significant variation between the different sets of parents. A one degree of freedom orthogonal contrast, between parents and crosses was also used to test the significance of average midparent heterosis.

Crosses were further partitioned into variation due to the GCA (additive) effect of males, the GCA effect of females and variation due to the SCA (non-additive) effect (male and female interaction). The general linear model used for the analysis of the crosses in the NCD II for one environment was as follows:

$$y_{ijk} = \mu + m_i + f_j + mf_{ij} + b_k + \epsilon_{ijk} \qquad \text{.....................................(1)}$$

In this model, y_{ijk} was the observed response to CMD; μ the general mean; m_i the GCA effect of the ith male; f_j the GCA effect of jth female; mf_{ij} the SCA effect of the ijth cross; b_k was the effect of the kth replicate; and ϵ_{ijk} the error associated with each observation (Singh and Chaudhary, 1985).

The analysis of variance for the combined analysis across environments was performed as given for an individual environment. The GXE interactions however, were further partitioned into their components.

The general linear model assumed for the NCD II population for the combined analysis across environments was:

$$y_{ijkl} = \mu + m_i + f_j + mf_{ij} + l_k + b_{l(k)} + ml_{ik} + fl_{jk} + mfl_{ijk} + \epsilon_{ijkl} \qquad \text{.............. (2)}$$

Here, y_{ijkl} was the observed response to CMD across the three environments; μ, m_i f_j and mf_{ij} were as described in equation (1) for an individual environment.

The l_k th effect was the kth environment. The $b_{l(k)}$ th effect was the kth environment nested in lth replicate. The ml_{ik} th effect was the ith male in the kth environment; and fl_{jk} was the effect of the jth female in the kth environment. The fml_{ijk} th effect was the ijth cross in the kth environment and $\in_{ijkl}$ the residual associated with each observation (Beil and Aitkins, 1967).

Combining ability estimates were determined for individual environments and across environments using least square means.

GCA of a clone was estimated as

$$g_i = \overline{X}_{i.} - \overline{X}_{..} \qquad \text{...(3)}$$

and the SCA of a cross was estimated as

$$s_{ij} = \overline{X}_{ij.} - \overline{X}_{i.} - \overline{X}_{.j} + \overline{X}_{..} \qquad \text{..(4)}$$

Here $\overline{X}_{ij.}$ was an F_1 mean, $\overline{X}_{i.}$ was the mean of crosses of the ith clone; $\overline{X}_{.j}$ was the mean of crosses of the jth clone and $\overline{X}_{..}$ is the mean of all the F_1 crosses (Beil and Aitkins, 1967).

The standard errors of these effects were calculated using the methods described by Singh and Chaudhary (1985) for a single environment and Cox and Frey (1984) for the combined environment.

The SE for GCA was

$$SE_{GCA_M} = \sqrt{\frac{MSE(m-1)}{rfm}} \text{ for males in a single environment or, ...(5)}$$

$$SE_{GCA_f} = \sqrt{\frac{MSE(f-1)}{rfm}}$$ for females in a single environment or, ...(6)

$$SE_{GCA_m} = \sqrt{\frac{MS_{ml}(m-1)}{mfrl}}$$ for males in the combined environment .... (7)

or,

$$SE_{GCA_f} = \sqrt{\frac{MS_{fl}(m/f-1)}{mfrl}}$$ for females in the combined environment (8)

The SE for SCA was

$$SE_{SCA} = \sqrt{\frac{MSE(m-1)(f-1)}{rmf}}$$ for a single environment..............(9)

or,

$$SE_{SCA} = \sqrt{\frac{MS_{mfl}(m-1)(f-1)}{mfr(l)}}$$ for the combined environment ......(10).

In equations (5) to (10), MSE was the mean square error of the crosses, m and f were the number of males or females, r the number of replicates and $r(l)$ the number of replicates and environments. MS_{ml} and MS_{fl} were the mean squares of males or females by environment interaction and MS_{mfl} was the mean square of the male by female by environment interaction.

The GCA and SCA effects were tested for significance in two tailed tests as described by Cox and Frey (1984) and the LSD was determined as,

$LSD_{GCA} = t_{(df,0.05)} x SE_{GCA}$ and ....(11)

$LSD_{SCA} = t_{(df,0.05)} x SE_{SCA}$ (Singh and Chaudhary, 1985) ....(12).

Negative and significant effects where considered as contributing to resistance and positive and significant effects were considered as contributing to susceptibility.

3.1.4.2 4X4 Diallel analysis

Genotypes were partitioned into the variation due to test genotypes (parents and crosses) and checks using the GLM procedure in SAS (SAS, 1999). Test genotypes were further partitioned into parents and crosses. A one degree of freedom orthogonal contrast between parents and crosses was used to test for the presence of significant average midparent heterosis.

The genetic analysis of the crosses was based on Griffing's method 1, in which the analysis is based on data from a complete diallel (crosses selfs and reciprocals) and using model 1, which is valid when the genotypes are fixed (Griffing, 1956b). Reciprocal effects were further partitioned into the maternal and non-maternal effects (Cockerham and Weir, 1977). The analysis was performed on individual environments and a combined analysis over all environments using the diallel-SAS programmes written by Kang (1994) for a single environment and Zhang and Kang (1997) for the combined environment.

The general linear model for an individual environment was;

$$Y_{ijk} = \mu + g_i + g_j + s_{ij} + r_{ij} + b_k + \in_{ijk} \quad \text{...(13)}$$

In this model, Y_{ijk} was the observed response to CMD; μ was the general mean; g_i the general combining ability of the ith parent; g_j the general combining ability of the jth parent; s_{ij} the specific combining ability associated with the ijth cross; r_{ij} the reciprocal effect associated with the ijth cross. The b_k th effect was the effect of the kth replicate and $\in_{ijk}$ the residual effect associated with each observation (Kang,

1994). The reciprocal effect r_{ij} was further partitioned into the maternal effect of the ith parent m_i, the maternal effect of the jth parent m_j and the non-maternal effect n_{ij} of the ijth cross (Cockerham and Weir, 1977).

The general linear model for the combined analysis was;

$$Y_{ijkl} = \mu + g_i + g_j + s_{ij} + r_{ij} + l_k + b_{l(k)} + gl_{ik} + gl_{jk} + sl_{ijk} + rl_{ijk} + \in_{ijkl} \quad \text{.........(14)}$$

In this model; Y_{ijkl} was the observed response to CMD across the three environments, μ, g_i, g_j, s_{ij} and r_{ij} and its partitions m_i, m_j and n_{ij} were as described in equation (13) for the individual environment analysis. The effect l_k th effect was the kth environment; $b_{l(k)}$ th effect of the lth replicate nested in the kth environment. The gl_{ik} th effect was the general combining ability of the ith parent in the kth environment; the gl_{jk} th effect was the general combining ability of the jth parent in the kth environment and the sl_{ijk} th effect was the specific combining ability associated with the ijth cross in the kth environment. The rl_{ijk} th effect was the reciprocal effect associated with the ijth cross in the kth environment and $\in_{ijkl}$ the residual effect associated with each observation (Kang *et al.*, 1999). The interaction of the reciprocal effects with the environment was also further partitioned into the maternal by environment interaction (ml_{ik} and ml_{jk}) and non-maternal by environment (nl_{ijk}) interaction .

Estimates of GCA, SCA, reciprocal maternal and non-maternal effects of the crosses were generated by the SAS programme with least square means. The computational formulae, which generated the estimates, were based on Cockerham's statistical model (1963) for Griffing's Methods, which was reviewed by Zhang and Kang (1997).

3.1.4.3 Relative importance of general and specific combing ability

The relative importance of GCA and SCA in predicting progeny performance can be determined from the ratio of the additive variance to total genetic variance. Since the genotypes were considered fixed, estimation of variance components was not appropriate. The analogous mean square components of the fixed effects were therefore estimated to compute the ratios (Baker, 1978; Kang, 1994). The mean square components of the fixed GCA effects of males, females, and the SCA effect of the female by male interaction were estimated from the variance components of the random effects associated with these fixed effects using the MIVQUE0 option of the VARCOMP procedure in SAS.

For the NCD II the ratio was estimated from the relationship;

$$\frac{GCA}{GCA+SCA}. \qquad \text{...(15)}$$

Here, GCA was the sum of the GCA mean square components due to female and GCA mean square components due to male. SCA was the mean square component due to the female by male interaction (Cukador-Olemedo *et al.*, 1997).

For the diallel analyses, the ratio was estimated from the relationship

$$\frac{2GCA}{2GCA+SCA}. \qquad \text{...(16)}$$

where GCA and SCA are the mean square components of the GCA and SCA effects respectively (Kang, 1994). Mean square component of the GCA and SCA effects in the diallel were also estimated from the expected mean squares as described by Singh and Chaudhary (1985). The GCA component (θ_{GCA}) and SCA component (θ_{SCA}) in one environment were estimated as described by Singh and Chaudhary (1985) as follows;

$$\theta_{GCA} = \frac{1}{(p-1)} \sum g_i^{\ 2} = \frac{MS_{GCA} - MS_{\epsilon}}{2r} \quad \text{.................................} \quad (17)$$

and

$$\theta_{SCA} = \frac{2}{p(p-1)} \sum \sum s_{ij}^{\ 2} = M \frac{S_{SCA} - MS_{\epsilon}}{r} \quad \text{.................................} \quad (18)$$

Where, MS_{GCA}, MS_{SCA} and MS_{ϵ} were the mean square for GCA, SCA and Error and r was the number of replicates.

For the combined analysis, the GCA component (θ_{GCA}) and SCA component (θ_{SCA}) were estimated as described by Zhang and Kang (1997) as follows:

$$\theta_{SCA} = \frac{1}{(p-1)} \sum g_i^{\ 2} = \frac{MS_{GCA} - MS_{GCAx\epsilon}}{2re} \quad \text{..............................} \quad (19)$$

and

$$\theta_{SCA} = \frac{2}{p(p-1)} \sum \sum s_{ij}^{\ 2} = \frac{MS_{SCA} - MS_{SCAx\epsilon}}{re} \quad \text{...........................} \quad 20).$$

Where, MS_{GCA}, MS_{SCA}, MS_{ϵ} and r were as described for an individual environment, MS_{GCAXE}, MS_{SCAXE} and *e* were the mean square for GCA X E, SCA X E and number of environments respectively.

Pearson correlation was performed on the parent means and GCA effects to compare per se performance of the parents with their GCA effects and their topcross performance in the NCD II experiment.

3.1.4.4 Heritability

Heritability across environments and the exact confidence intervals were estimated using the methods described by Knapp *et al.*, (1985) based on progeny mean. Heritabilty across environments for each experiment was therefore estimated as,

$$H^2 = 1 - \frac{MS_{CXE}}{MS_{cross}} \quad \text{...} (21)$$

where MS_{cross} and MS_{CXE} were the mean squares of the crosses and crosses by environment across environments respectively. The exact 90% (1-α =0.90) confidence limits were determined as

$$1 - [F_{0.05:df'',df'}(\frac{MS_{cross}}{MS_{CXE}})]^{-1}, \text{ upper limit} \quad \text{.....................................} (22)$$

$$1 - [F_{0.95:df'',df'}(\frac{MS_{cross}}{MS_{CXE}})]^{-1}, \text{ lower limit} \quad \text{.....................................} (23)$$

where $F_{0.05:df''df'}$ and $F_{0.95:df''df'}$ were the tabulated values of F at 0.05 and 0.95 for the appropriate degree of freedom in each experiment, MS_{cross} and MS_{CXE} were as described above.

3.1.4.5 Heterosis

Least square means were used to estimate average heterosis in each experiment and in the individual crosses. Heterosis of a cross was determined as the difference between the mean of a cross and the mean of the two parents (mid-parent), or the mean of the better parent (high parent), expressed as a percentage of the parent mean. Significance of average mid-parent heterosis was estimated by a one degree of freedom orthogonal contrast (parent versus cross) from the analysis of variance of the test genotypes. Significance of heterosis in an individual cross was determined

using a least significant difference (LSD) between the parent and cross mean (Dixon *et al.*, 1990), given below

$$LSD_{0.05} = t_{(df,0.05)} x \sqrt{\left(\frac{1}{ra} + \frac{1}{rb}\right) MS_{\epsilon}} \qquad (24)$$

where, $t_{(df,\ 0.05)}$ was the tabular value of *t* at the 0.05 level of significance with the appropriate degrees of freedom, *r* was the number of replicates and environments, *a* and *b* were the number of experimental units in a cross and parental mean respectively and MS_{ϵ} was the pooled error mean square. Since the average disease severity scores per replicate were used for the GLM analysis to generate the least square means used in the estimation of heterosis, the number of experimental units in the F_1 or parent means was 2.

3.2 Genetic relationships among the various sources of resistance to CMD

The genetic relationship between the different sources of resistance was assessed based on the phenotypic similarities of the parental responses to CMD and the segregation patterns of the F_1 progenies with respect to their response to CMD. The experimental procedure and CMD assessment were as described in section 3.1 above.

3.2.1 Area under the disease progress curve (AUDPC) of the sources of resistance to CMD

The mean CMD severity scores in the shoot tip and whole plant at 6 and 12 or 20 WAP of the parental and check clones which represent the sources of resistance were used to estimate the area under their disease progress curve (AUDPC) values in each environment and across environments. AUDPC was calculated using the method described by Jeger and Viljanen-Rollinson (2001) which allows estimates of AUDPC with values from two assessments in the equation,

$$AUDPC = T + \frac{\ln(\frac{y_0}{y_T})}{r} \quad \text{...} (25)$$

Here, y_0 was the CMD value at 6 WAP, and T was 12 or 20 weeks. For Ibadan 1999 and Mokwa 1998 environments, y_T was the CMD value at 12 WAP while for Ibadan 1998 and across environments it was 20 WAP and r the rate.

The rate r, was determined as

$$r = \frac{\ln(\frac{y_T}{1-y_T}) - \ln(\frac{y_0}{1-y_0}}{T} \quad \text{...} (26).$$

AUDPC values were then subjected to Proc Rank procedure in SAS (SAS, 1999) to assign the lowest rank value to the least (most resistant) AUDPC values. Spearmans correlation (SAS, 1999) was performed on the assigned ranks to test the association of the AUDPC values across environments.

3.2.2 Principal component analysis (PCA) of sources of resistance to CMD

Principal component analysis (PCA) was performed on the mean CMD incidence and severity scores of the various sources of resistance for the different scoring dates in the three environments, using the correlation matrix of the means (SAS, 1999). A scatterplot of the PCA scores of the first and second principal components was generated to explore the genetic similarities among the parental clones. The aim was to group the clones according to their CMD responses.

3.2.3 Gene complementarity among various sources of resistance to CMD based on the distribution of the F_1 disease severity scores

3.2.3.1 Transgressive segregation for resistance to CMD

The mean disease severity scores of individual F_1 progenies of the various sources of resistance at 12 WAP (largest variation for CMD severity) in an environment, were used to construct a frequency table of the distribution of disease severity scores in the three environments. The percentage of positive transgressive segregants in the F_1 cross was determined from the distribution of disease severity scores. A positive transgressive segregant was defined as an F_1 progeny at least one disease severity score lower than the average disease severity score of the better parent in a cross. Positive transgressive segregants were determined for each cross in each environment and across environments.

3.2.3.2 Cochran-Mantel-Haesnzel

The Cochran-Mantel-Haesnzel ANOVA statistic (SAS, 1999), was used to determine significant differences in the mean distribution of the F_1 disease severity scores among the various sources of resistance and to test for reciprocal differences.

3.2.3.3 Number of effective factors (NE) contributing to resistance to CMD

The minimum number of independently segregating effective factor pairs responsible for resistance to CMD was estimated in the segregating F_1 populations using a modification of the Castle-Wright formula (Castle, 1922; Wright, 1968),

$$NE = \frac{(P_1 - P_2)^2}{8(Var_{F_2} - Var_E)} \quad \text{..........} (27)$$

where NE was the number of effective factors by which the parents in a cross differ; P_1 and P_2 were the mean values of the better parent and poorer parent respectively,

Var_{F2} , the variance of the segregating population and Var_E the environmental variance estimated from the uniform population. The assumptions for an unbiased estimate using this formula are that:

i. all segregating loci contribute equally to the trait,

ii. no linkage exist among loci affecting the trait,

iii. no dominance, or the degree and direction of dominance of plus factors are similar for all loci,

iv. all plus factors are contributed by one parent and all minus factors contributed by the other parent,

v. no epistasis occurs among alleles at contributing loci and environmental, and

vi. genotypic variances are independent and combine additively to give total variability.

Lawrence and Frey (1976), suggested the use of the range of the F_2 population in the estimation of effective factors when the parents did not represent the genotypic extremes for the segregating loci and when the variance of the F_2 contained some non-additive components. Since cassava is a heterozygous crop, the F_1 is genetically equivalent to an F_2 of a homozygous crop (Magoon, 1967; Liu, 1998). Hence the formula used to estimate the minimum number of effective factors NE was;

$$NE = \frac{R^2}{8\sigma^2_g} \quad \text{......} \quad (28)$$

where R was the range of the F1 segregates in a cross, σ^2_g is the genetic variance(Lawrence and Frey, 1976). The genetic variance σ^2_g was estimated from the linear function of the observed phenotypic variance and the environmental

variance, which was estimated from the homozygous population (Falconer and Mackay, 1997). In this study, the cassava parents are homogenous vegetatively propagated cuttings and no two plants of a single cloned genotype have the same phenotypic effect. The mean estimate of the variances of the two parents in a cross was, therefore, used as the environmental variance of homozygous population (Wricke and Weber, 1986; Falconer and Mackay, 1997).

Therefore, the genetic variance was

$$Var_g = Var_{F_1} - \left(\frac{Var_{P_1} + Var_{P_2}}{n} \right) \quad \text{.......... (29)}$$

where Var_g was the genetic variance; Var_{F1} was the F_1 variance; Var_{P1} was the variance of the better parent P_1, Var_{P2} was the variance of the worse parent P_2 and n the number of observations in a parental mean. The number of factors affecting the CMD resistance trait contributed by each parent in a cross in each environment and across environments was estimated by using the procedure of Lawrence and Frey which is an extension of the Castle-Wright formula. Where little or no genetic variance was obtained, the number of effective factors was not estimated.

The number of favourable factors in the poorer parents were calculated as follows (Dixon *et al.*, 1991)

$$np = \left\{ \frac{(\overline{X}_b - \overline{X}_{P_b}) + (\overline{X}_{P_w} - \overline{X}_w)}{2} \right\} x \frac{NE}{R} \quad \text{.......... (30)}$$

where np was the number of plus (favourable) factors contributed by the poorer parent, $\overline{X}_b$ = the mean score of the best F_1 progeny of a cross, $\overline{X}_{P_b}$ = mean score of the better parent of a cross, $\overline{X}_{P_w}$ = mean score of the poor parent of the cross $\overline{X}_w$ = mean score of the poorest F_1 progeny of a cross. The number of favourable

factors in the better parent (nb) were obtained by subtracting np from NE, and because the number of factors estimated was the minimum, the number was approximated to the next larger interger.

3.3. Determination of DNA markers associated with resistance to CMD

Bulk segregant analysis (BSA) and linkage mapping were used to determine molecular markers linked to resistance to CMD in a resistant landrace using RAPD, SSR, and AFLP marker systems.

The BSA was carried out in three stages. In the initial screening, parental DNA and bulked DNA, were screened with 142 RAPD primers, 150 SSR, and 128 AFLP primer pairs. Primers that were polymorphic between the parents and the two bulks were selected for the second level screening. In the secondary screening, parents, bulks and the members of each bulk were screened with the selected primers. Primers that detected consistent differences between the resistant and susceptible DNA samples were then selected for the tertiary screening. The entire mapping population and the 23 cassava clones used in the diallel and NCD II studies were screened.

3.3.1 Genetic material and experimental procedure

The mapping population consisted of 69 F_1 progenies from a cross between the improved clone TMS I30555 (susceptible to CMD) and the resistant landrace TME 7 (Oko-Iyawo). The F_1 progenies were developed and evaluated in the NCD II experiments described in section 3.1.1 and 3.1.3 above. In March 2000, cuttings of the progenies and parents were planted in nursery beds and watered twice a week to produce young leaves for DNA extraction. The 23 cassava genotypes used as parents and checks in the diallel and NCD II experiments (Table 3.1) were included to confirm the potential of a DNA marker association with CMD resistance as a molecular marker for resistance to CMD in the landraces.

3.3.2 Resistance screening and selection of plants for bulk segregant analysis

The plants were evaluated for symptom severity as described in section 3.1.3, at 6, 12, 20, and at 50 WAP during the 1998/99 season and at 6 and 12 WAP during the 1999 season in Ibadan. Disease severity scores of whole plants and shoot tips (from the shoot apex to the first fully expanded leaf) were assessed. At 12 WAP during the 1999 growing season, the first 10 leaves of each plant were evaluated for symptom severity.

In March 2000, cuttings of the plants were made in a nursery bed to generate young leaves for DNA extraction. At 3 WAP, the shoot tips were excised for DNA extraction, and to enhance symptom severity. The plants were then assessed twice a week till the twelfth week for a final confirmation of resistance or susceptibility.

The mean disease severity scores based on shoot tip, whole plant, and the first 10 leaves for the individual plants were determined from the data collected. Ten highly resistant (HR) F_1 genotypes and ten highly susceptible (HS) genotypes of the mapping population were selected randomly to make up the two bulks for BSA.

3.3.3 DNA extraction

The DNA extraction kit, DNeasy™ from Qiagen was used for DNA extraction. The extraction was done according to the manufacturers' instructions (Qiagen, 1999). Briefly, 100 mg of fresh leaves from the shoot apices was collected into 1 ml eppendorf tubes and ground to a fine powder in liquid nitrogen. The extraction buffer (400 µl buffer AP1) plus 4 µl of Rnase (100 mg/ml) was added. The slurry was mixed with the pestle used to grind the sample; the sample was incubated at 65°C for 10 minutes. The tubes were inverted three times during the incubation period to facilitate lyses of the cells.

After the incubation, 130 µl of the buffer AP2, which precipitates detergents, proteins and polysaccharides was added, mixed, and incubated on ice for 5 minutes, followed by centrifugation at 14,000 rpm for 5 minutes. The lysate was then applied to a QIAshredder spin column, and centrifuged for 2 minutes at 14,000 rpm to remove precipitates and cell debris. The flow-through was collected, and transferred to a fresh tube and 225 µl of buffer AP3 and 450 µl of ethanol was added and mixed gently to precipitate the DNA. The solution was then applied to the DNeasy mini spin column and centrifuged for 1 minute at 8,000 rpm to collect the DNA onto the matrix of the spin column. The column was then placed in a collection tube and washed twice with 500 µl buffer AW plus ethanol. The first wash was at 8,000 rpm for 1 minute and the second wash at 14,000 rpm for 2 minutes.

The columns were allowed to dry at room temperature for about 30 minutes, then were placed in 1.5 ml eppendorf tubes. Pre-warmed eluting buffer (AE) was applied at 100 µl to each tube, incubated for 5 minutes at room temperature, and centrifuged for 1 minute at 8,000 rpm to elute the DNA. The elution step was repeated to give a total volume of 200 µl.

After extraction, the DNA quality was assessed on a 0.8% agarose gel in 0.5X tris borate EDTA (TBE) buffer (Appendix II). The DNA concentration was estimated with a Hoefer TKO 100 mini fluorometer and the purity checked by spectrophotometry.

3.3.4 DNA quantification

3.3.4.1. Agarose gel electrophoresis

Agarose solution of 0.8 % (w/v) concentration was prepared by weighing 1.6 g of gel-grade agarose (Sigma) into 200 ml of 0.5X TBE buffer. The emulsion was boiled in a microwave oven and allowed to cool to 50-55°C. For every 100 ml of agarose solution, 5 µl of ethidium bromide solution (10 mg/ml) was added. The solution was then poured into a horizontal gel electrophoresis tray (Owl E-D20) which was

mounted in a gel casting tray and fitted with two 50 teeth-combs (2 mm wide). After the gel had solidified, the tray was removed from the gel caster, and placed in its electrophoresis tank. The tank was filled with 900 to 1,000 ml of 0.5X TBE buffer, up to about 2 mm above the top of the gel, and then the combs were removed carefully from the moulded wells.

The extracted DNA samples were diluted (1:10) in tris EDTA (TE) buffer (Appendix III) and 10 µl aliquots mixed with 2.5 µl of loading dye (Appendix IV). Three concentrations of *Arabidopsis* DNA, were loaded with the samples as checks. The *Arabidopsis* DNA (100 ng/µl Gibco) was also diluted at 1:10 and 5 µl, 10 µl and 15 µl aliquots mixed with 1/3 volume of loading dye and loaded alongside the samples.

The gel was run at a constant voltage (40V) till the marker dye was 3/4 down the length of the gel. The gel was then visualised under UV-light on a transilluminator, and documented with the aid of Grab-It gel documentation computer software. The fluorescence intensities of the DNA samples were compared with those of the standards to give an indication of their concentration. The DNA samples were also checked for smearing. The presence of smearing indicated that the DNA had sheared during extraction; such a sample was discarded and fresh DNA of that sample extracted.

3.3.4.2 Estimation of DNA concentration and purity

The Hoefer TKO 100 mini fluorometer was used to estimate the concentration of the isolated DNA samples. It allows direct estimations of DNA concentrations in ng/µl by incorporating a dye, Hoechsts 33258 that binds DNA only. The fluorescence of Hoechsts 33258 in the presence of DNA depends on the AT content (Hoefer Scientific, 1992).

Prior to sample assay, the instrument was first switched on for 30 minutes to stabilise its readings. A standard DNA, 100 ng of Tomato or *Arabidopsis* DNA (Gibco BRL) was used to calibrate the instrument before samples were analysed. Each DNA sample (2 µl) was analysed in 2 ml of freshly prepared assay buffer (Appendix III).

Spectrophotometry readings at $OD_{260\ nm}$ and $OD_{280\ nm}$ were also taken to estimate the purity of the isolated DNA. The ratio of $OD_{260\ nm}/OD_{280\ nm}$ provides an estimate of the purity of the isolated DNA, which should be between 1.8 and 2.0 (Sambrook *et al.*, 1989).

3.3.5 Determination of CMD virus strain causing symptoms in the mapping population

The virus strain(s) causing the symptoms in the population was examined using the PCR method described by Zhou *et al.* (1997). DNA samples of the mapping population were tested with primers designed to identify the African cassava mosaic virus (ACMV), the East African cassava mosaic virus (EACMV) and both EACMV and the Uganda variant (UgV) of EACMV (Table 3.3).

Each 25µl reaction was made up of 2 µl DNA (1:40 dilution), 2.5 µl 10 X PCR reaction buffer, 2.5 µl $MgCl_2$ (25 mM stock), 2.5 µl 5% Tween-20, 2.0 µl dNTP's, dATP, dCTP, dGTP and dTTP (2.5 mM/µl stock), 1 µl each of the forward and reverse primers (5.0 pM/µl), 1U *thermus aquaticus* (*Taq*) polymerase and sterile ultra-pure water.

The PCR reactions were performed in a 96-well thermal cycler PTC-200 DNA engine (MJ Research Inc. Watertown Massachusetts, USA.). The reaction cycles were as reported by Zhou *et al.* (1997). The first cycle consisted of 1 minute denaturing at 94°C, 2 minutes primer annealing at 52°C, and 3 minutes extension at 72°C. This was followed by 35 cycles of 1 minute at 94°C, 1 minute at 52°C, and 1.33 minutes at

72°C. The final cycle consisted of 5 minutes at 72°C. PCR products were analysed on a 1.2% agarose gel in TBE as described in section 3.3.4.1, alongside a 1 kb DNA ladder (Gibco BRL, Gathersburg, Mel, USA).

Table 3.3 Nucleotide sequences of DNA primers used in polymerase chain reaction for the detection of cassava mosaic begomoviruses strain in F_1 mapping population

Primer	Primer Type	Forward and reverse	Sequence
1	ACMV Specific	ACMV-F1	5'-TTC AGT TAT CAG GGC TCG TAA-3'
		ACMV-R1	5'-GAG TGC AAG TTG ACT CAT GA-3'
2	ACMV Specific	ACMV-AL1/F	5' GCG GAA TCC CTA ACA TTA TC 3'
		ACMV-AR0/R	5' GCT CGT ATG TAT CCT CTA AGG CCT G 3'
3	EACMV specific	UV-AL1/F1	5' TGT CTT CTG GGA CTT GTG TG 3'
		EACMV-CP/R	5' ACT CTA TGR GTA ATR CCY GA 3'
4	EACMV or UgV	EACMV UV-AL1/F1	5' TGT CTT CTG GGA CTT GTG TG- 3'
		EACMV UV-AL1/R1	5' AAC CTA TCC CCG ATG CTC AT 3'
5	Ug	UV-AL1/F1	5' TGT CTT CTG GGA CTT GTG TG 3'
		ACMV-CP/R3	5' TGC CTC CTG ATG ATT ATA TGT C 3'

3.3.6 Bulk segregant analysis

DNA samples of bulks, parents and individual F_1s were standardised to 10 ng/µl. For the AFLP analysis, bulks were prepared from successfully digested, ligated, and preamplified templates.

3.3.6.1 Random amplified fragment length polymorphism (RAPD) analysis

Equal aliquots (10 µl) of each of the 10 DNA samples constituting a bulk group were combined in an eppendorf tube to make up the bulk DNA sample. A total of 142 RAPD primers (Operon, Alamanda) were used to screen the parentals and bulks. Each 25 µl reaction contained 25ng DNA, 2.5 µl of the primer (2 pmol/µl), 2.5 µl of dNTP's (2.5mM stock), 2.5 µl $MgCl_2$ (25mM/µl stock), 2.5 µl 10x Taq polymerase buffer (Promega), 2.5 µl of 5% Tween-20, 2.5 µl DNA (10 ng/µl), and 1U Taq polymerase enzyme (Promega) in sterile ultra-pure water. PCR was performed in the Perkin Elmer, GeneAmp thermocycler. The cycling profile used for amplification consisted of a 3 minutes initial denaturation at 94°C followed by 45 cycles of 94°C for 1 minute, 35°C for 1 minute, and 72°C for 2 minutes, a final extension for 7 minutes at 72°C, and then stored at 4°C until they were used. The PCR products were analysed on a 2% agarose gel in TBE as described in section 3.3.4.1 and 3.3.5.

3.3.6.2 Amplified fragment length polymorphism (AFLP) analysis

AFLP analysis was performed with three rare cutting restriction enzymes (6 base pair recognition sites), *Eco*R1, *Apa1* and *Pst1* and two frequent cutting (4 base pair recognition sites) restriction enzyme *Mse1* and *Taq1*, to generate *Eco*R1/*Mse1*, *Apa1*/*Taq1*, and *Pst1*/*Taq1* DNA fragments. The DNA fragments were ligated onto their respective adapters and used as templates for plus 1 and plus 3 amplification reactions. The sequences of the adapters, core primers sequences and the plus 1 or plus 3 extensions for the different restriction enzymes are given in Table 3.4.

Table 3.4. Nucleotide sequences of AFLP adapters, primers and primer extensions

	Adapter and primer core sequences		
	Adapters		
Mse1	5' GAC GAT GAG TCC TGA G 3'		
	5' TAC TCA GGA CTC AT 3'		
Taq1	5' GACGATGAGTCCTGAC 3		
	5' CGG TCA GGA CTC AT 3'		
Apa1	5' TCGTAGACTGCGTACA GGCC -3'		
	5' TGTACGCAGTCTAC 3'		
EcoR 1	5' CTC GTA GAC TGC GTA CC 3'		
	5' AAT TGG TAC GCA GTC 3'		
Pst1	5- CTCGTAGACTGCGTACATGCA-3		
	5- TGTACGCAGTCTAC-3		
	Primers	+1	+3
Mse1 + E	'5- GATGAGTCCTGAGTAA + E -3'	C	CAA, CAC,CAG, CAT, CTA, CTC, CTG, CTT
Taq1 + E	'5- CGATGAGTCCTGACCGA + E -3'	A, C	CAC, CAG, CAT, CTA, CTG, CTT, GAC, GTC
Apa1 + E	'5- GACTGCGTACAGGCCC + E -3'	A, C	ATT, CTA, CTG, GCA, TTG
*Eco*R1 + E	'5- AGACTGCGTACCAATTC + E -3'	A	AAC, AAG, ACA, ACT, ACC, ACG, AGC, AGG
Pst1 + E	'5- GACTGCGTACATGCAG + E -3'	A	ACA, ACC, ACT, AGC

E +1 or +3 nucleotide extension

For the *Eco*R1/*Mse1* analysis, the AFLP Analysis System I kit purchased from Life Technologies (Gibco BRL, Gathersburg, Mel, USA) was used. This kit is designed for use with plants having genomes ranging in size from 5. 3 x 10^8 to 6.3 x 10^9 bp and is suitable for cassava which has a genome size of 772 mega base-pairs (7.7 x 10^8) in the haploid genome (Awoleye *et al.*, 1994).

For the *Apa1/Taq1* and *Pst1/Taq1* analysis, restriction enzymes, buffers and oligonucleotides were purchased separately from different companies (Roche and Phamarcia) and the reaction performed according to the methods described by Vos *et al.* (1995) and Wong *et al.* (1999).

Restriction of genomic DNA

EcoR1/*Mse1*

The digestion mixes were set up in sterile 200 µl PCR tubes. Each 25 µl was made up of 5 µl of a 5X reaction buffer, 250 ng of each of DNA samples, 2.5 µl of *Eco*R1 and *Mse1* enzyme mix (1 U/µl) and AFLP-grade water. The tubes were flicked gently to mix the reaction mix, then spun down with a mini strip-tube centrifuge to collect the content. The digestion reaction was performed with the PTC-200 DNA Gene engine at 37°C for three hours. The restriction enzymes were then inactivated at 70°C for 15 minutes, allowed to cool at room temperature, and then placed on ice prior to the ligation reaction.

Apa1/Taq1 and *Pst1/Taq1*

Each 50 µl restriction reaction contained 500 ng DNA, 5 µl 10X reaction buffer for the specific enzymes, 0.05 mM DTT, 10 ng BSA and 5 U each of enzyme. The *Taq1* digestion preceded the *Apa1* or *Pst1* digestion at 65°C for three hours, after which the *Apa1* or *Pst1* enzyme together with their incubation buffer was added and

digestion continued at 37°C overnight. All reactions were performed with the PTC-200 DNA Gene engine.

Ligation of adapters

*Eco*R1/*Mse1*

A 24 μl of adapter ligation solution plus 1 μl T4 DNA ligase (1 U) was added to each digest giving a total reaction volume of 50μl. The tubes were mixed gently at room temperature, centrifuged briefly to collect the contents, and incubated at 20°C in the PCR machine for 2 hours. After the ligation, 10 μl aliquots of each sample was checked on a 1 % agarose gel to ensure complete digestion then stored at -20°C. The digestion and ligation were repeated for samples that showed evidence of incomplete digestion.

Apa1/Taq1 and *Pst1/Taq1*

Prior to ligation, the adapters were prepared by adding equimolar amounts of their respective reverse and forward single strands in sterile ultra-pure water to the required working concentration. The double stranded adapters were verified on 1% agarose gel before use.

