Chapter	#

CASSAVA

Subtitle

G. Thottappilly[1], M. Fregene[2], T. Makeshkumar[3], L.A. Calvert[2] and M. Cuervo[2]

Affiliation

[1] Mahyco Research Foundation, A.G. Heights, Road No. 12, Banjara Hills, Hyderabad- 500034, A.P., India, E-mail: g.thottappilly@sify.com ; [2]Centro Internacional de Agricultura Tropical (CIAT), AA 6713, Cali, Colombia, E-mail: m.fregene@cgiar.org ; [3]Central Tuber Crops Research Institute (CTCRI), Sreekariyam, Trivandrum, Kerala, India, E-mail: makeshkumar@yahoo.com

Introduction:

Cassava, (*Manihot esculenta*, Euphorbiaceae), one of the main tuber crops, is the staple or subsistence food for about one fifth of the world's population (Edison, 2000). Production and consumption of cassava is expected to rise in the next 20 years due to human population growth (Scott *et al.,* 2000).

Resistance to viruses is particularly important in a vegetatively propagated crop like cassava that becomes virus infected during propagation over years. Eighteen different viruses have been reported from cassava (Calvert and Thresh, 2002; Thottappilly *et al*., 2003).

Situation in Africa: Although nine viruses are reported from cassava in Africa (Thottappilly *et al.,* 2003), with the three new *Begomovirus* species affecting

Deleted:

1

cassava in Africa, viz. *East African cassava mosaic Cameroon virus*, *East African cassava mosaic Malawi virus* and *East African cassava mosaic Zanzibar virus* (Fauquet *et al*., 2003), the total number of viruses rises to 12. Cassava mosaic disease (CMD) is the main biotic constraint in cassava production and the most important threat to food security in sub-Saharan Africa (Thottappilly, 1992). Originally one geminivirus was regarded as the causal agent of CMD. However, recent studies have shown that several similar, but distinct whitefly-transmitted geminiviruses cause CMD in Africa (Fauquet *et al*., 2003) and they occur singly or in combinations. At least six different *Begomovirus* species are reported from Africa. Consequently, if not otherwise specified, the viruses causing CMD in Africa are referred to here as cassava mosaic begomoviruses (CMBs). From 1988 to present, a major pandemic of an unusually severe form of CMD has been speading throughout East and Central Africa, causing massive losses and affecting the region's food security (Otim-Nape *et al*., 1997; Legg *et al*., 2001).

Breeding for resistance has been considered a feasible strategy for the control of CMD (Thresh *et al*., 1994; Calvert and Thresh, 2002; Thottappilly *et al*., 2003). However, the performance of resistant varieties can only be guaranteed under certain conditions. Increased inoculum pressure, agroecological changes, vector population explosion or immigration of a new vector biotype can have a significant impact on the expression of resistance in cassava.

Use of resistant genotypes of cassava

The search for resistance to CMD started in the 1920s, but the most rewarding programmes began at Amani in Tanzania during the late 1930s (Jennings, 1994) and later in Madagascar, where all local varieties and many diverse cassava accessions were screened (Cours-Darne, 1968). Varieties such as "Bouquet de la Reunion", "Java 12/28", and "Criolina" were identified and released to farmers. However, since more effective resistance was needed, these were crossed with several wild species including the tree cassava species, *M. glaziovii* (Nichols, 1947; Cours, 1951; Jennings, 1957, 1994). The hybrids had non-tuberous roots but some plants, though infected by CMD, showed only mild and transient symptoms of CMD. At Amani, three backcrosses to cassava were made to restore root quality and maintain resistance to CMD (Nichols, 1947). Intercrosses between third backcross selections produced hybrids which combined good quality roots and effective virus resistance (Jennings, 1994). Open-pollinated seeds from these hybrids sent to many African countries including Nigeria yielded clone 58308 (Ekandem, 1970; Hahn *et al.,* 1980, 1989; Jennings, 1994). This clone had poor root yield but high resistance to CMD. It was used extensively at the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria (Hahn *et al.,* 1989) to develop resistant genotypes. The IITA program combined high levels of resistance to CMD, good root yield and root quality to produce the TMS cassava lines which are currently the most widely deployed source of CMD resistance in Africa and until recently the best sources of CMD resistance. Seeds

Deleted: a

Deleted:

Deleted: were

Deleted: where

Deleted: was selected and utilized

of CMD-resistant genotypes and *in-vitro* virus-free clones were sent by IITA to over 30 national programmes in Africa for evaluation and selection under specific agroecologies (Ng *et al.,* 1992; Mahungu *et al.,* 1994).

The genetics of currently deployed CMD resistance in the TMS lines derived from *M. glaziovii* is polygenic, and this involves recessive genes that are additively inherited, with a heritability of over 60% (Hahn *et al.* 1989; Jennings, 1994; Mahungu *et al.* 1994; Mba and Dixon, 1997). CMD incidence on the resistant genotypes is usually low, 20% incidence in genotype 30395 as against 100% on susceptible Isunikankiyan after 6 months of growth under high disease pressure. Similar results are reported from Côte d'Ivoire between resistant Garimoshi (30% incidence) and moderately resistant CB (90%) within six months (Fargette and Vie, 1995). In Uganda, the percent incidence on improved genotypes such as TMS 30572, TMS 60142, TMS 30337 and TMS 30395 was less than 30% during ten months of growth. In contrast, the local susceptible genotypes such as Ebwanateraka and Senyonjo had 100% incidence (Otim-Nape *et al*., 1998).

The first resistant varieties retained the broad-based polygenic resistance derived from *M. glaziovii*. Resistant cassava genotypes show certain features that distinguish them from susceptible ones. They were largely tolerant to CMD infection, incurring little or no yield loss. Epidemiological studies and field evaluation of the resistant genotypes reveal that they are not readily infected (Hahn *et al.,* 1980), and if infected, show mild symptoms, which may be restricted to some shoots (Jennings, 1960; Fargette *et al.,* 1996). Some of the resistant genotypes are characterised by transient or mild symptoms when infected by CMBs (Jennigs, 1994; Thresh *et al*., 1994), while others develop conspicuous symptoms that are restricted to a few leaves or shoots (Thresh *et al*., 1998b). Virus concentration in resistant genotypes was reported to be low and a significant correlation was shown between symptom severity and CMD titre among resistant genotypes (Fargette *et al*., 1996). However, the severity of symptoms expressed was not necessarily a reflection of virus concentration in some of the genotypes (Obge *et al.,* 2003).

Fauquet and Fargette (1986) identified six different components of resistance to CMD: Field resistance (percentage of infected plants), vector resistance (number of adult whiteflies per plant), inoculation resistance, virus resistance (virus content estimated by ELISA), symptom severity, and virus diffusion resistance (development of symptoms over time). I AM UNABLE TO GET THE ORIGINAL REFERENCE> The reference I have is Fauquet C., and Fargette D. 1990. African cassava mosaic virus. Etiology, Epidemiology and Control. Plant Disease 74(6): 404-411.

