CHAPTER 1

GENERAL INTRODUCTION

Cassava (*Manihot esculenta* Crantz) is one of the major staple foods in Tanzania and is ranked only second to maize (FAO, 2003). Cassava provides food and income to resource-poor farmers particularly on marginal or submarginal lands. Yields however, very low, owing to many production constraints. Cassava mosaic disease (CMD) caused by cassava mosaic geminiviruses (CMGs) constitute the major limiting factors causing yield losses as high as 100% or total abandonment of the crop in some areas (Thresh *et al*., 1994). Geminiviruses constitute a large group of plant viruses whose genome consists of a single-stranded DNA (ssDNA) circle contained in a small, twinned isometric particle (Hanley-Bowdoin *et al*., 1999) and cause severe disease and considerable damage to crops world-wide, including tomato, cotton, maize, bean and cassava. The genome organization and biological properties such as insect vector and host range allow geminiviruses to be divided into four genera (*Begomovirus*, *Curtovirus*, *Mastrevirus*, *Topocuvirus*) (Padidam *et al*., 1995; Fauquet *et al*., 2000; Fauquet and Stanley, 2003). Recently, phylogenetic analyses have also demonstrated a geographical basis for the evolution and divergence of geminiviruses, which are broadly divided into Old World (Eastern Hemisphere) and New World (Western Hemisphere) groups (Rybicki, 1994; Padidam *et al*., 1995). Most members in the genus *Begomovirus* have bipartite genomes, referred to as DNA-A and DNA-B components of 2.6-2.8 kbp in size, while a few species consist of only a single genomic component, resembling DNA-A.

A region of approximately 200 nucleotides common to both genomic components of bipartite begomoviruses contains *cis*-acting signals required for viral DNA replication and transcription. The viral DNA-A plus strand encodes the coat protein gene (CP/AV1). The DNA-A minus strand encodes three overlapping genes, of which Rep/AC1 (replication-associated protein) is essential for the replication of both genomic components (Hanley-

Bowdoin *et al*., 1999; Laufs *et al*., 1995; Unseld *et al*., 2004). T*r*AP/AC2 is required for the *trans*-activation of plus strand gene transcription from both DNA-A and DNA-B components, and the product of *REn*/AC3, which is not essential for infection, enhances viral DNA-Accumulation by an unknown mechanism (Haley *et al*., 1992; Hong and Stanley, 1995; Castillo *et al*., 2003, 2004). The two gene products *NSP*/BV1 and *MP*/BC1 encoded by DNA-B on the plus and minus strands respectively, are involved in viral spread (cell-to-cell) and symptom production (Frischmuth, 1999; Sanderfoot and Lazarowitz, 1995; Qin *et al*., 1998). Irrespective of their genome size and segmentation, all begomoviruses are transmitted by the whitefly *Bemisia tabaci* (Gennadius).

Because of the geminiviruses' importance and the relative ease with which their DNA genomes can be cloned, many geminiviruses are now being characterized. All begomoviruses that infect cassava are typical of the majority of members of the genus, having genomes of bipartite nature (Stanley, 1983; Stanley and Gay, 1983). Based on an approach, in which sequence homology demarcation of species is set at 89% for the DNA-A components of begomoviruses, six African and two Indian cassava mosaic geminiviruses (CMG) species are recognized (Fauquet and Stanley, 2003). These are *African cassava mosaic virus* (ACMV), *East African cassava mosaic virus* (EACMV), *East African cassava mosaic Cameroon virus* (EACMCV), *East African cassava mosaic Malawi virus* (EACMMV), *East African cassava mosaic Zanzibar virus* (EACMZV), *South African cassava mosaic virus* (SACMV), *Indian cassava mosaic virus* (ICMV) and *Sri-Lankan cassava mosaic virus* (SLCMV). The impact of cassava mosaic disease (CMD) caused by CMGs has drastically increased in recent years as a result of changes in agricultural practices, and dispersal of whitefly vector biotypes that have provided new opportunities for the viruses to adapt to different environmental conditions. The much wider dissemination of begomoviruses provides a greater opportunity for mixed infection, allowing recombination to play an important role in begomovirus diversity at a relatively frequent rate (Padidam *et al*., 1999). For example, recombination between ACMV and EACMV as well as synergism was probably responsible for the severe outbreak of a CMD pandemic in Uganda (Zhou *et al*., 1997; Pita *et al*., 2001). There is an intriguing possibility