Each 10 μl ligation reaction mix was made up of 5 pmol of either *Apa1* or Pst 1 adapter and 50 pmol of the *Taq1* adapter, 1 ul 10X one-phor-all buffer (Pharmacia), 0.05 mM DTT, 10 ng BSA, 12 mM ATP, 1 U T4 DNA ligase and 2.5 U each of the restriction enzymes were added to each 50 μl restriction reaction. The reactions were then incubated at 37°C for three hours and the enzymes inactivated at 85°C for 30 minutes. The samples were also checked on agarose gel as described for the Eco R1/*Mse1* reactions to ensure complete digestion and stored at -20°C.

Preamplification Reactions

*Eco*R1/*Mse1*

The preamplification reaction was performed with 5 µl of the undiluted restriction ligation, 40 µl of preamplification mix, 5 µl 10X PCR buffer plus $MgCl_2$ (both supplied with the kit) and 1 U *Taq* DNA polymerase (Roche). The use of undiluted template for preamplification or passing the ligation mix through a column is known to improve the quality of selective amplification (Chavarriaga-Aguirre *et al.*, 1999). The 51 µl reactions were covered with 10 µl of mineral oil and the preamplification was performed for 20 cycles at 94°C for 30 seconds, 56°C for 60 seconds, and 72°C for 60 seconds in the PTC-200 Gene engine. At the end of the reaction, the block temperature was maintained at 4°C until the samples were removed from the PCR machine.

After the PCR reaction, 10 µl aliquots were run on 3% agarose gel to ensure amplification. The resistant and susceptible DNA bulks were then prepared by combining 10 µl each of the preamplified DNAs of the members of each bulk. The DNA templates for the selective amplification were then prepared as 1:20 dilutions of the preamplified DNA with TE buffer (supplied with the kit).

Apa1/Taq1 and *Pst1/Taq1*

The *Apa1/Taq1* fragments were preamplified with either, *Apa1*+C/*Taq1*+A primers, *Apa1*+A/*Taq1*+C primers, or *Apa1*+C/*Taq1*+C primers. The *Pst1/Taq1* fragments were amplified with *Pst1*+A/*Taq1*+C primers. Each 50 µl reaction contained 5 µi of the restriction/ligation reaction, 75 pmol each of the *Pst1*+1 or *Apa1*+1 and *Taq1*+1 primers, 2 µl dNTP's (5 mM stock, Promega), 5 µl 10X PCR buffer (Promega), 35 mM $MgCl_2$ (25 mM stock, Promega) and 1 U of *Taq* polymerase. The preamplification PCR reaction profile for the *Apa1/Taq1* was 94°C for 2 minutes, followed by 35 cycles of 94°C for 30 seconds, 50°C for 30 seconds, and 72°C for 60 seconds. At the end of the reaction, the block temperature was maintained at 4°C until the samples were removed from the PCR machine. The *Pst1/Taq1* preamplification was

performed with the profile used for the *Eco*R1/*Mse1* reactions. The PCR reactions were then checked on agarose, diluted, and used to prepare bulk DNA as described for the *Eco*R1/*Mse1* reactions.

Selective amplification reactions

*Eco*R1/*Mse1*

Mse1 and *Eco*R1 primers with a total of eight plus 3 nucleotide extensions each (Table 3.4) were used in all possible combinations (64) to screen the parents and bulked DNA. Each PCR reaction contained 2.5 µl of the 1:20 dilution, 2.0 µl l of 10X PCR buffer (plus 15 mM $MgCl_2$l), 0.6 µl $MgCl_2$ (25 mM stock), 0.5 µl *Eco*R1 primer (10 ng/µl), 4.5 µl of *Mse*1 primer mix (6.7 ng/ul with 0.9 mM dNTP's) and 0.5 U *Taq* polymerase (Roche).

The selective amplification was carried out for one cycle at 94°C for 30 seconds, 65°C for 30 seconds, and 72°C for 60 seconds. This was followed by 12 cycles where the annealing temperature was lowered by 0.7°C for each cycle, giving a touch down phase of 13 cycles, then continued with 23 cycles at 94°C for 30 seconds, 56°C for 30 seconds, and 72°C for 60 seconds. At the end of the reaction, the block temperature was maintained at 4°C until the samples were removed from the PCR machine.

Apa1/Taq1 and Pst1/Taq1

The *Apa1*+C/*Taq1*+A templates of the parents and bulks were screened with all possible combinations of the *Apa1*+3 and *Taq1*+3 primers (Table 3.4). The *Apa1*+A/*Taq1*+C templates were screened with six of the primer combinations involving *Apa1*+ATT and *Taq1*+CAC, *Taq1*+CAG, *Taq1*+CAT, *Taq1*+CTA, *Taq1*+ CTG or *Taq1*+ CTT. The *Apa1*+C/*Taq1*+C templates were screened with 12 of the

primer combinations involving *Apa1* + CTA or CTG and *Taq1* + CAC, CAG, CAT, CTA, CTG or CTT.

The *Pst*1+A/*Taq*1+C templates were screened with 24 of the primer combinations involving *Pst1*+ ACA or *Pst1*+ ACC and *Taq1*+CAC, *Taq1*+CAG, *Taq1*+CAT, *Taq1*+CTA, *Taq1*+CTG or *Taq1*+CTT. Each 20ul PCR reaction mix contained 2.5 µl of the 1:20 diluted preamplification reaction, 2.0 µl 10X PCR buffer, 2.0 µl $MgCl_2$ (25 mM/µl), 1 µl *Apa1* or *Pst1* plus three primers (5 ng/µl stock), 1 µl *Taq1* primers (30 ng/µl stock), 0.8 µl dNTP's (5 mM stock) and 0.5 U *Taq* polymerase (Promega).

The selective amplification for the *Apa1*/*Taq1* templates was carried out for one cycle at 94°C for 60 seconds, 65°C for 30 seconds, and 72°C for 60 seconds. This was followed by 12 cycles where the annealing temperature was lowered by 0.7°C for each cycle, giving a touch down phase of 13 cycles. This was followed by 23 cycles at 94°C for 30 seconds, 56°C for 30 seconds, and 72°C for 60 seconds. At the end of the reaction, the block temperature was maintained at 4°C until the samples were removed from the PCR machine. The profile for the *Pst1*/*Taq1* was the same as that used for the *Eco*R1/*Mse*1 reactions. The PCR products were analysed immediately or stored at -20°C for a few days before analysis.

3.3.6.3 Simple Sequence Repeats (SSR) analysis

A total of 186 SSR previously reported (Chavarriaga-Aguirre *et al.*, 1998; Mba *et al*, 2001) were used to screen the parents and two bulks. Each 25 µl reaction contained 2.5 µl DNA (10 ng/ul), 2.5 µl each of the forward and reverse primers (2.0 uM each), 1 µl dNTPs (5 mM stock), 2.5 µl 10X PCR buffer, 1 or 1.5 µl $MgCl_2$ (25 mM) and 0.75-0.8 units of *Taq* polymerase. The PCR profile involved an initial denaturation for 5 minutes at 94°C. The first cycle then had a denaturation at 94°C for 30 seconds, annealing at 65°C for 1 minute, and extension at 72°C for 1 minute. This was followed by 10 cycles, which were similar to the first cycle, but the annealing

temperature was lowered by 0.7°C for each cycle. Thereafter, the annealing temperature was maintained at 55°C for 1 minute, for 25 cycles. The final extension was 72°C for 7 minutes. At the end of the reaction, the block temperature was maintained at 4°C until the samples were removed from the PCR machine.

3.3.6.4 Sequencing gel electrophoresis

AFLP selective amplification products and SSR amplification products were analysed on 6% polyacrylamide (Appendix IV) sequencing gels, using either an Owl S2S sequencing apparatus with gel size 35 X 45 cm or a Gibco Life Technologies Model S2 with gel size 31.0 X 38.5 cm.

Prior to sequencing gel electrophoresis, a pair of clean glass plates (a long and short plate) was washed with soap, followed by several rinses with tap water, then a 10-30 minute rinse in deionised water with agitation. When the plates were dry, they were cleaned with absolute ethanol. One plate was treated with 400 ul of a siliconizing agent (Appendix IV) for 5 minutes then the excess was wiped off gently with about 2 ml ethanol. The other plate was also swabbed with alcohol then treated with 1 ml of a gel binding solution (Appendix IV). The plates were then assembled on a gel caster with a pair of 0.4 mm spacers placed between them and clamped along their vertical sides. For the 31.0 X 38.5 cm gel, about 70 ml of the 6% polyacrylamide gel (Appendix IV) was poured between the two plates and for the 35 X 45 cm gel about 90 ml was used. When the gel had filled the plates, the flat surface of a 0.4 mm shark-tooth comb was inserted, clamped, and left to polymerise for at least 1 hour.

When the gel had polymerised, the clamps and comb were removed; the gel cassette was washed with deionised water and mounted on the appropriate sequencing gel apparatus and the upper and lower trays of the tank were filled with about 500 ml of 1X TBE. With a 50 ml syringe, the moulded well was washed with the running buffer to remove gel pieces and excess urea. The tank was closed and the gel prewarmed

at 60 W for at least 30 minutes till the temperature of the gel was 50°C. After pre-warming, the wells were flushed again with buffer to get rid of urea, and then the combs were fixed in the wells. Thereafter, the samples were loaded (within 15 minutes).

Prior to loading, 1/3 volume of formamide denaturation dye (Appendix IV) was added to each PCR product, denatured for 3 minutes at 95°C, and immediately placed on ice. About 8 µl of denatured sample was applied to the combs. A 50 bp marker (Promega) with 16 DNA fragments ranging from 50 to 800 bp in exactly 50 bp increments was loaded alongside the samples. For the 31.0 X 38.5 cm gel, 48 samples including the marker were loaded per gel and 72 for the 35 X 45 cm gel. The electrophoresis was continued at 60 W with the gel temperature at 50°C for 1.5-2 hours for the 31.0 X 38.5 cm gel, and 2.5-3 hours for the 35 X 45 cm gel.

3.3.6.5 Silver staining

The AFLP bands were visualised using the commercially available silver staining kit (Promega part No. Q4132). The silver staining procedure involves three stages. A fixing stage removes electrophoresis buffer and urea from the gel and prevents diffusion of small extension products. This is followed by staining the gel in a solution of $AgNO_3$ (0.1 %). It is then developed in a staining solution made of Na_2CO_3 (3 %) in the presence of formaldehyde and sodium thiosulphate which reduces the silver into metallic silver (Promega, 2000). The procedure was carried out according to the manufacturer's instructions. Reagents used for silver staining are listed in Appendix I.

After electrophoresis, the combs and spacers were removed and the two plates separated. The gel was placed (gel side up) in a plastic tray and enough fixer was added to completely cover the gel, and agitated gently for 20 minutes. This was followed with three washes in deionised water for 3 minutes each. The plate was then transferred to another tray containing enough stainer to completely cover the gel

and agitated for 30 minutes on a shaker. The gel was then washed briefly (5 to 10 sec) in deionised water to wash off excess silver, and transferred to a tray containing cold developer. The gel was then developed until the bands were visible, and transferred back into the fixer for 2 minutes to stop the reaction. It was then washed twice in deionised water, placed in an upright position, and allowed to dry.

3.3.6.6 Gel analysis, marker scoring and nomenclature

Agarose gels were documented under UV light with the Grabit gel documentation software and then scored with the Gelworks advanced documentation system. Polyacrylamide gels were also documented directly from the glass plates with the Grabit gel documentation software or by scanning. The sequencing gels were scored directly on the glass plate with the aid of a light box.

For the primary screening of the parents and bulks, the total number of bands and the number of polymorphic bands, i.e., bands clearly present in one parent or bulk and absent in the other, were recorded. In genotyping the mapping population, only the polymorphic bands were scored. Each lane containing DNA from a specific F_1 plant was scored for the presence or absence of polymorphic bands based on its similarity with the parents. When the polymorphic band was present in TMS I30555 (P_1) and absent in TME7 (P_2), the marker was scored as '1' for F_1 plants that possessed the marker band, while those F_1 plants that lacked the marker band were scored as '2'. Conversely, when the polymorphic band was absent in P_1 but present in P_2, the marker was scored as '1' for F_1 plants that lacked the band; and '2' for F_1 individuals that possessed the band. Faint bands that could not be scored with confidence were treated as missing data and were assigned scores of '0'. When the NCDII and diallel parental and check clones were scored, polymorphic markers were scored as "1" for present or "0" for absent.

The marker sizes were estimated by extrapolating their sizes from the migration distance of the 50 bp ladder for the AFLP and SSR gels and from the 1 kb marker for the RAPD gels. Markers were named according to the primer from which the marker was obtained, and its relative molecular weight. For example, AFLP marker E-ACC/M-CTC-250 indicated that the marker was amplified with the primer combination of *Eco*R1+ACC and *Mse1*+CTC and its molecular weight relative to the 50 bp marker was 250 bp. SSR markers were identified based on their assigned lab number and their expected molecular weight. When more than one allele was present, the alleles were identified with alphabets.

3.3.7 DNA markers associated with CMD resistance in F_1 population

3.3.7.1 Genetic linkage mapping

After the initial screening for the BSA, primers that were polymorphic between the bulks and parents were used to genotype the entire mapping population. The *Eco*R1/*Mse1* +3 primers (E-ACC/M-CTC, E-AAC/M-CAT, E-ACA/M-CAC, E-AAG/M-M-CTC, E-ACA/M-CAT, E-AAG/M-CTG, E-ACT/M-CAG, E-ACC/M-CAT, and E-ACC/M-CTC) were used to genotype the parents and F_1 population. Three *Pst1*/*Taq1* primers P-ACC/T-CTA, P-ACT/T-CTT, and P-ACC/T-CAG and 15 SSR (Table 3.5.) primers were also used to genotype the parents and F_1 population, to generate data for linkage analysis. PCR reactions, gel electrophoresis, developing and documented were done as described in sections 3.3.6.4, 3.3.6.5 and 3.3.6.6 above.

For the SSR analysis, PCR products of the mapping population were multiplexed in sets of three for each gel according to their base pair (bp) expected product sizes, with the products with a smallest bp loaded first and that with the largest loaded last.The CMD severity classes of the progenies were treated as a putative phenotypic locus designated CMDRL and included in the linkage analysis with the DNA markers such that the relationship between resistance to CMD and the markers

could be identified on the map. Plants that were classified as resistant (CMD scores of 1 and 2) were treated as identical to the resistant parent and were assigned a score of 2 while those that were classified as susceptible (CMD scores 3 to 5) were assigned a score of 1.

The markers were subjected to chi-square analysis to test their conformity to a 1:1 inheritance ratio to identify single-dose restriction fragments (SDRFs). SDRFs are DNA markers that are present in one parent and absent in the other and segregate in a 1:1 ratio (absence: presence) in the progeny. They represent the segregation equivalent of an allele at a heterozygous locus in a diploid or an allopolyploid genome or a simplex allele in an autopolyploid (Wu *et al.*, 1992). SDRFs are suitable for linkage analysis in an F_1 population when there is the presence of a number of unique segregating polymorphisms (heterozygosity) and normal meiosis in either or both parents. It results in two separate linkage maps based on male and female (Fregene *et al.,* 1997; Williams, 1998). The remaining markers, which did not conform to the 1:1 ratio, were then tested for their conformity to 3:1 ratio, which is expected for the segregation of double dose markers on homoelogous chromosomes of an allo or autopolyploid (Wu *et al.*, 1992). Double dose markers represent the duplex (double simplex condition) at a heterozygous locus.

Different strategies for linkage analysis were tested. These were generating separate female and male maps which is valid for heterozyous species (Fregene *et al.,* 1997; Williams, 1998); single combined map, inclusion of inverted scores of the female and male mapping data before linkage analysis (Fregene *et al.*, 1997), and tested for both separate and combined maps.

The data were analysed with the MAPMAKER version 3.0 computer programme (Lander *et al.*, 1987), using the markers generated in the study. Three CMD responses; mean shoot tip severity score (MST), mean whole plant severity score

(MWP) and mean leaf severity score (MLS) were included in the data set as three quantitative traits. The Kosambi mapping function (Kosambi, 1944) was employed for converting recombination fractions to map distances in centiMorgans (cM). A two-point analysis was first performed to assign loci to groups using minimum lod scores of 3.0 and maximum recombination fraction frequency of 0.33. This function inferred linkage between markers. The multipoint likelihood function was used to compare loci order in a linkage group. Initially a subset of markers was compared to determine the best order. Then the remaining markers in the group were tried in every interval using the 'multipoint/try' function. Locus order was then produced using the MAP function of MAPMAKER. Unlinked markers were further assessed by 'two-point/pairwise' function with reference to the already ordered linkage groups. The linkage groups were then assigned onto chromosomes and a framework map generated for further analysis.

Table 3.5 Description of cassava SSR loci and their primer pairs used in genetic linkage analysis

Lab No	SSR loci	Type of repeat	Forward primer	Reverse primer	Size (bp)	$MgCl_2$
SSR6	SSRY6	(CA) 7 (N) 51 (CA) 17 (N) 47 (CA) 15	TTTGTTGCGTTTAGAAAGGTGA	AACAAATCATTACGATCCATTTGA	298	1.5
SSR7	SSRY7	(CT) 26	TGCCTAAGGAAAATTCATTCAT	TGCTAAGCTGGTCATGCACT	250	1.5
SSR30	SSRY28	(CT) 26 (AT) 3 AC(AT) 2	TTGACATGAGTGATATTTTCTTGAG	GCTGCGTGCAAAACTAAAAT	180	1.5
SSR41	SSRY40	(GA) 16	TGCATCATGGTCCACTCACT	CATTCTTTTCGGCATTCCAT	231	1.5
SSR50	SSRY49	(GA) 25	TGAAAATCTCACTGGCATTATTT	TGCAACCATAGTGCCAAGC	300	1.5
SSR52	SSRY51	(CT) 11 CG(CT) 11 (CA) 18	AGGTTGGATGCTTGAAGGAA	GGATGCAGGAGTGCTCAACT	298	1.5
SSR103	SSRY91	(GA) 16	GTCTGCATGGCTCGATGAT	TGCCTGCTTCATATGTTTTTG	300	1
SSR108	SSRY95	(CT) 19 (CA) 16 CC (CA) 2 CC(CA) 3	CATGATTTGGATTTTGGAATGA	CAAAAGAAGCAACCTTCAGCA	282	1
SSR115	SSRY102	(GT) 11	TTGGCTGCTTTCACTAATGC	TTGAACACGTTGAACAACCA	179	1.5
SSR119	SSRY106	(CT) 24	GGAAACTGCTTGCACAAAGA	CAGCAAGACCATCACCAGTTT	270	1.5
SSR124	SSRY110	(GT) 12	TTGAGTGGTGAATGCGAAAG	AGTGCCACCTTGAAAGAGCA	247	1.5
SSR132	SSRY113	(GA) 19	TTTGCTGACCTGCCACAATA	TCAACAATTGGACTAAGCAGC	187	1.5
SSR157	SSRY135	(CT) 16	CCAGAAACTGAAATGCATCG	AACATGTGCGACAGTGATTG	253	1.5
SSR70	SSRY170	(TA) 5 (N) 71 (CT) 24	TCTCGATTTGGTTTGGTTCA	TCATCCTTGTTGCAGCGTTA	299	1.5
SSR105	SSRY179	(GA) 28	CAGGCTCAGGTGAAGTAAAGG	GCGAAAGTAAGTCTACAACTTTTC TAA	226	1.5

(Adapted from Mba *et al.*, 2001)

3.3.7.3 Quantitative trait loci (QTL) analysis

Simple phenotypic correlation and regression analysis were used to determine the association between the markers and the CMD responses. The CMD responses, (mean shoot tip, mean whole plant, and mean leaf severity scores at 12 WAP) were the dependent variables while the marker data were the independent variables for the regression analysis. Associations between markers and the CMD response traits were declared significant on a per marker basis at a significance threshold of p = 0.005 (Lander and Botstein, 1986). This stringent threshold was adopted to avoid type 1 error (Dudley, 1993).

3.3.8 DNA markers associated with CMD resistance in various sources of resistance

Following the segregation and QTL analysis, markers associated with resistance to CMD were tested on the 23 cassava genotypes used as parents and checks in the NCD II and Diallel experiments described in section 3.1 above.

Chapter Four

Results

4.1 Analysis of variance and combining ability analyses

4.1.1. 3X18 North Carolina Design II mating scheme

The analysis of variance for CMD symptom severity in the NCD II mating scheme is presented in Table 4.1. The results showed significant variation ($p<0.01$) among environments, replicates nested in environments and genotypes in each of the individual environments and across environments. Significant variation among the genotypes was due to the variation among checks and among test genotypes. The orthogonal contrasts between the checks and test genotypes were significant ($p<0.01$) in the individual environments but not across environments.

The significant variation among the test genotypes was due to significant variation ($p<0.01$) among the parents and crosses in Ibadan 1998 and 1999 and across environments. In the Mokwa 1998 environment, significant variation among the test genotypes was due to the parents only.

Variation among the 21 parents was due to the male parents, which varied significantly ($p<0.01$) in each environment and across environments, as well as to the female parents, which was significant only in the individual environments. The orthogonal contrast female versus male parents was significant ($p<0.05$) in Mokwa 1998 and across environments.

Table 4.1 Analysis of variance for CMD severity at 12 weeks after planting (WAP) among all genotypes in 3X18 NCD II mating scheme, evaluated in three environments

Source of Variation	Across Environment		Individual Environments	Mokwa 1998	Ibadan 1998	Ibadan 1999
	df	MS	df (‡)	MS	MS	MS
Environment (E)	2	59.17**				
Replicates within E	3	1.40**	1	1.18**	2.98**	0.05**
Genotypes in population (G)	78	1.39**	78(77)	0.36**	0.49**	1.06**
Checks (Ck)	3	7.72**	3(2)	2.46**	2.71**	3.48**
Test Genotypes (TG)	74	1.12**	74	0.30**	0.38**	0.96**
Ck versus TG	1	1.32	1	2.57**	1.97**	1.56**
Parent (P)	20	3.17**	20	0.91**	0.89**	1.90**
Female (F)	2	1.64	2	0.55**	0.38*	1.24**
Male (M)	17	3.51**	17	0.99**	0.99**	2.08**
F versus M	1	0.46*	1	0.23*	0.07	0.19
Susceptible (S)	5	1.08	5	0.36**	0.68**	0.51**
Resistant (R)	14	0.29	14	0.11**	0.16*	0.63**
R versus S	1	53.98**	1	15.15**	12.55**	26.47**
Crosses (C)	53	0.31**	53	0.04	0.19**	0.37**
F (GCA)	2	1.40	2	0.18**	2.28**	0.42**
M (GCA)	17	0.57*	17	0.06*	0.14	0.91**
F x M (SCA)	34	0.11*	34	0.02	0.09	0.13**
P vs C	1	3.10	1	1.77**	0.54*	13.22**
GxE	155	0.28**				
Ck x E	5	0.07				
TG x E	148	0.26**				
Ck vs TG x E	2	2.71**				
P x E	40	0.26**				
F x E	4	0.26**				
M x E	34	0.28**				
F vs M x E	2	0.14				
S x E	10	0.36**				
R x E	28	0.36**				
R vs S x E	2	1.01**				
C x E	106	0.15**				
F (GCA) x E	4	0.54*				
M (GCA) x E	34	0.27*				
F x M (SCA) x E	68	0.07				
P vs CxE	2	6.20**				
Error (genotypes)	233	0.07	78(77)	0.04	0.09	0.07
Error (crosses)	159	0.06	53	0.03	0.08	0.08
$\frac{GCA}{GCA + SCA}$		0.76		1.00	0.94	0.84

(‡) df of genotypes and checks in Mokwa 1998 was 77 where check, 91/02324 was missing
* Significantly different from zero at the 0.05 probability level
** Significantly different from zero at the 0.01 probability level

Further partitioning of the parents into resistant and susceptible parents revealed significant differences among parents for each group in the individual environments but not across environments. The orthogonal contrast between resistant and susceptible parents, however, was significant ($p<0.01$) in all three environments and across environments

The reaction of the F_1 crosses to CMD symptom severity was significant ($p<0.01$) in Ibadan 1998 and 1999, and across environments. This was attributed to the GCA effects of the females in the Ibadan 1998 and 1999 environments and the GCA effects of the males in Ibadan 1999 and across environments. The SCA effect (female by male interaction) was also significant across environments and in Ibadan 1999. Although there was no significant variation among the crosses in Mokwa 1998, the GCA effect of the females and males was significant.

The orthogonal contrast of the parent versus crosses, which is a test of average heterosis, was significant in the individual environments but not across environments.

The significant GXE interaction ($p<0.01$) was due to all the component interactions except for the check by environment, the parent contrast, female versus male by environment, and the SCA effect (female by male) by environment interactions.

The relative importance of GCA and SCA estimated by the genetic ratios, $\frac{GCA}{GCA+SCA}$ were 0.76, 1.0, 0.84 and 0.96 across environments, in Mokwa 1998, Ibadan 1998 and Ibadan 1999 environments respectively. Heritability based on progeny means across environments was 0.516 with lower and upper 90% confidence interval of 0.268 and 0.668.

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4.1.2. 4X4 Diallel mating scheme

The analysis of variance for the 4X4 diallel involving the resistant genetic stock clone 58308 and three improved cassava clones (I30001, I30555 and I30572) also used as females in the 3X18 NCD II mating is presented in Table 3.2.

There was significant variation ($p<0.01$) among environments but replicate within environment was significant only in Mokwa 1998. The variation due to test genotypes was also significant ($p<0.01$) in the individual environments and across environments. This was attributed to the significant variation due to checks and test genotypes. The contrast check versus test genotypes was significant in the Mokwa 1998 environment only.

The results further showed that significant variation ($p<0.01$) among the test genotypes in all the three environments and across environments was due to variation among parents and crosses. The test for average heterosis, parent versus cross contrast, was significant in all of the three environments but not across environments.

The Griffing's analysis of variance (based on method 1 for a complete diallel, and model 1 for fixed genotypes) for the crosses within and across environments revealed that GCA effects of the parents were significant in the individual environments but not across environments. SCA effects also contributed significantly to the variation among crosses in the Ibadan 1999 and Mokwa 1998 environments. Reciprocal effects were significant only in the Ibadan 1998 environment and this was due to significant maternal effect.

Table 4.2 Analysis of variance for CMD severity at 12 weeks after planting (WAP) among all genotypes in 4X4 diallel mating scheme, evaluated in three environments

Source of Variation			Across Environment		Individual Environments			
						Mokwa 1998	Ibadan 1998	Ibadan 1999
			df	MS	df	MS	MS	MS
Environment (E)			2	9.00**				
Replicates within E			3	0.14	1	0.25**	0.08	0.08
Genotypes in population (G)			23	1.47**	23(21) ‡	0.46**	0.67**	0.78**
	Checks(Chk)		3	6.74**	3‡	2.72**	3.26**	1.95**
	Test Genotypes (TG)		19	0.65**	19(18) ‡	0.14**	0.27**	0.62**
	Parent (P)		3	1.08*	3 (2)‡	0.11**	0.51**	0.81*
	Cross (C)		15	0.37*	15	0.15**	0.17**	0.37*
		GCA	3	0.79	3	0.47**	0.39**	0.51*
		SCA	6	0.40	6	0.1**	0.04	0.56*
		Reciprocal	6	0.13	6	0.05	0.19*	0.12
		Maternal	3	0.11	3	0.06*	0.36**	0.06
		Non-maternal	3	0.15	3	0.03	0.02	0.17
	P vs C		1	2.36	1	0.09*	1.33**	3.73**
	Chk vs TG		1	0.36	1	1.69**	0.14	0.56
G x E			43	0.21*				
	Chk x E		5	0.17				
	TG x E		36	0.19**				
	P x E		4	0.11				
	C x E		30	0.16**				
		GCA x E	3	0.47**				
		SCA x E	6	0.23				
		R x E	6	0.21				
		M x E	3	0.35*				
		N x E	3	0.06				
	P vs C x E		1	1.16**				
	Chk vs TG x E		1	1.09**				
Error (genotypes)			66	0.11	23(21) ‡	0.02	0.08	0.23
Error (cross)			45	0.08	15	0.02	0.05	0.16
$\frac{2GCA}{2GCA+SCA}$				0.19		0.39	0.76	0.10

( ) df of genotypes, test genotypes and parents in Ibadan 1998 are 21, 18 and 3 respectively where 58308 was missing

‡ df of genotypes, test genotypes checks and parents in Mokwa 1998 are 20, 17 and 2 respectively where 58308 and 91/02324 were missing

* Significantly different from zero at the 0.05 probability level

** Significantly different from zero at the 0.01 probability level

Despite the lack of significance of the reciprocal effect in Mokwa 1998, significant maternal effects were detected. The partitioning of the reciprocal effects into its components revealed significant maternal effect in the Mokwa 1998 and Ibadan 1998 environments.

The significant genotype by environment interaction ($p<0.05$) was due to the interactions between environment and test genotypes, crosses, GCA effect and maternal effects, parent versus cross and the check versus test genotypes.

The relative importance of GCA and SCA estimated by the genetic ratios, $\frac{2GCA}{2GCA+SCA}$ were 0.19, 0.39, 0.76 and 0.10 for the combined environment analysis, the Mokwa 1998, the Ibadan 1998 and the Ibadan 1999 environments respectively. Heritability based on progeny means was 0.568 with lower and upper 90% confidence interval of 0.028 and 0.786.

4.1.3 Combining ability effects of 3X18 NCD II mating scheme

The GCA effects and means of the parental clones in the three environments and across environments are presented in Table 4.3. Mean disease severity scores of the clones varied across and within each environment.

In this study, negative GCA effects were desirable for resistance. Significant and negative values of the clones indicated contribution towards resistance while significant and positive values indicated a contribution towards susceptibility. Similarly, negative SCA values of a cross indicated that the cross was more resistant than average and significant and positive SCA effect indicated a cross was more susceptible than average.

Table 4.3 Mean‡ CMD severity scores and GCA effects for CMD symptom severity of parents in the 3X18 NCD II mating scheme, evaluated in three environments

Parent	Across environments Mean+	Across environments GCA	Mokwa 1998 Mean	Mokwa 1998 GCA	Ibadan 1998 Mean	Ibadan 1998 GCA	Ibadan 1999 Mean	Ibadan 1999 GCA
Female								
I30001	1.20	0.13	1.00	0.08	1.15	0.29*	1.45	0.02
I30555	2.23	-0.04	1.95	-0.02	1.85	-0.11	2.88	0.02
I30572	1.55	-0.09	1.10	-0.06	1.94	-0.17*	1.60	-0.04
Mean	1.66		1.35		1.65		1.98	
SE	0.12	0.06	0.25	0.02	0.22	0.04	0.18	0.04
LSD		0.258		0.165		0.165		0.083
Male								
I4(2)1425	1.96	-0.06	1.20	-0.04	1.87	0.16	2.80	-0.31*
I60142	1.63	-0.20	1.00	-0.19*	1.30	-0.06	2.60	-0.34*
I90257	1.48	-0.26*	1.30	-0.14	1.54	-0.01	1.60	-0.63*
TME1	1.48	-0.09	1.10	-0.04	1.58	-0.07	1.75	-0.17
TME2	2.68	0.55*	2.85	0.23*	2.13	0.44*	3.06	0.98*
TME4	1.18	-0.02	1.00	0.02	1.21	-0.08	1.34	-0.01
TME5	1.20	0.06	1.10	0.04	1.20	0.14	1.30	0.01
TME6	1.37	-0.07	1.25	-0.04	1.48	-0.19	1.38	0.01
TME7	1.12	-0.03	1.10	0.05	1.11	-0.05	1.15	-0.07
TME8	1.36	-0.12	1.30	0.00	1.08	-0.06	1.70	-0.29*
TME9	1.15	-0.05	1.05	0.14	1.25	-0.03	1.15	-0.27*
TME10	2.76	0.05	1.93	-0.01	2.78	0.09	3.55	0.07
TME11	1.44	-0.09	1.29	0.12	1.73	-0.22	1.30	-0.18
TME12	1.39	-0.09	1.30	0.03	1.43	-0.12	1.45	-0.20
TME14	1.37	0.06	1.10	0.03	1.30	0.09	1.70	0.06
TME31	2.77	0.20	3.05	-0.03	2.17	-0.04	3.10	0.67*
TME41	3.15	0.01	2.55	-0.10	3.05	-0.11	3.85	0.24*
TME117	3.46	0.17	2.65	-0.05	3.49	0.09	4.25	0.48*
Mean	1.83		1.56		1.76		2.17	
SE	0.12	0.06	0.25	0.02	0.22	0.04	0.18	0.04
LSD		0.238		0.235		0.235		0.144

* Significantly different from zero at the 0.05 probability level

** Significantly different from zero at the 0.01 probability level

‡mean disease score ≤ 2 = resistant; mean disease scores > 2=susceptible.

Negative GCA effects were estimated for the resistant clone TMS I30572 in all environments but its effect was significant only in the Ibadan 1998 environment. Although the resistant clone TMS I30001 had positive GCA in all environments and across environments, its effect was significant only in Ibadan 1998. All the improved clones used as males TMS I4(2)1425, TMS I60142 and TMS I90257 had negative and significant GCA effects in Ibadan 1999. Negative and significant GCA effects were also estimated for clones TMS I60142 in the Mokwa 1998 environment and for TMS I90257 across environments. Negative and significant GCA effects were also detected for the resistant landraces TME8 and TME9 in the Ibadan 1999 environment.

The susceptible landrace TME2 had significant and positive GCA in all three environments and across environments, while the susceptible landraces TME31, TME41 and TME117 had significant and positive GCA effects only in the Ibadan 1999 environment. The susceptible improved clone TMS I30555 used as female, did not contribute significantly to susceptibility in any environment.

The correlation analysis between parental means and GCA revealed significant and positive linear relationships in the Ibadan 1999 environment ($r=0.551$, $p<0.05$) and across environments ($r=0.507$, $p<0.01$).

The mean disease severity scores and specific combining ability effects of the crosses for environments with significant variation for the effect are presented in Table 4.4. Negative SCA effects were also desirable for resistance. The most resistant cross over environments was TMS I30572 X TMS I90257 which had the lowest mean of 1.48 and largest negative SCA effect of -0.43 while the TMS I30555 X TME2 with a mean of 2.57 and largest positive SCA of 0.63 was the most susceptible cross across environments.