In the field TMS resistant cassava lines would be infected with CMD and the first few leaves show good symptoms, especially during rainy season. Then the plant would produce several leaves without symptoms. Then one or two leaves

Deleted:

Deleted: c

will show symptoms, probably due to new infection. Again the plant would produce several leaves without symptoms. In the susceptible varities, once the plant is infected almost all leaves would show symptoms. Based on this observation, it could be speculated that this is due to the inhibition of long distance transport function. Once a leaf becomes infected, the virus spreads effectively cell to cell but not to other leaves. Therefore it appears that cell to cell movement is not inhibited and the recessive gene affects long distance transport of the virus.

The resistance has been effective for more than 40 years in East and West Africa and there is no breakdown till now due to the emergence of resistance-breaking strains of CMBs. Interestingly the use of resistant varieties contributed in overcoming the recent pandemic of EACMV-UG in Uganda, where IITA genotypes have been widely used (Otim-Nape *et al.,* 1994).

More recently, classical genetic analysis and molecular mapping confirmed the polygenic nature of the *M. glaziovii* source of resistance to CMD (Akano *et al.,* 2002). Bulk segregant analysis (BSA) (Michelmore *et al*., 1991) of a BC_1 population derived from TMS30572 identified a simple sequence repeat marker SSRY40 on linkage group D of the TMS30572-derived genetic map of cassava that explains 48% of the phenotypic variance of CMD resistance at P<0.001 (Fregene, 2000). The resistance gene(s) associated with SSRY40 was also shown to be recessive. The linkage group D of the TMS30572- derived genetic map shows reduced recombination and a high number of markers, evidence of inter-specific introgression. The gene controlling resistance to the *M. glaziovii* source of resistance has been desginated CMD1. Although highly resistant varieties of this type are available in many countries, they are not always widely grown due to the lack of adequate quantities of planting material and in many countries farmers continue to grow local varieties including some that may have little or no resistance to CMD. This explains why the disease is widespread in many areas, causing serious losses as during the current pandemic in East Africa.

CMBs are not always fully systemic and uninfected cuttings can be obtained from some branches of infected plants, especially those of resistant varieties not expressing symptoms. The resistant/ tolerant genotypes often have two categories of plants: symptomless and symptomatic plants (Jennings, 1994; Fargette *et al.* 1996). Among the latter, at least some branches of infected plants of resistant varieties are free from virus through 'reversion' under natural conditions (Storey and Nichols, 1938; Njock *et al.* 1996; Fargette *et al.,* 1994). The extent of reversion depends on the genotype, and is regarded as a component of the resistance of cassava to CMBs (Fargette *et al.,* 1996; Thresh *et al.,*1998a,b; Fondong *et al.,* 2000). Reversion has been exploited to select and produce healthy cuttings for CMD epidemiological studies in Côte d'lvoire (Fargette *et al.,* 1985, 1988). In resistant genotypes, cuttings obtained from the lower portions of the main stem are more likely to grow into virus-infected plants than cuttings from

4

Deleted:

Deleted: Genetic analysis of field evaluation of CMD resistance confirmed the polygenic nature of the *M. glaziovii* source of resistance.

Deleted: the

Deleted:

Deleted:

the upper portion of the main stem and stem branches (Cours, 1951; Njock *et al.,* 1994).

The partial systemicity of CMBs is probably associated with the reversion phenomenon (ability to provide uninfected cuttings even when infected). Reversion has probably prevented total infection of the vegetative stock of resistant genotypes. For example, 96.0, 95.7, and 23.7% symptomless plants were recorded for resistant genotypes TMS 30001, TMS 30395, and TMS 30572, respectively, after 6 years of vegetative propagation (Hahn *et al.,* 1980). Recent studies showed that restriction of virus movement into axillary buds is an important aspect of resistance in CMD (Ogbe *et al.* 2002). This probably explains reversion phenomenon in which infected stem of resistant genotypes could sprout into healthy plants in subsequent generation and why reversion of resistant genotypes is such a feature of resistant gene.

Resistance to virus infection differs from resistance to the whitefly vector. Fargette *et al*. (1996) found that cassava genotypes differed widely in whitefly infestation. In contrast, Hahn *et al*. (1980) observed similar number of whiteflies on resistant and susceptible genotypes and thus inferred that resistance to the vector was unlikely. Nevertheless, variation in the suitability of cassava as a host for *B. tabaci* has been noted (Legg, 1994). This variation could be exploited in breeding for resistance to the vector, since several studies have shown a correlation between whitefly population and disease incidence (Leuschner, 1977; Otim-Nape *et al.* 1998). More recently, a cassava genotype, MECU72, showing resistance to the whitefly vector has been identified (Bellotti 2004 pers communications), it would be interesting to see if this genotype can be infected with CMBs under natural conditions in the field.

As IITA was deploying these resistant materials, the concern to diversifying resistance and expanding the gene pool was recognised and pursued (Dixon *et al.* 2001). A novel source of resistance was recently identified in a Nigerian cassava landrace (TME-3) that confers immunity to CMD (Dixon *et al.* 2001; Fregene *et al.* 2001a). AFLP analysis of the CMD resistant land races and the TMS lines reveal significant genetic dis-similarity between them reducing the possibility that the land races are escapes from the back cross derivatives with the *M. glaziovii* source of CMD resistance (Fregene *et. al*., 2000) Genetic analysis of field evaluations of two crosses involving the CMD resistant local Nigerian varieties TME 3 and TME4 revealed a major dominant gene control for the new extreme resistance source (Akano *et al*., 2002). The dominant CMD resistance gene has been designated *CMD2* and it is flanked by SSRY28 and GY1 at 9 and 8cM respectively. Since then 2 additional markers, NS158 (SSR marker) and RME-1 (SCAR marker) at <2cM and <1cM to the gene respectively and explaning more than 90% of phenoytypic variation for resistance have been identified.

Deleted:

Deleted:

Deleted:

Deleted:

Deleted:

Deleted:

Deleted: ¶

Deleted: the other

Deleted:

Considerable progress has been made in developing a comprehensive molecular genetic map (Fregene *et al*., 1997) and a clustering of cassava accessions into groups having differential resistance has been achieved (Fregene *et al*., 2000). Furthermore, progress has been reported in localizing resistance genes (Akano *et al.,* 2002). This provides opportunity to apply marker-assisted breeding for efficient selection of this trait. The advantage of marker assisted selection (MAS) is that it enables the breeder to eliminate at an early stage CMD susceptible genotypes, which in the case of the heterozygous CMD resistant land races is 50%, reducing the costs of disease evaluation by half.