that additional CMG species remain to be identified, since comprehensive sampling and characterization work has only been done for materials collected from a fraction of the geographical range affected by CMD (Legg and Fauquet, 2004).

Since different viruses have very different biological characteristics often with gross differences in the severity of the disease (Harrison *et al*., 1997; Fondong *et al*., 2000; Pita *et al*., 2001), there is an obvious advantage to be gained from understanding which virus species, strains and mixtures occur and how they are distributed in the major cassava producing countries.

The main goal of this study was to conduct molecular characterization of CMGs infecting cassava in Tanzania with the following specific objectives;- i) to identify CMGs and their strains in CMD-infected plants from different geographical areas in Tanzania, ii) to provide a molecular analysis of provisionally characterized CMGs isolates, iii) to determine and compare CMG DNA sequences with sequences obtained from GenBank, iv) to characterize and define behaviour of CMG in mixed infections, v) to develop a molecular diagnostic technique for identification of CMGs in single and mixed infections in Tanzania, vi) to determine molecular factors involved in symptom severity of CMG.
The following experiments were conducted to achieve the above objectives and are presented in this thesis (Chapters 3-7). –Collection of CMD-infected cassava samples and cuttings from all the major cassava-growing areas in Tanzania and the use of Polymerase Chain Reaction (PCR) and Restriction Fragment Length Polymorphism (RFLP) to charaterize the CMGs. Results of this work are presented in Chapter 3. Viral DNA samples that displayed unique RFLP patterns were chosen for further molecular analysis. Chapter 4 describes a molecular analysis (cloning, sequencing and infectivity assay) of a defective viral DNA molecule that was found naturally-occuring in the field in a mixed infection with EACMV. To determine molecular factors that are involved in CMG symptom expression, two novel single-stranded DNA satellite molecules were isolated and characterized. The results of this experiement are presented in Chaper 5. Chaper 6 describes the results of sequence analysis of DNA-A and DNA-B of CMG collections from

Tanzania. A molecular technique of characterizing geminiviruses using plant DNA stored on FTA® cards is described in Chapter 7. Issues arising from each research chapter are discussed in Chapter 8 (general discussion). The research chapters are preceded by Chapter one and two that deals with the general introduction and literature review, respectively.
All research chapters are written following the recent style of the Journal of General Virology.

1.1 REFERENCES

Castillo, A. G., Collinet, D., Deret, S., Kashoggi, A. & Bejarano, E. (2003). Dual interaction of plant PCNA with geminivirus replication accessory protein (REn) and viral replication protein (Rep). *Virology* **312,** 381-394.

Castillo, A. G., Kong, L. J., Hanley-Bowdoin, L. & Bejarano, E. R. (2004). Interaction between a geminivirus replication protein and the plant sumoylation system. *Journal of Virology* **78,** 2758-2769.

FAO. (2003). Cassava production data 2002. (http:/www.fao.org)

Fauquet, C. M., Maxwell, D. P., Gronenborn, B. & Stanley, J. (2000). Revised proposal for naming of geminiviruses. *Archives of Virology* **145/8,** 1744-1761.

Fauquet, C. M. & Stanley, J. (2003). Geminivirus classification and nomenclanture: progress and problems. *Annals of Applied Biology* **142,** 165-189.

Fondong, V. N., Pita, S. T., Rey, M. E. C., de Kochko, A., Beachy, R. N. & Fauquet, C. M. (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.