Table 4.4 Mean CMD severity scores and SCA effects for CMD symptom severity of crosses in 3X18 design II crosses in environment with significant variation for SCA

Cross	Across Environments Mean	Across Environments SCA	Ibadan 1999 Mean	Ibadan 1999 SCA
I30001 X I4(2)1425	2.09	-0.03	2.34	-0.17
I30001 X I60142	1.84	-0.28*	2.12	-0.36*
I30001 X I90257	1.96	-0.16	2.48	0.30
I30001 X TME1	1.95	-0.17	2.25	-0.40*
I30001 X TME10	2.2	0.08	2.99	0.10
I30001 X TME11	1.92	-0.20	2.45	-0.19
I30001 X TME117	2.12	0.00	2.98	-0.31
I30001 X TME12	1.91	-0.21	2.64	0.02
I30001 X TME14	2.28	0.16	3.23	0.35*
I30001 X TME2	2.7	0.58*	3.81	0.02
I30001 X TME31	2.36	0.24*	3.72	0.23
I30001 X TME4	2.2	0.08	2.89	0.08
I30001 X TME41	2.21	0.09	3.25	0.19
I30001 X TME5	2.09	-0.03	2.66	-0.16
I30001 X TME6	2.09	-0.03	2.8	-0.03
I30001 X TME7	2.2	0.08	2.92	0.19
I30001 X TME8	2	-0.12	2.47	-0.06
I30001 X TME9	2.04	-0.08	2.75	0.20
I30555 X I4(2)1425	2	0.05	2.78	0.27
I30555 X I60142	1.74	-0.21	2.65	0.17
I30555 X I90257	1.74	-0.21	2.04	-0.15
I30555 X TME1	1.8	-0.09	2.56	-0.09
I30555 X TME10	2.14	0.13	2.98	0.09
I30555 X TME11	2.04	0.09	3.03	0.39*
I30555 X TME117	2.17	0.21	3.4	0.10
I30555 X TME12	1.69	-0.24*	2.49	-0.13
I30555 X TME14	1.97	0.02	2.78	-0.10
I30555 X TME2	2.57	0.62*	3.89	0.09
I30555 X TME31	2.06	0.11	3.36	-0.14
I30555 X TME4	1.78	-0.17	2.51	-0.30
I30555 X TME41	1.83	-0.13	2.85	-0.22
I30555 X TME5	2.23	0.28*	3.14	0.30
I30555 X TME6	1.93	-0.02	2.96	0.13
I30555 X TME7	1.84	-0.13	2.58	-0.20
I30555 X TME8	1.75	-0.20	2.44	-0.09
I30555 X TME9	1.83	-0.13	2.4	-0.15
SE	0.10	0.09	0.20	0.16
LSD		0.216		0.317

* Significantly different from zero at the 0.05 probability level

** Significantly different from zero at the 0.01 probability level

Negative and significantly different from zero at the 0.05 probability level

Table 4.4 Cont

Cross	Across Environments Mean	SCA	Ibadan 1999 Mean	SCA
I30572 X I4(2)1425	1.69	-0.22	2.36	-0.09
I30572 X I60142	1.79	-0.11	2.62	0.19
I30572 X I90257	1.48	-0.43*	1.98	-0.15
I30572 X TME1	1.95	0.05	3.08	0.49*
I30572 X TME10	1.77	-0.13	2.63	-0.20
I30572 X TME11	1.74	-0.17	2.37	-0.21
I30572 X TME117	2.19	0.29*	3.45	0.21
I30572 X TME12	2.08	0.18	2.66	0.10
I30572 X TME14	1.91	0.04	2.58	-0.24
I30572 X TME2	2.34	0.44*	3.63	-0.11
I30572 X TME31	2.15	0.24*	3.34	-0.10
I30572 X TME4	1.93	0.02	2.97	0.22
I30572 X TME41	1.97	0.06	3.03	0.03
I30572 X TME5	1.84	-0.06	2.63	-0.14
I30572 X TME6	1.73	-0.17	2.66	-0.11
I30572 X TME7	1.86	-0.04	2.68	0.01
I30572 X TME8	1.86	-0.04	2.61	0.14
I30572 X TME9	1.96	0.05	2.44	-0.05
SE	0.10	0.09	0.20	0.16
LSD		0.216		0.317

* Significantly different from zero at the 0.05 probability level
** Significantly different from zero at the 0.01 probability level
Negative and significantly different from zero at the 0.05 probability level

Significant and negative SCA was detected for TMS I30001 X TMS I60142 in Ibadan 1999 and across environments, for TMS I30001 X TME1 in Ibadan 1999 and also for TMS I30555 X TME12 across environments.

Significant and positive SCA effects were estimated for all the three crosses involving TME2, TMS I30001 X TME31, TMS I30555 X TME5, TMS I30572 X TME117 and TMS I30572 X TME31 across environments, and TMS I30001 X TME14, TMS I30555 X TME11 and TMS I30572 X TME1 in Ibadan 1999.

4.1.4 Combining ability and maternal effects in the 4X4 Diallel mating scheme

The parental means, GCA and maternal effects of the four parents used in environments with significant variation for these effects are given in (Table 4.5). Significant and negative GCA effects were detected for the clones TMS I30572 and clone TMS I30001 in Mokwa 1998 and also for TMS I30572 in Ibadan 1998. Significant and positive GCA effects were also detected for the clones TMS I30555 in Mokwa 1998 and 58308 in Ibadan 1998. The maternal effect of 58308 was also significant and positive in the Mokwa 1998 and Ibadan 1998 environments.

In Mokwa 1998 significant and negative SCA effects were detected for the crosses TMS I30001 X TMS I30555, TMS I30555 X TMS I30572 and in Ibadan 1999 environment, for the crosses TMS I30001 X 58308 and TMS I30555 X TMS I30572 (Table 4.6). The self crosses TMS I30001 X TMS I30001, TMS I30555 x TMS I30555, and 58308 X 58308 had significant and positive SCA effects in Mokwa 1998 environment, and in Ibadan 1999, TMS I30001 X TMS I30001, 58308 X 58308 and I30572 X 58308 were also significant and positive.

Table 4.5 Mean, GCA and maternal (MAT) effects for CMD symptom severity of parents in 4X4 diallel crosses in environments with significant variation for their effects

	Mokwa 1998			Ibadan 1998			Ibadan 1999‡		
Clone	Mean	GCA	MAT	Mean	GCA	MAT	Mean	GCA	MAT
I30001	1.00	-0.18*	-0.02	1.01	-0.07	-0.13	1.34	0.00	
I30555	1.95	0.21*	-0.03	2.01	0.10	-0.12	2.35	0.23	
I30572	1.10	-0.10*	-0.03	1.63	-0.18*	0.09	1.95	-0.03	
58308	x	0.07	0.09*	x	0.16*	0.17*	1.13	-0.20	
Mean	1.35			1.55			1.69		
SE	0.25			0.20			0.34		
LSD		0.086	0.086		0.136	0.136		0.245	
LSD $_{gi-gj}$		0.156			0.237			0.411	
LSD $_{mi-mj}$			0.156			0.237			

x Not estimated due to missing plot
‡ Maternal effect not significant in Ibadan 1999 environment
* Significantly different from zero at the 0.05 probability level
** Significantly different from zero at the 0.01 probability level

Table 4.6 Mean CMD severity scores and SCA effects for CMD symptom severity of crosses in 4X4 diallel in environments with significant variation for their effects

Cross	Mokwa 1998		Ibadan 1999	
	Mean	SCA	Mean	SCA
I30001 X I30001	1.23	0.18*	3.14	0.64*
I30001 X I30555	1.24	-0.12*	2.40	-0.08
I30001 X I30572	1.15	0.03	2.51	-0.02
I30001 X 58308	1.18	-0.09	2.08	-0.54*
I30555 X I30555	2.16	0.34*	3.07	0.13
I30555 X I30572	1.35	-0.18*	2.33	-0.34*
I30555 X 58308	1.42	-0.04	2.80	0.29
I30572 X I30572	1.24	0.04	2.43	0.01
I30572 X 58308	1.36	0.11	2.66	0.35*
58308 X 58308	1.55	0.29*	2.00	0.35*
Mean	1.40		2.50	
SE	0.11		0.38	
LSD $_{sii}$		0.156		0.410
LSD $_{sij}$		0.116		0.308
LSD $_{sii-sjj}$		0.208		0.548
LSD $_{sii-sij}$		0.224		0.612
LSD $_{sii-sjk}$		0.180		0.474
LSD $_{sij-sik}$		0.180		0.474
LSD $_{sij-skl}$		0.146		0.388

* Significantly different from zero at the 0.05 probability level
** Significantly different from zero at the 0.01 probability level
Negative and significantly different from zero at the 0.05 probability level

The significant reciprocal effect in Ibadan 1998 was due to the negative and significant effect of three reciprocal crosses 58308 X I30001, I30572 X I30555 and 58308 X I30555.

4.1.5 Heterosis for resistance to CMD in F_1 crosses

In the NCD II experiment, average values for mid-parent and high-parent heterosis were generally positive and indicated that the F_1 crosses were generally more susceptible than their mid or high parents except in the Mokwa 1998 environment where the F_1 differed from the mid-parent value by -4.95%.

Significant and negative heterosis was estimated in only two crosses involving clone TMS I30555 as the female parent, and TME117 and TME41 as the male parents. The most heterotic cross, which contributed significantly to the average midparent heterosis, was TMS I30555 X TME41. It had negative and significant mid parent heterosis of -31.94% across environments and in Mokwa 1998, both mid-parent and high-parent heterosis of -48.44% and -40.51% were negative and significant. The next most heterotic cross was TMS I30555 X TME117, which also had negative heterosis in all environments. Its mid-parent heterosis of -50.00% and high-parent heterosis of -41.03% were significant in Mokwa 1998.

Generally, the crosses in the diallel experiment had positive heterotic effects for both mid-parent and high-parent heterosis and were generally more susceptible than their mid-parent or high-parents. Negative heterosis was detected only in the self cross TMS I30572 X TMS I30572 in Ibadan 1998 and for most crosses in the Mokwa environment, however, none of these negative heterotic effects were significant.

4.2 Genetic relationships among the various sources of resistance to CMD

4.2.1 Relationships among sources of resistance based on area under the disease progress curve (AUDPC) for CMD

Estimated AUDPC values of the cassava clones used as parents and checks and their respective rankings in each and across environments for CMD resistance responses are shown in Table 4.7.

Clone 91/02324 and TME9 had the smallest AUDPC values for the shoot-tip and whole plant severity and were ranked 1 (i.e. best resistant clones) across environments. In Ibadan 1998 and 1999, TME9 consistently had a rank of 1 for shoot tip severity but ranked 4 for whole plant in Mokwa 1998. The clone 91/02324 also ranked 3 for whole plant severity in the Ibadan 1999 environment.

The next best clone was the resistant improved line TMS I30001, which ranked 3 across environments for both shoot tip and whole plant, and also ranked 1 for shoot tip severity in the individual environments and 4 for whole plant severity in Ibadan 1998. In the Ibadan 1998 environment, I60142 also ranked 1 for shoot tip and whole plant severity and in Mokwa 1998, for shoot tip severity.

In the Ibadan 1998 environment, the resistant landraces TME5, TME7, TME8 TME11 TME12 and TME14 ranked 1 for shoot tip severity, while in Ibadan 1999, resistant landraces, TME4 and TME5 ranked 1 for shoot tip severity in Ibadan.

Based on their AUDPC values and ranks across environments and in Ibadan 1998 and 1999, the results further showed that the resistant landraces were generally more resistant than improved clones TMS I30572 and TMS I4(2)1425. However, in Mokwa 1998, TMS I30572 had lower ranks for both shoot tip and whole plant severity, indicating that it was more resistant than resistant landraces, TME5, TME7, TME8, TME9, TME12 and TME14.

Table 4.7 Area under the disease progress curve (AUDPC) of cassava clones based on mean CMD severity scores and their ranks for CMD resistance.

Clone	Across environments		Mokwa1998		Ibadan 1998		Ibadan 1999	
	Shoot Tip Severity							
	AUDPC	Rank	AUDPC	Rank	AUDPC	Rank	AUDPC	Rank
58308	x	x	x	x	x	x	12.00	1
91/02324	20.00	1	x	x	20.00	1	12.00	1
TMS I30001	22.60	3	12.00	1	20.00	1	12.00	1
TMS I30555	33.40	17	27.73	17	20.00	1	32.80	19
TMS I30572	29.31	15	13.28	7	28.23	16	18.50	15
TMS I4(2)1425	31.79	16	18.97	15	30.46	17	26.40	17
TMS I60142	27.91	13	12.00	1	20.00	1	22.30	16
TMS I90257	24.75	8	16.43	13	23.88	13	16.00	12
TME1	26.49	11	17.17	14	23.52	12	16.30	13
TME2	39.88	18	40.47	19	35.64	18	30.80	18
TME4	25.75	10	12.00	1	22.52	11	12.00	1
TME5	24.66	7	15.59	10	20.00	1	12.00	1
TME6	26.87	12	12.00	1	26.68	14	14.30	9
TME7	23.3	4	14.55	8	20.00	1	16.80	14
TME8	23.36	5	15.45	9	20.00	1	14.10	8
TME9	20.00	1	12.00	1	20.00	1	12.00	1
TME10	45.88	19	26.17	16	42.32	20	41.70	20
TME11	28.03	14	12.00	1	27.14	15	15.20	11
TME12	25.32	9	16.30	12	20.00	1	14.30	9
TME14	23.65	6	16.03	11	20.00	1	12.00	1
TME31	51.20	20	50.61	20	40.78	19	47.50	21
TME41	53.27	21	38.10	18	44.56	21	56.90	22
TME117	68.05	22	51.29	21	65.09	22	63.32	23
	Whole Plant Severity							
	AUDPC	Rank	AUDPC	Rank	AUDPC	Rank	AUDPC	Rank
58308	x	x	x	x	x	x	12.00	1
91/02324	20.00	1	x	x	20.00	1	14.71	3
TMS I30001	24.51	3	12.00	1	23.60	4	18.09	4
TMS I30555	38.52	17	33.79	16	35.12	16	49.63	19
TMS I30572	33.09	14	15.11	5	35.65	17	24.42	11
TMS I4(2)1425	35.59	16	20.75	15	33.82	15	43.73	17
TMS I60142	33.11	15	12.00	1	27.78	10	38.62	16
TMS I90257	27.37	6	19.12	10	27.53	9	23.92	9
TME1	31.56	13	19.10	9	29.66	13	26.47	13
TME2	45.19	18	47.98	19	42.40	18	45.29	18
TME4	28.95	9	12.00	1	26.19	8	23.34	8
TME5	27.78	8	16.77	8	24.43	7	21.93	7
TME6	30.39	10	19.85	11	28.73	11	24.30	10
TME7	27.05	5	16.61	7	23.60	4	20.52	5
TME8	27.64	7	19.93	12	20.00	1	28.32	15
TME9	20.00	1	13.81	4	20.00	1	12.00	1
TME10	51.66	19	38.99	17	50.81	20	55.42	20
TME11	31.41	12	20.38	14	32.27	14	21.27	6
TME12	31.19	11	20.18	13	29.44	12	25.98	12
TME14	25.91	4	16.33	6	24.30	6	27.07	14
TME31	54.72	20	54.48	21	45.78	19	57.5	21
TME41	57.28	21	44.93	18	55.47	21	70.48	22
TME117	70.38	22	52.73	20	70.58	22	72.52	23

x Not estimated due to missing plot

Table 4.8 Spearman's rank correlation coefficient for AUDPC values for shoot-tip and whole plant CMD symptom severity

	Across environments	Mokwa 1998	Ibadan 1998	Ibadan 1999
		Shoot-tip		
Across Env		0.678**	0.835**	0.886**
Mokwa 1998	0.678**		0.573**	0.744**
Ibadan 1998	0.835**	0.573**		0.727**
Ibadan 1999	0.886**	0.744**	0.727**	
		Whole Plant		
Across Env		0.754**	0.951**	0.880**
Mokwa 1998	0.754**		0.757**	0.741**
Ibadan 1998	0.951**	0.757**		0.792**
Ibadan 1999	0.880**	0.741**	0.792**	

As was expected, the susceptible improved clone (TMS I30555) and the susceptible landraces (TME2, TME10, TME31, TME41 and TME117) had the largest AUDPC values and had ranks higher than the resistant clones. With the exception of whole plant severity in Mokwa 1998, TME117 was the least resistant and most susceptible clone. TMS I30555 was the least susceptible followed by TME2 or TME10 depending on the environment.

The rank correlation coefficients among the AUDPC values showed significant and positive linear associations among the AUDPC values in the different environments for both shoot-tip and whole plant CMD symptom severity (Table 4.8). The correlation coefficient (r) ranged between 0.57 to 0.89 for shoot tip CMD severity and between 0.74 to 0.95 for whole plant CMD severity.

4.2.2. Relationships among sources of resistance based on principal component analysis (PCA) of CMD responses

Eigenvalues (proportion of the total variance) and eigenvectors for the first three principal components (PC1, PC2 and PC3) for the CMD resistance responses among the 23 parental and check clones are presented in Table 4.9. The eigenvalues for the first three principal components accounted for 78.87% of the total variation.

Table 4.9 Eigenvectors and eigenvalues as a proportion of total variance of the first three principal components for the CMD resistance responses of the 23 parental and check clones

Environment	CMD Response	PC1	PC2	PC3
Mokwa 1998	MST6WK	0.218	-0.058	0.238
	MCMD6WK	0.238	0.003	0.186
	MST12WK	0.232	-0.075	0.132
	MCMD12WK	0.228	-0.058	0.176
	ST6WKI	0.082	0.389	0.205
	CMD6WKI	0.163	0.047	0.304
	ST12WKI	0.231	-0.047	0.142
	CMD12WKI	0.201	-0.118	-0.032
Ibadan 1998	MST6WK	0.229	-0.006	-0.198
	MCMD6WK	0.227	-0.002	-0.230
	MST12WK	0.228	-0.008	-0.196
	MCMD12WK	0.225	0.001	-0.236
	MST20WK	0.218	-0.089	-0.013
	MCMD20WK	0.238	-0.052	-0.015
	ST6WKI	0.048	0.387	-0.230
	CMD6WKI	0.099	0.339	-0.185
	ST12WKI	0.041	0.411	-0.239
	CMD12WKI	0.106	0.323	-0.169
	MST20WKI	0.101	0.320	0.126
	MCMD20WKI	-0.007	0.317	0.362
Ibadan 1999	MST6WK	0.239	-0.067	-0.076
	MCMD6WK	0.237	-0.030	0.014
	MST12WK	0.237	-0.082	0.087
	MCMD12WK	0.231	-0.043	0.099
	ST6WKI	0.195	-0.053	-0.146
	CMD6WKI	-0.123	0.153	0.279
	ST12WKI	0.151	0.161	0.252
	CMD12WKI	-0.138	0.024	0.111
	Eigenvalues (%)	57.33	13.78	7.76

MST6WK	Mean shoot-tip CMD severity at 6 WAP
MST12WK	Mean shoot-tip CMD severity at 12 WAP
MCMD6WK	Mean whole plant CMD severity at 6 WAP
MCMD12WK	Mean whole plant CMD severity at 12 WAP
MST20WK	Mean shoot-tip CMD severity at 20 WAP
MCMD20WK	Mean whole plant CMD severity at 20 WAP
ST6WKI	Mean shoot-tip CMD incidence at 6 WAP
ST12WKI	Mean shoot-tip CMD incidence at 12 WAP
CMD6WKI	Mean whole plant CMD incidence at 6 WAP
CMD12WKI	Mean whole plant CMD incidence at 12 WAP

The first PC (PC1), which accounted for 57.33% of the total variation gave higher weights to severity of shoot tip and whole plant at 6 and 12 WAP in Mokwa, 6, 12 and 20 WAP in Ibadan 1998, and 6 and 12 WAP in Ibadan 1999. In the Mokwa 1998 environment, PC1 also gave a high weight for shoot tip incidence at 12 WAP. The second principal component (PC2), which accounted for 14% of the total variation, gave higher weights to shoot tip and whole plant incidence at 6, 12 and 20 WAP in the Ibadan 1998 environment. The third principal component (PC3) also gave high weightings to shoot tip severity at 6 WAP and whole plant incidence at 6 WAP in Mokwa 1998. In the Ibadan 1998 environment, whole plant severity at 6 and 12 WAP, shoot tip incidence at 6 and 12 WAP, and whole plant incidence at 20 WAP also had high weightings in PC3, but PC3 accounted for only 8% of the total variation.

The scatter-plot of the first versus second principal component scores for the CMD response of the various sources of resistance in the three environments is presented in Fig 4.1. Since 58308 and 91/02324 were not present in all environments they were excluded from the analysis. The results showed that, generally, the resistant clones grouped together and were different from the susceptible. The first resistant group was made up of the resistant landraces TME8, TME9, TMS I30001, TME7 and TME12. TMS I90257clustered together with TME1 and TME6. The resistant landraces TME5 and TME14 formed another group, and TME11 clustered together with TMS I60142 and TMS I30572. The resistant landraces TME4 and the improved clone TMS I4(2)1425 formed single groups. TMS I4(2)1425's group was close to the largest susceptible group made up of clones TME10 only, TME31 and TME2. The susceptible landrace TME117 formed a single group away from all the other susceptible groups. TME41 and TMS I30555 also formed single susceptible groups.

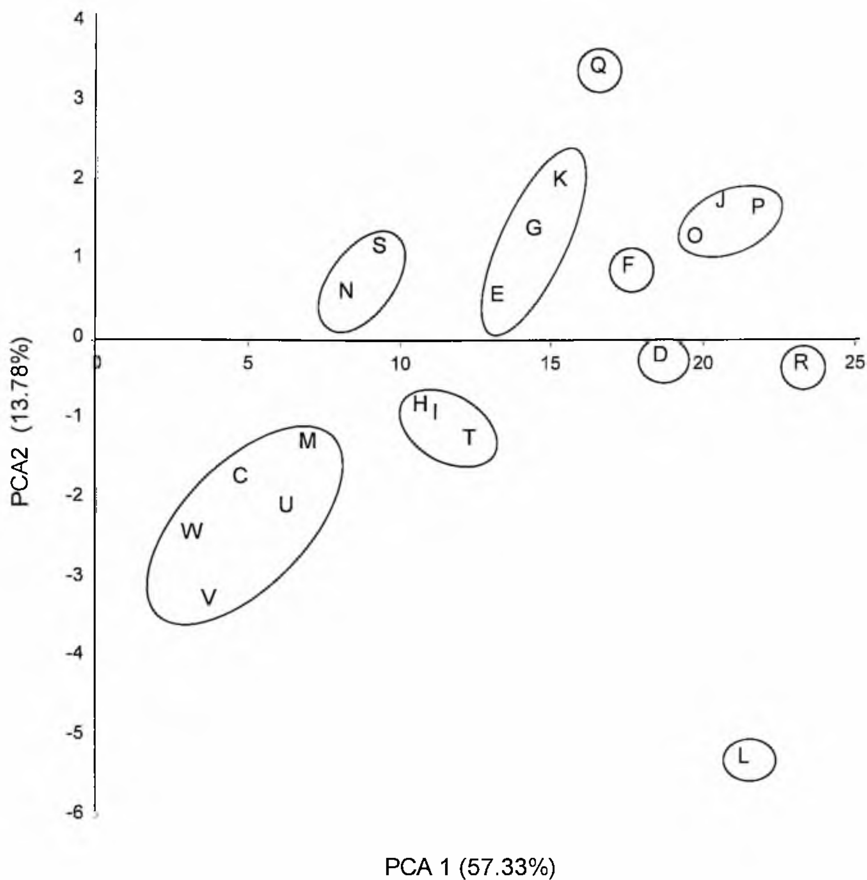

Fig 4.1 Plot of first principal component versus second principal component analysis of parents and checks across environments

Alphabeths represent clones;

C=TMS I30001,	D=TMS I30555,	E=TMS I30572,	F=I4(2)1425,
G=TMS I60142,	H=TMSI90257,	I=TME1,	J=TME10,
K=TME11,	L=TME117,	M=TME12,	N=TME14,
O=TME2,	P=TME31,	Q=TME4,	R=TME41,
S=TME5,	T=TME6,	U=TME7,	V=TME8,
W=TME9			

4.2.3 Complementarity of genes for resistance to CMD among various sources of resistance

4.2.3.1 Frequency distribution of CMD severity scores in F_1 progenies of various sources of resistance

The frequency distributions of the mean disease severity scores of the F_1 progenies in crosses between susceptible parents, resistant parents and between resistant and susceptible parents are presented in Tables 4.10, 4.11 and 4.12 respectively. With scores of 1 and 2 taken as resistant, and scores of 3 to 5 taken as susceptible, the proportion of resistant to susceptible progenies varied from cross to cross and across environments but higher levels of resistance were generally obtained in the Mokwa 1998 environment. In all the three environments, there were generally fewer plants in the extremely susceptible class with the disease severity score of 5 for the various crosses.

The susceptible by susceptible crosses TMS I30555 X TME41 and the self cross TMS I30555 X TMS I30555 had about 50% of their progenies in the resistant classes of 1 and 2 in Ibadan 1998 and 1999. The other crosses in this category had over 60% of their progenies being susceptible (Table 4.10).

Susceptible progenies were detected in all the resistant by resistant crosses in at least one environment (Table 4.11). The best resistant by resistant cross was TMS I30572 X TMS I90257 which had relatively fewer susceptible progenies across environments (9%), in Ibadan 1998 (10.8%), in Ibadan 1999 (24%) and in Mokwa 1998 (0%). The worst resistant by resistant cross was TMS I30572 X TME12 with 32.9% of its progenies being susceptible across environments, 14.3% in Mokwa 1998, 37.2% in Ibadan 1988, and 56.2% in Ibadan 1999.

Table 4.10. Frequency distribution of F_1 disease severity scores (based on mean disease severity scores of progenies) in an environment and mean number of progenies (N) in crosses involving susceptible parents.

	Across environments						Mokwa 1998						Ibadan 1998						Ibadan 1999					
	CMD severity scores					N	CMD severity scores					N	CMD severity scores					N	CMD severity scores					N
Cross	1	2	3	4	5		1	2	3	4	5		1	2	3	4	5		1	2	3	4	5	
I30555 X I30555	2	10	4	1	0	17	3	9	4	1	0	17	1	13	4	2	0	20	1	5	2	6	2	16
I30555 X TME2	24	3	10	18	1	56	49	7	10	4	0	70	22	2	15	10	1	50	0	1	6	39	3	49
I30555 X TME10	47	7	12	11	2	79	100	9	15	3	0	127	36	3	8	16	2	65	5	8	14	13	4	44
I30555 X TME31	20	4	4	8	0	36	41	2	3	2	0	48	18	6	4	2	0	30	2	4	6	19	0	31
I30555 X TME41	48	9	5	12	1	75	81	4	3	2	0	90	52	6	2	7	1	68	10	17	9	26	2	64
I30555 X TME117	10	2	3	3	1	19	21	3	1	0	0	25	10	1	3	2	1	17	0	3	4	8	1	16

Table 4.11 Frequency distribution of F_1 disease severity scores (based on mean disease severity scores of progenies) in an environment and mean number of progenies (N) in crosses involving resistant parents.

Cross	Across environments						Mokwa 1998						Ibadan 1998						Ibadan 1999					
	CMD severity scores					N	CMD severity scores					N	CMD severity scores					N	CMD severity scores					N
	1	2	3	4	5		1	2	3	4	5		1	2	3	4	5		1	2	3	4	5	
I30001 X I30001	8	2	1	1	0	12	9	2	1	0	0	12	5	1	2	2	0	10	1	1	2	3	0	7
I30001 X I30572	29	9	1	0	0	39	35	6	0	0	0	41	18	14	4	0	0	36	4	10	6	6	0	26
I30001 X I58308	6	3	0	0	0	9	7	2	0	0	0	9	3	6	1	0	0	10	2	4	1	1	0	8
I30001 X I4(2)1425	5	5	2	1	1	14	10	5	1	0	0	16	1	5	3	2	1	12	3	4	3	2	1	13
I30001 X I60142	21	6	6	3	0	36	40	3	2	0	0	45	12	7	10	6	0	35	10	7	7	3	0	27
I30001 X I90257	6	4	2	1	0	13	12	2	1	0	0	15	3	6	1	2	0	12	2	4	4	2	0	12
I30001 X TME1	3	3	2	1	0	9	6	3	1	0	0	10	2	3	3	1	0	9	2	3	2	1	0	8
I30001 X TME4	12	3	4	5	0	24	23	2	6	2	0	33	10	3	4	4	1	22	4	4	2	9	0	19
I30001 X TME5	15	9	6	4	1	35	28	8	3	1	1	41	13	7	9	4	1	34	3	11	5	8	0	27
I30001 X TME6	9	4	4	2	0	19	16	5	2	1	0	24	7	4	5	2	0	18	3	2	5	3	1	14
I30001 X TME7	15	7	6	7	0	35	27	8	3	2	0	40	12	7	10	5	0	34	6	5	5	13	1	30
I30001 X TME8	6	8	1	1	0	16	12	9	2	0	0	23	4	7	2	1	0	14	2	7	0	3	1	13
I30001 X TME9	30	8	9	9	1	57	50	8	8	1	0	67	27	7	13	6	0	53	13	10	5	20	2	50
I30001 X TME11	8	4	2	3	0	17	12	4	2	1	0	19	7	5	2	2	0	16	5	3	1	5	0	14
I30001 X TME12	8	2	1	2	1	14	15	2	0	1	0	18	6	3	1	1	1	12	3	2	1	3	1	10
I30001 X TME14	4	2	1	2	0	9	8	1	1	1	0	11	3	2	3	1	0	9	1	2	0	4	1	8
I30572 XI30001	21	6	2	1	0	30	25	5	0	0	0	30	13	9	7	2	0	31	7	7	3	7	0	24
I30572 X I30572	10	2	1	0	0	13	11	3	0	0	0	14	7	1	3	0	0	11	3	1	0	3	0	7
I30572 X 58308	35	20	7	2	0	64	43	19	2	0	0	64	18	23	16	7	0	64	10	14	6	17	1	48
I30572 X I4(2)1425	33	5	4	3	0	45	60	5	2	0	0	67	26	3	5	3	0	37	13	6	5	5	0	29
I30572 X I60142	15	3	3	3	0	24	28	1	0	0	0	29	13	1	4	2	0	20	4	6	5	6	0	21
I30572 X I90257	32	7	2	2	0	43	53	4	0	0	0	57	28	5	2	2	0	37	14	11	3	5	0	33

Table 4.11. continued

Cross	Across environments CMD severity scores 1	2	3	4	5	N	Mokwa 1998 CMD severity scores 1	2	3	4	5	N	Ibadan 1998 CMD severity scores 1	2	3	4	5	N	Ibadan 1999 CMD severity scores 1	2	3	4	5	N
I30572 X TME1	6	1	0	2	0	9	10	2	1	0	0	13	5	1	0	1	0	7	2	0	0	4	1	7
I30572 X TME4	44	7	5	13	1	70	76	6	4	0	0	86	44	5	7	8	0	64	13	11	5	31	2	62
I30572 X TME5	37	6	5	8	0	56	61	2	5	1	0	69	37	5	6	8	0	56	12	11	3	16	1	43
I30572 X TME6	32	5	4	6	0	47	51	3	3	0	0	57	33	2	5	2	0	42	11	9	4	15	1	39
I30572 X TME7	20	3	3	5	0	31	32	4	4	0	0	40	20	2	3	4	0	29	9	3	3	11	1	27
I30572 X TME8	47	10	3	11	0	71	91	7	1	2	0	101	34	11	4	11	0	60	15	12	3	21	1	52
I30572 X TME9	157	21	37	44	2	261	237	33	54	10	1	335	136	15	34	39	3	227	97	14	22	84	1	218
I30572 X TME11	52	20	3	10	0	85	84	25	2	7	0	118	52	14	2	4	0	72	20	21	6	19	0	66
I30572 X TME12	45	12	10	17	1	85	69	21	10	5	0	105	42	7	10	18	1	78	24	8	11	28	2	73
I30572 X TME14	41	15	7	12	1	76	66	20	5	3	0	94	42	8	7	12	1	70	16	16	8	21	1	62
58308 X I30001	4	3	2	0	0	9	5	2	0	0	0	7	2	4	6	0	0	12	4	1	1	0	0	6
58308 X I30572	18	16	6	2	0	42	22	18	4	1	0	45	11	11	10	4	1	37	6	10	5	9	0	30
58308 X 58308	2	2	1	0	0	5	3	2	1	0	0	6	1	3	1	1	0	6	1	3	0	1	0	5

Table 4.12 Frequency distribution of F_1 disease severity scores (based on mean disease severity scores of progenies) in an environment and mean number of progenies (N) in crosses involving resistant and susceptible parents.

	Across environments						Mokwa 1998						Ibadan 1998						Ibadan 1999					
	CMD severity scores					N	CMD severity scores					N	CMD severity scores					N	CMD severity scores					N
Cross	1	2	3	4	5		1	2	3	4	5		1	2	3	4	5		1	2	3	4	5	
I30001 X I30555	28	12	4	1	0	45	35	10	1	0	0	46	13	17	11	2	0	43	7	15	7	7	0	36
I30001 X TME2	9	4	9	7	1	30	22	5	9	1	0	37	6	6	11	6	1	30	0	0	6	15	1	22
I30001 X TME10	13	5	8	5	0	31	26	4	3	1	0	34	10	5	11	3	1	30	3	6	9	10	0	28
I30001 X TME31	5	1	3	2	0	12	10	3	1	0	0	14	5	1	4	0	1	11	0	0	3	7	0	10
I30001 X TME41	16	5	4	7	0	32	30	9	1	0	0	40	13	4	7	4	0	28	4	3	4	16	1	28
I30001 X TME117	12	6	4	4	0	25	22	9	1	0	0	32	11	6	5	4	0	26	3	2	5	8	0	18
I30572 X I30555	19	9	5	1	0	34	24	9	1	0	0	34	9	9	12	2	0	32	4	14	2	6	0	26
I30572 X TME2	18	3	7	8	0	36	40	5	6	0	0	51	15	1	8	5	1	30	0	2	6	19	0	27
I30572 X TME10	37	5	9	6	0	57	55	7	2	0	0	64	41	2	10	3	0	56	15	6	16	14	1	52
I30572 X TME31	37	7	9	14	1	68	64	9	4	1	0	78	38	10	9	9	1	67	9	2	13	32	3	59
I30572 X TME41	49	3	4	14	1	71	94	7	3	1	1	106	37	1	7	8	0	53	16	2	3	33	1	55
I30572 X TME117	53	15	14	25	1	108	92	20	5	1	0	118	63	9	18	15	1	106	3	15	19	58	3	98
I30555 X I30001	24	12	6	1	0	43	30	11	3	0	0	44	12	14	12	4	1	43	5	10	5	17	1	38
I30555 X I30572	21	10	3	0	0	34	24	12	1	0	0	37	15	6	7	0	0	28	8	4	2	7	0	21
I30555 X 58308	13	8	2	1	0	24	15	8	1	0	0	24	9	8	3	3	0	23	2	7	0	6	1	16

Table 4.12 Continued

	Across environments						Mokwa 1998						Ibadan 1998						Ibadan 1999					
	CMD severity scores					N	CMD severity scores					N	CMD severity scores					N	CMD severity scores					N
Cross	1	2	3	4	5		1	2	3	4	5		1	2	3	4	5		1	2	3	4	5	
I30555 X I4(2)1425	6	4	1	2	0	13	12	5	1	0	0	18	4	3	1	2	0	10	2	3	1	4	0	10
I30555 X I60142	25	5	3	4	0	37	47	4	1	1	0	53	23	3	1	3	0	30	5	8	7	9	0	29
I30555 X I90257	15	8	1	1	0	25	35	6	0	0	0	41	5	10	2	2	0	19	5	8	2	2	0	17
I30555 X TME1	25	10	5	4	0	44	45	5	3	0	0	53	24	10	6	2	0	42	5	16	5	10	0	36
I30555 X TME4	34	7	3	7	1	52	56	5	3	1	0	65	31	5	4	5	0	45	14	10	2	14	2	42
I30555 X TME5	19	6	7	8	1	41	37	4	6	1	0	48	18	7	7	6	2	40	3	8	8	17	1	37
I30555 X TME6	53	9	8	14	2	86	86	9	6	1	0	102	60	2	7	10	2	81	13	15	10	32	3	73
I30555 XTME7	49	11	5	10	2	77	84	11	5	3	1	104	39	12	4	4	2	61	25	11	5	23	3	67
I30555 X TME8	43	16	4	7	0	70	77	13	3	1	0	94	37	15	4	5	0	61	14	19	4	16	0	53
I30555 XTME9	27	3	4	7	0	41	40	3	4	3	0	50	27	1	1	7	0	36	14	5	6	10	1	36
I30555 X TME11	29	13	5	9	1	57	58	6	7	2	0	73	24	17	4	4	0	49	4	15	4	20	2	45
I30555 X TME12	40	5	4	6	0	55	72	4	3	1	0	80	36	2	4	4	0	46	13	10	6	14	0	43
I30555 X TME14	29	7	3	5	0	44	68	7	6	3	0	84	18	3	3	5	0	29	2	11	1	8	1	23
58308 X I30555	3	6	3	1	0	13	4	7	2	0	0	13	2	3	5	2	0	12	0	4	0	3	0	7

The crosses involving resistant and susceptible parents had progenies falling into all classes in the Ibadan 1998 and Ibadan 1999 environments (Table 4.12). The crosses involving the resistant parent TMS I30001, however, had fewer plants in the resistant classes of 1 and 2 in Ibadan 1999. The crosses TMS I30001 X TME2 and TMS I30001 X TME31 had no resistant progenies in that environment. Another poor cross in Ibadan 1999 was TMS I30572 X TME2 with only 4 out of 54 progenies having the resistant score of 2. The best cross between a susceptible and resistant parent was the cross TMS I30555 X TMS I90527, with 96%, 100%, 78% and 76% of its progeny being resistant across environments, in Mokwa 1998, Ibadan 1998, and Ibadan 1999 respectively.