The new source of resistance is controlled by a single dominant gene (Akano *et. al*., 2002) reminiscent of plant R genes whose product play a key role in recognizing a hypersensitive response (HR), a localized cell death and tissue necrosis at the site of pathogen ingress. Other conspicuous features of the new source of CMD resistance are the lack of observable disease symptoms and the suppression of virus accumulation in infected cells of cassava leaves (Rossel *et al*., 1994; F.Ogbe, personal communication). Several improved lines have been developed using this new source of resistance. Since then, several additional land races from all over West Africa, with possible additional sources of resistance to CMD, have been identified (Dixon, 2004). They, putatively, represent seven different resistance genes, but this needs to be confirmed by further studies.

To test the use of CMD2 for resistance breeding, a pilot experiment was set up in 2000. Six crosses, and reciprocals, over 2,400 genotypes, were made between two cassava land races from Nigeria that carry *CMD2*, and a susceptible Nigerian land race and 2 elite cassava varieties from IITA, one tolerant and the other susceptible to CMD. The crosses were evaluated in Ibadan (Nigeria), at high CMD pressure area and molecular marker analysis conducted with SSR marker NS158 tightly associated with *CMD2*. Marker analysis alone was able to predict CMD resistance with 95% accuracy. Based on this result, a molecular marker-assisted breeding (MAB) of resistance to CMD was initiated at CIAT using the markers NS158 and RME1. The MAB project is a pre-emptive measure in case the disease is accidentally introduced into Latin America, as Neo-tropical cassava germplasm are very susceptible to CMD (Okogbenin *et al*., 1998). The MAB scheme at CIAT currently involves crossing CMD resistant parents to CIAT's elite cassava parents (by agro-ecology) and the embryo rescue of seeds followed by multiplication *in vitro* as well as molecular analysis. Genotypes shown to be resistant are transferred to the screen house for hardening and to the regular breeding program, samples of resistant genotypes are also shipped as *in vitro* plants to collaborators in Africa and India. In 2003, 2315 genotypes were processed but the capacity for MAB at the moment is 5000 seeds, the current cost of MAB per genotype is US$0.5 (Fregene *et al*., 2004).

MAB of CMD and the cassava green mites (CGM) has also recently been initiated in Tanzania to transfer the concept to National programs. Tanzania is the

---

Deleted: 2001b

Deleted:

Deleted: AFLP analysis of the CMD resistant land races and the TMS lines reveal significant genetic dis-similarity between them reducing the possibility that the land races are escapes from the back cross derivatives with the *M. glaziovii* source of CMD resistance (Fregene *et. al*., 2000). Besides, t

Deleted:

Deleted: In a project launched in 1996 by Centro Internacional de Agricultura Tropical (CIAT), Cali, Colombia and IITA with Rockefeller Foundation funding, genes controlling both sources of resistance to CMD (CMD1 and CMD2) have been tagged. Markers for gene tagging came from a molecular genetic map of cassava earlier constructed at CIAT (Fregene *et al.*, 1997).

Deleted:

Deleted:

fourth largest producer of cassava with average yields of 8 tons/ha compared to 10 tons/ha for Africa. The breeding project employs elite cassava parents that have been bred for resistance to CMD, the TME3 source, using molecular markers at CIAT, and a source of resistance to CGM from the wild species, *M. esculenta* sub spp *flabellifolia*. The improved introductions will be crossed to farmer preferred local varieties and molecular markers associated with CMD and CGM will be used to eliminate progeny that do not carry the resistance genes, leaving a largely reduced breeding population for more careful agronomic evaluation under typical farmer's conditions.

Mechanism of the resistance of CMD2 has been studied at the Donald Danforth center for Plant Science, St Louis (USA) and the DSMZ-Plant Virus Division at Braunschweig (Germany). Protoplast cultures of TME3 and TMS117 were transformed, by electroporation, with infectious virus clones and grown for 24h, after which the protoplast cultures were harvested and subjected to Southern analysis using an infectious virus DNA clone as probe. The results reveal that ACMV could replicate equally in both clones discarding interference with replication as the resistance mechanism (C. Fauquet, personal communication). However when infectious virus clones were introduced into resistant and susceptible varieties via microprojectile bombardment, both groups of varieties became infected but infection in the resistant genotypes did not become systemic, suggesting that interference with movement of the virus is the principal mode of resistance in the TME3 source (Winter *et al*., 2004).

The serial analysis of gene expression (SAGE) was used to analyze the gene expression pattern in a bulk of 40 genotypes each of CMD resistant and susceptible genotypes drawn from a gene mapping progeny (Fregene *et al*., 2004). Messenger RNA used for the SAGE analysis came from plants that have been exposed to heavy disease pressure over a period of two years in the field. One hundred and seventy five transcripts were expressed 3 to12 times more in the resistant bulk compared 94 transcripts found 3-5 times in the susceptible bulk implying that many more genes have been switched on in the resistant bulk in response to virus infection. The SAGE analysis of bulks of CMD resistant and susceptible cassava genotypes have identified genes known to be involved in systemic acquired resistance (SAR) response to disease in plants.

There is also an interest to clone CMD2 for use in genetic transformation (Fregene *et al*., 2001a; Fregene and Puonti-Kaerlas, 2002). The gene could be used to incorporate to the desirable local/improved cultivars through genetic enginnering. A high resolution map of the region of the cassava genome bearing the CMD2 gene was developed towards positional cloning of CMD2 using a full-sib population of 1690 individuals (Moreno *et al*., 2004). A bacterial artifical chromosome (BAC) library with more than 70,000 clones, with an average size of 110kb, and a more than 10X coverage of the cassava genome was also developed. The BAC library was screened with the two markers closest to CMD2, RME1 and NS158, and positive clones were used to construct contigs.

The end of the contigs were sequenced and then mapped, as single nucleotide polymorphisms (SNPs), in the recombinants, 112 in total of the fine mapping population. BAC clones that flank CMD2 have been identified and sub-cloned for use in genetic complementary experiments to identify the clones that carry the gene.

ASIA AND THE PACIFIC REGION:

A cassava mosaic disease was reported in India and Sri Lanka. In order to differentiate screening and resistance breeding work against begomoviruses in Africa and in India, we prefer to distinguish CMD in India as Indian cassava mosaic disease (ICMD), although symptoms are identical with CMD in Africa and India. Two disinct begomoviruses, viz. *Indian cassava mosaic virus* (Hong *et al*., 1993) and *Sri Lankan cassava mosaic virus* (Saunders *et al*., 2002) cause CMD in Asia.

ICMD is a serious constraint to cassava production in India. This disease is reported to be widespread in South India mainly in Kerala, Tamil Nadu and Andhra Pradesh (Narasimhan and Arjunan, 1976). However due to the systemic nature of the disease, and frequent and common use of infected cuttings, this disease spread to other areas of the country.

