Molecular Characterization of Viruses Causing Mosaic Disease in Banana (*Musa spp.*) and Standardization of Technique for their Detection

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Abstract

To accomplish the objectives of the thesies entitled, "*Molecular Characterization of Viruses Causing Mosaic Disease in Banana (Musa spp.) and Standardization of Technique for their Detection*", the studies on survey, collection, maintenance of virus culture; transmission, host range, electron microscopic studies; molecular characterization of mosaic viruses (Cucumber mosaic virus & Banana bract mosaic virus) infecting banana; expression of CMV coat protein (CP) in *E. coli*, rasing of polyclonal antibody and standardization of efficient, sensitive, reliable protocol for the detection of mosaic viruses were performed.

Banana is an important fruit crop, especially in developing countries. Cucumber mosaic virus (CMV) is the most widespread virus in banana cultivars after banana bunchy top virus. Cucumber mosaic virus (CMV) infection was more prevalent in banana plantations of Uttar Pradesh followed by Karnataka and Maharashtra. However, samples showing typical banana bract mosaic virus (BBrMV) symptoms were collected from Karnataka. Host range and mechanical transmission studies showed that unlike many other CMV isolates, our CMV isolates (Karnataka, Maharashtra and Uttar Pradesh) were less competent in mechanical transmission. CMV isolates under study seem to have adapted themselves well to banana. Two primer pairs for CMV (CP & MP gene) and one pair for BBrMV (CP gene) were amplified the respective genes, which were cloned and sequenced. The sequence of CMV CP (Accession No. AM055602, AM158321 and DQ640743), MP (Accession No DQ642018, DQ642019 and DQ642017) and BBrMV CP (Accession No. EF654655 have been submitted to EMBL database. Our findings based

on symptomatology, virus particle morphology, nucleic acid properties and high degree of CP & MP sequence identity, phylogenetic analysis reveled that isolates under study belongs to subgroup IB of *cucumovirus* group. Similarly, symptomatology, nucleic acid properties and high degree of CP sequence identity confirmed that BBrMV isolate under study belong to *potyvirus* group

RT-PCR based limit detection and slot blot hybridization experiments we inferred that CP transcript can be more effectively used for diagnosis of CMV epidemiology. Furter, RT-PCR based detection of coat protein and movement protein genes of CMV in banana leaf tissue were standardized with respect to different annealing temperatures, magnesium concentrations, RNA transcript limit and polyclonal antibody based IC-RT-PCR. Similarly to RT-PCR based detection of CMV, protocol for BBrMV detection on the basis of coat protein gene of BBrMV from banana leaf tissue were also standardized.

Polyclonal Antibody raised against expressed coat protein of CMV was found to be reliable for the detection of virus in crude sap of banana plant by Ouchterlony Double Diffusion test and immunocapture RT-PCR. The well characterized coat protein (CP) gene of CMV and BBrMV could be used for transformation study with a view to generate transgenic against to CMV and BBrMV. The availability of standardized RT-PCR can be exploited for specific detection of CMV and BBrMV present in very low concentration in infected banana plants.