4.2.3.2 Transgressive segregants in F_1 progenies of various sources of resistance

Positive transgressive segregant (PTS), individual progenies with a mean CMD severity scores at least one disease severity score lower than the better parent, were desirable for resistance and are presented for the different cross combinations in Table 4.13. PTS were detected in six susceptible by susceptible crosses, 14 resistant by susceptible crosses and eight resistant by resistant crosses. The highest frequencies of PTS across environments were detected in six susceptible by susceptible crosses, which ranged from 20.83% in the self, I30555XI30555 to 72% in I30555XTME41. Similarly, the lowest frequency of TS was detected in the resistant by resistant cross TMS I30572 X TMS I60142 (6.17%), and I30572XTME14 (8.83%).

Table 4.13 Frequency of positive transgressive in percentages (PTS) with disease severity scores at least one score better than the better parent in a cross in three environments

Cross	Across environments		Mokwa 1998		Ibadan 1998		Ibadan 1999	
Sus x Sus	PTS	N	PTS	N	PTS	N	PTS	N
I30555 X I30555	20.83	18	15.50	17	5.50	20	41.50	16
I30555 X TME2	42.5	56	80.5	70	45.0	50	2.0	49
I30555 X TME10	56.3	79	80.0	127	57.5	65	31.5	44
I30555 X TME31	57.8	36	91.5	48	63.5	30	18.5	31
I30555 X TME41	72.0	75	95.0	90	77.0	68	44.0	64
I30555 X TME117	59.8	19	98.0	25	62.0	17	19.5	16
Sus x Res								
I30555 X I30572	32.33	34			55.00	28	42.00	21
I30555 X I4(2)1425	33.00	13			49.50	10	49.50	10
I30555 X I60142	15.17	37					45.50	29
I30555 X I90257	18.83	25			26.50	19	30.00	17
I30555 X TME1	23.33	44			58.00	42	12.00	36
I30555 X TME8	8.50	70					25.50	53
I30555 X TME11	16.33	57			49.00	49		
I30555 XTME14	3.00	44					9.00	23
Res x Sus								
I30572 X I30555	15.2	34			29.0	32	16.5	26
I30572 X TME2	18.2	36			54.5	30		
I30572 X TME10	34.0	57			74.0	56	28.0	52
I30572 X TME31	23.7	68			57.0	67	14.0	59
I30572 X TME41	33.3	71			71.0	53	29.0	55
I30572 X TME117	21.0	108			60.0	106	3.0	98
Res x Res								
I30572 X I30572	36.50	13			67.00	11	42.50	7
I30572 X I4(2)1425	36.17	45			71.50	37	37.00	29
I30572 X I60142	6.17	24					18.50	21
I30572 X I90257	39.17	43			76.00	37	41.50	33
I30572 X TME1	36.50	9			76.50	7	33.00	7
I30572 X TME8	9.83	71					29.50	52
I30572 X TME11	23.83	85			71.50	72		
I30572 X TME14	8.83	76					26.50	62

N number of progenies in an environment and across environments
Sus Susceptible
Res Resistant

4.2.3.3 Cochran-Mantel-Haenzel (CMH) statistic test of association

The CMH test based on the mean distribution of F_1 progeny disease severity scores of the sources of resistance, revealed significant differences among crosses between TMS I4(2)1425 and the resistant landraces TME12, TME14, and TME9 as males and the resistant clone TMS I30572 as the female (Table 4.14). Significant differences were also detected among crosses between TMS I60142 and TME5 as the male parents and TMS I30555. The resistant improved clone TMS I90257 exhibited significant difference from the resistant landraces, TME5 when these were crossed with TMS I30555 and with TME9, TME12 and TME14 when these were crossed with TMS I30572. TME12 and TME11 were significantly different based on their crosses with TMS I30572, while TME5, TME8, TME12 and TME14 were significantly different based on their crosses with TMS I30555.

The results showed that susceptible clone TMS I30555 was significantly different from resistant clones TMS I30001, 58308 and TMS I30572 when these were crossed with TMS I30555. The susceptible clones TME2, TME31 and TME117 were also different from the resistant clones TMS I4(2)1425 and TMS I90257 when these were crossed with TMS I30572 (Table 4.15). The results further showed that TME2 was significantly different from TMS I60142 based on their crosses with TMS I30001 and TMS I30555 and different from TMS I90257, TME1, TME4, TME6, TME7, TME8, TME9 and TME11 from their crosses with TMS I30555. Significant difference between TME2 and TME11 was also detected when they were crossed with TMS I30572. The susceptible clone TME117 was also significantly different from TME5, TME6, TME8 and TME11 when these were crossed with TMS I30572.

The results further showed that TME2 was significantly different from TME10 and TME41 from their crosses with TMS I30555 and that TME117 and TME10 were significantly different from their crosses with TMS I30572 (Table 4.16).

Table 4. 14 Chi-square values for differences in the mean distribution of disease severity scores of F_1 progenies in crosses involving resistant male parents when crossed with each of the female parents I30001 resistant to CMD, I30555 susceptible to CMD and I30572 resistant to CMD

	Female parent			
Resistant male	58308	I30001	I30555	I30572
I30001 vs 58308	0.00	0.49	0.00	1.18
I30001 vs I30572	0.01	1.92	0.83	0.27
I30572 vs 58308	0.00	0.08	0.63	1.76
I4(2)1425 vs I60142		1.31	0.79	1.09
I4(2)1425 vs I90257		0.48	1.68	0.26
I4(2)1425 vs TME1		0.00	0.37	0.65
I4(2)1425 vs TME11		0.11	0.00	0.89
I4(2)1425 vs TME12		0.08	1.23	6.11*
I4(2)1425 vs TME14		0.00	0.75	4.06*
I4(2)1425 vs TME4		0.02	0.29	2.83
I4(2)1425 vs TME5		0.05	0.39	1.17
I4(2)1425 vs TME6		0.24	0.02	0.66
I4(2)1425 vs TME7		0.00	0.20	1.40
I4(2)1425 vs TME8		0.76	0.86	1.02
I4(2)1425 vs TME9		0.15	0.15	4.62*
I60142 vs I90257		0.09	0.18	2.25
I60142 vs TME1		0.89	0.22	0.00
I60142 vs TME11		0.63	1.85	0.15
I60142 vs TME12		0.49	0.07	0.93
I60142 vs TME14		0.82	0.00	0.34
I60142 vs TME4		1.27	0.21	0.14
I60142 vs TME5		1.39	4.09*	0.02
I60142 vs TME6		0.45	1.14	0.11
I60142 vs TME7		2.18	0.40	0.01
I60142 vs TME8		0.05	0.01	0.05
I60142 vs TME9		1.04	0.39	0.34
I90257 vs TME1		0.37	0.81	1.36
I90257 vs TME11		0.15	2.67	2.22
I90257 vs TME12		0.11	0.04	8.44**
I90257 vs TME14		0.31	0.24	6.18*
I90257 vs TME4		0.36	0.67	4.53*
I90257 vs TME5		0.34	4.93*	2.47
I90257 vs TME6		0.07	1.76	1.69
I90257 vs TME7		0.64	0.94	2.69
I90257 vs TME8		0.01	0.32	2.27
I90257 vs TME9		0.18	0.95	6.78**
TME1 vs TME11		0.06	0.98	0.11
TME1 vs TME12		0.04	0.64	0.31
TME1 vs TME14		0.00	0.18	0.10
TME1 vs TME4		0.00	0.00	0.03
TME1 vs TME5		0.02	3.11	0.02
TME1 vs TME6		0.15	0.45	0.09
TME1 vs TME7		0.01	0.03	0.00
TME1 vs TME8		0.62	0.20	0.05
TME1 vs TME9		0.07	0.05	0.09

* P-value <0.05
** P-value <0.01

Table 4.14 Continued

Resistant male	Female parent			
	58308	I30001	I30555	I30572
TME4 vs TME5		0.01	2.91	0.44
TME4 vs TME6		0.14	0.43	0.78
TME4 vs TME7		0.03	0.03	0.10
TME4 vs TME8		0.58	0.20	0.70
TME4 vs TME9		0.08	0.04	0.07
TME5 vs TME6		0.12	1.55	0.06
TME5 vs TME7		0.09	2.88	0.06
TME5 vs TME8		0.58	5.70*	0.01
TME5 vs TME9		0.05	2.01	1.11
TME6 vs TME7		0.36	0.30	0.20
TME6 vs TME8		0.17	1.56	0.02
TME6 vs TME9		0.03	0.15	1.60
TME7 vs TME8		1.00	0.47	0.12
TME7 vs TME9		0.30	0.00	0.30
TME8 vs TME9		0.33	0.44	1.72
TME11 vs TME12		0.00	3.26	4.29*
TME11 vs TME14		0.05	1.88	2.07
TME11 vs TME4		0.05	0.92	1.19
TME11 vs TME5		0.03	0.79	0.09
TME11 vs TME6		0.02	0.13	0.00
TME11 vs TME7		0.17	0.76	0.27
TME11 vs TME8		0.28	2.48	0.03
TME11 vs TME9		0.00	0.47	2.72
TME12 vs TME14		0.04	0.12	0.36
TME12 vs TME4		0.04	0.62	0.68
TME12 vs TME5		0.02	6.23*	2.21
TME12 vs TME6		0.02	2.30	2.76
TME12 vs TME7		0.13	1.04	0.93
TME12 vs TME8		0.21	0.19	2.98
TME12 vs TME9		0.00	0.91	0.65
TME14 vs TME4		0.00	0.17	0.06
TME14 vs TME5		0.02	4.31*	0.90
TME14 vs TME6		0.13	1.15	1.36
TME14 vs TME7		0.01	0.37	0.29
TME14 vs TME8		0.52	0.00	1.31
TME14 vs TME9		0.06	0.36	0.00

* P-value <0.05
** P-value <0.01

Table 4.15 Chi-square values for differences in the mean distribution of disease severity scores of F_1 progenies in crosses involving resistant and susceptible male parents when crossed with each of the female parents I30001 resistant to CMD, I30555 susceptible to CMD and I30572 resistant to CMD

	Female parent			
Male resistant source	58308	I30001	I30555	I30572
I30001 vs I30555	0.99	0.08	6.37*	1.10
I30555 vs 58308	0.59	0.46	5.22*	0.02
I30555 vs I30572	1.54	2.51	10.99**	1.68
I4(2)1425 vs TME10		0.00	0.00	1.30
I4(2)1425 vs TME117		0.14	0.16	8.83**
I4(2)1425 vs TME2		1.10	1.62	6.63*
I4(2)1425 vs TME31		0.01	0.04	5.98*
I4(2)1425 vs TME41		0.04	0.15	2.01
I60142 vs TME10		2.33	1.51	0.01
I60142 vs TME117		0.83	2.14	1.80
I60142 vs TME2		7.63**	8.84**	1.48
I60142 vs TME31		1.34	1.93	0.99
I60142 vs TME41		1.28	0.51	0.03
I90257 vs TME10		0.74	2.18	2.70
I90257 vs TME117		0.18	3.07	11.46**
I90257 vs TME2		3.20	8.93**	9.05**
I90257 vs TME31		0.55	2.77	8.23**
I90257 vs TME41		0.32	1.08	3.44
TME1 vs TME10		0.01	0.71	0.02
TME1 vs TME117		0.07	1.50	0.63
TME1 vs TME2		0.98	7.92**	0.58
TME1 vs TME31		0.02	1.16	0.34
TME1 vs TME41		0.01	0.08	0.00
TME2 vs TME31		0.77	2.42	0.13
TME2 vs TME4		1.96	7.86**	1.19
TME2 vs TME5		2.81	1.01	2.77
TME2 vs TME6		3.03	6.34*	3.32
TME2 vs TME7		1.92	8.74**	1.46
TME2 vs TME8		4.33*	13.22**	3.44
TME2 vs TME9		4.00	5.88*	1.20
TME4 vs TME41		0.00	0.07	0.07
TME10 vs TME11		0.21	0.03	0.12
TME10 vs TME12		0.17	2.87	2.22
TME10 vs TME14		0.01	1.55	0.89
TME10 vs TME5		0.13	1.15	0.00
TME10 vs TME6		0.43	0.04	0.08
TME10 vs TME7		0.00	0.56	0.05
TME10 vs TME8		1.14	2.09	0.02
TME10 vs TME9		0.36	0.31	1.07
TME11 vs TME117		0.00	0.24	7.50**
TME11 vs TME2		2.26	4.15*	4.75*
TME11 vs TME31		0.16	0.04	4.23*
TME11 vs TME41		0.03	0.59	0.62
TME12 vs TME2		1.77	13.00**	0.21
TME12 vs TME31		0.12	3.17	0.01
TME12 vs TME41		0.02	1.23	1.17
TME14 vs TME31		0.02	1.97	0.44
TME14 vs TME41		0.01	0.48	0.27

* P-value <0.05
** P-value <0.01

Table 4.15 Continued

Male resistant source	58308	Female I30001	parent I30555	I30572
TME31 vs TME4		0.05	1.05	0.75
TME31 vs TME5		0.09	0.35	2.24
TME31 vs TME6		0.30	0.26	2.76
TME31 vs TME7		0.01	0.91	0.99
TME31 vs TME8		0.86	2.55	2.97
TME31 vs TME9		0.20	0.62	0.73
TME41 vs TME5		0.00	2.55	0.17
TME41 vs TME6		0.11	0.19	0.40
TME41 vs TME7		0.07	0.01	0.01
TME41 vs TME8		0.53	0.62	0.31
TME41 vs TME9		0.05	0.00	0.35
TME117 vs TME12		0.00	3.17	0.34
TME117 vs TME14		0.06	2.18	1.43
TME117 vs TME4		0.06	1.29	1.95
TME117 vs TME5		0.04	0.03	4.15*
TME117 vs TME6		0.03	0.52	4.72*
TME117 vs TME7		0.23	1.16	1.92
TME117 vs TME8		0.33	2.73	5.46*
TME117 vs TME9		0.00	0.88	2.62

* P-value <0.05
** P-value <0.01

Table 4. 16 Chi-square values for differences in the mean distribution of disease severity scores of F_1 progenies in crosses involving susceptible male parents when crossed with each of the female parents I30001 resistant to CMD, I30555 susceptible to CMD and I30572 resistant to CMD

Male parent	Female		
	I30001	I30555	I30572
TME2 vs TME31	0.77	2.42	0.13
TME2 vs TME41	2.47	8.09**	1.64
TME10 vs TME2	1.71	5.38*	2.84
TME10 vs TME31	0.00	0.13	2.26
TME10 vs TME41	0.11	0.40	0.15
TME31 vs TME41	0.08	0.74	1.22
TME10 vs TME117	0.28	0.36	4.19*
TME117 vs TME2	3.01	0.88	0.00
TME117 vs TME31	0.19	0.08	0.19
TME117 vs TME41	0.04	1.01	2.77

* P-value <0.05
** P-value <0.01

4.2.3.4. Number of effective factors

The minimum number of effective factors segregating for resistance to CMD in crosses between the various sources of resistance and susceptible parents is presented in Tables 4.17 to 4.19. The number of factors responsible for resistance varied from cross to cross and from environment to environment. Where little (less than 0.2) or no genetic variance was detected, the number of effective factors was not estimated.

Among the crosses involving resistant parents (Table 4.17), the number of effective factors ranged from 2 to 3 across environment, 2 to 4 in Mokwa 1998, 2 to 6 in Ibadan 1998 and from 2 to 3 in Ibadan 1999. In most cases, both parents contributed effective factors to the cross. Generally both resistant parents contributed equal numbers of effective factors except for a few cases were the better parent contributed more.

Effective factors for crosses between resistant and susceptible parents ranged from 2 to 4 across environments, 2 to 7 in Mokwa 1998, 2 to 6 in Ibadan 1998, and 2 to 7 in Ibadan 1999 (Table 4.18). Generally, both the resistant and susceptible parents contributed effective factors. In the majority of cases, the resistant parent contributed more factors to resistance. However, in six crosses with TMS I30001 as the resistant parent and seven crosses with TMS I30555 as the susceptible parent, both parents contributed equally in at least one environment.

In crosses between susceptible parents, the effective factors ranged from 2 to 4 across environments, 2 to 5 in Ibadan 1998 and 2 to 4 in Ibadan 1998 (Table 4.19). Generally, both parents contributed equally to resistance, although in some cases, TMS I30555 the better parent, contributed more.

Table 4.17 Number of effective factors (NE), number of effective factors contributed by better parent (NBP) and number of effective factors contributed by poorer parent (NPP) in F_1 crosses between susceptible parents

Cross	Across environments			Mokwa 1998			Ibadan 1998			Ibadan 1999		
	NE	NBP	NPP	NE	NBP	NPP	NE	NBP	NPP	NE	NBP	NPP
I30555 x I30555	2	1	1	-	-	-	-	-	-	2	1	1
I30555 x TME2	2	1	1	2	1	1	3	2	1	-	-	-
I30555 x TME10	2	1	1	-	-	-	3	2	1	2	1	1
I30555 x TME31	2	1	1	-	-	-	2	1	1	2	1	1
I30555 x TME41	4	2	2	-	-	-	5	3	2	3	2	1
I30555 x TME117	3	2	1	-	-	-	2	1	1	4	3	1

- not estimated due to little or no genetic variance

Table 4.18 Number of effective factors (NE), number of effective factors contributed by better parent (NBP) and number of effective factors contributed by poorer parent (NPP) in F_1 crosses between resistant parents

Cross	Across environments			Mokwa 1998			Ibadan 1998			Ibadan 1999		
	NE	NEBP	NEPP	NE	NEBP	NEPP	NE	NEBP	NEPP	NE	NEBP	NEPP
I30001 x I30001	2	1	1	-	-	-	2	1	1	2	1	1
I30001 x I30572	2	1	1	-	-	-	-	-	-	2	1	1
I30001 x I58308	2	1	1	-	-	-	-	-	-	2	1	1
I30572 x I30001	3	2	1	-	-	-	6	4	2	2	1	1
I30572 x I30572	2	1	1	-	-	-	-	-	-	2	1	1
I30572 x I58308	2	1	1	-	-	-	-	-	-	2	1	1
I58308 x I30001	2	1	1	-	-	-	-	-	-	2	1	1
I58308 x I30572	2	1	1	-	-	-	-	-	-	2	1	1
I58308 x I58308	2	1	1	-	-	-	-	-	-	2	1	1
I30001 x I4(2)1425	2	1	1	2	1	1	2	1	1	3	2	1
I30001 x I60142	2	1	1	2	1	1	2	1	1	2	1	1
I30001 x I90257	2	1	1	2	1	1	2	1	1	3	2	1
I30001 x TME1	2	1	1	2	1	1	2	1	1	2	1	1
I30001 x TME4	2	1	1	2	1	1	2	1	1	2	1	1
I30001 x TME5	2	1	1	4	2	2	2	1	1	2	1	1
I30001 x TME6	2	1	1	2	1	1	2	1	1	2	1	1
I30001 x TME7	2	1	1	2	1	1	2	1	1	2	1	1
I30001 x TME8	2	1	1	2	1	1	2	1	1	2	1	1
I30001 x TME9	2	1	1	2	1	1	4	1	1	2	1	1
I30001 x TME11	2	1	1	2	1	1	4	1	1	2	1	1
I30001 x TME12	2	1	1	2	1	1	2	1	1	2	1	1
I30001 x TME14	2	1	1	2	1	1	2	1	1	2	1	1

- not estimated due to little or no genetic variance

Table 4.18 Continued

Cross	Across environments			Mokwa 1998			Ibadan 1998			Ibadan 1999		
	NE	NEBP	NEPP	NE	NEBP	NEPP	NE	NEBP	NEPP	NE	NEBP	NEPP
I30572 x I4(2)1425	2	1	1	-	-	-	3	1	1	2	1	1
I30572 x I60142	2	1	1	-	-	-	2	1	1	2	1	1
I30572 x I90257	3	2	1	-	-	-	4	2	2	2	1	1
I30572 x TME1	2	1	1	2	1	1	2	1	1	2	1	1
I30572 x TME4	2	1	1	2	1	1	3	2	1	2	1	1
I30572 x TME5	2	1	1	2	1	1	2	1	1	2	1	1
I30572 x TME6	2	1	1	2	1	1	2	1	1	2	1	1
I30572 x TME7	2	1	1	2	1	1	3	2	1	2	1	1
I30572 x TME8	2	1	1	4	2	2	2	1	1	2	1	1
I30572 x TME9	2	1	1	4	2	2	2	1	1	2	1	1
I30572 x TME11	2	1	1	3	2	1	-	-	-	2	1	1
I30572 x TME12	2	1	1	3	1	1	2	1	1	2	1	1
I30572 x TME14	2	1	1	2	1	1	2	1	1	2	1	1

- not estimated due to little or no genetic variance

Table 4.19 Number of effective factors (NE), number of effective factors contributed by better parent (NBP) and number of effective factors contributed by poorer parent (NPP) in F_1 crosses between resistant and susceptible parents

Cross	Across environments			Mokwa 1998			Ibadan 1998			Ibadan 1999		
	NE	NEBP	NEPP	NE	NEBP	NEPP	NE	NEBP	NEPP	NE	NEBP	NEPP
I30001 x I30555	3	2	1	-	-	-	-	-	-	3	2	1
I30572 x I30555	2	1	1	-	-	-	-	-	-	2	1	1
I58308 x I30555	2	1	1	-	-	-	-	-	-	2	1	1
I30001 x TME2	2	1	1	2	1	1	2	1	1	-	-	-
I30001 x TME10	2	1	1	-	-	-	3	2	1	2	1	1
I30001 x TME31	2	1	1	-	-	-	2	1	1	2	1	1
I30001 x TME41	2	1	1	-	-	-	2	1	1	2	1	1
I30001 x TME117	2	1	1	-	-	-	2	1	1	2	1	1
I30572 x TME2	3	2	1	3	2	1	4	2	2	3	2	1
I30572 x TME10	4	3	1	-	-	-	6	4	2	3	2	1
I30572 x TME31	4	2	1	3	3	1	2	1	1	5	3	2
I30572 x TME41	2	1	1	-	-	-	2	1	1	2	1	1
I30572 x TME117	5	4	1	-	-	-	3	2	1	7	6	1
I30555 x I30001	2	1	1	-	-	-	3	1	2	2	1	1
I30555 x I30572	2	1	1	-	-	-	-	-	-	2	1	1
I30555 x I58308	2	1	1	-	-	-	-	-	-	2	1	1
I30555 x I4(2)1425	4	2	2	7	4	3	-	-	-	2	1	1
I30555 x I60142	2	1	1	-	-	-	2	1	1	2	1	1
I30555 x I90257	3	2	1	-	-	-	3	2	2	3	2	1

- not estimated due to little or no genetic variance

Table 4.19 Continued

Cross	Across environments			Mokwa 1998			Ibadan 1998			Ibadan 1999		
	NE	NEBP	NEPP	NE	NEBP	NEPP	NE	NEBP	NEPP	NE	NEBP	NEPP
I30555 x TME1	3	2	1	-	-	-	5	3	2	2	1	1
I30555 x TME4	2	1	1	-	-	-	2	1	1	2	1	1
I30555 x TME5	2	1	1	3	2	1	2	1	1	2	1	1
I30555 x TME6	3	2	1	-	-	-	2	1	1	3	2	1
I30555 x TME7	4	3	1	4	3	2	5	3	2	2	1	1
I30555 x TME8	3	2	1	-	-	-	5	3	2	2	1	1
I30555 x TME9	2	1	1	2	1	1	2	1	1	2	1	1
I30555 x TME11	3	2	1	3	2	1	5	2	2	3	2	1
I30555 x TME12	3	2	1	-	-	-	5	3	2	2	1	1
I30555 x TME14	3	2	1	5	3	2	2	1	1	2	1	1

not estimated due to little or no genetic variance

4.3 Determination of DNA markers associated with resistance to CMD

4.3.1 Resistance screening and selection of plants for bulk segregant analysis

The mean disease severity scores for shoot tips, whole plant, leaf severity, and the presence or absence of ACMV viruses of the F_1 plants in the mapping population is presented in Table 4.20. Based on these results, plants were classified as highly resistant (HR), resistant (R), susceptible (S), and highly susceptible (HS). A total of ten plants from the highly resistant and highly susceptible classes respectively were selected for BSA. The plants that made up the resistant bulk were: 9, 11, 22, 29, 36, 40, 43, 45, 54, and 59, while those that made up the susceptible bulk were: 1, 8, 28, 31, 33, 42, 56, 62, 63 and 67.

4.3.2 Genomic DNA quality and concentration

The DNA extraction method yielded genomic DNA of good quality and quantity. There was no smearing when checked by agarose gel (0.8%) electrophoresis. On average, 254 ng of DNA per 100 mg of fresh leaves was obtained based on the estimates given with the flourometer. The ratio of OD_{260nm} to OD_{280nm} determined by spectrophotometry ranged from 1.86 to 2.00 for the samples.

4.3.3 Cassava mosaic virus strains in mapping population

The ACMV specific primer 1 (ACMV-F1/ACMV-R1) and 2 (ACMV-ALF/ACMV-ARO/R.) detected ACMV in 61 and 37 samples, respectively (Table 4.20). The ACMV specific primer 1 detected the virus in 29 resistant samples (24 HR and 5 R samples) while ACMV specific primer 2 detected the virus in only nine resistant samples (8 HR and 1 R). Thus ACMV 1 detected the virus in 20 more samples. ACMV specific primer 2, on the other hand, detected the virus in one resistant sample, sample 48, which was not detected by ACMV specific primer 1.

Table 4.20 Reaction of F_1 plants in mapping population to CMD severity in their shoot tips (MST), whole plant (MWP), mean severity of first 10 leaves plant (MLS), total mean response presence or absence of ACMV virus and their classification (Class)

Plant number	MST	MWP	MLS	Mean	ACMV 1	ACMV 2	Status
1	4	4.5	3.8	4.10	1	1	HS
2	2	4	2.9	2.97	1	1	S
3	1.5	1.5	1.3	1.43	0	0	HR
4	1	2	1.1	1.37	1	1	HR
5	3.5	4	3.8	3.77	1	1	HS
6	1	1	1	1.00	1	0	HR
7	1	2	1	1.33	1	0	HR
8	3.5	4.5	3.4	3.80	1	1	HS
9	1	1	1	1.00	1	0	HR
10	1	1	1	1.00	1	1	HR
11	1	1	1.1	1.03	1	0	HR
12	1.5	2	1	1.50	1	0	R
13	2.5	3.5	1.2	2.40	1	0	R
14	3	4	3.9	3.63	1	1	HS
15	4	4	3.4	3.80	1	1	HS
16	4	4	3	3.67	1	1	HS
17	1	1	1	1.00	0	0	HR
18	1	2.5	1.3	1.60	1	0	R
19	4	4	3.9	3.97	1	1	HS
20	3.5	4	3.7	3.73	1	1	HS
21	1	1.5	1.2	1.23	1	1	HR
22	1	1	1	1.00	1	1	HR
23	3	4	4	3.67	1	1	HS
24	3.5	4	3.2	3.57	1	1	HS
25	3	3.5	3.2	3.23	1	0	S
26	3.5	4	3	3.50	1	1	HS
27	4	4	3.2	3.73	1	0	HS
28	4.5	4	3.7	4.07	1	1	HS
29	1	1	1	1.00	1	0	HR
30	1.5	1.5	1	1.33	1	0	HR
31	4.5	4.5	4.3	4.43	1	1	HS
32	2	4	2.4	2.80	1	1	S
33	4.5	4	3.5	4.00	1	0	HS
34	3.5	4	3.4	3.63	0	0	HS

S, susceptible HS, highly susceptible
R, resistant HR, highly resistant
ACMV1 ACMV specific primer (ACMV-F1/ACMV-R1)
ACMV2 ACMV specific primer (ACMV-AL/F/ACMV-AR0/R)
1=Presence of virus product
0=Absence of virus product

Table 4.20 continued

Plant number	MST	MWP	MLS	Mean	ACMV 1	ACMV 2	Status
35	1	1	1	1.00	1	1	HR
36	1	1	1	1.00	0	0	HR
37	1	1.5	1.1	1.20	0	0	HR
38	3.5	4	3.5	3.67	1	1	HS
39	1.5	2	1.3	1.60	1	1	R
40	1	1	1	1.00	1	0	HR
41	2.5	4	2.2	2.90	1	1	S
42	4.5	4	3.6	4.03	1	1	HS
43	1	1	1	1.00	0	0	HR
44	1	1.5	1	1.17	1	0	HR
45	1	1	1	1.00	1	0	HR
46	1	1.5	1	1.17	1	0	HR
47	1	1	1	1.00	1	0	HR
48	1	1	1	1.00	0	1	HR
49	1	1	1.1	1.03	1	0	HR
50	1	1.5	1.2	1.23	1	1	HR
51	2	2	1	1.67	1	0	R
52	1	1	1	1.00	0	0	HR
53	1.5	3	1.2	1.90	1	1	S
54	1	1	1	1.00	1	0	HR
55	3.5	3.5	1.2	2.73	1	1	S
56	4	4	3.3	3.77	1	1	HS
57	3.5	4	3.8	3.77	1	1	HS
58	1	1	1.1	1.03	1	0	HR
59	1	1	1	1.00	1	0	HR
60	2	3	2.5	2.50	1	1	S
61	3.5	4	3.4	3.63	1	0	HS
62	4.5	4.5	4.3	4.43	1	1	HS
63	4	4	4	4.00	1	1	HS
64	3.5	4	3.3	3.60	1	1	HS
65	1	1	1	1.00	1	0	HR
66	3.5	3.5	2.7	3.23	1	1	S
67	4	4.5	3.7	4.07	1	1	HS
68	1	1	1	1.00	1	1	HR
69	1	1	1.1	1.03	1	0	HR

S, susceptible — HS, highly susceptible
R, resistant — HR, highly resistant
ACMV1 — ACMV specific primer (ACMV-F1/ACMV-R1)
ACMV2 — ACMV specific primer (ACMV-AL/F/ACMV-AR0/R)
1= — Presence of virus product
0= — Absence of virus product

The ACMV primer ACMV 1, also detected the virus in 32 susceptible samples (24 HS and 8 S) and ACMV 2 detected the virus in 28 susceptible samples (21 HS and 7 S). The virus was not detected in six resistant samples, 3, 17, 36, 37, 43 and 52 and in one susceptible sample 34 by either of the two ACMV specific primers. Plate 4.1 shows the amplification products of some of the samples showing the presence of ACMV with the two primers. The EACMV specific primer and the EACMV/UgV primer did not detect the presence of the virus in any of the samples.

4.4 Screening parents and bulks for polymorphic markers

4.4.1 Random amplified fragment length polymorphism analysis

A total of 403 markers were recorded from 107 of 142 RAPD primers which were used to screen the parents and bulks. The number of bands per primer ranged from 1-8, averaging 4 markers per primer. Forty-eight of the 107 primers were polymorphic between the two parents and three of these exhibited polymorphism between the two bulks. The three primers, however, did not distinctively distinguish between the resistant and susceptible DNAs when used to screen the individual member of the two bulks. An example of the amplification products using RAPD primers, K11, K14, K15, K16, and M05, on the parents and bulks is shown in plate 4.2. In this example, all the primers except primer K15 were polymorphic between the two parents and none of the primers were polymorphic between the bulks.

Plate 4.1 Amplification products of section of mapping population showing the presence of ACMV with two ACMV specific primers
Panel 1: ACMV specific primer ACMV-F1/ACMV-R1F1/R1
Panel 2: ACMV specific primer ACMV-AL/F/ACMV-AR0/R
Lane 1: 1 Kb marker
Lane 2 to 45: Example of F1 progenies with or without ACMV PCR products

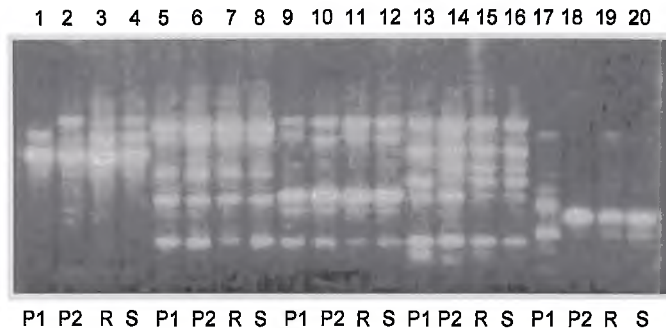

Plate 4.2 Amplification profile generated by some RAPD primers on, P1 (TMS I30555), P2 (TME7), R (resistant bulk) and S (susceptible bulk).
Lanes 1-4, Operon Primer K11;
Lanes 5-8 Operon Primer K14;
Lanes 9-12, Operon Primer K15;
Lanes 13-16, Operon Primer K16;
Lanes 17-20 Operon Primer M05.

4.4.2 Amplified fragment length polymorphism analysis

The average number of bands and the percentage polymorphism between the parents and the bulks for the different AFLP systems is shown in Table 21.

With the *Eco*R1/*Mse*1 analysis, amplification products were obtained from 57 primer pairs of the 64 used to screen the two parents (TMS I30555 and TME7) all of which were polymorphic between the two parents. The number of bands detected after the silver staining ranged from 23 to 108, giving an average of 68.5 bands per primer pair. A total of 3,906 bands were generated, out of which 843 were polymorphic averaging 14.8 polymorphic bands per primer pair, and an overall of 21.58% polymorphism. For the bulked DNAs, amplification products were obtained from 54 of the *Eco*R1/*Mse*1 +3 primer combinations. These yielded a total of 2,860 bands averaging 51.07 per primer pair, of which only 119 bands were polymorphic, averaging 2.13 polymorphic bands per primer pair and giving 4.16% level of polymorphism between the bulks.

The *Apa*1+C/*Taq*1+A templates screened with the 40 combinations of *Apa*1+3 and *Taq*1+3 primers yielded a total of 1,903 bands from 35 primer pairs. The total number of bands ranged from 19 to 93, averaging 54 bands per primer. There were 401 bands, which were polymorphic between the parents, giving an average of 7.36 polymorphic bands per primer pair and 21.13% polymorphism on the whole for this system. A total of 314 of the bands were polymorphic between the two bulks, giving 16.5% polymorphism among the bulks. However, when the were used to screen the members of the two bulks, the results were very erratic, and none of the polymorphic primer pairs showed a clear distinction between the two groups.