Yield losses up to 88 per cent in highly susceptible cultivar 'Kalikalan' and 17 to 36 per cent in improved varieties released by CTCRI were reported (Malathi *et al.,* 1985). ICMV is transmitted by the whitefly *Bemisia tabaci* and the extent of spread by this vector in field varies (Palaniswami *et al*., 1996). Spread through the vector is reported to be very low in improved cultivars (Chacko and Thankappan, 1973; Hrishi *et al.,* 1977). Whether this low spread is due to the field resistance of these varieties or due to the inefficiency of the vector is not clearly understood. Recently it was reported that only cassava biotype of *B. tabaci* transmit ICMV (Palaniswami *et al*., 2004).

Resistance Breeding in India:

In India cassava breeding is mainly carried out at the Central Tuber Crops Research Institute (CTCRI), Thiruvananthapuram, as well as in the State Agricultural Universities through the All India Co-ordinating centres (Abraham *et al.,* 2000; Unnikrishnan *et al.,* 2002). During the last four decades of research at CTCRI, a large number of cassava varieties with varying reaction to ICMV have been released (Nair *et al.*, 1998).

CTCRI has a rich collection (1638) of indigenous (854) and exotic (784) cassava germplasm (Pillai *et al*., 2004a) of which only 113 accessions were found to be free from ICMD. Genetic diversity among these ICMD free accessions were assessed in terms of esterase isozyme polymorphism which showed ten distinct clusters with the number of accession in each group ranged from 1 to 27 and similarity between different clusters ranged from 15-50 per cent (Pillai *et al.,* 1995,1999).

Screening of land races of cassava in India showed low variability for reaction to ICMD and most of them were susceptible (Pillai *et al.,* 2004b). One hundred and thirteen germplasm accessions of cassava were evaluated against ICMD at TNAU and found that eighteen genotypes were field resistant, thirteen were field tolerant, thirty eight were susceptible and forty four were highly susceptible (Ramaiah *et al.,* 1993).

Graft transmission studies have shown that four germplasm accessions possess a high degree of resistance as compared to the highly susceptible cultivar 'Kalikalan' (Rajendran *et al.,* 1995).

Recently, cassava variety MNga-1 and *Manihot caerulescence* were identified as resistant to ICMV (Unnikrishnan *et al.,* 2002; Sheela *et al.,* 2004). MNga-1 is a breeding line from IITA, designated as TMS3001 there, received via CIAT in 1994 has been continuously evaluated for CMD for the past 10 years and showed 0 to 1% infection at field level while other lines exhibited 3 to 67% infection (Unnikrishnan *et al.,* 2002). Since 2001, more intensive resistance breeding programme was undertaken through intervarietal and interspecific hybridization programme. In Intervarietal hybridization programme MNga-1 was used as ICMV resistance donor parent and crosses made with released varieties and promising selections from indigenous germplasm. Evaluation of seedling population for ICMV showed that open pollinated populations showed lesser ICMV incidence (15-34%) than the crosses (37%) (Unnikrishnan *et al.,* 2002). Similarly, hybrid progenies obtained from crosses with Ambakadan X MNga-1, showed resistance to ICMV at seedling and first clonal stage (Rajendran *et al*., 2004).

Reactions of Indian cassava cultivars to ICMV :

Since 1963, when intensive breeding work started in India, nearly 20 varieties were released from CTCRI, Kerala Agricutlural University and Tamil Nadu Agricultural University. The response of these cultivars to ICMV varies from susceptible to field tolerant (Shanmugavelu *et al*., 1987; Thamburaj, 1990, Joseph *et al*., 1990; Nair *et al.,* 1998,). Although large collection of germplasm is available, no immune or highly field resistant cultivars were released. Field tolerant Indain cultivars were H 97, H 165, CO-1, CO-2,CO-3, ME120 and MVD-1.

A number of indigenous cultivars like Kalikalan, Ariyan and Burmah were found 100 per cent infected by the virus. The clones CE-9 (1310), CE-14 (1315), CE-92 (2171) and CE-101 (2350) had high degree of resistance. A high degree of tolerance was exhibited by M4 (CE 687) (Jos and Sreekumari, 1994).

Wild genetic resources :

The genus *Manihot* comprises 98 species and all are natives of new world tropics. Presently 8 species are maintained at CTCRI of which *M. glaziovii*, shows resistance to CMD in Africa (Jennings 1972, 1977; Doughty, 1958).

In interspecific breeding programme, *M. glaziovii, M. caerulescence, M. tristis, M. flasellifolia, M. peruviana* and *M. pseudoglaziovii* were used for evolving ICMV resistant lines. Among them, accessions of *M. caerulescence* exhibited high level of resistance and were used as donor parents for transferring resistance to elite Indian cultivars (Sheela *et al.,* 2002, 2004). Among the crosses, one interspecific hybrid cassava with *M. caerulescence* (CMC-1) showed complete resistance to ICMV for the past twelve years of evaluation while other field tolerant interspecific hybrids showed varying degrees of incidence during the same periods. This hybrid is now being back crossed with elite cassava cultivars for recovering quality attributes (Unnikrishnan *et al.,* 2002; Sheela *et al.,* 2002, 2004).

In order to identify new sources of ICMD resistance, 44 wild *Manihot* accessions were tested, of which 14 accessions belonging to *M. caerulescence, M. carthaginensis, M. dichotoma* and *M. pseudoglaziovii* were resistant to ICMV (Unnikrishnan *et al.,* 2004). Similarly evaluations of back cross hybrids, three BC hybrids showed no ICMD symptom (Unnikrishnan *et al.,* 2004).

Resistance against Vector:

Preference of whitefly to cassava varieties and their reaction to ICMV were shown not to be related. Varieties with green petioles and soft leaves were preferred to those with red or red-green petioles and coarse leaves. Erect leaf orientation had double the number of whiteflies than horizontal or downward ones (Nair and Daniel, 1983). The finding could be exploited in breeding. Spread of ICMV by the vector is reported to be low in improved cultivars(Hrishi *et al*., 1977). Leuschner (1977) reported from Nigeria a close relationship between population density of *Bemisia* sp., and the development of CMD. The extent of spread of ICMD in the field in different varieties was found to vary (Chacko and Thankappan, 1973).

Field spread of ICMD varied with cultivars. In hybrids H 43 and H 226 the spread was 30 and 15 per cent respectively, while in H 97, H 165, Sree Visakham, Sree Sahya and M4 it was less than 5 per cent. However, the spread was as high as 52 per cent in Kalikalan. Nair (1981) could achieve reduction in whitefly population through insecticidal sprays, but the field spread of ICMD could not be reduced.