Frischmuth, T. (1999). Genome of DNA viruses. In: Mandahar, C. L. Molecular Biology of Plant Viruses, Ed. Kluwer Academic Publisher, Boston, USA.

Harrison, B. D., Zhou, X., Otim-Nape, G. W., Liu, Y. & Robinson, D. J. (1997). 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.

Hanley-Bowdoin, L., Settlage, S. B., Orozco, B. M., Nagar, S. & Robertson, D. (1999). Geminiviruses: Models for plant DNA replication, transcription, and cell cycle regulation. *Critical Reviews in Plant Sciences* **18**, 71-106.

Haley, A., Zhan, X. C., Richardson, K., Head, K, K. & Morris, B. (1992). Regulation of the activities of African cassava mosaic promoters by the AC1, AC2, and AC3 gene products. *Virology*, **188**, 905-909.

Hong, Y. & Stanley, J. (1995). Regulation of African cassava mosaic virus complimentary-sense gene expression by N-terminal sequences of the replication-associated protein AC1. *Journal of General Virology* **76**, 2415-2422.

Laufs, J., Jupin, I., David, C., Schumacher, S., Heyraud-Nitschke, F. & Gronenborn, B. (1995). Geminivirus replication-genetic and biochemical characterization of Rep protein function, a review. *Biochemie* **77,** 765-773.

Legg, J. P. & Fauquet, C. M. (2004). Cassava mosaic geminiviruses in Africa. *Plant Molecular Biology* (In press).

Padidam, M., Beachy, R. & Fauquet, C. M. (1995). Classification and identification of geminiviruses using sequence comparisons. *Journal of Virology* **76,** 249-263.

Padidam, M., Sawyer, S. & Fauquet, C. M. (1999). Possible emergence of new geminiviruses by frequent recombination. *Virology* **265,** 218-225.

Pita, S., Fondong, V. N., Sangare, Otim-Nape, G. W., Ogwal, S. & Fauquet, C. M. (2001). Recombination, pseudorecombination and synergism of geminiviruses are determinant keys to the epidemic of severe cassava mosaic disease in Uganda. *Journal of General Virology* **82**, 655-665.

Qin, S., Ward, B. R. & Lazarowitz, S. (1998). The bipartite geminivirus coat protein aids function in viral movement by affecting the accumulation of viral single-stranded DNA. *Journal of Virology* **72,** 9247-9256.

Sanderfoot, A. A. & Lazarowtz, S. G. (1995). Cooperation in viral movement: The geminivirus virus BL1 movement protein interacts with BR1 and directs it from the nuclear to the cell periphery. *The Plant Cell* **7**, 1185-1194.

Rybicki, E. P. (1994). A phylogenetic and evolutionary justification for three genera of geminiviridae. *Archives of Virology* **139,** 49-77.

Stanley, J. (1983). Infectivity of the cloned geminivirus genome requires sequences from both DNAs. *Nature* **301**, 643-645.

Stanley, J. & Gay, M. R. (1983). Nucleotide sequence of cassava latent virus DNA. *Nature* **301**, 260-362.

Thresh, J. M., Fargette, D. & Otim-Nape, G. W. (1994). Effects of African cassava mosaic virus on the yield of cassava. *Tropical Science* **26**, 34-37.

Unseld, S., Frischmuth, T. & Jeske, H. (2004). Short deletions in nuclear targeting sequences of African cassava mosaic virus coat protein prevent geminivirus twinned particle formation. *Virology* **318,** 90-101.

Zhou, X., Liu, Y., Calvert, L., Munoz, C., Otim-Nape, G. W., Robinson, D. J. & Harrison, B.D (1997). Evidence that DNA-A of a geminivirus associated with severe cassava mosaic disease in Uganda has risen by interspecific recombination. *Journal of General Virology* **78,** 2101-2111.