The *Apa*1+A/Taq1+C templates screened with the 6 primer combinations (*Apa*1+ATT and Taq1+ CAC, CAG, CAT, CTA, CTG or CTT) yielded a total of 475 bands ranging from 62 to 88 bands per primer pair, with an average of 79 bands per primer pair. A

total of 73 of the 475 bands were polymorphic between the parents, giving 15.4% polymorphism between the parents and 8 were polymorphic between the bulks, giving 1.68% polymorphism between the bulks.

Table 4.21 Average number of bands, level of polymorphism among parents (TMS I30555, TME7) and bulks with different AFLP systems

Preamplification Template	Average number of bands	Level of Polymorphism (%)	
		Parents	Bulks
*Eco*R1+A/*Mse*+C	68.5	21.58	4.16
*Apa*1+C/Taq1+A	54.4	21.13	16.50
*Apa*1+A/Taq1+C	79.2	15.4	1.68
*Apa*1+C/Taq1+C	45.3	17.81	1.43
*Pst*1+A/Taq1+C	51	9.87	0.3

The *Apa*1+C/Taq1+C templates screened with 12 primer combinations (involving *Apa*1 + CTA or CTG and Taq1 + CAC, CAG, CAT, CTA, CTG or CTT) yielded a total of 494 bands for the parents, averaging 45 bands per primer pair. The number of polymorphic bands between the parents ranged from 3 to 18, averaging 8.8 polymorphic bands per primer pair, and the total number of polymorphic bands was 88 giving 17.81% polymorphism. For the bulks, a total of 491 bands were scored and 8 were polymorphic between the bulks, giving 1.43% polymorphism.

The *Pst* 1+ A/Taq 1+C templates screened with 24 of the primer combinations involving *Pst*1+ ACA or ACC and Taq1 + CAC, CAG, CAT, CTA, CTG or CTT yielded a total of 1,165 bands ranging from 20 to 83 bands and an average of 51 bands per primer. A total of 115 of the total number of bands were polymorphic between the two parents, giving 9.87% polymorphism between the parents, while three of the bands were polymorphic between the two bulks, giving 0.3% polymorphism.

4.4.3 Simple Sequence Repeats

The number of bands (microsatelliite alleles) generated from the SSR markers ranged from 1 to 3 per sample. Fifteen markers were polymorphic for both the parents and the bulks. One SSR primer SSR30, which had three alleles (a, b, and c) among the two parents, consistently distinguished between the two DNA bulks and the members of each bulk group. The polymorphic marker (allele "a"), designated as SSR30-180, was present in the resistant landrace TME7 but absent in the susceptible improved line I30555. The band was also present in all the members of the resistant bulk, but absent in the members of the susceptible group (Plate 4.3).

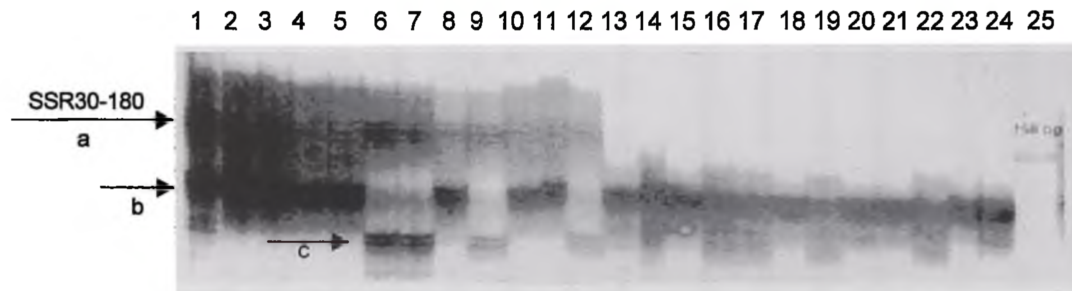

Plate 4.3 Amplification products of SSR primer 30 in parents, bulks and bulk members.
Lane 1, TME7;
Lane 2, resistant bulk;
Lanes 3 to 12 samples 9, 11, 22, 29, 36, 40, 43, 45, 54 and 59;
Lane 13, TMS I30555;
Lane 14, susceptible bulk,
Lane 15 to 24 samples 1, 8,14,19, 23, 42, 57, 60, 62 and 63;
Lane 25, 50bp marker.
Long arrow showing allele a, marker SSR30-180
Shorter arrows showing two other alleles b and c

4.5 Association of DNA markers with CMD resistance in F_1 mapping population

4.5.1 Genetic Linkage analysis

The nine *Eco*R1/*Mse*1+3 primers, three *Pst*1/Taq1+3 primers and 15 SSR primers, which were used in genotyping the F_1 mapping population, gave a total of 138 polymorphic bands for linkage analysis. The resistant male parent TME7 contributed 73 and the susceptible female parent TMS I30555 contributed 65 markers of the 138 loci generated from the AFLP and SSR analysis.

Plates 4.4 and 4.5 show examples of sections of the F_1 mapping population following selective amplification with the primer E-AAC/M-CAT and P-ACC/T-CTA. An example of the amplification products of three SSR primers, SSR6, SSR70 and SSR7, on a section of the F_1 mapping population is also shown in plate 4.6.

Prior to linkage analysis, the markers were subjected to chi-square analysis to test their conformity to the 1:1 and 3:1 inheritance ratios. The chi square analysis revealed that at the 5 and 1 % probability levels, 38 (58.46%) of the markers contributed by TMS I30555 and 44 (60.27%) contributed by the resistant parent TME7 were segregating for the1:1 segregation ratio of single dose markers. The results further showed that 10 (15.38%) of the markers donated by the female and four markers (5.48%) donated by the male parents were significant for 3:1 segregation ratio of double dose markers (Tables 4.22 and 4.23).

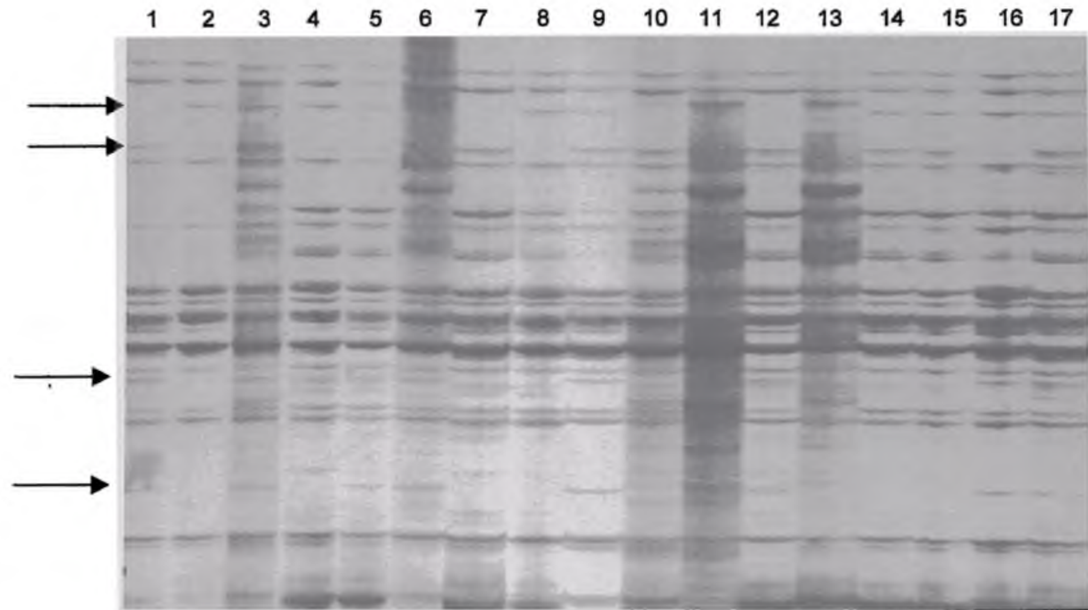

Plate 4.4 A cross section of AFLP products of parents TMS I30555 and TME7 and progenies following amplification with primers E-ACC/M-CAT. Arrows showing polymorphic bands

Lane 1: TMS I30555

Lane 2: TME7

Lane 3 to 17: F_1s 1 to 15

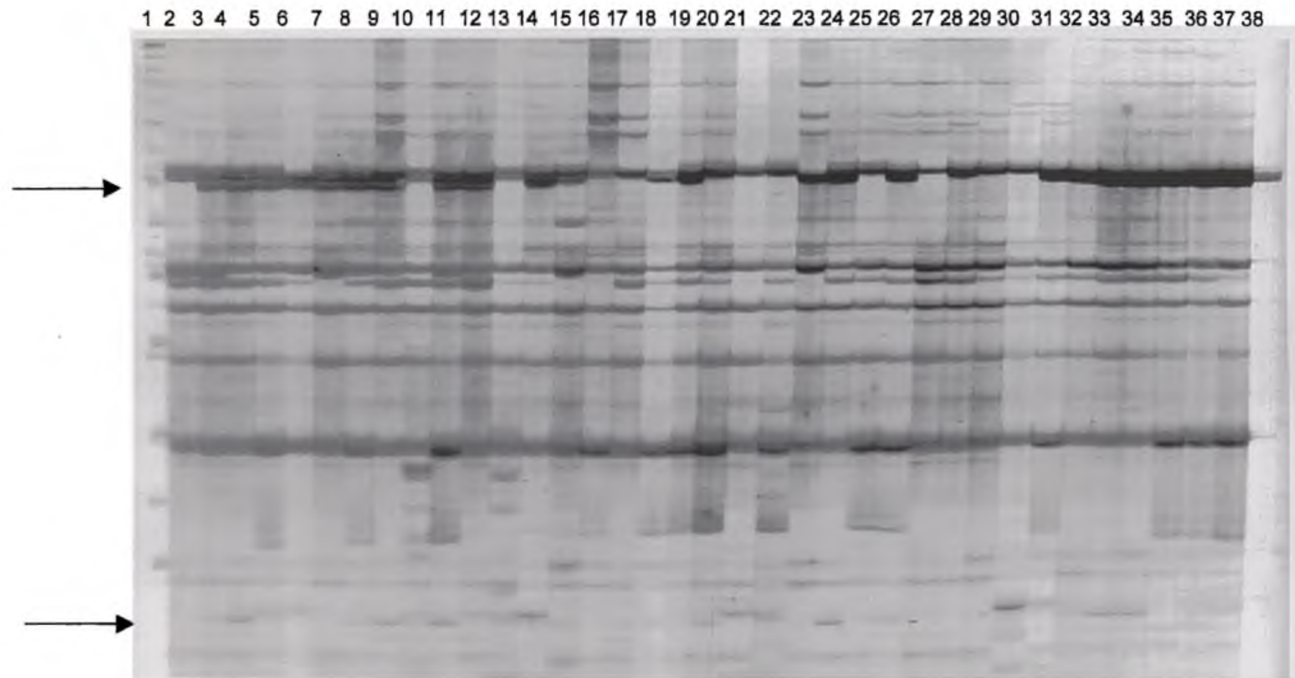

Plate 4.5. Section of AFLP products for primer P-ACC/T-CTA. Arrows showing polymorphic bands

Lane 1: 50bp marker;

Lane 2: TMS I30555;

Lane 3: TME7;

Lanes 4 to 38; F_1 plants 36 to 69

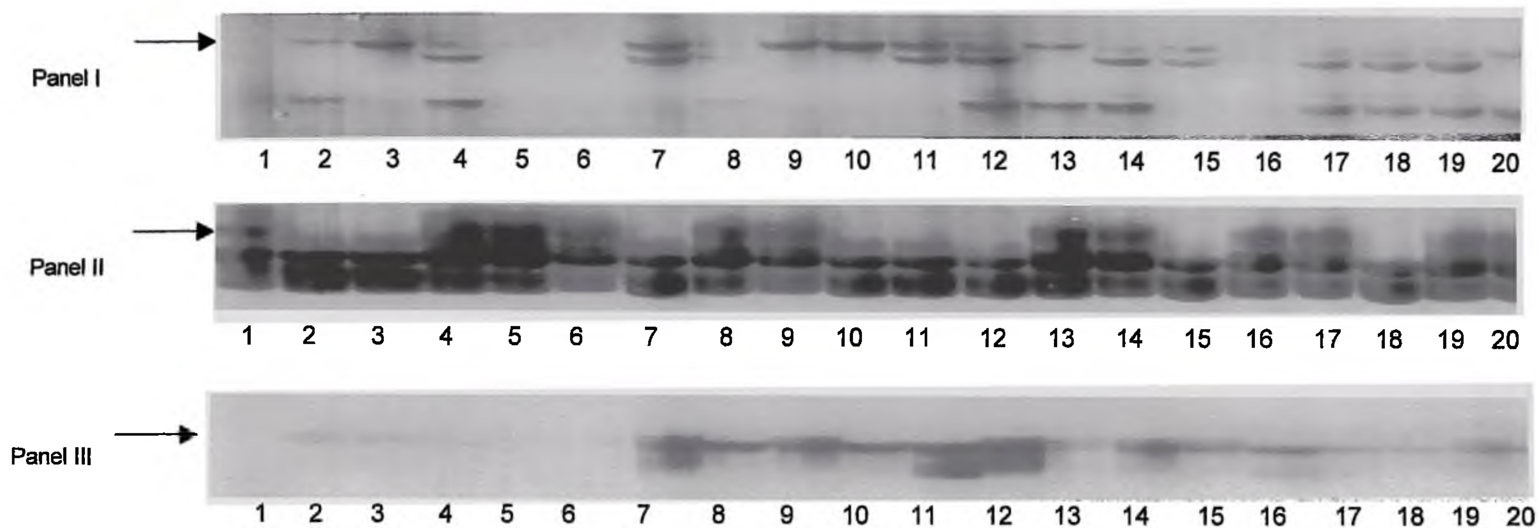

Plate 4.6. Example of multiplexing PCR products of three SSR primers
Panel I: SSR6, Panel II: SSR70, Panel III: SSR7
Lane 1: TMS I30555
Lane 2: TME7
Lanes 3 to 2: F_1 population, samples 1 to 17
Arows showing polymorphic bands

Table 4.22 Segregation analysis for conformity to 1:1 and 3:1 segregation ratio of polymorphic DNA markers scored in mapping population contributed by P1 I30555

Allele	Chi value for 1:1 ratio	Chi value for 3:1 ratio
SSR132-A	0.00	68.00
E-ACC/M-CAT-175	0.01	73.06
SSR124-2	0.01	65.06
SSR6/290	0.06	76.24
E-ACC/M-CAT-130	0.13	57.52
E-ACA/M-CAT-130	0.13	79.54
E-ACC/M-CAT-500	0.36	90.45
SSR56-B	0.37	48.49
E-ACA/M-CAT-145	0.47	51.88
E-ACC/M-CTC-175	0.53	46.12
P-ACC/T-CTA-210	0.53	46.12
E-ACC/M-CAT-364	0.56	90.25
P-ACT/T-CTT-440	0.71	99.84
P-ACT/T-CTT-435	0.71	43.84
E-ACT/M-CAG-245	0.73	41.93
E-ACT/M-CAG-240	0.73	41.93
SSR157-B	0.75	40.02
E-ACT/M-CAG-210	0.94	103.76
E-ACC/M-CTC-650	0.94	103.76
SSR70/320	0.94	39.76
E-ACA/M-CAC-340	1.17	109.70
E-ACA/M-CAT-360	1.40	12.60
E-ACC/M-CTG-450	1.52	32.06
E-ACC/M-CAT-775	2.31	80.26
E-AAG/M-CTG-220	2.45	130.80
E-ACA/M-CAC-650	2.45	26.80
E-ACT/M-CAG-190	2.52	25.09
P-ACC/T-CAG-380	2.52	25.09
E-AAG/M-CTG-560	2.88	135.53
E-AAG/M-CTG-182	3.26	142.04
P-ACT/T-CTT-520	3.26	142.04
E-AAG/M-CTC-170	3.76	19.06
E-ACA/M-CAC-545	3.76	147.06
SSR7/220	3.76	19.06
E-ACA/M-CAC-280	4.19	153.75
E-ACC/M-CTG-48	4.91	13.64
E-AAG/M-CTC-195	6.39	178.57
E-ACA/M-CAT-480	6.45	7.81

Tabulated chi value at 5% probability 3.84
Tabulated chi value at 1% probability 6.63

Table 4.22 Cont

Allele	Chi value for 1:1 ratio	Chi value for 3:1 ratio
E-ACT/M-CAG-208	7.12	184.47
E-ACC/M-CAT-370	7.67	7.67
E-ACA/M-CAT-305	7.81	5.23
E-ACA/M-CAT-160	7.90	6.58
E-ACA/M-CAT-240	9.94	3.76
E-ACC/M-CTC-210	9.94	3.76
SSR119-B	9.94	211.76
E-ACC/M-CTG-350	10.24	2.97
E-ACA/M-CAT-330	10.90	1.61
E-AAG/M-CTG-181	11.53	226.12
E-AAG/M-CTG-620	12.19	233.75
E-ACC/M-CAT-745	12.19	233.75
E-ACC/M-CAT-650	12.60	1.40
E-AAG/M-CTC-350	13.24	0.94
E-AAG/M-CTG-110	13.93	248.71
E-ACC/M-CAT-220	15.78	0.13
E-ACC/M-CTC-180	17.75	280.01
SSR41-A	17.75	280.01
P-ACCT/-CAG-340	18.28	280.13
E-ACA/M-CAT-155	18.69	221.77
P-ACC/T-CAG-175	20.43	0.73
P-ACC/T-CAG-240	23.53	322.12
E-ACC/M-CTC-215	25.94	339.76
P-ACC/T-CAG-170	39.76	19.06
SSR50-A	47.52	32.06
E-ACC/M-CAT-180	53.93	40.71
E-ACC/M-CTG-260	54.55	44.18

Tabulated chi value at 5% probability is 3.84
Tabulated chi value at 1% probability is 6.63

Table 4.23 Segregation analysis for conformity to 1:1 segregation ratio of polymorphic DNA markers scored in mapping population contributed by P2 TME7

Allele	Chi value for 1:1 ratio	Chi value for 3:1 ratio
E-ACC/M-CTG-180	0.00	66.00
E-AAG/M-CTG-550	0.01	65.06
E-ACC/M-CAT-725	0.01	65.06
E-ACC/M-CTC-450	0.01	65.06
E-ACT/M-CAG-175	0.01	71.06
SSR132-B	0.01	63.06
E-ACT/M-CAG-250	0.06	60.24
SSR41-B	0.13	57.52
E-AAG/M-CTG-850	0.24	52.94
E-ACA/M-CAT-150	0.24	84.94
P-ACC/T-CTA-190	0.24	84.94
SSR56-A	0.24	84.94
E-AAG/M-CTC-55	0.36	50.45
SSR115-A	0.36	50.45
P-ACC/T-CTA-145	0.37	88.49
SSR119-C	0.37	88.49
E-ACA/M-CAT-170	0.53	94.12
SSR6/340	0.53	46.12
SSR124-A	0.53	46.12
E-ACC/M-CTC-240	0.71	43.84
P-ACC/T-CTA-470	0.73	97.93
E-ACA/M-CAT-94	0.94	103.76
SSR30-180	1.17	37.70
E-AAG/M-CTG-180	1.47	113.88
E-ACA/M-CAT-118	1.47	33.88
E-ACC/M-CAT-95	1.75	32.01
SSR7/210	1.75	120.01
E-ACC/M-CAT-105	2.88	23.53
E-ACA/M-CAT-175	2.88	23.53
E-ACT/M-CAG-230	2.88	135.53
E-ACT/M-CAG-92	2.88	23.53
E-ACC/M-CAT-795	2.88	23.53
E-ACC/M-CTG-45	2.97	21.88
SSR119-A	3.36	140.43
P-ACT/T-CTT-175	3.76	19.06
E-ACT/M-CAG-95	4.31	16.25
E-ACC/M-CTC-225	4.76	159.06
P-ACT/T-CTT-110	5.23	13.93
E-ACC/M-CAT-570	5.88	171.53
E-ACA/M-CAC-290	5.88	11.53
SSR50-B	6.06	10.24
E-ACC/M-CAT-83	6.39	10.57
E-AAG/M-CTC-470	6.39	10.57
SSR50-C	6.58	177.33

Tabulated chi value at 5% probability is 3.84
Tabulated chi value at 1% probability is 6.63

Table 4.23 continued

Allele	Chi value for 1:1 ratio	Chi value for 3:1 ratio
SSR157-A	7.33	183.33
E-ACC/M-CTC-120	8.47	5.88
E-ACC/M-CTG-55	8.73	196.91
E-ACC/M-CTG-325	9.33	204.31
E-ACC/M-CTG-635	10.88	218.52
E-ACA/M-CAT-345	10.94	152.76
E-ACA/M-CAT-230	12.55	233.21
E-ACC/M-CAT-352	13.24	0.94
P-ACC/T-CTA-350	13.24	240.94
E-AAG/M-CTC-275	13.93	248.71
E-ACC/M-CTG-315	15.52	256.06
P-ACC/T-CTA-280	17.00	272.00
P-ACC/T-CAG-425	17.00	272.00
P-ACT/T-CTT-75	17.00	0.00
E-ACA/M-CAT-77	21.00	21.00
E-ACC/M-CTG-52	21.88	1.52
E-ACC/M-CTG-230	22.70	313.81
P-ACT/T-CTT-66	24.36	330.45
E-ACC/M-CTG-570	25.09	331.36
E-ACC/M-CTG-540	25.09	331.36
E-ACC/M-CTG-105	26.73	340.91
E-ACC/M-CTG-215	29.33	359.33
E-ACC/M-CTG-150	32.06	378.24
E-ACC/M-CTG-680	36.76	415.06
P-ACC/T-CAG-220	36.76	415.06
P-ACT/T-CTT-55	40.71	443.84
E-ACC/M-CTG-370	45.15	467.60
P-ACC/T-CAG-440	49.47	497.88
E-ACC/M-CTG-748	68.00	612.00

Tabulated chi value at 5% probability is 3.84
Tabulated chi value at 1% probability is 6.63

Initially, all 138 markers were subjected to linkage analysis. This generated seven linkage groups with about 80% of the markers in linkage group-I. The 96 markers segregating for the two genetic ratios were then tested with different linkage analysis strategies (i.e. single female and male maps, combined map, single maps with inverted loci, and combined maps with inverted loci). The most suitable results were obtained for the combined maps. With all 96 markers, only 36 markers mapped in 15 linkage groups and 12 groups had just two markers.

Since the backcross configuration of single-dose segregating markers is desirable in mapping heterozygous F_1 populations (Liu, 1998); the 14 markers segregating for the 3:1 ratio were eliminated and finally, 82 of the 138 markers (59.42%) and the converted CMD resistance locus CMDRL, were used to construct the linkage map.

The loci mapped in 14 linkage groups with six groups having only two markers each (Table 4.24). The 14 linkage groups ranged in size from 16.8 to 227.6 cM and the number of markers per linkage group correlated with the map distances per linkage group (r^2=0.98). The distance between adjacent loci in centimorgans on the map ranged from 14.9 to 73.2 cM with an average of 33.66 cM, even though the distance threshold set with the LOD of 3 and linkage criteria of 0.33 was 39.6 cM. On the whole, the genetic linkage map spanned 1,111.4 cM of the genome. The AFLP primer pair E-ACT/M-CAG contributed six markers in four linkage groups (I, V, VI and VII), which was the highest number of markers contributed by any of the AFLP primer pairs.

The SSR30-180 marker and an AFLP marker, E-ACC/M-CTC-225, flanked the converted CMDRL on linkage group-X (Fig. 4.2). Both markers were donated by the resistant parent TME7. The map distances that separated CMDRL from SSR30-180 and E-ACC/M-CTC-225 were 14.9 and 31.10 cM respectively.

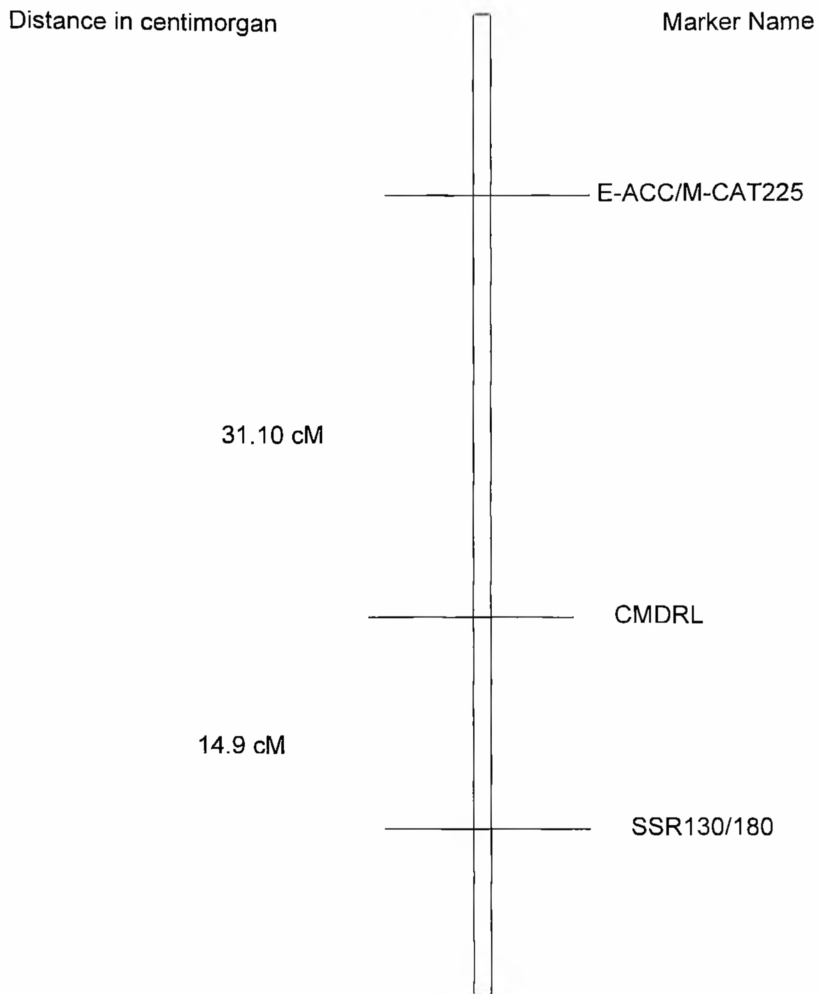

Fig. 4.2 Linkage group-X of the linkage map showing the location of the resistance to CMD, defined as the CMDRL

4.5.2 QTL analysis

Simple linear correlation revealed significant association between six markers, including CMDRL and the CMD response traits (Table 4.25). The marker SSR30-180 had the highest linear association after CMDL for all the response traits and their mean effect. Two markers SSR119-A and SSR119-C, which mapped on linkage group-XIII and were donated by the resistant male parent, had the next largest association with CMDRL and the CMDR traits. SSR30-180 and E-ACC/M-CTC-225 had positive correlation with CMDRL and negative correlation with the traits while, SSR119-A and SSR119-C had negative correlation with CMDRL and positive correlation with the responses. An unassigned marker, E-AAG/M-CTG-550, had a weak correlation ($p<0.05$) with mean whole plant severity.

Significant marker-trait association was detected by regression analysis for the four markers SSR30-180, E-ACC/M-CTC-225, SSR119-A and SSR119-C and the CMD resistance responses (Table 4.26). The r^2 values, which indicated the proportion of the phenotypic variation explained by the markers, were highest for SSR30-180 in all cases. It accounted for 57.41% of the phenotypic variation in the population for the mean response, followed by SSR119-C which accounted for 37.83%, SSR119-A accounted for 32.24% then E-ACC/M-CTC-225 accounted for 22.5% of the total phenotypic variation in the population for resistance to CMD.

Table 4.25 Simple linear correlation coefficient between markers and CMD responses

Marker	Linkage Group	CMDL	MST	MWP	MLS	Mean
E-ACC/M-CTC-225	X	0.453***	-0.413***	-0.481***	-0.531***	-0.486***
SSR30180	X	0.723***	-0.738***	-0.769***	-0.715***	-0.762***
SSR119-A	XIII	-0.548***	0.537***	0.588***	0.556***	0.577***
SSR119-C	XIII	-0.619***	0.610***	0.591***	0.617***	0.622***
CMDL	X	1.000****	-0.898***	-0.943***	-0.910***	-0.942***
E-AAG/M-CTG-550	UM	-0.228	-0.208	-0.275*	-0.241	-0.241

* $p < 0.05$ probability level
*** $p < 0.005$ probability level
UM: unassigned marker
MST: Mean shoot tips,
MWP: Mean whole plant,
MLS: Mean severity of first 10 leaves plant
Mean: Total mean response

Table 4.26 Association between markers SSR30-180 and E-ACC/M-CTC-225 with all CMD resistance traits (All traits), mean shoot-tip severity (MST), mean whole plant severity (MWP), and mean leaf severity (MLS) in mapping population

	Linkage Group	CMD response	r(%)	F-ratio	p-value
E-ACC/M-CTC-225	X	MST	15.79	13.75	0.0004
		MWP	22.01	20.19	0.0001
		MLS	27.13	26.32	0.0001
		Mean	22.5	20.74	0.0001
SSR30180	X	MST	53.76	80.07	<0.0001
		MWP	58.48	96.76	<0.0001
		MLS	50.43	70.19	<0.0001
		Mean	57.41	92.65	<0.0001
SSR119-A	XIII	MST	27.82	27.21	<0.0001
		MWP	33.59	35.4	<0.0001
		MLS	29.87	29.96	<0.0001
		Mean	32.24	33.36	<0.0001
SSR119-C	XIII	MST	36.36	39.78	<0.0001
		MWP	33.99	36.02	<0.0001
		MLS	37.18	41.25	<0.0001
		Mean	37.83	42.38	<0.0001
E-AAG/M-CTG-550	UM	MST	3.77	3.66	ns
		MWP	2.88	3.01	ns
		MLS	6.18	5.48	ns
		Mean	4.42	4.15	ns

UM= unassigned marker
Ns= not significant

4.6 Association of DNA marker SSR30-180 with CMD resistance in other cassava clones

There were differences between some resistant clones and TME7 with respect to the marker SSR30-180. The SSR primer SSR30 detected four different alleles (a, b, c, and d) among all the 23 cassava clones used as parents and checks in the genetic NCD II and diallel experiments as opposed to the three it detected between the two parents TMS I30555 and TME7. The resistant clones 58308, TMS I30572, and the landrace TME8, did not have the marker SSR180-30 (allele "a"). The primer amplified a different allele (allele "d") for these clones, suggesting that different alleles may be involved in cassava resistance to CMD (Plate 4.7). The susceptible clones TME31 and TME117 had alleles similar to those of TME7.

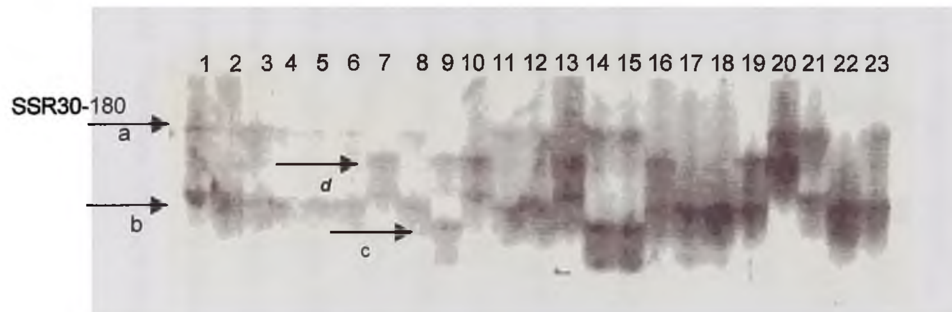

Plate 4.7 Amplification product of SSR 30 on 23 cassava clones

Lane 1: TME1	Lane 2: TME2	Lane 3: TME4	Lane 4: TME5
Lane 5: TME6	Lane 6: TME7	Lane 7: TME8	Lane 8: TME9
Lane 9: TME10	Lane 10: TME11	Lane 11: TME12	Lane 12: TME14
Lane 13: TME31	Lane 14: TME41	Lane 15: TME117	Lane 16: TMS I30001
Lane 17: TMS I30555	Lane 18: TMS I30572;	Lane 19: 58308	Lane 20: TMS I4(2)1425
Lane 21: TMS I60142	Lane 22: TMS I90257	Lane 23: 91/02324	

With alleles a, b and c as seen in plate 4.3 and new allele *d* occurring in some clones.

Chapter Five

Discussion

Resistance to CMD from the resistant genetic stock, clone 58308 which has been the main source of resistance to CMD in breeding is polygenic, recessive and inherited largely in an additive manner (Hahn and Howland, 1972). Polygenic inheritance has also been reported for some of the African landraces, which are potential new sources of resistance to the disease (Lokko *et al.*, 1998). However, a major dominant gene responsible for resistance in an African landrace has also recently been reported (IITA, 2000; Akano *et al.*, 2002). In this study, further progress was made in understanding the genetics of resistance to CMD from the 58308 source and among the landraces.

Genotype by environment interaction on inheritance of resistance

Fargette *et al.* (1994) had previously shown that CMD responses are environment dependent. The significant GXE detected for most of the genotypic effects in the NCD II experiment and GCA effects in the diallel indicate a lack of stability of the genetic effects measure across environments. The differences in ranks of the AUDPC estimates across environments further demonstrated the lack of stability of the CMD symptom severity across environments. The importance of GXE interaction detected in this study shows that none of the genotypes can be effectively assessed in any single environment. Therefore, multiple environmental evaluation, which has been recommended for traits with significant environmental interaction effect affecting them, is required to obtain precise genetic information (Zhang and Kang, 1997).

Relative importance of GCA/SCA

The relative importance of GCA or additive gene effect in predicting progeny performance was estimated from the ratios of the mean square components associated with variance of GCA and SCA. The closer this ratio is to 1, the greater the chances of predicting progeny performance based on GCA (Baker, 1978; Kang, 1994). From the results, GCA was relatively more important in determining progeny performance in the NCD II experiment and the GCA effect due to both the females and the males was important, although this was environment-dependent. The predominance of GCA in the NCD II was further reflected by the significant correlation between parental means and GCA, which implies that progeny performance can be predicted from the parental performance. The significance of the female by male interaction (SCA), however, suggests the presence of some non-additive components, which was detected in some crosses.

Results from previous diallel experiments indicated that resistance to CMD derived from clone 58308 was inherited largely in an additive manner (IITA, 1973; Hahn *et al.,* 1980; 1989; Msabaha, 1983). In the diallel experiment, additive gene effect which was due mainly to clone TMS I30572 and 58308, was important in the Ibadan 1998 environment. However, the magnitude of the ratios of the mean square components associated with variance of GCA and SCA, across environments, in Mokwa 1998 and Ibadan 1999 indicate a predominance of SCA which implies that non-additive genetic effect was more important in determining progeny performance. A predominance of SCA is expected among crosses involving inbred lines (Matzinger and Kempthorne, 1956). Although cassava is a heterozygous species, three of the parents used in the diallel experiment are known to have common ancestry and, therefore, the assumption of no inbreeding did not hold. The population could therefore be likened

to a diallel cross among inbred lines and explains the predominance of SCA in the diallel experiment.