Physiological basis of Resistance:

Varying levels of susceptibility and tolerance to ICMD have been identified among cassava cultivars, based on disease severity index, disease spread and yield loss (Malathi *et al*., 1985). The factors contributing to this variation in susceptibility are not known. Neither the inoculum source nor the whitefly population harboured in the cultivars was found to have correlation with susceptibility (Nair and Daniel, 1983). Cassava has high concentration of cyanoglucosides (linamarin and lotaustralin) present in all plant tissues, especially

Deleted:

in leaves. The physiological role of these compounds is not clear, although they are believed to be involved in repelling or inhibiting pathogens and pests (Conn,1980). The   cyanoglucosides in leaves and the activity of cyanide metabolizing enzymes were studied in ICMV susceptible and tolerant cassava cultivars. The results showed that cyanoglucosides do not have any role in resistance to ICMV. Neither leaf cyanoglucoside content nor cyanide metabolism in leaf was related to ICMV tolerance. Mild disease symptoms were associated with decrease in cyanide levels and severe symptoms with an increase in cyanide content in all cultivars (Balanambisan and Malathi, 1993). Studies on the role of virus – induced proteins in the pathogenesis of ICMV were done for soluble and total protein profiles of healthy and diseased cassava leaves and the results show that higher amounts of protein could be solubilised from diseased leaves than from healthy ones. However, whether these proteins are host specific, found in response to virus infection or are associated with the virus has yet to be established (Balanambisan,1996).

SOUTH AND CENTRAL AMERICA:

Although seven viruses are reported from South and Central America, cassava frogskin disease (CFSD) is economically the most important. The causal agent of CFSD is not known, but a virus is suspected. CFSD was first reported in 1971 from southern Andean region of Colombia (Lozano and Nolt, 1989). The range of CFSD is increasing and it is becoming more frequent in areas of Colombia, Costa Rica, Venezuela and Brazil.

In the Amazon regions of Brazil and Colombia, differences in the reaction of varieties to CFSD were observed. Some varieties developed typical root symptoms. Other varieties or landraces often in the same field did not develop symptoms. This led to the idea that some cassava landraces may be resistant to CFSD.

In CFSD affected cassava, the root periderm and corky layers enlarge to form raised lip shaped fissures. Severely affected roots do not fill with starch, and yield losses can be 100% (Lozano & Nolt, 1989). In some cassava landraces including Secundina (accession CM 6014), CFSD affected plants are stunted and the leaves develop mosaic symptoms.

Cassava is propagated vegetatively, and all the plants grown from affected plants will have CFSD. The symptom severity is affected by temperature. As the temperatures increase there tends to be a decrease in symptoms. For example, CFSD affected Secundina grown at constant temperature of 30C will not develop the mosaic leaf symptoms. The masking of symptoms makes the disease harder to control, and the selection of resistant lines a multi-year task. Because of the inhibition of transmission by high temperatures, thermo-therapy followed by *in vitro* meristem culture can be used to eliminate the disease from infected plants (Maffla et al, 1984).

Evaluation of cassava for resistance to CFSD.

The Centro Internacional de Agricultura Tropical (CIAT) core collection, which consists of 640 cassava lines that are representative of the CIAT cassava collection that contains over 6000 lines, were tested for their reaction to CFSD. Five plants from each line were inoculated by grafting them with CFSD affected stem cuttings of the cassava line 5460-10. In the subsequent years, 10-20 plants per line were grown for 12 months and evaluated visually for root symptoms. The rating scale used was 1 for no symptoms, 2 for very mild symptoms, 3 for moderate symptoms, and 4 for severe symptoms. Those cassava lines with either moderate or severe symptoms were eliminated from the experiment. After five years, 121 cassava lines were still showing a good level of resistance to CFSD while 340 cassava lines were classified as susceptible to CFSD. Although the origins of cassava lines were from many countries throughout the world , 70% of the resistant landrace were from Brazil (31), Peru (18) and Colombia (18) and Paraguay (16).

After five years, the 66 lines that were resistant to CFSD and had good agronomic characteristics were grown at the CIAT experiment station at Santander de Quilichao, Cauca, Colombia. Only very mild or no symptoms were present on any of the lines during the next 3 years of testing. Representative plants from 66 lines were assayed for CFSD by grafting stem cuttings (rootstock) to Secundina (scion), and the new leaves were examined for mosaic symptoms. Plants from all of the lines were positive for CFSD. This confirmed that the cassava lines were resistant to the disease even though they were affected with CFSD. The transmission of CFSD in stem cutting is nearly 100%, and the plants remained infected over the course of many years. This indicates that the mechanism of resistance is tolerance to the virus.

The large percentage of accessions in the CIAT core collection that have resistance to cassava frogskin disease was surprising because they had never been selected for that trait. Although some lines do have more disease in some years, this is expected since the expression of symptoms is affected by temperature. After eight years of field trials, some lines never had any visible symptoms. Since these plants were still infected by CFSD, it was concluded that the tolerance appears stable. More than 55% of the lines selected for their resistance to CFSD came Brazil, Colombia and Peru from countries where CFSD is endemic. Tolerance as the mechanism of resistance usually occurs only after a long association of the pathogen and host. The earliest known cultivation of cassava is from the Brazilian Amazon region and this is also thought to be the origin of CFSD which implies a long association of this pathogen with its host. For the future, we need to understand the genetic basis of the resistance and develop

modern breeding tools to systemize the selection of resistant lines.

Concluding remarks:

Use of resistant cultivars, production and distribution of healthy planting material, improved cultural practices and eventually strategic use of transgenic crops could provide more sustainable solutions to cassava virus problems. Host plant resistance to viruses and vectors, eventually remain one of the most important means of disease control.

Yield losses due to CMBs may increase if virulent strain(s) reach new areas by natural spread or through the movement of infected planting material. The occurrence of different viruses or virus combinations in different regions undermines the effectiveness of resistance breeding programmes. Germplasm exchange is important for breeding purposes. However, it is essential to follow strict quarantine regulations when disseminating vegetative material. So far, there is no report of any virus of cassava being transmitted through seeds. Hence, currently this may be the safest way to exchange germplasm.

The CMBs that differ from each other in sequence identity are considered to be of independent origin. Rapid progress has been made recently in advancing the knowledge on CMBs as detailed in this chapter. It is obvious that whitefly-transmitted geminiviruses are becoming increasingly important with novel geminiviruses and new whitefly biotypes. For CMBs, this has been shown by many reports on the emergence of new virus types through recombination between and among virus species contributing to a high genetic diversity of begomoviruses (Padidam *et al.* 1999).

Acknowledgements:

Deleted:

REFERENCES

Abraham, K., Nair, S.G. and Naskar, S.K. 2000. Cassava breeding and varietal dissemination in India. Major achievements during the past 25-30 years of research. In *Sixth Asian Cassava Research Workshop*. 21-25 February 2000. Vietnam.

Akano, A.O., Dixon, A.G.O., Mba, C., Barrera, E. and Fregene. M. 2002. Genetic mapping of a dominant gene conferring resistance to cassava mosaic disease. *Theor. Appl. Genetics* 105:521-525.