In this study, negative GCA effects of a parent indicated that the F_1 crosses involving that parent are more resistant than the average F_1 crosses. Negative values of clones indicated a larger contribution towards resistance whiles positive significant value suggest a contribution towards susceptibility. The best general combiner in the NCD II, which contributed most to resistance, was the improved clone TMS I90572. The clone TMS I30572 was the best general combiner among the females and the landraces TME8 and TME9 were good general combiners in the Ibadan 1999 environment. TMS I30572 together with TMS I30001 were the best general combiners among the parents in the diallel experiment. TMS I30001, however, was a poor general combiner in the NCD II experiment. In both experiments TMS I30555 was a poor general combiner. The resistant genetic stock, clone 58308, was also a poor general combiner in the diallel and also had a significant but positive maternal effect. However, in earlier studies, 58308 exhibited good parental value for resistance to CMD (IITA, 1973). In another study conducted in three environments, the landraces TME8, TME9, and TME11 were the best general combiners for resistance to CMD while TMS I30001, TMS I30555, and TMS I30572 were poor general combiners (Lokko *et al.*, 1998).

The results of the present study compared with those from previous studies (IITA, 1973; Lokko *et al.*, 1998) show that the GCA of a clone is dependent on the genetic constitution of the population within which it is being tested as well as the environment. They further show that the resistant parents identified as potential sources of resistance for breeding need to be selected carefully after progeny testing to determine the most suitable parents.

The overall best general combiner detected in the NCD II experiment, TMS I90257 should be used in breeding programmes to enhance resistance to CMD. Other clones such as TMS I30572, TMS I60142 TMS I4(2)1425, the landraces TME8 and TME9 could also be used in breeding programmes to enhance resistance but the progenies would have to be tested in different environments to select resistant phenotypes. The high heritability estimates obtained in the two experiments shows that generally, significant progress in breeding for resistance to CMD can be made by selection. Ogbe, (2001) recommended TMS I30001 and TME8 as sources of resistance for breeding, based on their ability to express two types of resistance to CMD, resistance to virus movement and resistance to virus multiplication. However, based on the results of this study, TMS I30001 is a poor general combiner for resistance to CMD. It should, therefore, be used in combination with the resistant clones, which have been shown to have good combining ability.

Significant and negative SCA effects were desirable for resistance. A cross with significant and negative SCA implied that this cross was more resistant than the average GCA of the parent clones and a cross with significant and positive SCA implied that this cross was more susceptible than the average GCA of the parent clones. The three crosses with negative and significant SCA effects across environments were more resistant than expected based on the average performance of the parents and in Ibadan 1999, two crosses were more resistant than the average performance of the parents. Although two good general combiners were involved in one of these crosses, the parents in the other crosses were not the best general combiners. This implies that there is no general relationship between the GCA of a parent and the SCA of its cross. Furthermore, out of the six crosses that were more susceptible than the average of their parents across environments, only three had one of their parents as the poor general combiner (TME2). In Ibadan 1999, none of the parents involved in the three crosses with significant and positive SCA, were poor

general combiners. This shows that progeny testing is also required to select the best parental combinations for CMD resistance.

The results of the diallel also revealed that there is no direct relationship between the level of resistance in a parent and the SCA effect of its cross. TMS I30555 X TMS I30572 involving a good general combiner with negative and significant GCA (TMS I30572) and a poor general combiner (TMS I30555) with positive and significant GCA were more resistant in Mokwa 1998 than the average performance of the parents. In Ibadan 1999, two crosses TMS I30001 X 58308 and TMS I30555 X TMS I30572 were more resistant than the average performance of the parents while their reciprocals were not, which further suggests that progeny testing is required to determine resistant genotypes in a cross. The selfs of TMS I30555 and 58308, on the other hand, were more susceptible than the average performance of the parents in Mokwa 1998. Although it is expected that selfing in cassava reduces hybrid vigor, significant and positive SCA was the general trend in all the selfs and for the selfs TMS I30555 X TMS I30555 and 58308 X 58308 in all environments.

The significant reciprocal and maternal effects detected in the Ibadan 1998 environment and maternal effects detected in Mokwa 1998 environment were due to the significant and positive maternal effects of clone 58308. Maternal effect refers to the effect of the maternal genotype or tissues on a trait of its offspring, while non-maternal effect represent the effect on a trait of non-nuclear or extranuclear genetic factors or the interaction between products of nuclear genes and plasmagenes (Kang *et al.*, 1999). Maternal effect due to 58308 was also environment-dependent, therefore progenies with 58308 as the mother would have to be tested in different environments to select for higher levels of resistance to CMD.

Heritability of CMD

In this study, heritability was estimated from the mean square components of the crosses. These values represent estimates of narrow sense heritability since the genetic variances among half sibs equals to covariances of the half sibs, which represent primarily additive genetic variance contained in the phenotypic variance among the half sib families (Nguyen and Sleper, 1983). The results show that significant progress in breeding for resistance to CMD can be made from selection. These estimates of 51.6% and 56.6% in the NCD II and diallel respectively, are comparable to those reported in previous studies, which varied from 30% to 68% (Hahn et al. 1980a). The difference in the magnitudes of the heritability estimates indicates that the composition of the genotypes, and environment in which they are evaluated were also important.

Heterosis

The significance of the parents' mean squares in the two experiments is an indication of the diverse variability among the parents used in the study. Significant differences among the male parents and resistant parents in the NCD II experiment suggests that the African landraces could serve as potential sources of exotic genes for resistance. The significant and negative mid-parent and high parent heterosis estimated for CMD severity in some crosses is further evidence that the landraces could serve as good sources of resistance.

Heterosis occurs when there is allelic interaction or overdominance at some or all alleles, when the parents differ in gene frequency and when directional dominance exists (Kang, 1994; Falconer and Mackay, 1997). However, heterosis would vary from cross to cross. The significance of the contrast parent versus cross in the individual environments for both experiments suggests the presence of average mid-

parent heterosis. Heterosis was also environment dependent, therefore crosses need to be evaluated in several environments to determine the most heterotic cross.

Non–addivtive gene effect due to additive by additive epistatsis has been proposed as the cause of heterosis in self pollinating species (Gravois, 1994;) whiles in outcrossers both dominance effect and additive by additive epistatsis contribute to SCA causing heterosis (Holland, 2001). The means for the various crosses however, showed various intermediate values, which suggested polygenic inheritance and the absence of dominance. No dominance is assumed if the mean in a first generation cross is equal to the mid-parent (Hallauer and Miranda, 1988). These suggest that heterosis detected in some of the crosses may be due to allelic interaction (Xiao *et al.,* 1995; Falconer and Mackay, 1997; Yu *et al.,* 1998).

Genetic differences based on parental responses to CMD

The PCA 1 versus PCA 2 scatter-plots illustrated the genetic relationship among the clones, with genotypes within a cluster group being genetically related.

The results implied that the four resistant landraces TME7, TME8, TME9 and TME12 are genetically similar to TMS I30001. The tiers in the ranks of AUDPC values for either shoot tip or whole plant in at least one environment for these clones further suggest genetic similarities among them. Genetic similarities between TMS I90257 and the landraces TME1 and TME6 and between TMS I30572 and TMS I60142 and landraces TME11 were also established. These genetic similarities suggest that the clones in the same genetic group may exhibit the same level of resistance mechanism. As was expected, the susceptible clones were genetically distinct from the resistant clones.

Mode of inheritance of resistance to CMD

In many cases, resistance to plant viruses is under a simple genetic control involving a single dominant or recessive gene (Fraser, 1990). However, there are reports in

the literature indicating that resistance to some plant viruses is under complex genetic control (Hahn and Howland, 1972; McMullen *et al.*, 1994; Caranta and Plaloix, 1996; Melchinger *et al.*, 1998). This study also confirmed the polygenic mode of resistance to CMD.

The resistant parents used in this study did not represent the genotypic extremes but had varying levels of resistance in the different environments. Similarly, the susceptible parents exhibited varying levels of susceptibility. The segregating F_1 crosses between the various sources of resistance and susceptible parents and between the resistant and susceptible parents also exhibited varying levels of resistance and susceptibility to CMD, suggesting multiple factors with genes from both parents. The presence of resistant phenotypes in the crosses involving susceptible by susceptible parents imply that resistance to CMD in the clones studied is recessive. These results are similar to those of Hahn and Howland (1972) who reported polygenic and recessive inheritance for resistance to CMD. In crosses involving two resistant parents, the presence of susceptible progenies also suggest that the genes for resistance in these parents are non-allelic. Wang *et al.* (1998) tested allelism forresistance to the soybean mosaic virus and showed that if resistance genes from the two parents are non-allelic and not linked, a proportion of the progenies from their crosses would be susceptible.

Transgressive segregation

Transgressive segregation is due either to mutants induced by hybridity, the unmasking of recessive genes in heterozygote parents, or complementary action of genes with additive effects (Rick and Smith, 1953; Xu *et al.*, 1998). Since mutation induced by hybridity rarely happens (Xu *et al.*, 1998), the likely explanation for the transgressive segregants (TS) detected in this study would be the unmasking of recessive genes in heterozygote parents or complementary action of genes which were detected in the study. The presence of transgressive segregation in this study is

further evidence for the polygenic control of resistance to CMD among the cassava clones studied that involved different alleles in different parents and is cumulative for degree of resistance.

The lack of TS for CMD severity in the diallel population suggests that similar genetic mechanisms affect the cassava clones used as parents in the diallel (Fenster and Ritland, 1994). Another explanation for the lack of TS in the diallel is that there is linkage among loci. Linkage could also result in a reduced frequency of transgressive segregates for a trait (Lawrence and Frey, 1976).

Gene complementarity

The main purpose of testing for gene complementarity is to examine relationships among genes from different sources of a trait in order to determine whether the genes are allelic. Gene complementarity among sources of resistance to disease and pest have been reported in oats, soybean and sorghum (Dixon *et al.*, 1991; Fox *et al.*, 1997; Wang *et al.*, 1998).

Significant differences in the mean distribution of F_1 progeny disease severity scores of the 15 sources of resistance to CMD, when these were crossed with resistant clones TMS I30001, TMS I30572, and the susceptible clone TMS I30555, are an indication of allelic differences in their genes for resistance. As was expected, there were no significant differences between the resistant improved clones, which were derived from clone 58308. However, the improved clones I4(2)1425, I60142 and I90572 were different from three, one and five resistant landraces respectively. While no significant differences were detected due to reciprocal effects, the significant differences detected when either TMS I30555 or TMS I30572 was the female parent indicates that the expression of resistance is influenced by the nature of the female parent. The resistant landrace TME5 for instance, showed significant differences from three improved clones, TMS I4(2)1425, TMS I60142 and TMS I90572 and

landraces TME8, TME12 and TME14 when these were crossed with TMS I30555. This indicates the presence of minor allelic genes, which are influenced by specific crosses.

Significant differences between the resistant and susceptible clones imply that although the susceptible parents contributed some effective factors to resistance, their allelic effects were different from those of the resistant parents. Interestingly, the test did not reveal significant differences between susceptible clones TME2 and TME10, which were also different from TME117.

Allelic differences imply the possibility of TS and the presence of heterosis. Due to the polygenic and recessive nature of resistance to CMD reported earlier, Hahn *et al.* (1979) recommended population improvement and recurrent selection in breeding, as this would accumulate desirable gene combinations that would be difficult for the pathogens to circumvent in the long term. The use of non-allelic clones to induce TS would further increase resistance in a breeding population.

Number of effective factors

In this study, the parent clones did not represent the genotypic extremes. Furthermore, the analysis of variance revealed the presence of non-additive components, thus, the use of the range between the extreme F_1 progenies in the estimation of effective factors.

The minimum number of effective factors affecting a quantitative trait is expected to range from one to two times the haploid number of the species. However, linkage, gene interaction such as epistasis and the presence of transgressive segregants could cause a reduction in the estimated number of effective factors (Fenster and Ritland, 1994). Studies have shown that the absence of linkage assumed in the Castle-Wright formula is not usually attained (Zeng *et al.* 1992; Lynch and Walsh, 1998) and would account for an underestimation of the number of effective factors.

The few effective factors estimated for some crosses could be attributed to any of these factors since transgressive segregants and the presence of gene interaction from the significance of the SCA effects were detected. The number of effective factors was not estimated in crosses where there was little or no genetic variance, which may be due to the absence of differences in the genes or differences in gene action of the parents.

The number of effective factors estimated for the resistant by resistant crosses suggests that both the resistant improved clones and resistant landraces have equal potential in contributing resistant genes to their progenies. Furthermore, the presence of plus factors observed in progenies of resistant by resistant crosses indicates that the sources of resistance carry different genes that complement each other. The contribution of effective factors by both resistant and susceptible parents also indicates that resistance to CMD can be enhanced by continually combining elite genotypes regardless of their resistance status.

There are no previous reports on the number of effective factors responsible for resistance to CMD. Hahn *et al.* (1980b), inferred from the polygenic nature of resistance to CMD that resistance must be attributed to the combined action of the number of loci which are linked on a chromosome or a set of chromosomes of a genome or genomes. They, however, suggested that since cassava is genetically heterozygous and probably an allotetraploid, a study on the genetic mechanism of resistance to CMD would be complicated and difficult.

Virus strain causing CMD symptoms

The results of this study showed that ACMV was responsible for the disease symptom in the mapping population. Although mixed infections of ACMV and EACMV have been reported in West Africa (Ogbe *et al.*, 1999; Offei *et al.*, 1999), none was detected in the study using two primers. The ACMV primer ACMV-

F1/ACMV-R1 was more efficient in detecting the virus in the samples in that it detected the virus in more samples than the primer ACMV-AL F/ACMV-ARO R. However, the two primers did not detect the virus in a susceptible plant, which maybe due to a high concentration of DNA template, which would inhibit the amplification reaction.

The presence of the virus in some of the resistant samples suggests that field resistance was not necessarily an indication of resistance to virus infection. The A genome of the gemini-viruses encode a protein required for their replication and must recruit the remaining DNA replication mechanism from the host plant, while the B genome is responsible for spread and symptom production (Estessami *et al.,* 1991; Fontes *et al.*, 1992). Since DNA replication is part of the natural growth and development, it is possible that the virus is able to replicate and probably even spread in the resistant plant but the subsequent disease symptoms are inhibited.

Using field evaluation, axial bud inoculation and PCR, Ogbe (2001) concluded that field resistance as shown by lack of symptoms was not necessarily an indication of resistance to virus infection, but could be partly due to lack of virus multiplication, which suggests that field selection of resistance should be complemented with PCR or inoculation test.

Development of molecular markers associated with CMD resistance

The detection of multiple genes for virus resistance using segregation analysis alone is not efficient because of the differences due to genotype by environment interaction (McMullen and Louie, 1989). To determine molecular markers associated with resistance to CMD, three marker systems, RAPDs AFLPs and SSRs were employed, using bulk segregant analysis (BSA) and linkage analysis.

RAPD markers have the potential of detecting unique regions in the genome due to the random amplification of sites within the genome. In BSA, where, DNA samples are pooled from individuals which are similar for a particular trait, RAPD markers are particularly useful since it is expected that the low frequency allele(s) in the pooled DNA sample will not be amplified (Liu, 1999; Michelmore *et al.*, 1991). Using BSA, a RAPD marker was found to be linked with high cyanogenic potential (CNP) in cassava (Dossou-Yovo, 2000). In this study, however, none of RAPD markers assayed were able to detect any significant differences between the resistant and susceptible samples.

The AFLP experiments, as expected, generated several data points per assay. The different AFLP experiments were performed with BSA to generate large number of markers and the possibility of some association with resistance to CMD. Reamon-Buttner *et al.* (1998), for instance, detected nine AFLP markers tightly linked to the sex locus of the dioecious plant, *Asparagus officinalis* L, using the silver staining technique. In this study however, BSA did not reveal tight linkages between any AFLP marker and resistance to CMD.

Wong *et al.* (1999) reported that cassava has a GC rich genome, therefore, restriction enzymes such as *Taq1*, *Apa1* and *Pst1*, which recognise GC sites would be more suitable in AFLP analysis of cassava. The highest number of bands was detected with one of the *Apa1* experiments and the highest number of polymorphic bands was also obtained with another *Apa1/Taq1* experiment. The number of bands detected in this study with the silver staining detection technique which is affected by quality of reagents and water purity, were good compared with other AFLP studies on cassava which used radioactive detection (Fregene *et al.*, 2000; Roa *et al.*, 1997).

Wong *et al.* (1999) reported high levels of polymorphism among cultivars using the *Apa1/Taq1* restriction enzymes. Although the *Apa1/Taq1* experiment, generated the

highest number of polymorphism in the bulks, they gave very erratic results, which were not repeatable, thus no useful markers were detected for the subsequent genotyping of the entire mapping population. This could be due to the high degree of non-specific amplification due to the differences in the +3 selective primers and the selective amplification template, which can cause mismatches. Certain mismatches at the terminal 3' would allow amplification without a reduction in the amplification yield (Kwok *et al.*, 1990).

Mapping molecular markers associated with CMD resistance

The linkage analysis established associations between resistance to CMD and two markers, SSR30-180 and E-ACC/M-CAT225, and suggests the presence of a locus for resistance to CMD between the two markers. Both markers were donated by the resistant parent TME7; that shows that their linkage with CMD resistance is in coupling phase. The distance of 14.9 cM between SSR30180 and the converted resistance locus, however, suggests that the detected marker is not tightly linked to resistance. Its high association with resistance in the mapping population may be due to inheritance of that segment from TME7 in the resistant progeny. This indicates the need to saturate this region of the genome with more DNA markers, to obtain a marker that is more tightly linked to resistance to CMD.

While BSA and linkage analysis confirmed the presence of a locus associated with resistance to CMD on linkage group-X, the marker trait association test further showed the presence of another region on linkage group X-III associated with CMD resistance. The correlation coefficients for SSR30-180 and E-ACC/M-CTC-225 with CMD responses were negative while those for SSR119-A and SSR119-C were positive. This implies that the CMD resistance genes associated with these two regions of the genome are different.

The SSR marker SSR30-180 which explained about 58% of the total variation has also been identified in another cassava population from an African landrace, TME3 (known in Western Nigeria as 2nd Agric). In that study, the marker mapped on linkage group R of the male cassava framework map with a recombination distance of 8 cM from a dominant cassava resistance gene *CMD2* (IITA, 2000; Akano *et al.*, 2002). The differences in the two-recombination values may be due to the smaller population size in this study. The results of the two studies, however, imply that the resistant gene *CMD2* is related to the genes or a gene for resistance in TME7 and the other resistant clones with the marker. However, the presence of the marker in two susceptible clones also suggests that resistance genes in the landraces may be genetically related to non-resistance genes, with few differences in their sequences.

The differences between some resistant clones and TME7 with respect to the marker SSR/30-180 further suggest that different alleles may be involved in resistance to CMD. These differences could be utilised in breeding to enhance resistance, as was shown from the significant and negative SCA effects, significant and negative heterosis, and positive TS in some crosses.

Currently, further development of SSR markers involving untranslated regions of cassava ESTs for SSR repeats are underway (Mba *et al.*, 2001). Another project involves fine mapping the region of the cassava framework map at CIAT, followed by contig mapping with bacterial artificial chromosome (BAC) libraries (Fregene *et al.*, 2001). Since the markers, which had the highest association with CMD resistance were developed from microsatellite loci with CT repeats (Mba *et al.*, 2001), exploring more markers based on such repeats could facilitate efforts at fine mapping and isolating CMD resistance genes. Alternatively, high throughput markers such as EST obtained from cDNA clones of known resistance genes and SNPs, may be employed in mapping CMD resistance genes. Due to the high frequency of SNPs in the

genome, there is high possibility of them being closely associated with resistance (Lohmann *et al.*, 2000).

Applications of the cassava linkage map

In this study, three strategies for linkage analysis were tested and the single-combined map gave the most suitable results. In mapping heterozygous species, since markers generated in the F_1 progenies result from independent meiosis and crossing over in the maternal and paternal parents, individual maps are often constructed for each parent if the progeny numbers are large enough (Williams, 1998). When this was tested in this study, due to the number of markers for linkage analysis, the individual maps generated 5 and 7 linkage groups for the female and male respectively which was not adequate. Fregene *et al.* (1997) suggested that if a polyploid origin for the cassava genome is assumed, the expected number of linkage groups in cassava would depend on chromosome assortment. Eighteen groups are expected where preferential pairing is exclusive, up to 36 where there is mostly random pairing, and a number between 18 and 36 where there is a mixture of preferential and random pairing (Fregene *et al.*, 1997).

The 14 linkage groups generated for the single-combined map in this study indicate that the map is not condensed. Furthermore, the closest distance between any two adjacent markers was 14.9 cM. A dense map would have at least one marker in a 5 cM segment (Liu,1998). This again can be attributed to either insufficient number of markers or the size of the mapping population.

Fregene *et al.* (1997) further described a method to compensate for the random assignment of "present or absent" to alternate alleles at a locus and to detect linkage in repulsion. In their study, they included inverted scores of the female and male mapping data before linkage analysis. This technique also did give a condensed map, thus the single-combined map. A key observation in all three techniques

(separate male and female maps, single-combined map, and inclusion of inverted scores) for linkage analysis, resulted in the markers E-ACC/M-CTC-225 and SSR30-180 on the same linkage group and order with CMDL.

Despite the low saturation of the map, the information from it and the map itself can be utilised in cassava genetics. The loci were randomly distributed over linkage groups since the number of markers per linkage group correlated with the map distances per linkage group. Since the mapping population was segregating for resistance to CMD and several other traits, the markers could be used to map other traits of interest provided quantitative data are available on them. The markers could also be applied in studies with similar cassava populations. The AFLP primer pairs E-ACT/M-CAG which, contributed markers in four linkage groups could be used in experiments such as in diversity studies which require coverage of most of the genome. The map could also be used in linkage map pooling with other maps based on different parents such as the cassava framework map (Fregene *et al.*, 1997) or with one parent in common such as the population studied by Akano *et al.* (2002) to form a composite map of cassava.

A composite map could then be used in planning experiments, to construct a genome database, to compare QTL identities in different genetic backgrounds, and for comparative mapping with other species. Composite maps have been reported in *Arabidopsis thaliana* and in maize (Beavis and Grant, 1991; Hauge *et al.*, 1993). Comparative mapping is a strategy that uses information obtained from the study of a species with a simple genome to make inferences about the map position and function of genes in species with a complex genome (Brown, 1999). Comparative mapping has been used successful in human and mouse genome mapping to increase the efficiency of mapping (Liu, 1998). It has been suggested that comparative mapping has definite advantages in mapping plant genomes. For instance, genes in wheat with a genome size of 17,000 Mb would be mapped from

information obtained from a small member of the *Gramineae,* rice, with a genome size of 400 Mb (Gale and Devos, 1998; Liu 1998; Brown 1999).

Chapter Six

Conclusion

The genetic control of resistance to CMD in some African landraces and their relationship with the widely used resistant genetic stock, clone 58308, was evaluated in two genetic experiments in three environments in Nigeria. This provided information on the inheritance of resistance to CMD based on symptom severity in the cassava genotypes evaluated and showed how the different sources of resistance complement each other.

The importance of GXE interaction in the expression of resistance to CMD in the experimental material was demonstrated. The study showed that, depending on the population, both additive and non-additive gene effects were involved in the genetics of resistance to CMD. In the NCD II experiment involving the landraces, additive gene effect was more important while in the diallel involving clone 58308, both additive and non-additive gene effects were important. High estimates of heritability were obtained in both experiments, which shows that significant progress in breeding for resistance to CMD can be made by selection.

The best general combiner was I90257. This clone may be used as parent with any other cassava clone to obtain progenies with resistance to CMD. The clones I30572, TME8, and TME9, which also exhibited good general combining ability, may be used as parent to obtain progenies with resistance to CMD. However, due to the importance of GXE, presence of non-additive effects, the trend of heterosis, and the transgressive segregants detected, the best parent combinations should be decided after progeny testing in multiple environments.

The presence of multiple genetic factors or polygenic inheritance for resistance to CMD was also demonstrated in the study. The results showed that resistance to CMD in the clones studied is also recessive.

The study showed that both the resistant improved clones and resistant landraces have potential in contributing resistant factors to their progenies. The presence of PTS in the susceptible by resistant crosses and the contribution of effective factors by both resistant and susceptible parents indicate that resistance to CMD can be enhanced by continually combining elite genotypes. Allelic differences detected between some of the cassava clones implies that resistance to CMD in the breeding population can be further enhanced by making crosses with the clones with non-allelic resistant genes, to induce PTS which represent a potential source of novel genetic variations.

There is the need to saturate the region of the genome between the marker SSR30-180 and CMDL, and between SSR119-A and SSR119-C with more DNA markers, to obtain a marker, which is tightly linked with resistance to CMD. High throughput marker systems such as EST and SNPs could be applied.

The AFLP primer pair E-ACT/M-CAG, can be used in genetic experiments such as diversity studies which require coverage of most of the genome. The linkage map could be used to develop a consensus map of cassava that would be of importance to cassava research. This would allow researchers to identify informative experimental markers, QTL for trait of economic importance and generate a genomic database of cassava which could also be used for comparative mapping with other species.

References

Adejare, G.O. and R.H.A. Coutts, 1982. The isolation and characterisation of a virus from Nigerian cassava plants affected by the cassava mosaic disease and attempted transmission of the disease. *Phytopathologische Zeitschrift 103:198-210.*

Adejare, G.O. and R.H.A. Coutts, 1981. Eradication of cassava mosaic disease from Nigerian cassava clones by meristem-tip culture. *Plant Cell Tissue Organ Culture 1:25-32.*

Agyare-Tabbi, A., L.F. Pereira and L.R. Erickson, 1997. Isolation and characterisation of repetitive and microsatellite DNA sequences in cassava. *African Journal of Root and Tuber Crops 2:135-137.*

Ahiabu, R.K., Y. Lokko, K.E. Danso and G.Y.P. Klu, 1995. Mutagenesis for ACMV resistance in a Ghanaian cassava cultivar Bosom nsia. *Presented at the fourth. FAO/IAEA/Italy RCM on the improvement of basic food crops in Africa through plant breeding including the use of induced mutations.* Naples, Italy.

Ahloowalia, B.S., 1995. *In vitro* mutagenesis for the improvement of vegetatively propagated plants. Induced mutations and molecular techniques for crop improvement. *Proceedings IAEA/FAO International Symposium on the use of Induced Mutations and Molecular Techniques for crop Improvement, pg 531-541* IAEA, Vienna Austria.

Akano, A.O., A.G.O. Dixon, C.Mba, E. Barrera and M. Fregene, 2002. Genetic mapping of a dominant gene conferring resistance to the cassava mosaic disease (CMD). *Theoretical and Applied Genetics 105:521–525.*

Alagianagalingan, M.N. and K. Ramakrishaman, 1970. Studies on virus disease of tapioca (*M. esculenta* Crantz) carbohydrate metabolism. *Madgras Agric Journal 57:55-62.*

Allem A.C., 1994. The origin of *Manihot Esculenta* Crantz (Euphorbiaceae). *Genetic Resources and Crop Evolution 41:133-150.*

Allem A.C. and S.K. Hahn, 1991. Cassava germplasm strategies for Africa. In: *Ng. N.Q., P. Perrino, F. Attere and H. Zedan (Eds). Crop genetic resources of Africa vol. II. Proceedings of an international conference organised by the International Institute of Tropical Agriculture (IITA) and the National Research Concil Italy (NRC),in association with the International Board for Plant Genetic Resources (IBPGR) and the United Nation Environmental Programme (UNEP) held in Ibadan Nigeria 17-20 Oct 1998, pg 127-149*, Ibadan, Nigeria.

Amma, C. S. E., M.N. Sheela and P.K.T., Pillai, 1995. Combining ability heterosis and gene action for three major quality traits in cassava. *Journal of Root Crops 21(1):24-29*

Angel, F, D. Arias, J. Tohme, C. Iglesias and W. Roca 1993. Towards the construction of a molecular map of cassava (*Manihot esculenta* Crantz): comparison of restriction enzymes and probe source indetecting RFLPs. *Journal of Biotechnology 31:103-113.*

Arunachalam, V., 1976. Evaluation of diallel crosses by graphical and combining ability methods. *Indian Journal of Genetics and Plant Breeding, 36:358-366.*

Asiedu, R., K.V. Bai, R. Terauchi, A.G.O. Dixon and S.K. Hahn, 1992. Status of wide crosses in cassava and yam. *In: G. Thottappilly, L.M., Monti, D.R., Mohan and A.W., Moore (Eds). Biotechnology: Enhancing Research on Tropical Crops in Africa. CTA/IITA co-publication, pg. 63-68* IITA, Ibadan, Nigeria..

Awoleye F., M. van Duren, J. Dolezel and F.J. Novak, 1994. Nuclear DNA content in in vitro induced somatic polyploidisation cassava (*Manihot esculenta* Crantz) breeding. *Euphytica 76:195-202.*

Bai, K.B.V., 1982. Cytogenetics of tuber crops. In: *IITA (International Institute of Tropical Agriculture) Annual report for 1982*, Ibadan Nigeria.

Bai, K.B.V., 1987. Recent advances in cassava genetic and cytogenetics. In: Hershey C.H. (Ed.) *Cassava breeding a multidisciplinary review, Proceedings of a workshop held in the Philippines, 4-7 March 1985.* CIAT, Cali Colombia.

Bai, K.V., K. Asiedu and A.G.O. Dixon, 1993. Cytogenetics of *Manihot* species and interspecific hybrids. In: Roca, W.M., and A.M. Thro (Eds), *Proceedings of the first international meeting of the cassava biotechnology network. Cartagena de Indias Colombia, 25-28 August, 1992 pg 51-55. Working document No. 123.* CIAT, Cali, Colombia.

Baird, W.V., R.E. Ballard, S. Rajapakse and A.G. Abbott, 1996. Progress in *Prunus* mapping and application of molecular markers to germplasm improvement. *HortScience 31(7):1099-1106*

Baker, R.J., 1978. Issues in diallel analysis. *Crop Science 18(4):533-536.*

Barker, B., P. Zambryski, B. Staskawicz and S.P. Dinesh-Kumar, 1997. Signalling in plant-microbe interactions. *Science 276:726-733.*

Barrett, B.A. and K.K. Kidwell, 1998. AFLP-based genetic diversity assesment among wheat cultivars from the Pacific Northwest. Crop Science 38:1261-1271.

Beavis W.D and D. Grant, 1991. A linkage map based on information from four F2 populations in Maize (*Zea mays*). *Theoretical and Applied Genetics 82:636-644.*

Beckmann, J.S and M. Soller, 1986. Restriction fragments length polymorphism in plant genetic improvement. *Oxford Surveys of Plant Molecular and Cell Biology 3:197-250.*

Beeching, J.R., P. Marmey, M.C Gavalda, M. Noirot, H.R. Haysom, M.A. Hughes and A. Charrier, 1993. An assessment of genetic diversity within a collection of cassava (*Manihot esculenta* Crantz) germplasm using molecular markers. *Annals of Botany 72:515-520.*

Beil G.M and R.E. Aitkins, 1967. Estimates and specific combining abilities in F_1 hybrids for grain yield and its components in grain sorghum, *Sorghum vulgare* Pers. *Crop Science 7:225-228.*

Berrie, L.C., K.E. Palmer, E.P. Rybicki and M.E.C. Rey, 1998. Molecular characterisation of a distinct South African cassava infecting geminivirus. *Archives of Virology 143:2253-2260.*

Best, R. and G. Henry, 1994. Cassava Towards Year 2000. In. *International Network for cassava Genetic resources. Colombia, 18-23 August 1992. International Crop. Network series No. 10 International Plant Genetic Resources Inst., pg 12-15.* Rome, Italy.

Bock, K.R., 1994. Control of African cassava mosaic geminivirus by using virus-free planting material. *Tropical Science 34:102-109.*

Bock, K.R. and B.D. Harrison, 1985. African cassava mosaic virus. *Association of Applied Biologists Description of Plant Viruses* No. 297.

Bokanga, M., 1994. Processing cassava leaves for human consumption. *Acta Horticulturae 375:203-207.*

Bokanga, M., 1998. Cassava flour and chips: Commodities for African development in the 21st century. *Presented at the 7th Triennial symposium of the International society for tropical root crops, Africa Branch. 11-17 Oct. 1998, Cotonou, Bènin.* IITA, Ibadan, Nigeria.

Bonierbale, M., C. Guevara, A.G.O. Dixon, N.Q. Ng, R. Asiedu and S.Y.C. Ng, 1997a. Cassava. In: D. Fuccillo, L. Sears and P. Stapleton (Eds). *Biodiversity in trust conservation and use of plant genetic resources in CGIAR centres. Cambridge University Press. pg 1-20.* Cambridge, UK.

Bonierbale, M.W., R.L. Plaisted and S.D. Tanksley. 1993. A test of populations of alfalfa *(Medicago sativa)* using RAPD molecular the maximum heterozygosity hypothesis using molecular markers in tetraploid potatoes. *Theoretical and Applied Genetics 86:481–491.*

Bonierbale, M., A.C. Roa, M.M. Maya, M.C. Duque and J. Tohme, 1997b. Assessment of genetic diversity in Manihot species with AFLPs. *African Journal of Root and Tuber Crops 2:139.*

Botstein, D., R.L. White, M. Skolnick, and R.W. Davis, 1980 Construction of a genetic map in man using restriction fragment length polymorphism. *American Journal of Human Genetics 32:314-330.*

Brown, T.A. 1999. Genomes. *BIOS Scientific Publication, 472pg.* New York USA.

Brummer, E.C., 1999. Capturing heterosis in forage crop cultivar development. *Crop Science 39:943-954.*

Bubeck, D.M., M.M. Goodman, W.D. Beavis and D. Grant, 1993. Quantitative trait loci controlling resistance to gray leaf spot in maize. *Crop Science 33:838-847.*

Burr, B. and F.A. Burr, 1991. Recombinant inbreds for molecular mapping in maize: theoretical and practical considerations. *Trends in Genetics 7:55-60.*

Burrow, M.D. and T.K. Blake, 1998.. Molecular tools for the study of complex traits. In: A.H. Paterson (Ed) *Molecular dissection of complex traits. CRC Press LLC . pg 13-30.* Boca Raton, FL, USA.

Byrne, D., 1987. Breeding cassava. In: Russell (Ed), *Plant Breeding Reviews 2:73-133.*

Caetano-Anollés, D., B.J. Bassam, and P.M. Gresshoff. 1991. DNA amplification fingerprinting using very short arbitrary oligonucleotide primers. *Biotechnology 9: 553-557.*

Calvalho, I.J.M.B., J.C.M. Cascardo., P.S. Cimera., M.C.M Ribeiro., A.C. Allem and J.J. Fialho, 1993. Study of DNA polymorphism in *Manihot esculenta* and related species In: Roca, W.M., and A.M. Thro (Eds), *Proceedings of the first international meeting of the cassava biotechnology network. Cartagena de Indias Colombia, 25-28 August, 1992 pg 56-58. Working document No. 123.* CIAT, Cali, Colombia..

Calvert, L.A., M.I. Cuervo, M.D. Ospina, C.M. Fauquet, and B.C. Ramirez, 1996. Characterisation of cassava common mosaic virus and a defective RNA species. *Journal of General Virology. 77:525-530.*

Caranta, C. and A. Plaloix, 1996. Both common and specific factors are involved in polygenic resistance of pepper to several potyviruses. *Theoretical and Applied Genetic 92:15-20.*

Castle, W.E, 1921. An Improved method of estimating the number of genetic factors concerned in cases of blending inheritance. *Science 54:223.*

Changa, 1992. The non-systemicity component of resistance to the African cassava mosaic virus in cassava germplasm and its epidemiological implications. *MSc Thesis University of Ibadan 94 pg.*

Chavarriaga-Aguirre, P. M.M. Maya, M.W. Bonierbale, S. Kresovich M.A. Fregene, J. Tohme and G. Kochert, 1998. Microsatellites in cassava *(Manihot esculenta* Crantz): discovery, inheritance and variability. *Theoretical and Applied Genetics 97:493–501.*

Chavarriaga-Aguirre, P.M. M. Maya, J. Tohme, M.C. Duque, C. Iglesias, M.W. Bonierbale, S. Kresovich and G. Kochert, 1999. Using microsatellites, isozymes and AFLPs to evaluate genetic diversity and redundancy in the cassava core collection and to assess the usefulness of DNA-based markers to maintain germ-plasm collections. *Molecular Breeding 5:263–273.*

Chen, X., S. Temnykn, Y. Xu, Y.G. Cho and S.R. Mc Couch, 1997. Development of a microsatellite framework map providing genome coverage in rice (*Oryza sativa* L.) *Theoretical and Applied Genetics* 95:553-567.

Cock, J.H., 1985a. Cassava, new potential for a neglected crop. West View Press, Inc., Boulder, Colorado. USA pg 191.

Cock, J.H., 1985b. Cassava: A basic energy source in the tropics. In: *Cassava research production and utilisation: Preliminary edition. Cassava program, Centro International de Agriculture Tropical (CIAT), pg. 1-29.* CIAT, Cali, Colombia.

Cock, J.H and R.H. Howeler, 1978. The ability of cassava to grow on poor soils. In: *Jung G.A. (ed).* Crop tolerance to sub-optimal land conditions. *American Society of Agronomy, pg 145.* Madison, Wisconsin, USA.

Cockerham, C.C., 1963. Estimation of genetic variances. In: *Hanson, W.D. and H.F Robinson (ed). Statistical genetics and plant breeding. NRC Publication 982. National Academy of Science, National Research Council,. pg 53-93.* Washington DC, USA.

Cockerham, C.C., 1986. Modifications in estimating the number of genes for a quantitative character. *Genetics 114:659-664.*

Cockerham, C.C. and B.D. Weir, 1977. Quadratic analysis of reciprocal crosses. *Biometrics 33:187-203.*

Collins, N.C., C.A. Webb, S. Seah, J.G. Ellis, S.H. Hulbert and A. Pryor, 1998. The isolation and mapping of disease resistance gene analogs in maize. *Molecular Plant Microbe Interaction 11:968-978.*

Comstock, R.E. and H.F. Robinson, 1948. The components of genetic variance in populations of biparental progenies and their use in estimating the average dominance. *Biometrics 4:254-266.*

Concibido, V.C., D.A. Lange, R.L. Denny, J.H. Orf and D. Young, 1994. DNA marker analysis of loci underlying resistance to soybean cyst nematode (*Heterodera glycines* Ichinohe). *Crop Science 34:240-246.*

Correa, R.X, M.R. Costa, P.I. Good-God, V.A. Ragagnin, F.G. Faleiro, M.A. Moreira and E.G. de Barros, 2000. Sequence characterised amplified regions linked to rust resistance genes in common bean. *Crop Science 40:804-807.*

Cours-Darne, G., 1968. Improving cassava in Africa. *The Abidjan Conference. Agricultural Research Priorities for Economic Development in Africa. 2: 330-339.*

Cox, D.J. and K.L., Frey, 1984. Combining ability and selection of parents for interspecific oat matings. *Crop Science 24:963-967.*

Cukadar-Olemedo, B., J.F., Miller and J.J. Hammond, 1997. Combining ability of the stay green and seed moisture content in sunflower. *Crop Science 37:378-382.*

Danesh, D., S. Aarons, G.E. McGill and N.D. Young, 1994. Genetic dissection of oligogeneic resistance to bacterial wilt in tomato. *Molecular Plant Microbe Interaction 7:464-471.*

de Kochko, A.B., R.N, Verdaguer and C.M. Fauquet, 1998. Cassava vein mosaic virus (CsVMV), type species for a new genus of plant double stranded DNA viruses? *Archives of Virology, 143:945-962.*

Dixon, A.G.O, P.J. Bramel-Cox and T.L. Harvey, 1990. Diallel analysis of resistance in sorghum to greenbug biotype E: antibiosis and tolerance. *Crop Science 30:1055-1059.*

Dixon, A.G.O, R. Asiedu and M. Bokanga, 1994. Breeding cassava for low cyanogenic potential. Problems progress and prospects. *Acta Horticulturae 375:153-161.*

Dixon, A.G.O., P.J. Bramel-Cox and T.L. Harvey, 1991. Complementarity of genes for resistance to green bug (*Schizaphis graminum* (Rondani)), biotype E, in sorghum ((*Sorghum biocolor* (L)) Moench). *Theoretical and Applied Genetics 81:105-110.*

Dorosh, P., 1988. The economics of root and tuber crops in Africa. *Resource and Crop Management Program (RCMP) Research Monograph No.1 IITA,* Ibadan, Nigeria.

Dossou-Yovo, I, 2000. Identification de facteurs moleculaires controlant l'accumulation de glucosides cyanogeniques dans le manioc: *PhD Thesis University of Paris IV. 193 pg.*

Dudley, J.W., 1993. Molecular markers in plant improvement: Manipulation of genes affecting quantitative trait. Crop *Science 33:660-668.*

Ehrlich, H.A, R. Gibbs and H.H. Kazazian, 1989. Polymerase chain reaction. *Cold Spring Habour Press.* Cold Spring Harbour, NY, USA.

Estessami, P, K. Saunder, J. Watt and J. Stanley, 1991. Mutational analysis of complementary sense genes of ACMV DNA-A. *Journal of General Virology 72:1005-1012.*

Falconer, D.S. and T.F.C. Mackay, 1997. Introduction to quantitative genetics. *4th edition. Longman Scientific and Technical co.,* Essex, England. *464pg.*

FAO, 1997. FAO food outlook. *No. 3/4. pg. 21-25.*

FAO, 2000. http:// www.FAO.org

Fargette, D., L.T. Colon, R. Bouveau and C. Fauquet, 1996. Components of resistance of cassava to African cassava mosaic virus. *European Journal of Plant Pathology 102: 645-654.*

Fargette, D., C. Fauquet, E. Grenier and J.M. Thresh, 1990. The spread of African cassava mosaic virus into and within cassava fields. *Journal of Phytopathology 130: 289-302.*

Fargette, D., C. Fauquet and J.C. Thouvenel, 1988. Yield losses induced by *African cassava mosaic virus* in relation to the mode and the date of infection. *Tropical Pest Management* 34: 89–91.

Fargette, D., C. Fauquet and J.C. Thouvenel, 1985. Field studies on the spread of African cassava mosaic. *Annals of Applied Biology 106, 285-294.*

Fargette, D., M. Jeger, C. Fauquet, and L.D.C. Fishpool. 1994. Analysis of temporal disease progress of African cassava mosaic virus. *Phytopathology 84:91-98.*

Fargette, D. and K. Vié, 1988. Yield losses induced by African cassava mosaic virus in relation to mode and date of infection. *Tropical Pest Management 34:89-91.*

Fargette, D. and K. Vié, 1994. Modelling the temporal primary spread of African cassava mosaic virus into plantings. *Phytopathology 84: 378 – 382.*

Fargette, D. and K. Vié, 1995. Simulation of the effects of host resistance, reversion and cutting selection on incidence of cassava mosaic virus and yield losses in cassava. *Phytopathology 85: 370-375.*

Fauquet, C and R.N., Beachy, 1991. Cassava virus and genetic engineering. *International Cassava Trans Project (CTA) 30 pg.*

Fauquet, C. and D. Fargette, 1990. African cassava mosaic virus: aetiology, epidermiology and control. *Plant Diseases 74(6): 404-411.*

Fauquet, C., D. Fargette and J.C. Thouvenel, 1987a. Selection of healthy cassava plants obtained by reversion in cassava fields. In*: The International Seminar on African Mosaic Disease and its Control, Yamoussoukro, Côte d'Ivoire 4-8 May, 1987, . pg. 146-149.* CTA, FAO, ORSTOM, IITA, IAPC publication. Wageningen The Netherlands.

Fauquet, C., D. Fargette and J.C. Thouvenel, 1987b. The resistance of cassava to African cassava mosaic. In: *The International Seminar on African Mosaic Disease and its Control, Yamoussoukro, Côte d'Ivoire 4-8 May 1987. pg 183-188.* CTA, FAO, ORSTOM, IITA, IAPC publication. Wageningen, The Netherlands.

Fauquet, C., D. Fargette and J.C. Thouvenel, 1988. Some aspects of the epidermiology of African cassava mosaic virus in Ivory Coast. *Tropical Pest Management 34: 92-96.*

Fenster C.B. and K. Ritland, 1994. Quantitative genetics of mating system divergence in the yellow monkeyflower species complex. *Heredity 73:422-435.*

Feyt, H., 1976. Etude critque de l'analyse des croisements dialles au moyen de la simulation. Annales de l'Amelioration des Plantes *26:173-193.*

Fondong, V.N., F.S. Pita, M.E.C. Rey, A. de Kochko, R.N. Beachy and C.M. Fauquet, 2000. Evidence of synergism between African cassava mosaic virus and a new double-recombinant geminivirus infecting cassava in Cameroon. *Journal of General Virology 81:287-297.*

Fontes, E.P.B, V.A Luckow, L. Hanley-Bowdoin 1992) A geminivirus replication protein is a sequence specific DNA binding protein. *Plant Cell 4:597-608.*

Fox, S.L., P.D. Brown and J. Chong, 1997. Inheritance of crown rust resistance in four accessions of *Avena sterilis* L. *Crop Science 37:342-345*

Fraser, R.S.S., 1990. Disease resistance mechanisms. *In*: C.L. Mandachar and C.L. Mandachar (eds) *Plant Viruses Volume II. Pathology, pg 321-345, CRC Press, Boca Raton, FL., USA.*

Fregene, M.A., 1996. Phylogeny of cassava (*Manihot esculenta* Crantz) and its wild relatives based on restriction fragment length polymorphism (RFLP) analysis. *PhD Thesis University of Ibadan, Ibadan Nigeria.*

Fregene, M., F. Angel, R. Gomez, F. Rodriguez, P. Chavarriaga, M. Bonierbale, W. Roca and J. Tohme, 1997. A molecular genetic map of cassava (*Manihot esculenta* Crantz). *Theoretical and Applied Genetics 95:431-441.*

Fregene, M.A., F. Angel, R. Gomez, F. Rodriguez, M. Mayer, J. Tohme, M. Bonierbale, C. Iglesias, and W.M. Roca, 1994. A linkage map of cassava (*Manihot esculenta* Crantz) based on RFLP and RADP markers. In: *International Cassava Biotechnology Network: Proceedings of the 2nd. International Scientific. Meeting, Bogor Indonesia 22-26 August 1994.*

Fregene, M., A. Bernal, M. Duque, A. Dixon and J. Tohme, 2000. AFLP analysis of African cassava (*Manihot esculenta Crantz*) germplasm resistant to the cassava mosaic disease (CMD). *Theoretical and Applied Genetics 100: 678-685*

Fregene, M., E. Okgbenin, C. Mba, F. Angel, M.C. Suarez, J. Guitierez, P. Chavarriaga, W. Roca, M. Bonierbale and J. Tohme, 2001. Genome mapping in cassava improvement:: Challenges, achievements and opportunities. *Euphytica 120: 159-165.*

Fritz, A.K., S. Caldwell and W.D. Worall, 1999. Molecular mapping of Russian wheat aphid resistance from triticale accession PI38156. *Crop Science 39:1707-1710.*

Gale, M.D and K.M. Devos, 1998. Plant comparative genetics after 10 years. *Science 282:656-659.*

Garcia, G.M., H.T., Stalker, E. Shroeder and G. Kochert, 1996. Identification of RAPD, SCAR and RFLP markers tightly linked to nematode resistance genes introgressed from *Arachis cardenasii* into *Arachis hypogaea*. *Genome 39:836-845.*

Gardener, C.O. and S.A. Eberhart, 1966. *Biometrics 22(3):439-452.*

Geddes, A. M., 1990. The relative importance of crop pests in sub-Saharan Africa. *Bulletin 36; Chatham Natural Resource Institute (NRI).*

Gentzbittel L., S. Mouzeyar, S. Badaoui, E. Mestries, F. Vear, D.T. de Labrouhe, P. Nicolas, 1998. Cloning of molecular markers for disease resistance in sunflower, *Helianthus annuus* L. *Theoretical and Applied Genetics 96:519-525.*

Gibson,. R.W., 1994. Long-term absence of symptoms in heat-treated African cassava mosaic geminivirus infected resistant cassava plants. *Tropical Science 34:154-158.*

Gilbert, N.E., 1973. Biometrical Interpretation. Clarendon Press, Oxford. pg 125.

Giovannoni, J.J., R.A. Wing, M.W. Ganal,. S.D. Tanksley, 1991. Isolation of molecular markers from specific chromosomal intervals using DNA pools from existing mapping populations. *Nucleic Acids Research 19(23):6553-6558.*

Gomez, R. F. Angel, M.W. Bonierbale, F. Rodriguez, J. Tohme and W.M. Roca, 1996. Inheritance of random amplified polymorphic DNA markers in cassava (*Manihot esculenta* Crantz) *Genome 39:1039-1043.*

Grattapagalia, D., C. Costa de Silva, and N.M.A. Nassar, 1996. Strict apomix, in cassava (*Manihot esculenta* Crantz). *Canadian Journal of Plant Science 76:379-382*

Gravois, K.A., 1994. Diallel analysis of head rice percentage, total milled rice percentage, and rough rice yield. *Crop Science 34:42-45.*

Griffing, B., 1956a. A generalized treatment of the use of diallel crosses in quantitative inheritance. *Heredity 10: 31-50.*

Griffing, B., 1956b. Concept of general and specific combining ability in relation to diallel crossing systems. *Australian Journal of Biological Science 9: 463-493.*

Gulick, P., C. Hershey and J. Esquinas-Alcazar, 1983. Genetic Resources of cassava and wild relatives. IBPGR report 82/III. *International Board for plant genetic resource,* Rome Italy.

Hahn, S.K. 1978. Breeding cassava for resistance to cassava mosaic disease and bacterial blight in Africa. In: H. Maraite and Y.A. Meyer (Eds), *Diseases of Tropical Food Crops, Proceedings. International Symposium at the University Catholique Louvain pg 211-219.* Louvain La Neuve, Belgium.

Hahn, S.K. and A.K. Howland, 1972. Breeding for resistance to cassava mosaic disease. In: Terry, E.R (Ed) *Proccedings, Cassava mosaic workshop, Ibadan Nigeria pg 37-39.* IITA, Ibadan Nigeria.

Hahn, S.K., A.K. Howland and E. R. Terry, 1980a. Correlated resistance of cassava to mosaic and bacterial blight disease. *Euphytica 29:305-311*

Hahn S.K., C. John, G. Isoba and T. Ikoun, 1989. Resistance breeding in root and tuber crops at the International Institute for Tropical Agriculture (IITA), Ibadan Nigeria. *Crop Protection 8:147-168.*

Hahn, S.K., E.R. Terry and K. Leuschner, 1980b. Breeding cassava for resistance to cassava mosaic virus disease. *Euphytica 29:673-683*

Hahn, S. K., E.R. Terry, K. Leuschner, I.O. Akobundu, C. Okali and R. Lal, 1979. Cassava improvement in Africa. *Field Crops Research. 2:193-226.*

Haley, S.D., L.K. Afanador, P.N. Miklas, J.R. Stavely and J.D. Kelly. 1994. Heterogeneous inbred populations are useful as source of near-isogenic lines for RAPD marker localisation. *Theoretical and Applied Genetics 88:337-342.*

Haley, S.D., P.N. Miklas, J.R. Stavely, J. Byrum and J.D. Kelly, 1993. Identification of RAPD markers linked to a major rust resistance gene block in common bean. *Theoretical and Applied Genetics 86:505-512.*

Hallauer A.R. and J.B. Miranda, 1988. Quantitative genetics in maize breeding. *2nd Ed Iowa State University 468pg.* Ames, USA.

Hamada, H., M.G. Pertino, and T. Kakunaga. 1982. A novel repeated element with Z-DNA- forming potential is widely found in evolutionary diverse eukaryotic genomes. *Proceedings, National Academy of Science (USA) 79:6465-6469.*

Harrison, B.D., Y.L. Liu, X. Zhou, D.J. Robinson, L. Calvert, C. Munoz and G.W. Otim-Nape, 1997b. Properties, differentiation and geographical distribution of geminiviruses that cause cassava mosaic disease. *African Journal of Root and Tuber Crops 2:19-22.*

Harrison, B.D. and D.J. Robinson, 1988. Molecular variation in vector-borne plant viruses: epidemiological significance. *Philosophical Transactions of the Royal Society of London 321:447-462.*

Harrison, B.D., X. Zhou, G.W. Otim-Nape, Y. Liu and D.J. Robinson, 1997a. Role of a novel type of double infection in the geminivirus-induced epidemic of severe cassava mosaic in Uganda. *Annals of Applied Biology 131:437-448.*

Hauge, B.M, S.M. Haney, S. Cartinhour, J.M. Cherry, H.M. Goodman, M. Koornneof, P. Stam, C. Chang, S. Kempin, L. Medrano and E.M. Meyerowitz, 1993. An intergrated genetic /RFLP map of *Arabidopsis thaliana* genome. *The Plant Journal 3(5):745-754* .

Hayman, B.I., 1954a. The analysis of variance of diallel tables. *Biometrics 10: 235-244.*

Hayman, B.I., 1954b. The theory and analysis of diallel crosses. *Genetics 39: 789-809*

Haysom, H.R., T.L.C. Chan and M.A. Hughes, 1994. Phylogenetic relationships of *Manihot* species revealed by restriction fragment length polymorphism. *Euphytica 76:277-234.*

Hershey C., 1987. Cassava germplam resources. *Proceedings workshop on cassava breeding: A multidisciplinary review. The Philippines, 4-7 March 1985.* CIAT, Cali Colombia.

Hoefer Scientific, 1992 Operating instructions TKO 100 minifluorometer. Hoefer Scientific Instruments, San Francisco CA., USA. 50pg.

Holland, J.B., 2001. Epistasis and plant breeding. In: *Janick, J. (Ed). Plant Breeding Reveiws. 21:27-91.*

Holloway, J. and S. Knapp, 1994. GMendel 3.0.Users Guide. knapps@css.orst.edu

Hong, Y.G., D.J. Robinson and B.D. Harrison, 1993. Nucleotide sequence evidence for the occurrence of three distinct whitefly-transmitted geminiviruses in cassava. *Journal of General Virology 74: 2437-2443.*

Horvat, F. and M. Verhoyen, 1981. Cytological modifications and presence of virus-like particles in cells of *Nicotinana benthamiana* (Domin) and *Manihot utilissima* (Pohl.) infected with the geminivirus isolated from cassava infected with cassava African mosaic disease. *Parasitica 37: 121-130.*

Hussain, A., W. Bushuk., H. Ramirez and W. Roca, 1987. Identification of cassava (*Manihot esculenta* Crantz) cultivars by electrophoretic patterns of esterase enzymes. *Seed Science Technolgy 15: 19-21.*

Iglesias C.A and C.H. Hershey, 1991. True cassava seed: research for a production alternative. In: Ofori, F and S.K. Hahn (Eds).*Tropical root crops in a developing economy. Proceedings 9th symposium of the international society of tropical crops 20-26 Oct 1991, Accra, Ghana. pg. 164-171.* Wageningen, The Netherlands.

IITA, 1973. In house report 1972. Root and tuber crop improvement programme. *International Institute for Tropical Agriculture, Ibadan Nigeria pg 17-23.*

IITA, 1988,1980,1997, 1998, 2000. Annual Report of the International Institute of Tropical Agriculture. *IITA Ibadan Nigeria.*

IITA, 1990. Cassava in tropical Africa. A reference manual. *Chayce Publication Services, United Kingdom Edition.* Baling & Mansell International, Wisbech UK. 196 pg.

Janessen, P., R. Coopman, G. Huys, J. Swings, M. Bleeker, P. Voss, M, Zabeau and K. Kersters, 1996. Evaluation of the DNA fingerprinting method AFLP as a new tool in bacteria taxonomy. *Microbiology 142:1881-1893.*

Jeffrey, A.J., V. Wilson, and S.L. Thein. 1985. Hypervariable minisatellite regions in human DNA. *Nature 314:67-73.*

Jeger, M.J and S.L.H. Viljanen-Rollinson, 2001. The use of area under the disease progress curve (AUDPC) to assess quantitative disease resistance in crop cultivars. *Theoretical and Applied Genetics 102:32-40.*

Jennings, D.L., 1960. Observations on virus diseases of cassava in resistant and susceptible varieties: I Mosaic disease. *Empire Journal of Experimental Agriculture* 28: 23–34.

Jennings, D.L., 1977. Inheritance of linked resistance to African cassava mosaic and bacteria blight. In: *Proceedings Cassava Protection workshop. CIAT. Colombia pg 45-50.*

Jennings, D.L., 1976. Breeding for resistance to African cassava mosaic. *In: African cassava mosaic Report of an interdisciplinary workshop held at Muguga, Kenya, pg. 60-87.* IITA, Ibadan, Nigeria

Jennings, D.L., 1994. Breeding for resistance to African cassava mosaic virus in East Africa. *Tropical Science 34:110-122.*

Jennings, D.L. and C.H. Hershey, 1985. Cassava breeding: a decade of progress from international programmes. In: *Russell G.E. (Ed.) Progress in Plant Breeding I. Butterworths, Cambridge pg 89-116.*

Jinks, J.L. and B.I. Hayman, 1953. The analysis of diallel crosses. *Maize Genetics Cooperative Newsletter* 27: 48-54.

Jos, J.S., and N.G. Nair, 1979. Pachytene pairing in relation to pollen fertility in five cultivars of cassava. *Cytologia (Tokyo) 44:813-820.*

Jos, J.S., K.V. Bai and N.G. Nair, 1984. Unreported symptoms associated with mosaic disease in cassava. *Indian Phytopathology* 37:696-699.

Kaiser, W.J. and L.R. Teemba, 1979. Use of tissue culture and thermotherapy to free East African cassava cultivars of African cassava mosaic and cassava brown streak disease. *Plant Disease Reporter 63:780-784.*

Kaiser, W.J. and R. Louie, 1982. Heat therapy of cassava infected with African cassava mosaic disease. *Plant Disease 66: 475-477.*

Kanazin, V., L.F. Marek, and R.C. Shoemaker, 1996. Resistance gene analogs are conserved and clustered in soybean. *Proceedings of National Academy of Science (USA) 93:11746-11750.*

Kang, M.S. 1994. Applied quantitative genetics. *2477 Creekside Drive Baton Rouge. 96 pg.* Baton Rogue, FL., USA.

Kang, M.S., 1998. Using Genotype by Environment interaction for crop cultivar development. *Advances in Agronomy, 62:199-258.*

Kang, M.S, A. Kushairi, Y. Zhang and R. Magari, 1999. Combining ability for rind puncture resistance in maize. *Crop Science 39:368-371.*

Kawano, K., 1978. Genetic improvement of cassava (*Manihot esculenta* Crantz) for productivity. *Tropical Agriculture Research Series. II. Ministry of Agriculture and Forestry, Japan. 21 pg.*

Kawano, K., 1980. Cassava. In Hybridisation of crop plants. American Society of Agronomy and Crop Science Society of America, pg 255-233. Madison, Wisconsin. USA.

Kelly, J.D., 1995. Use of random amplified polymorphic DNA markers in breeding for major gene resistance to plant pathogens. *HortScience 30: 461-465.*

Kempthorne, O. 1956. The theory of the diallel cross. *Genetics, 41: 451-459.*

Kempthorne, O. and R.N. Curnow, 1961. The partial diallel cross. *Biometrics, 17: 229-250.*

Kiehne, K., and D.B. Neale, 1998. DNA pooling strategy for saturation mapping in outcrosses. *Molecular Breeding 4:179-185.*

Knapp, S.J., W.W. Stoup and W.M. Ross, 1985. Exact confidence intervals for heritability on a progeny means basis. *Crop Science 25:192-194.*

Kosambi, D. D. 1944. The estimation of map distances from recombination values. *Annals of Eugenics 12:172-175.*

Kwok, S. D.E. Kellogg, N. McKinney, D. Spasic, L. Goda, C. Levenson and J.J. Sninsky, 1990. Effects of primer-template mismatches on the polymerase chain reaction: human immunodeficiency virus type 1 model studies. *Nucleic Acids Research 18 (4): 999-1005.*

Laminski, S., E.R. Robinson and V.M. Gray, 1997: Application of molecular markers to describe South African elite cassava cultivars. *African Journal of Root and Tuber Crops 2:132-134.*

Lancaster, P.A. and J.E. Brooks, 1983. Cassava leaves as human food. *Economic Botany 37(3):33-348.*

Lande, R., 1981. Minimum number of genes contributing to quantitative variation between and within populations. *Genetics 99:541-553.*

Lander, E.S. and D. Botstein, 1986. Mapping complex genetic traits in humans: New method using a complex RFLP linkage map *Cold Spring Harbor Symposia on Quantitative Biology 51: 49-62.*

Lander, E.S., P. Green, J. Abrahamson, A. Barlow, M.J. Daly, S.E. Lincoln, and L. Newberg, 1987. MAPMAKER: An interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. *Genomics 1:174-181.*

Lawrence, P.L and K.J. Frey, 1976. Inheritance of grain yield in oats species crosses *Avena sativa* L. x *A. sterilis* L. *Egyptian Journal of Genetics and Cytology 5:400-409.*

Lefevre, F. and A. Charrier, 1993. Isozyme diversity within African *Manihot* germplasm. *Euphytica 66:73-80.*

Legg, J.P., 1994. *Bemisia tabaci*: The whitefly vector of cassava mosaic geminiviruses in Africa: An ecological perspective. *African Crop Science Journal* 2: 437-448.

Leonards-Schippers, C., W. Gieffers, R. Schaufe-Pregl, E. Ritter, S.J. Knapp, F. Saamini and C. Gebhardt, 1994. Quantitative resistance to *Phytphthora infestans* in potato: a case study of QTL mapping in an allogamous plant species. *Genetics 137:67-77.*

Leuschner, K., 1978. Whiteflies: biology and transmission of African cassava mosaic disease. In. T. Brekelbaum, A. Belloti and T.C. Lozano (Eds.). *Proceedings of the Cassava Protection Workshop, CIAT, Cali, Colombia 7-12 November 1977, pg 51-58.* CIAT, Colombia:

Littell, R.C., G.A. Miliken, W.W. Stoup and R.D. Wolfinger, 1996. SAS system for mixed models. *SAS Institute Inc., Cary, North Carolina, USA. 633 pg.*

Liu, B. H., 1997. Statistical Genomics: Linkage mapping and QTL analysis. CRC Press LLC, 611 pg. Boca Raton, Florida, USA.

Liu, B. H., 1998. Computational tools for study of complex traits. In A.H. Paterson (Ed) *Molecular dissection of complex traits. CRC Press LLC pg 43-79.* Boca Raton, Florida, USA

Lohmann, S. L. Lehmann and K. Tabiti, 2000. Fast and flexible single nucleotide polymorphism (SNP) dtection with the LightCycler System. *Biochemica 4:23:28.*

Lokko, Y., A.G.O. Dixon and S.K. Offei, 1998. Combining Ability of resistance to the cassava mosaic virus disease. In M.O. Akorada and J.M. Ngeve (Eds). Root Crops in the 21st Century. *Proceedings of the 7th Triennial conference of the International Society for Tuber and Root Crops, Africa Branch,. 11 –17 Oct. 1998 Cotonou, Benin, pg 438-442.* IITA Ibadan, Nigeria.

Lozano, J.C. 1992. Overview of integrated control of cassava diseases. *Fitopatologia Brasileira 17:18-22.*

Lynch, M. and B. Walsh, 1998. Genetic Analysis of Quantitative traits. *Sinauer Associates Inc.,* Sunderland, MA., USA *985 pg.*

Magoon, M.L. 1967. Recent trends in cassava breeding in India. In: University of west Indies. In*: E.A. Tai, W.B. Charles, P.H. Haynes, E.F. Iton, K.A. Leslie (Eds) Proceedings first International Symposium of Tropical Root Crops, held at the University of West Indies, vol 2 pg 100-117* St. Augustine, Trinidad.

Magoon, M.L., and R.K., Krishnan, 1977. Extending the frontiers of genetic improvement in cassava. In: Leaky L.A. (ed.). *Proceedings third symposium of the international society for tropical root crops. IITA Ibadan, Nigeria, 2-9 Th. Dec 1973, pg. 14-18.*

Magoon, M.L., R. Krishnan and K.V. Bai, 1969. Morphology of pachytene chromosome and meiosis in *Manihot esculenta* Crantz. *Cytologia 34:612-626.*

Maluszynski, M., B.S. Ahloowalia, B. Sigurbjornsson, 1995. Application of *in vivo* and *in vitro* mutation techniques for crop improvement. *Euphytica 85:303-315.*

Mandal, R.C., 1993. Tropical root and tuber crops. Cassava (tropical), sweetpotato, aroids, yam yam beans and coleus. *Agrobotanical Publishers pg 130-133.* Bikane, India

Manly, K.F. and E.H. Cudmore Jr., 1996. New versions of MapManager genetic mapping software. *Plant genome IV (Abs) pg105.*

Marmey, P. J.R., Beeching., S. Hammon and A. Charrier, 1994. Evaluation of cassava (*Manihot esculenta* Crantz) germplasm using RAPD markers. *Euphytica 74:203-209.*

Mather, K. and J.L. Jinks, 1982. *Biometrical Genetics: The Study of Continuous Variation. Longman, London. 396 pg.*

Matzinger, D.F. and O. Kempthorne, 1956. The modified diallel table with partial inbreeding and interactions with environment. *Genetics 41:822-833.*

Mba, R.E.C, P. Stephenson, K. Edwards, S. Melzer, J. Nkumbira, U. Gullberg, K. Apel, M. Gale, J. Tohme and M. Fregene, 2001, Simple sequence repeat (SSR) markers survey of the cassava (*Manihot esculenta* Crantz) genome: towards an SSR-based molecular genetic map of cassava *Theoretical and Applied Genetics 102:21–31.*

McIntosh, M.S., 1983. Analysis of combined experiments. *Agronomy Journal 75:153-155.*

McMullen, MD, M.W. Jones, K.D. Simcox and R. Louie. 1994 Three genes control resistance to wheat streak mosaic virus in the maize inbred Pa405. *Molecular plant. Microbe Interaction 7:708-712.*

McMullen, M.D. and R. Louie. 1989. Linkage of molecular markers to a gene controlling the symptom response in maize to maize dwarf mosaic virus. *Molecular plant. Microbe Interaction 2(6):309-314.*

Melchinger, A.E. 1990. Use of molecular markers in breeding for oligogenic disease resistance. *Plant Breeding 104:1-19.*

Melchinger, A.E., L. Kuntze, R. K. Gumber, T.L. Übberstedt and E. Fuchs 1998. Genetic basis of resistance to sugarcane mosaic virus in European maize germplasm *Theoretical and Applied Genetics 96:1151-1161.*

Michelmore, R.W, 1995 Molecular approaches to manipulation of disease resistance genes. *Annual Review of Phytopathopathology 15:393-427.*

Michelmore, R. W., I. Paran and R.V. Kesseli, 1991. Identification of markers linked to disease-resistant genes by bulked segregant analysis: A rapid method to detect markers in specific genomic regions by using segregating populations. *Proceedings of National Academy of Science (USA) 88:9828-9832.*

Mignouna, H.D. and A.G.O Dixon, 1997. Genetic relationships among cassava clones with varying levels of resistance to the African mosaic disease using RAPD markers. *African Journal of Root and Tuber Crops 2 (1&2):28-32.*

Miklas, P.N., L. Afanador and J.D. Kelly, 1996. Recombination-facilitated RAPD marker-assisted selection for disease resistance in common bean. *Crop Science 36:86-90.*

Miklas, P.N., J.R. Stavely, and J.D. Kelly, 1993. Identification and potential use of a molecular markers for rust resistance in common bean. *Theoretical and Applied Genetics 85:745-749.*

Moll, R.H., W.S. Salhuana and H.F. Robinson, 1962. Heterosis and genetic diversity in variety crosses of maize. *Crop Science 2:197-198.*

Morgante, M. and A. M. Olivieri, 1993. PCR-amplified microsatellites as markers in plant genetics. *Plant Journal 1:175-182.*

Msabaha, M.A.M., 1983. Genetic relationship between resistance to mosaic and bacterial blight disease, in cassava. (*M. esculenta* Crantz). *PhD Thesis University of Ibadan. 215 pg.*

Msikita, W., B. James, E. Nnodu, J. Legg, K. Wydra and F. Ogbe, 2000 Disease Control in Cassava Farms IPM Field Guide for Extension Agents. *International Institute of Tropical Agriculture, Ibadan Nigeria. 26 pg.*

Muehlbauer, G.J, J.E., Specht, M.A. Thomas-Compoton, P.E, Staswick, and R.L Bernard, 1988. Near-isogenic lines-A potential resource in the integration of conventional and molecular marker linkage maps. *Crop Science 28:729-735.*

Multize, D.K. and R.J. Barker, 1985. Evaluation of biometrical methods for estimating the number of genes. *Theoretical and Applied Genetics 69:559-566.*

Nassar, N.M.A., 1994. Development and selection of apomixis in cassava. *Canadian Journal of Plant Science 74(4)857-858.*

Ng, S.Y.C., G. Thottappilly and H.W. Rossel, 1992. Tissue culture in disease elimination and micropropagation. *In:* G. Thottappilly, L.M. Monti, D.R. Mohan Raj, A.W. Moore (eds.) *Biotechnology: Enhancing Research on Tropical Crops in Africa*, pg *171-182, CTA/IITA co-publication. IITA, Ibadan, Nigeria.*

Nguyen, H.T. and D.A Sleper, 1983. Genetic variability of seed yield and reproductive characters in tall fescue. *Crop Science 23:621-626.*

Nian C.A., S. Abraham, L.A. Bia and A. Kariachen, 1976. Lipid metabolism in cassava mosaic infected *Manihot esculenta* Crantz. *4th Symposium. Tropical Root Crops. CIAT, Cali Colombia 1976.* Cali, Colombia.

Nichols, R.F.W., 1947. Breeding cassava for virus resistance. *East African Agricultural Journal 15:154-160.*

Njock, T.E., 1994. Epidemiology of disease recovery phenomenon of ACMV in resistant and susceptible cassava clones. *PhD Thesis, University of Ibadan, Ibadan, Nigeria. 194pg.*

Njock, T.E., G.I. Atiri, G. Thottappilly and J.M. Thresh, 1996. Disease development and recovery in resistant and susceptible cassava genotypes infected by African cassava mosaic geminivirus. *African Plant Protection 2:97-102.*

Njock, T.E., G.I. Atiri, G. Thottappilly and S.K. Hahn, 1994. Distribution and disease recovery of African cassava mosaic virus (ACMV) in stems of resistant and susceptible cassava. *In: Proceedings of the fifth Triennial Symposium of the International Society of Tropical Root Crop, Africa Branch (ISTRC-AB)*, pg *149-152.* IITA, Ibadan, Nigeria.

Ocampo, C., C.H. Hershey C.A. Iglesias and M. Iwansa, 1993. Esterase Isozyme fingerprinting of CIAT cassava germplasm collection In: Roca, W.M., and A.M. Thro (Eds), *Proceedings of the first international meeting of the cassava biotechnology network. Cartagena de Indias, Colombia, 81-89 August, 1992 pg 51-55. Working document No. 123.* CIAT, Cali, Colombia.