Balanambisan. 1996. Pathogenesis related proteins in cassava mosaic disease. In *Tropical tuber crops: Problems, prospects and future strategies.* Ed. Kurup et al. Oxford & IBH, New Delhi. Pp375-379.

Balanambisan and Malathi,V.G. 1993. Studies on Cyanoglucosides in relation to cassava mosaic disease. *J. Root Crops*. **19**: 122-126.

Calvert, L. and Thresh, J.M. (2002) The viruses and virus diseases of cassava. In: *Cassava: Biology, Production and Utilization*. R.J. Hillocks, J.M. Thresh and A. Bellotti (eds.)*,* pp. 237-260, *CAB International*, Wallingford, UK.

Chacko, C.I. and Thankappan, M. 1973. Survey and study of virus diseases of different tuber crops and their control. 3. studies on the current season spread of cassava mosaic under field conditions. *Annual Report, CTCRI*, Trivandrum. Pp.46-49.

Conn,E.E. 1980. Cyanoglucosides. In *Secondary plant products* (Eds. E.A.Bell and B.V.Charlwood). Springer – Verlag, New York. Pp 461-487.

Cours, G. 1951. Le Manioc a Madagascar. Memoires de l'Institute Scientifique de Madagascar. *Serie B, Biologie Vegetale* 3: 203-400.

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

Dixon, A. 2004. Conventional cassava breeding: Lessons learmt. A new source of resistance to cassava mosaic disease (CMD). In: Alves and Tohme. Adding Value to a Small-Farmer Crop: Proceedings of the Sixth International Scientific Meeting of the Cassava Biotechnology Network. 8-14 March 2004, CIAT, Cali Colombia. Book of Abstracts Pp4.

Dixon, A.G.O., Whyte, J.B.A., Mahungu, N.M. and Ng, S.Y.C. 2001. Tackling the cassava mosaic disease (CMD) challenge in sub-saharan Africa: The role of host-plant resistance and germplasm deployment. In: Cassava, an ancient crop for modern times: Food, health, culture, N.J. Taylor, F. Ogbe and C.M. Fauquet (eds), pp. S8-05, Donald Danforth Plant sciences Center, St. Louis, USA.

Doughty,L.R.1958. Cassava breeding for resistance to mosaic and brown streak virus. *East Afr.Agric. Forestry Res. Org. Annual Report*. Pp.48-55.

Edison, S. 2000. Tuber crops–scope for value addition. In: *The Hindu Survey of Indian Agriculture* 2000, pp. 77-82, Hindu, Chennai, India.

Ekandem, M.J. 1970. Cassava research in Nigeria before 1967. Federal Report of Agric. Research, Ibadan, Nigeria. Memo No. 103.

Fargette, D. and Vié, K. 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.

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

Fargette, D., Fauquet, C. and Thouvenel, J.C. 1985. Field studies on the spread of African cassava mosaic. *Ann. appl. Biol.* 106: 285-294.

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

Fargette, D., Thresh, J.M. and Otim-Nape, G.W. 1994. The epidemiology of African cassava mosaic geminivirus: reversion and the concept of equilibrium. *Trop. Science* 34: 123-133.

Fauquet C., and Fargette D. 1990. African cassava mosaic virus. Etiology, Epidemiology and Control. Plant Disease 74(6): 404-411.

Fauquet, C.M., Bisaro, D.M., Briddon, R.W., Brown, J.K., Harrison, B.D., Rybicki, E.P., Stenger, D.C. and Stanley, J. 2003. Revision of taxonomic criteria for species demarcation in the family Geminiviridae, and an updated list of begomovirus species*. Arch Virol.* 148: 405-421.

Fondong, V.N. Thresh, J.M. and Fauquet, C. 2000. Field experiments in Cameroon on cassava mosaic virus disease and the reversion phenomenon in susceptible and resistant cassava cultivars. *Internat. J. Pest Management* 4: 211-217.

Fregene, M. 2000. Marking progress: collaboration to improve cassava. In Kinley D. (ed) Synergies in Science. CGIAR (Consultative Group on International Agricultural Research). Washington DC,USA pp6-7.

Fregene, M. and Puonti-Kaerlas, J. 2002. Cassava biotechnology. In: Cassava: Biology, Production and Utilization. R.J. Hillocks, J.M. Thresh and A.C. Bellotto (eds), Chapter 10, pp. 179-207. CABI Publishing, Wallingford, UK.

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

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

Fregene, M., Matsumura, H., Akano, A., Dixon, A. and Terauchi, R. 2001a. Progress towards cloning the single dominant gene conferrring immunity to cassava mosaic disease (CMD). In: *Cassava, an ancient crop for modern times: Food, health, culture,* N.J. Taylor, F. Ogbe and C.M. Fauquet (eds), pp. S5-09, Donald Danforth Plant Sciences Center, St. Louis, USA.

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

Fregene M. , Okogbenin E.,, Marin J., Moreno I., Ariyo O., Akinwale O., Barrera E., Ceballos H., and Dixon A. 2004. Molecular Marker Assisted Selection (MAS) of Resistance to the Cassava Mosaic Disease (CMD). *Molecular Breeding* (submitted).

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

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

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

Hrishi, N., Rajendran, P.G., Kamalam, P., Maini, S.B., Nair, N.G., and Lal, S.S. 1977. Genetic improvement of cassava for yield and quality: Screening hybrids/varieties against cassava mosaic disease. *Annu. Report,* pp. 15-16, Central Tuber Crops Res. Inst., Trivandrum, India.

Jennings, D.L. 1957. Further studies in breeding cassava for virus resistance. *East Afr. Agric. J.* 22: 213-219.

Jennings, D.L. 1960. Observations on virus diseases of cassava in resistant and susceptible varieties: I mosaic disease. *Emp. J. Experimental Agric.* 28: 23-34.

Jennings,D.L.1972. Breeding for resistance to cassava mosaic in Africa. *Proc. Cassava Mosaic Workshop*. IITA,Ibadan,Nigeria. Pp.40-42.

Jennings,D.L.1977. Inheritance of linked resistance to African Cassava Mosaic and bacterial blight. *Proc. Cassava Protection Workshop*, CIAT,Cali, Colombia. Pp 45-50.

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

Jos, J.S. and Sreekumari, M.T. 1994. Genetic resources in Cassava. In *Advances in Horticulture. Vol.8 – Tuber Crops* Ed. K.L. Chadha and G.G. Nayar. Malhotra Publishing House, New Delhi pp.17-31.

Joseph, J., Nayar, G.G., Rajendran, P.G., Jos, J.S., Easwari Amma, C., Vasudevan, K., Unnikrishnan, M. and Naskar, S.K. 1990. Recent progress in cassava varietal improvement in India. *Proc. 3rd Asian Cassava Workshop.* 22-27 October 1990. Malang, Indonesia.

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

Legg, J.P., Okao-Okuja, G., Mayala, R. and Muhinyuza, J.B. 2001. Spread into Rwanda of the severe cassava mosaic virus disease pandemic and associated variant of East Africa cassavamosaic virus (EACMV-UG). *Plant Pathol.* 50: 796.

Leuschner, K. 1977. Whiteflies: Biology and transmission of African cassava mosaic disease. In: *Proceedings Cassava Protection Workshop*, T. Brekelbaum, A. Belloti and J.C. Lozano (eds.). pp.51-58, CIAT, Cali, Colombia.

Lozano, J.C. and Nolt, B.L. (1989) Pest and pathogens of cassava. Pages 174-175 in: Plant Protection and Quarantine: Selected Pests and Pathogens of Quarantine Significance Vol. 2 R.P. Kahn, ed., CRC Press, Pr. Inc., Boca Raton FL.

Maffla, G., Roa, J.C. and Roca, W.M. 1984. Erradicacion de la enfermedad cuero de sapo de la yuca Manihot esculenta, pro medio del dultive de meristemos. Efecto de la termoterapia, y del tamano del explante sobre la tasa de saneamiento. In: Perea, D. & Angarita, Z.A. eds. Congreso Nacional de Cultivo de Tejidos Vegetales, Bogotá, Colombia, 1984. Memorias. Bogoata, U. Nacional de Colombia. Pp. 171-175.

Mahungu, N.M., Dixon, A.G.O. and Kumbira, 1994. Breeding cassava for multiple pest resistance in Africa. *Afric. Crop Science J.* 2: 539-552.

Malathi,V.G., Nair,N.G and Shantha,P. 1985. Cassava mosaic disease. *Tech. Bulletin series* – **5.** CTCRI, Thiruvananthapuram. 18pp.

Mba, R.E.C. and Dixon, A.G.O. 1997. Heritability estimates for some newly developed cassava clones in Nigeria. *African J. Root and Tuber Crops*, 2: 56-58.

Michelmore RW, Paran I, Kesseli RV (1991). Identification of markers linked to disease-resistance genes by bulked segregant analysis:a rapid method to detect markers in specific genomic regions by using segregating populations. *Proc Natl Acad Sci USA.* 88(21): 9828-32.

Moreno M., Tomkins J., Fregene M. 2004. Positional cloning of CMD2 the gene that confers high level resistance to the cassava mosaic disease (CMD). In: Alves and Tohme. Adding Value to a Small-Farmer Crop: Proceedings of the Sixth International Scientific Meeting of the Cassava Biotechnology Network. 8-14 March 2004, CIAT, Cali Colombia. Book of Abstracts Pp163.

Nair,N.G.1981. Relationship between cassava mosaic disease spread and whitefly (*Bemisia tabaci* Gen.) population under different insecticide treatments. *J.Root Crops.* **7**: 15-19.

Nair,N.G and Daniel,R.S. 1983. Preference of *Bemisia tabaci* Gen. to cassava varieties and their reaction to cassava mosaic disease. *J.Root Crops.* **9**: 45-49.

Nair, S.G., Unnikrishnan, M., Sheela, M.N., Abraham, K., Vimala, B., Sreekumari, M.T. and Easwari Amma, C.S. 1998. Tuber crops varieties released by CTCRI. *Technical Bulletin Series* : **24**, CTCRI, India. pp.6-14.

Narasimhan,V. and Arjunan,G. 1976. Mosaic disease of Cassava - Loss in yield and tuber splitting. *Indian Phytopath*. **29**: 428 - 429.

Ng, S.Y.C., Thottappilly, G. and Rossel, H.W. 1992. Tissue Culture in disease elimination and micropropagation, In: *Biotechnology: Enhancing Research on Tropical Crops in Africa.* G. Thottappilly, L.M. Monti, D.R. Mohan Raj, A.W. Moore (eds.), pp. 171-182, CTA/I.I.T.A. co-publication. IITA, Ibadan, Nigeria.

Nichols, R.F.W. 1947. Breeding cassava for virus resistance. *East Afr. Agric. J.* 15: 154-160.

Njock, T.E., Atiri, G.I., Thottappilly, G. and Hahn, S.K. 1994. Distribution and disease recovery of African cassava mosaic virus (ACMV) in stems of resistant and susceptible cassava. In: *Proc. Fifth Triennial Symp. Intern. Soc. Tropical Root Crop-African Branch (ISTRC-AB)*, pp.149-152. Fifth Triennial ISTRC-AB Symposium, Kampala, Uganda, 22-28 November, 1992.

Njock, T.E., Atiri, G.I., Thottappilly, G. and Thresh, J.M. 1996. Disease development and recovery in resistant and susceptible cassava genotypes infected by African cassava mosaic geminivirus. *African Plant Protection* 2: 97-102.

Ogbe F.O., Dixon, A.G.O., G.I. Atiri, A.G.O. and Thottappilly, G.. 2002. Restriction of virus movement into axillary buds in an important aspect of resistance in cassava to *African cassava mosaic virus*. *J. Phytopath.* 150: 546-552.

Ogbe, F.O., Atiri, G.I., Dixon, A.G.O. and Thottappilly, G. 2003. Symptom severity of cassava mosaic disease in relation to concentration on *African cassava mosaic virus* in different cassava genotypes. *Plant Pathol.* 52, 84-91.

Okogbenin E, Porto MCM, Dixon AGO (1998). Influence of planting season on incidence and severity of African cassava mosaic disease in the sub humic zone of Nigeria. In: Akoroda MO Ekanayake IJ (1998) Root Crops and Poverty Alleviation. IITA, Ibadan, Nigeria. Pp388-392.

Otim-Nape, G.W., Bua, A. and Baguma, Y. 1994. Accelerating the transfer of improved production technologies: Controlling African cassava mosaic virus disease epidemic in Uganda. *Afric. Crop Science J.* 2: 479-495.

Otim-Nape, G.W., Bua, A., Thresh, J.M., Baguma, Y., Ogwal, S., Ssemakula, G.N., Acola, G., Byabakama, B. and Martin, A. 1997. Cassava mosaic virus disease in Uganda: The current pandemic and approaches to control. NARO, NRI, ODA publication, 65pp.

Otim-Nape, G.W., Thresh, J.M., Bua, A., Baguma, Y. and Shaw, M.W. 1998. Temporal spread of cassava mosaic virus disease in a range of cassava cultivars in different agro-ecological regions of Uganda. *Ann. appl. Biol.* 133: 415-430.

Padidam, E., Sawyer, S. and Fauquet, C.M. 1999. Possible emergence of new geminiviruses by frequent recombination. *Virology* 265: 218-225.

Palaniswami,M.S., Binu,A., Lisha Vijayan,S and Henneberry.T.J. 2004. Whitefly *Bemisia tabaci*: Biotypes, *Indian Cassava Mosaic Virus* and its biocontrol agents. In *Sixth International Scientific meeting of the Cassava Biotechnology Network*, 8-14 March 2004, CIAT, Colombia. Abstracts pp.87.