Offei, S. K., M. Owuna-Kwakye and G. Thottappilly, 1999. First report of East African cassava mosaic begomovirus in Ghana. *Plant Disease 83:877.*

Ogbe, F.O., 2001. Survey of cassava begmoviruses in Nigeria and the response of resistant cassava genotypes to African cassava mosaic begmovirus infection. *PhD Thesis University of Ibadan, Nigeria. 197 pg.*

Ogbe, F.O., G.I. Atiri, D. Robinson, A.G.O. Dixon, S. Winter, F.M. Quin and G. Thottappilly, 1999. First report of East African cassava mosaic begomovirus in Nigeria. *Plant Disease 83(4):398.*

Ogbe, F.O., E.C. Nnodu and S.O, Odurukwe, 1996. Control of African cassava mosaic disease incidence and severity. *Tropical Science 36(2):174-181.*

Ori, N., Y. Eshed, I. Paran, G. Presting, D. Aviv, S. Tanksley, D. Zamir, and R. Fluhr, 1997. The *I*2C family from wilt disease resistance locus I2 belongs to the nucleotide binding, leucine-rich repeat superfamily of plant resistance genes. *The Plant Cell 9:521-532.*

Oslen, K.M. and B.A. Schaal 1999. Evidence on the origin of cassava: Phylogeography of *Manihot esculenta. Proceedings of the National Academy of Science USA 96:5586-5591.*

Otim-Nape, G.W., A. Bua and Y. Baguma, 1994b. Accelerating the transfer of improved production technologies: Controlling *African cassava mosaic virus* disease epidemic in Uganda. *African Crop Science Journal 2: 479-495.*

Otim-Nape, G.W., J. Shaw and M. Thresh, 1994a. Effects of African cassava mosaic geminivirus on the growth and yield of cassava in Uganda. *Tropical Science 34:43-54.*

Otim-Nape, G.W., J.M. Thresh, A. Bua, Y. Baguma and M.W. Shaw, 1998. Temporal spread of cassava mosaic virus disease in a range of cassava cultivars in different agro-ecological regions of Uganda. *Annals of Applied Biology 133:415-430.*

Paterson, A., J. DeVerna, B. Lanini, and S. Tanksley. 1990. Fine mapping of quantitative trait loci using selected overlapping recombinant chromosomes, in an interspecies cross of tomato. *Genetics 124:735-742.*

Paterson, A.H, E.S. Lander, J.D., Hewitt, S. Peterson, S.E. Lincoln and S.D. Tanksley. 1988. Resolution of quantitative trait into mendelian factors by using a complete RFLP linkage map. *Nature 335:721-726.*

Pedersen, J.F., F.A. Haskins, H.J., Gorz and R. Brittons, 1983. Variability for traits used to estimate silage quality in forage sorghum hybrids. *Crop Science 23:376-379.*

Pillay, M., and S.T., Kenny, 1996. Random amplified polymorphic DNA (RAPD) markers in hops, *Humulus lupulus*: level of genetic variability and segregation in F_1 progeny. *Theoretical and Applied Genetics 92:334-339.*

Pooni, H.S., J.L., Jinks, and R.K. Singh, 1984. Methods of analysis and the estimation of the genetic parameters from a diallel set of crosses. *Heredity* 52 (2):243-253.

Porto, M.C., R. Asiedu, A.G.O. Dixon, and S.K. Hahn, 1994. An agroecological oriented introduction of cassava germplasm from Latin America into Africa. In: Ofori, F and S.K. Hahn (Eds).*Tropical root crops in a developing economy. Proceedings 9th symposium of the international society of tropical crops 20-26 Oct 1991, Accra, Ghana.* Wageningen, The Netherlands.

Powell, W., G.C. Machray and J. Provan, 1996b Polymorphism revealed by simple sequence repeats. *Trends in Plant Science 1:215-222.*

Powell, W., M. Morgante, C. Andre, M. Hansafey, J. Vogel, S. Tingey and A. Rafalski, 1996a. The comparison of RFLP, RAPD, AFLP and SSR (microsatellite) markers for germplasm analysis. *Molecular Breeding 2:225-238.*

Promega, 2000. Technical manual silver staining ™ DNA sequencing system. Promega Corporation, Madison, WI. USA. 24 pg.

Qiagen, 1999. DNeasy ™ Plant Mini Handbook for DNA isolation from plant tissue. QIAGEN Ltd. Crawley, West Sussex, UK. 22 pg.

Rajendran, P.G., 1989. Combining ability in cassava. *Journal of Root Crops. 15(1):15-18..*

Raji, A.A., 1995. Evaluation of pest and disease reactions and trait association in some landraces of cassava. *MSc Thesis, University of Ibadan, Ibadan, Nigeria, 86 pg.*

Ramirez, H., A. Hussain, W. Roca and W. Bushuk, 1987. Isoenzyme electrophorogram of sixteen enzymes in five tissues of cassava (*Manihot esculenta* Crantz) varieties. *Euphytica 36: 39-48.*

Reamon-Buttner, S.M., J. Schondelmaier and C. Jung, 1998. AFLP markers tightly linked to the sex locus in *Asparagus officinalis* L. *Molecular Breeding 4:91-98.*

Rhode, W. 1996. Inverse sequence-tagged repeat (ISTR) analysis a novel and universal PCR-based technique for genome analysis in the plant and animal kingdom. *Journal of Genetics &. Breeding 50:249-261.*

Rick, C.M., and P.G. Smith, 1953. Novel variation in tomato species hybrids. *The American Naturalist 88:359–373.*

Roa, A., P. Chavarriaga-Aguirre, M.C. Durque, M.M. Maya, M.W. Bonierbale, C. Iglesias and J. Tohme, 2000. Cross-species amplification of cassava (*Manihot esculenta*) (Euphorbiaceae) microsatellites: Allelic polymorphism and degree of relationship. *American Journal of Botany 87(11):1647-1655.*

Roa, A., M.M. Maya, M. Durque, M., J. Tohme, A. Allem, and M. Bonierbale, 1997. AFLP analysis of relationships among cassava and other *Manihot* species. *Theoretical Applied Genetics 95:741-750*

Robinson, D.J., B.D. Harrison, J.C. Sequeira, and G.H. Duncan, 1984. Detection of strains of African cassava mosaic virus by nuclei acid hybridisation and some effects of temperature on their multiplication. *Annals of Applied Biology 105:483-493.*

Rooney, W.L., D.Z. Skinner and J.O. Fritz, 1997. Combining ability for protein degradability in Alfalfa. *Crop Science 37:128-131.*

Ross, W.M, H.J. Gorz, F.A. Haskins, G.H. Hookstra, J.K. Rutto and R. Ritter, 1983. Combining ability effects for forage residue traits in grain sorghum hybrids. *Crop Science 23:97-101.*

Rossel, H.W., R. Asiedu, and A.G.O. Dixon 1992. Resistance of cassava to African cassava mosaic virus: What really pertains. *Tropical Root Tuber Crops Bulletin 6:2.*

Rusell, J.R., J.D. Fuller, M. Macaulay, B.G. Hatz, A. Jahoor, W. Powell and R. Waugh, 1997. Direct comparison levels of genetic variation among barley accessions detected by RFLP's, AFLP's, SSR's and RAPD. *Theoretical and Applied Genetics 95:714-722.*

Sambrook, J., E.F. Fritsch, and T. Maniatis, 1989. Molecular cloning: a laboratory manual. 2nd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, USA.

Sanchez G., S. Restrepo, M. Duque, M. Fregene, M.W. Bonierbale and V. Verdier, 1999. AFLP assessment of genetic variability in cassava accessions (*Manihot esculenta* Crantz) resistant and susceptible to the cassava bacterial blight (CBB) *Genome 42:163-172.*

SAS, 1997. SAS/STAT Software. Changes and enhancements through Release version 6.12. *SAS institute, Cary, North Carolina, USA. pg 1028.*

SAS Institute, 1999. SAS companion for Microsoft windows environment, version 6, *1st ed. SAS Institute, Cary, North Carolina, USA.*

Sax, K. 1923 The association of size differences with seed-coat patterns and pigmentation in *Phaseolus vulgaris. Genetics 8:552-560.*

Schaal, B., L.J.C.B. Carvalho, T. Prinzie, K. Olsen, P. Olson, G. Cabral and M. Hernandez, 1997. Phylogenetic relationships among Manihot species. Africa. *African Journal of Root and Tuber Crops 2:147-149.*

Schöpke, C., R. Carcano, R.N. Beachy and C. Fauquet, 1997. Plant regeneration from transgenic and non-transgenic suspension cultures of cassava (*M. esculenta* Crantz). *African Journal of Root and Tuber Crops 2:194-195.*

Second, G., A.C. Allem, L. Emperarie, C. Ingram, C. Columbo, R.A. Mendes and L.J. C.B. Carvalho, 1997. AFLP based *Manihot* and cassava numerical taxonomy and genetic structure analysis in progress: Implications for dynamic conservation and genetic mapping. *African Journal of Root and Tuber Crops 2:140-146.*

Seif, A.A., 1982. Effect of cassava mosaic virus on yield of cassava. *Plant Disease 66:661-662.*

Seo, Y.W., J.W. Johnson, and R.L. Jarret, 1997. A molecular marker associated with H21 Hessian fly resistance gene in wheat. *Molecular Breeding 3:177-181.*

Shen, K.A., B.C. Meyers, M.N. Islam-Faridi, D.B. Chin, D.M. Stelly, R.W. Michelmore, 1998. Resistance genes candidates identified by PCR with degenerate oligonucleotide primers map to clusters of resistance genes in lettuce. *Molecular Plant Microbe Interaction 11:815-823.*

Singh, R.K. and B.D. Chaudhary, 1985. Biometrical methods in quantitative genetics analysis. Kalyan Publications 3rd Ed. Pg 205-220.

Silvestre, P. and M. Arraudeaus, 1983. Le manioc. *ACCT, coll. Techniques, Agricoles et Productions Tropicales.* Maisonneuve & Larose, Paris, France.

Soller, M. and J.S. Beckmann, 1983. Genetic polymorphism in varietal identification and genetic improvement. *Theoretical and Applied Genetics. 67:25-33.*

Sprague, G.F., and L.A. Tatum, 1942. General versus specific combing ability in single crosses of corn. *Journal of the American Society of Agronomy 34:923-932.*

Stam, P. 1993. Construction of integrated genetic linkage maps by means of a new computer package: JoinMap. *Plant Journal 3: 739-744.*

Stanley, J., T. Frischmuth and S. Ellwood, 1990. Defective DNA amelioration symptoms of geminivirus infection in transgenic plants. *Proceedings National Academy of Science (USA) 87:6291-6295.*

Staskawicz, B.J, F.M. Ausbel, B.J. Barker, J.G. Ellis and J.D.G. Jones, 1995. Molecular genetics of plant disease resistance. *Science 268:661-667.*

Staub, J.E, J.C. Serquen and M. Gupta, 1996. Genetic markers, mapconstruction and their application in plant breeding. *HortScience 31(5):729-740.*

Storey, H.H. and R.F.W. Nichols, 1938. Studies on the mosaic disease of cassava. *Annals of Applied Biology, 25:790-806.*

Suiter, .A., J.F. Wendell, and J.S.S. Case. 1983. Linkage-1. A PASCAL computer program for the detection and analysis of genetic linkage. *Journal of Heredity 74: 203-204.*

Tanksley, S.D., 1993. Mapping polygenes. *Annual Review of Genetics 27:205-233.*

Tanksley, S.D., R. Bernatzsky, N.L. Lapitan, and J.P. Prince, 1988. Conservation of gene repertoire but not gene order in pepper and tomato. *Proceedings of National Academy of Science USA 85: 6419-6423.*

Tanksley, S.D., N.D. Young., A.H Paterson and M.W. Bonierbale, 1989. RFLP mapping in plant breeding: New tools for an old science. *Bio/Technology 7:257-264.*

Terry, E.R. and S.K. Hahn, 1980. The effect of cassava mosaic disease on growth and yield of a local and an improved variety of cassava. *Tropical Pest Management 26:34-37.*

Théberge, R.L., F.E Caveness, K.M. Lema, and J.S. Yaninek, 1985. Common African pests and diseases of cassava, yam, sweetpotato and cocoyam. IITA, Ibadan, Nigeria. 108pg.

Thankappan, M. and C.I. Chacko, 1976. Effect of cassava mosaic on the different plant parts and tuber yield in cassava. *Journal of Root Crops* 2: 45-47.

Thoday, J.M. 1961. Location of polygenes. *Nature 191:368-370.*

Thomas ,C.M, D.A. Jones, M. Parniske, K. Harrison, P.J. Balint-Kurt, K. Hatzixanthis and J.D.G. Jones, 1997. Characterisation of the tomato Cf-4 gene for resistance to *Cladosporium fulvum* identifies sequences that determine recognition specificity in Cf-4 and Cf-9. *The Plant Cell 9:2209-2224.*

Thomas, J. E. P.R. Massalski and B.D. Harrison, 1986. Production of monoclonal antibodies to African cassava mosaic virus and differences in their reactivities with other white-fly transmitted geminiviruses. *Journal of General Virology 67:2739-2748.*

Thresh, J.M., D. Fargette and G.W. Otim-Nape, 1994a. Effect of African cassava mosaic geminivirus on yield of cassava. *Tropical Science 34:26-42.*

Thresh, J.M., L.D.C Fishpool, G.W. Otim-Nape and D. Fargette, 1994b. African cassava mosaic diseases: an underestimated and unsolved problem. *Tropical Science 34:3-14.*

Thresh, J.M. and G.W. Otim-Nape, 1994. Strategies for controlling African cassava mosaic geminivirus. *Advances in Disease Vector Research* 10: 215-236.

Thresh, J.M., G.W. Otim-Nape and D. Fargette, 1998b. The components and deployment of resistance to cassava mosaic virus disease. *Integrated Pest Management Reviews 3:209-224.*

Thresh, J.M., G.W. Otim-Nape, J.P. Legg and D. Fargette, 1997. *African cassava mosaic virus* disease: The magnitude of the problem. *African Journal of Root and Tuber Crops 2:13-18.*

Thresh, J.M., G.W. Otim-Nape and D. Fargette, 1998a. The control of African cassava mosaic virus disease: phytosanitation and/or resistance? In: A. Hadidi, R.K. Khetarpal and H. Koganezawa (eds.*), Plant Virus Disease Control. pg 670-677, APS Press.* St. Paul. Minnesota, USA.

Umemura, Y. and K. Kawano, 1983. Field assessment and inheritance of resistance to cassava bacterial blight. *Crop Science 23:1127-1132.*

Vogel, J.M., A. Rafalski, W. Powell, M. Morgante, C. Andre. M. Hanafey, and S.V. Tingey. 1996. Application of genetic diagnostics to plant genome analysis and plant breeding. *HortScience 31:1106-1107.*

Vos, P., R. Hogers, M. Bleeker, M. Reijans, T. Van de Lee, M. Hornes, A. Frijters, J. Pot, J. Peleman, M. Kuiper and M. Zabeau, 1995. AFLP: a new technique for DNA fingerprinting. *Nucleic Acid Research 21:4407-4414*

Vuylsteike, D., 1981. Onderzoek op enkele agronomiche en fysiologische aspekten van cassava en zoete bataat. *MSc Thesis, Katholiete Universiteit,* Leuven, Belgium..

Wang, G.L, D.J., Mackill, J.M., Bonman, S.R., Couch, M.C., Champoux and R.J. Nelson. 1994. RFLP mapping of genes conferring complete and partial resistance to blast in a durably resistance rice cultivar. *Genetics 136:1421-1434.*

Wang, Y, R.L. Nelson and Y, Hui. 1998. Genetic analysis of resistance to soybean mosaic virus in four soybean cultivars from China. *Crop Science 38:922-925.*

Wang, G.L, D.L. Ruan, W.Y. Song, S. Sideris, L. Chen, L.Y. Pi, S. Zhang, Z. Zhang, C. Fauquet, B.S. Gaut, M.C. Whalen and P.C, Ronald. 1998. Xa21D encodes a receptor-like molecule with a leucine-rich repeat domain that determines race specific recognition and is subject to adaptive evolution. *The Plant Cell 10:765-769.*

Wanyera N.W.M., 1993. Phylogenetic relationships among cultivated cassava (*Manihot esculenta* Crantz) and two wild *Manihot* species. *PhD Thesis University of Ibadan, Ibadan Nigeria 193 pg.*

Welsh, J. and M. McClelland, 1990. Fingerprinting genomes using PCR with arbitrary primers. *Nucleic Acid Research 18:7213-7218.*

West, C.E., F. Pepping and C.R. Temalilwa, 1988. The composition of foods commonly eaten in East Africa. *Wageningen Agriculture University. The Netherlands.*

Wiiliams, C.G., 1998. QTL mapping in outbreed pedigrees. *In; Paterson A.H (Ed). Molecular dissection of complex traits CRC Press CLL, pg 81-94,* USA.

Willams, J.G.K., A.R. Kubelik, K.I. Livak, J.A. Rafalski and S.V. Tingey, 1990. DNA polymorphism amplified by arbitrary primers are useful as genetic markers. *Nucleic Acid Research 18:6531-6535.*

Wong, H.L. H.H. Yeoh, S.W. Lim, 1999. Customisation of AFLP analysis for cassava varietal identification. *Phytochemistry 50:919-924*

Wricke, G. and W.E. Weber, 1986. Quantitative genetics and selection in plant breeding. Walter de Gruyter, New York, USA. 406pg.

Wright,. S., 1968. Evolution and Genetics of Populations, vol. 1. Genetic and Biometric Foundations. *University of Chicago Press, Chicago, USA.*

Wu, K.K, W. Burnquist, M.E. Sorrels, T.L. Tew, P.H. Moore, S.D. Tanksley, 1992. The detection and estimation of linkage in polyploids using single dose restriction fragments. *Theoretical and Applied Genetics* 81:471-476.

Xiao, J., J. Li, L. Yuan and S.D. Tanksley, 1995. Dominance is the major genetic basis of heterosis in rice as revealed by QTL analysis using molecular markers. *Genetics 140:745–754.*

Xu, Y., S. R. McCouch, and S. Zongtan 1998. Transgressive Segregation of Tiller Angle in Rice Caused by Complementary Gene Action. *Crop Sciences 38:12–19.*

Yaninek, J.S., G.J. de Moraes, and R.H. Markham, 1989. Handbook on the cassava green mite *Mononychellus tanajoa* in Africa. *International Institute of Tropical Agriculture, Ibadan, Nigeria. 210 pg.*

Young, N.D., 1996. QTL mapping and quantitative disease resistance in plants. *Annual. Review of Phytopathology 34:479-501.*

Young, N.D., D. Zamir, M.W. Gana, and S.D. Tanksley, 1988. Use of near isogenic lines and simultaneous probing to identify DNA markers tightly linked to the Tm-2a gene in tomato. *Genetics 120:579-585*

Yu, S.B., J.X Li, Y.E. Tan, Y.J. Gao, X.H. Li, O.F. Zhang and M.A Saghai-Maroof, 1998. Importance of epistasis as the genetic basis of heterosis in an elite rice hybrid. *Proceedings National Academy of Science (USA) 94:9226-9231.*

Zeng, Z., 1992. Correcting the bias of Wright's estimate of the number of genes affecting a quantitative character: A further improved method. *Genetics 131:987-1001.*

Zeng, Z.B., D. Houle, and C.C. Cockerham, 1990. How informative is Wright's estimator of the number of genes affecting a quantitative character? *Genetics 126:235-247.*

Zhang, G., E.R. Angels, M.L.P. Abenes, G.S. Klush and N. Huang, 1996. RAPD and RFLP mapping of the bacteria blight resistance gene xa-13 in rice. *Theoretical and Applied Genetics 93:65-70.*

Zhang, Y. and M.S. Kang, 1997. Diallel-SAS: A SAS program for Griffing's diallel analyses. *Agronomy Journal 89:176-182.*

Zhou, X., Y. Liu, L. Calvert, C. Munoz, G.W. Otim-Nape, D.J. Robinson and B.D. Harrison, 1997. Evidence that DNA-A of a geminivirus associated with severe cassava mosaic disease in Uganda has arisen by inter-specific recombination. *Journal of General Virology 78:2101-2111.*

Appendix I

Appendix Ia The physical and chemical properties of soil on which genetic experiments were planted

Soil parameter	Site Mokwa 1998	Ibadan 1998	Ibadan 1999
Part sand (%)	76	89	82
Size silt (%)	9	5	13
Clay (%)	15	6	5
pH (H_2O 1:1)	5.4	6.1	5.6
Organic C (%)	0.7	0.96	1.69
Total N (%)	0.032	0.086	0.104
C/N ratio	8.4	11	16
Bray-1 P (mg/Kg)	0.4	5.2	18.2
Exchangeable Ca (cmol (+)/Kg)	1	1.2	3.5
Exchangeable Mg (cmol (+)/Kg)	0.3	0.3	0.7
Exchangeable K (cmol (+)/Kg)	0.03	0.2	0.4
Exchangeable Na (cmol (+)/Kg)	0.1	0.1	0.3
Exchangeable Mn (cmol (+)/Kg)	0.02	0.1	0.2
Exchangeable Acid (cmol (+)/Kg)	0.3	0.3	0.5
Effective cation exchange capacity (ECEC)	1.7	2.1	5.4
Cu (ppm)	0.5	0.8	3.1
Zn (ppm)	Trace	3	19.3
Mn (ppm)	30.4	37.1	89.6
Fe (ppm)	2.9	10.6	16.8

Appendix Ib Rainfall and temperature data for the Mokwa 1998/1999, Ibadan 1998/1999 and Ibadan 1999/2000 growing seasons*

	Mokwa 1998/1999					Ibadan1998/1999					Ibadan 1999/2000				
	Total		Temperature °C			Total		Temperature °C			Total		Temperature °C		
Month	Rainfall (mm)	No Rainy Days	Min	Max	Mean	Rainfall (mm)	No Rainy Days	Min	Max	Mean	Rainfall (mm)	No Rainy Days	Min	Max	Mean
JUN	376.6	11	22.4	32.4	27.4	158.1	17	22.9	31	26.9	255.8	20	22.4	30.6	26.5
JUL	154.5	6	22.8	31.3	27.05	71.9	17	22.8	28.3	25.5	267	19	22.6	29.5	26.1
AUG	121	5	28.3	30.3	29.3	50.8	14	22.2	28.1	25.1	99.3	12	22.3	28.7	25.5
SEP	224.8	6	21.4	31.4	26.4	143.6	16	23	29	26	180.4	17	22.2	28.9	25.6
OCT	153.3	9	21.1	33	27.05	178.7	17	23.7	30.6	27.2	309.2	18	22.3	30.1	26.2
NOV	0	0	16	35.1	25.55	31.7	5	24.3	33.2	28.7	36.9	3	22.8	32.3	27.5
DEC	0	0	16.9	37	26.95	11.4	5	21.7	32.4	27	0	0	10.6	17.2	13.9
JAN	0	0	17.3	34.8	26.05	0	0	22.5	33.4	28	11.7	2	22.1	33.2	27.6
FEB	0	0	21.1	37.5	29.3	86.3	7	22.5	34.3	28.4	0	0	19.7	34.7	27.2
MAR	0	0	23.9	38.6	31.25	105.5	11	23.2	32.7	28	96.5	5	22.4	35.8	29.1
APR	38.5	3	25.1	38.6	31.85	176.8	14	23.4	32.4	27.1	123.5	10	22.9	32.8	27.9
MAY	139	8	23.2	34.2	28.7	130.8	18	22.9	32.3	27.6	87.3	7	22.6	32	27.3
JUN	148.3	6	23.7	33.6	28.65	255.8	20	22.4	30.6	26.5	163.9	11	21.7	30.1	25.9
JUL	215.5	6	23.5	31.6	27.55	267	19	22.6	29.5	26.1	231.6	12	21.4	28.4	24.9

* The data were made available by the Agroclimatology Unit of the International Institute of Tropical Agriculture, Ibadan, Nigeria.

Appendix IIa Pearson Correlation Coefficients for CMD severity responses in genetic material across environments

	MST6WK	MCMD6WK	MST12WK	MCMD12WK	MST20WK	MCMD20WK	ST6WKI	CMD6WKI	ST12WKI	CMD12WKI	ST20WKI	CMD20WKI
MST6WK		0.923**	0.866**	0.865**	0.670**	0.644**	0.849**	0.666**	0.761**	0.697**	0.635**	0.509**
MCMD6WK	0.923**		0.816**	0.879**	0.607**	0.588**	0.869**	0.823**	0.737**	0.755**	0.618**	0.486**
MST12WK	0.866**	0.816**		0.931**	0.803**	0.840**	0.699**	0.534**	0.894**	0.757**	0.740**	0.702**
MCMD12WK	0.865**	0.880**	0.931**		0.702**	0.712**	0.744**	0.637**	0.876	0.865**	0.709**	0.622**
MST20WK	0.670**	0.607**	0.803**	0.702**		0.868**	0.532**	0.391**	0.711**	0.610**	0.912	0.739**
MCMD20WK	0.644**	0.589**	0.840**	0.713**	0.868**		0.530**	0.403**	0.793**	0.664**	0.807**	0.8942**
ST6WKI	0.848**	0.869**	0.699**	0.744**	0.532**	0.530**		0.838**	0.774**	0.770**	0.614**	0.490**
CMD6WKI	0.666**	0.822**	0.534**	0.636**	0.390**	0.403**	0.838**		0.635**	0.704**	0.520**	0.397**
ST12WKI	0.761**	0.737**	0.894**	0.875**	0.711**	0.793**	0.774**	0.635**		0.897**	0.767**	0.760**
CMD12WKI	0.697**	0.755**	0.757**	0.865**	0.609**	0.664**	0.770**	0.704**	0.897**		0.666**	0.659**
ST20WKI	0.635**	0.618**	0.739**	0.708**	0.912**	0.807**	0.614**	0.520	0.767**	0.666**		0.828
CMD20WKI	0.509**	0.485**	0.702**	0.621**	0.738**	0.894**	0.489**	0.397**	0.760**	0.659**	0.828**	

MST6WK	Mean shoot-tip CMD severity at 6 WAP	ST6WKI	Mean shoot-tip CMD incidence at 6 WAP
MST12WK	Mean shoot-tip CMD severity at 12WAP	ST12WKI	Mean shoot-tip CMD incidence at 12WAP
MST20WK	Mean shoot-tip CMD severity at 20 WAP	ST20WKI	Mean shoot-tip CMD incidence at 20 WAP
MCMD6WK	Mean whole plant CMD severity at 6 WAP	CMD6WKI	Mean whole plant CMD incidence at 6 WAP
MCMD12WK	Mean whole plant CMD severity at 12WAP	CMD12WKI	Mean whole plant CMD incidence at 12WAP
MCMD20WK	Mean whole plant CMD severity at 20 WAP	CMD20WKI	Mean whole plant CMD incidence at 20 WAP

Appendix IIb Pearson Correlation Coefficients for CMD severity responses in genetic material in Mokwa 1998

	MST6WK	MCMD6WK	MST12WK	MCMD12WK	ST6WKI	CMD6WKI	ST12WKI	CMD12WKI
MST6WK		0.891**	0.726**	0.760**	0.937**	0.695**	0.771**	0.703**
MCMD6WK	0.891**		0.683**	0.780**	0.890**	0.901**	0.785**	0.786
MST12WK	0.726**	0.683**		0.926**	0.571**	0.387**	0.952**	0.811**
MCMD12WK	0.760**	0.780**	0.926**		0.612**	0.500**	0.931**	0.937**
ST6WKI	0.937**	0.890**	0.571**	0.612**		0.829**	0.732**	0.686**
CMD6WKI	0.695**	0.901**	0.387**	0.500**	0.829**		0.631**	0.644**
ST12WKI	0.771**	0.785**	0.952**	0.931**	0.732**	0.631**		0.912**
CMD12WKI	0.703**	0.786**	0.811**	0.937**	0.686**	0.644**	0.912**	

MST6WK Mean shoot-tip CMD severity at 6 WAP
MST12WK Mean shoot-tip CMD severity at 12WAP
MCMD6WK Mean whole plant CMD severity at 6 WAP
MCMD12WK Mean whole plant CMD severity at 12WAP
ST6WKI Mean shoot-tip CMD incidence at 6 WAP
ST12WKI Mean shoot-tip CMD incidence at 12WAP
CMD6WKI Mean whole plant CMD incidence at 6 WAP
CMD12WKI Mean whole plant CMD incidence at 12WAP

Appendix IIc Pearson Correlation Coefficients for CMD severity responses in genetic material in Ibadan 1998

	MST6WK	MCMD6WK	MST12WK	MCMD12WK	MST20WK	MCMD20WK	ST6WKI	CMD6WKI	ST12WKI	CMD12WKI	ST20WKI	CMD20WKI
MST6WK		0.944**	0.854**	0.815**	0.671**	0.644**	0.900**	0.736**	0.688**	0.593**	0.635**	0.509**
MCMD6WK	0.944**		0.775**	0.813**	0.607**	0.589**	0.911**	0.883**	0.655**	0.641**	0.618**	0.486**
MST12WK	0.854**	0.775**		0.898**	0.804**	0.840**	0.713**	0.522**	0.897**	0.743**	0.740**	0.702**
MCMD12WK	0.815**	0.813**	0.898**		0.702**	0.713**	0.700**	0.609**	0.870**	0.842**	0.709**	0.622**
MST20WK	0.671**	0.607**	0.804**	0.702**		0.868**	0.532**	0.391**	0.711**	0.610**	0.912**	0.739**
MCMD20WK	0.644**	0.589**	0.840**	0.713**	0.868**		0.530**	0.403**	0.793**	0.664**	0.807**	0.894**
ST6WKI	0.900**	0.911**	0.713**	0.700**	0.532**	0.530**		0.914**	0.689**	0.612**	0.614**	0.490**
CMD6WKI	0.736**	0.883**	0.522**	0.609**	0.391**	0.403**	0.914**		0.564**	0.609**	0.520**	0.397**
ST12WKI	0.688**	0.655**	0.897**	0.870**	0.711**	0.793**	0.689**	0.564**		0.895**	0.767**	0.760**
CMD12WKI	0.593**	0.641**	0.743**	0.842**	0.610**	0.664**	0.612**	0.609**	0.895**		0.666**	0.659**
ST20WKI	0.635**	0.618**	0.740**	0.709**	0.912**	0.807**	0.614**	0.520**	0.767**	0.666**		0.828**
CMD20WKI	0.509**	0.486**	0.702**	0.622**	0.739**	0.894**	0.490**	0.397**	0.760**	0.659**	0.828**	

MST6WK Mean shoot-tip CMD severity at 6 WAP
MST12WK Mean shoot-tip CMD severity at 12WAP
MST20WK Mean shoot-tip CMD severity at 20 WAP
MCMD6WK Mean whole plant CMD severity at 6 WAP
MCMD12WK Mean whole plant CMD severity at 12WAP
MCMD20WK Mean whole plant CMD severity at 20 WAP

ST6WKI Mean shoot-tip CMD incidence at 6 WAP
ST12WKI Mean shoot-tip CMD incidence at 12WAP
ST20WKI Mean shoot-tip CMD incidence at 20 WAP
CMD6WKI Mean whole plant CMD incidence at 6 WAP
CMD12WKI Mean whole plant CMD incidence at 12WAP
CMD20WKI Mean whole plant CMD incidence at 20 WAP

Appendix IId Pearson Correlation Coefficients for CMD severity responses in genetic material in Ibadan 1999

	MST6WK	MCMD6WK	MST12WK	MCMD12WK	ST6WKI	CMD6WKI	ST12WKI	CMD12WKI
MST6WK		0.910**	0.836**	0.829**	0.694**	0.501**	0.591**	0.473**
MCMD6WK	0.910**		0.834**	0.878**	0.680**	0.547**	0.561**	0.549**
MST12WK	0.836**	0.834**		0.928**	0.613**	0.535**	0.785**	0.557**
MCMD12WK	0.829**	0.878**	0.928**		0.609**	0.557**	0.747**	0.675**
ST6WKI	0.694**	0.680**	0.613**	0.609**		0.658**	0.724**	0.710
CMD6WKI	0.501**	0.547**	0.535**	0.557**	0.658**		0.569**	0.641**
ST12WKI	0.591**	0.561**	0.785**	0.747**	0.724**	0.568**		0.785**
CMD12WKI	0.473**	0.549**	0.557**	0.675**	0.710**	0.641**	0.785**	

MST6WK Mean shoot-tip CMD severity at 6 WAP
MST12WK Mean shoot-tip CMD severity at 12WAP
MCMD6WK Mean whole plant CMD severity at 6 WAP
MCMD12WK Mean whole plant CMD severity at 12WAP
ST6WKI Mean shoot-tip CMD incidence at 6 WAP
ST12WKI Mean shoot-tip CMD incidence at 12WAP
CMD6WKI Mean whole plant CMD incidence at 6 WAP
CMD12WKI Mean whole plant CMD incidence at 12WAP

Appendix III

Stock solutions and Buffers.

EDTA (0.5 M) pH 8.0	
EDTA	186.1 g
NAOH	18 g
Deionised water	1 L
Adjust pH to 8.2 Autoclave	
TE (Tris EDTA)	
10mMTris-HCl (pH 8.0) 0.1mM EDTA	
Tris-HCl 1M pH 8.0	5 ml
EDTA 0.5 M	1 ml
Sterile deionised water up to 500	
Tri Borate EDTA 10X	
Tris base	108 g
EDTA 0.5M	40 ml
Boric Acid	55 g
Deionised water up to 1 L	
Tris-HCl 1M	
Tris base	121.1 g
HCl pH 7.4	70 ml
...........pH 7.6	60 ml
...........pH 8.0	42 ml
Deionised water up to 1 L	
TNE 10X	
Tris base	121.1 g
EDTA-Na_2	3.7 g
NaCl	58.4 g
Fluorometer Assay Buffer	
Hoechst stock solution	10 µl
10X TNE buffer	10 ml
Sterile deionised water	90 ml

Appendix IV

Gel electrophoresis reagents.

Gel loading buffer	
bromophenol blue,	0.25 %
glycerol in water	40 %
Siliconising agents	
Commercial automobile windscreen cleaner Rain-X	1000 µl per plate
Sigma cote or Gel slick	600 µl per plate
Gel Binding Solution	
Bind silane	3 µl
Acetic Acid 95%	1 ml
Ethanol 5%	
6% Acrylamide	
40% Acrylamide:bisacrylamide (19:1)	75 ml
Urea (7M)	210 g
10X TBE buffer	50 ml
Deionised water up to 500ml and stored a $4^{O}C$.	
To 75 ml of gel before use	
10% Ammonium persulphate	300 µl
Temed	35 µl
Formamide dye	
Bromophenol blue	0.25 %,
xylene cyanol in H_20	0.25 %
EDTA (0.5 M) 10 mM	20 µl
Formamide	98 %
Deionised water (sterile) up to 1000 µl	

Appendix V

Silver staining reagents.

Fix/stop solution	
(10% glacial acetic acid):	
Glacial acetic acid	200 ml
Deionised water	1,800 ml
Staining solution	
Silver Nitrate ($AgNO_3$)	2 g (1 packet)
Formaldehyde 37%	3 ml (1 vial)
Deionised water	2 L
Developing solution	
Sodium carbonate (Na_2CO_3)	60 g (1 packet)
Deionised water	2 L
Chill at 4°C. Just before use	
Formaldehyde 37%	3 ml (1 vial)
Sodium thiosulfate (10mg/ml)	400 µl (vial)