Palaniswami,M.S., Nair,R.R., Pillai,K.S and Thankappan,M. 1996. Whiteflies on cassava and its role as vector of cassava mosaic disease in India. *J.Root Crops*, **22**: 1-8.

Pillai, Santha V., Potty, V.P., Sundaresan, S. and Dinesh, V. 1999. Genetic diversity in ICMD-free accessions of cassava expressed in terms of esterase isozyme polymorphism. In *Tropical tuber crops –Food Security and Nutrition* Ed. Balagopalan *et al.,* Oxford & IBH Publishing Co., New Delhi pp. 238-242.

Pillai, Santha V., Sheela,M.N., Thankappan,M., Mohankumar,C.R. and Lakshmi,K.R. 1995. Collection, evaluation, cataloguing and conservation of germplasm of cassava. *Annual Report 1994-1995.* CTCRI, India. Pp 14-15.

Pillai, Santha V., Nair,R.R and Sreekumar,J. 2004a. Collection, conservation, cataloguing and evaluation of cassava germplasm. *Annual Report 2002-03*. CTCRI, Thiruvananthapuram, India. Pp 23-24.

Pillai, Santha V., Sumarani, G.D., Manjusha, P. and Sundaresan, S. 2004b. Molecular diversity in the land races of cassava in India. In Sixth *International Scientific meeting of the Cassava Biotechnology Network*, 8-14 March 2004, CIAT, Colombia. Abstracts pp.45.

Rajendran, P.G., Nair, S.G., Easwari Amma, C.S., Vasudevan, K. and Sreekumari, M.T. 1995. Recent progress in cassava varietal improvement in India. In *Cassava Breeding, Agronomy Research and Technology transfer in Asia.* Proc. of Fourth Asian region workshop held at Trivandrum. Ed. R.H. Howler, pp.84-96.

Rajendran.P.G, Mohan. C and Sreekumar. J. 2004. Breeding True Cassava Seed Progeny for Mosaic Disease (CMD) Resistance. *Gene Conserve*. **11**: 154-186. http://www.geneconserve.pro.br/artigo_20.htm

Ramaiah, M., Ramasamy, N., Jayarajan, R. and Ramadas, S. 1993. Evaluation of cassava genotypes for resistance against Indian cassava mosaic disease, *J. Root Crops* **19**:60-61.

Rossel HW., Changa CM., and Atiri G. I. 1994. Quantification of resistance to African Cassava Mosaic Virus (ACMV) in IITA-improved, mosaic-resistant cassava breeding material. In: Akoroda M. O. Root crops for food security in Africa. Proceedinds of the Fifth Triennial Symposium of the International Society for Tropical Root Crops - Africa Branch held at Kampala, Uganda. 22-28November 1992.

Saunders, K., Salim, N., Mali, V.R., Malathi, V.G., Briddon, S.R., Markham, P.G. and Stanley, J. 2002. Characterisation of *Sri Lankan cassava mosaic virus* and *Indian cassava mosaic virus*: Evidence for acquisition of a DNA B component by a monopartite begomovirus. *Virology* 293: 63-74.

Scott, G.J., Best, R., Rosegrant, M., and Bokanga, M. 2000. Roots and tubers in the global food system : A vision statement to the year 2020 (including Annex.) A Co-publication of CIP, CIAT, IFRI, IITA and IPERI, International Potato Centre, Lima, Peru.

Shanmugavelu, K.G., Thamburaj, S. and Abdul Khader, J.B.M. 1987. New varieties of horticultural crops. *South Indian Horticulture*, Tamil Nadu Agr. Univ., Coimbatore. pp.12.

Sheela, M.N., Easwari Amma, C.S., Unnikrishnan, M. and Nair, S.G. 2002. Expression of cassava mosaic tolerance in interspecific back cross progeny of cassava (*Manihot esculenta* Crantz.). In *International Conference on Vegetables*, Nov. 11-14, 2002, Bangalore. Abstracts pp. 58.

Sheela, M.N., Unnikrishnan, M., Edison, S. and Easwari Amma, C.S. 2004. *Manihot caerulescence - A new source of resistance to cassava mosaic disease (ICMD). In Sixth International Scientific meeting of the Cassava Biotechnology Network*, 8-14 March 2004, CIAT, Colombia. Abstracts pp.48.

Storey, H.H. and Nichols, R.F.W. 1838. Studies of the mosaic disease of cassava. *Ann. appl. Biol.* 25: 790-806.

Thamburaj, S. 1990. Research accomplishments in tapioca at Tamil Nadu Agricultural University. *Green Book on Tapioca. pp.16-18.*

Thottappilly, G. 1992. Plant virus diseases of importance to African agriculture. *J. Phytopathol.* 134: 264-288.

Thottappilly, G., Thresh, J.M., L.A. Calvert and Winter, S. 2003. Cassava. In: Virus and virus-like diseases of major crops in developing countries, G. Loebenstein and G. Thottappilly (eds), Pages 107-165, Kluwer Academic publishers, 800 pp.

Thresh, J.M., Otim-Nape, G.W. and Jennings, D.L. 1994. Exploiting resistance to African cassava mosaic virus. *Aspects Appl. Biol.* 39: 51-60.

Thresh, J.M., Otim-Nape, G.W. and Fargette, D. 1998a. The control of African cassava mosaic disease : phytosanitation and/or resistance? In: Plant Virus Disease Control , A. Hadidi, R.K. Khetarpal and H. Koganezawa (eds). Pp. 670-677, American Phytopathological Society, St. Paul, Minnesota.

Thresh, J.M., Otim-Nape, G.W. and Fargette, D. 1998b. The component and deployment of resistance to cassava mosaic virus disease. Integrated Pest Manag. Rev. 3: 209-224.

Unnikrishnan, M., Easwari Amma, C.S., Santha V. Pillai, Sheela, M.N., Anantharaman, M. and Nair, R.R. 2004. Varietal improvement programme in cassava. *Annual Report 2002-2003*, CTCRI, India. pp.24-26.

Unnikrishnan, M., Easwari Amma, C.S., Sreekumari, M.T., Sheela, M.N. and Mohan, C. 2002. Cassava germplasm conservation and improvement in India. In *CIAT-VII Asian Cassava Research Workshop*, Oct. 28. Nov. 1, 2002, Thailand Program & Abstract p.4

Winter S., Ariyo O., Koerbler M., Dietrich K., Dixon A. 2004. In: Alves and Tohme. Adding Value to a Small-Farmer Crop: Proceedings of the Sixth International Scientific Meeting of the Cassava Biotechnology Network. 8-14 March 2004, CIAT, Cali Colombia. Book of Abstracts Pp97.

2