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EFFECT OF *Fusarium mangiferae* ON MANGO DISEASE IN AZAMGARH DISRICT OF U.P., INDIA

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ABSTRACT

This study provides further direct evidence of involvement of different strains of *F. mangiferae* in malformation diseases of mango in the Azamgarh district of U.P., India. *Fusarium* isolates were made by PCR amplification using primers specific to the translation elongation factors 1α and β- tubulin gene of *F. mangiferae*. Further, SDS-PAGE and RAPD profiles showed genetic variability among isolates of *F. mangiferae*.

KEYWORDS : *Fusarium mangiferae*, Colony Character, Genetic Variability, Mango Malformation, PCR Confirmation

Mango malformation is the most threatening disease to mango, causing heavy losses of mango fruits every year. Malformation recognized from the Darbhanga district in Bihar by Maries, (1981) is one of the most censorious diseases of mango in the natural world, causing 60-70% of the damage to mango fruits, and may be up to 100% (Mishra et.al.,2009). It is recognized as a disease (Summanwar et al., 1966) and a physiological disorder (Ansari et al., 2012). In India it was reported that the development of floral and vegetative malformation in mango is due to the involvement of *F. moniliforme* with the disease (Summanwar et al., 1966). Recently, we reported that *Fusarium* sp. displayed the most morphological resemblance to the accepted standard features of F. mangiferae via morphological characterization (Ansari et al., 2012). In this study, the extension of the same work has been carried over to further characterized. *F. mangiferae* isolates from mango of the Eastern part of the U.P. via PCR amplification using primers specific to translation elongation factor 1α and β-tubulin *F. mangiferae*. Further, genetic variation among the isolates of *F. mangiferae* was identified through SDS-PAGE and RAPD profile.

MATERIALS AND METHODS

Fusarium isolates from mango trees (Ansari et al., 2012) were purified by adopting the Keitt method (Keitt,1951). The genomic DNA was isolated by means of the CTAB (N-acetyl-N, N, N-trimethyl-ammonium bromide) method (1999) to perform PCR. The 2 regions were dissected from gDNA using the primer set EF1 + EF2 [5'-atgggtaagg a(a/g)gacaaga c-3' and 5'-gga(g'a)gtacc agt(g/c)atcat gtt-3'; (O'Donnell et al.,1998)] and T1+T2[5'-aacatgegtg agattgtaag t-3' and 5'-tagtgaccct tggcccagtt g-3'; (O'Donnell and Cigelnik,1997)]. The SDS-PAGE analysis was performed with the same procedure mentioned earlier (Aly et al., 2003). The RAPD markers study was performed by following the method described by (Quellet and Seifert, 1993). The presence and absence of unique and shares polymorphic as well as monomorphic products were used to generate a similarity coefficient. The similarity coefficient was then used to construct a diagram by the unweight pair group method with arithmetical average (UPGMA).

RESULTS AND DISCUSSION

The isolates of *Fusarium* were obtained from mango plants showing bluish-white colony character. The PCR amplification gives rise to the expected size amplicon of 0.7 kband 0.6 kb with the primer set EF1+EF2 for the genes encoding translation elongation factor 1α (EF 1 α) and β-tubulin, respectively. In the SDS-PAGE analysis, the isolate-1 showed lesser similarity coefficient (0.11) and great variability (99.89%) followed by isolate-9, which exhibited (99.87%) variability. The sub-cluster of isolate-2 and -5, isolate-3 and -7, and isolate-4 and-8 were observed to share similarity of 0.53, 0.45, and 0.40, respectively, and exhibited 99.47%, 99.55%, and 99.60% variability, respectively. In cluster A, B, and C, differential similarity was detected that could explain the variability among the clusters with respect to sub-clusters. The RAPD profile revealed that isolate-2 was the least similar to the remaining isolates with a similarity coefficient of 0.1429 and showed greater variability (99.86%). The isolate-10 and-9 showed

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maximum similarity of 0.3095% (99.69% variability) followed by isolate-3 and-1 (showed 0.2982% similarity and 99.70% variability) and isolate-6 and-5 (showed 0.2778% similarity and 99.72% variability). Isolates formed clusters and sub-clusters. In cluster A, isolate-4 shares similarity of 0.2123% (99.79% variability) with sub-cluster al of siolate-3 and isolate-1. In cluster B, Isolate-8 shares similarity of 0.1186% (99.78% variability) with sub-cluster b1. In cluster C, isolate-7 showed 0.2254% similarity (99.77% variability) with respect to sub-cluster c1 of isolate-5 and isolate-6.

Out report on distinguishing features of *Fusarium* (Ansari et al., 2012) such as growth, colony colours, septate hypha, microconidia, macroconidia and transverse sections of hyphae were found to be specific to features of *F. mangiferae*. All the studied isolates were in conformity with the earlier used standard and displayed highly similar characteristics defined for *F. mangiferae* (Britz et al., 2002). Here, the extension of same work has been carried over to characterized *F. mangiferae* further on the basis of DNA and protein profile. In this study, a purified isolate of *Fusarium* from mango plants of the tarai region showed bluish-white colony colour. The entire EF 1α nucleotide sequences were evaluated via the BLAST search tool to individuals within the database of *Fusarium* identification (Geiser et al., 2004). A comparison was made for nucleotide sequences for β-tubulin to those in the database of the National Centre for Biotechnology Information (NCBI) to attain first round identifications. In the present study, PCR products) 0.7kb and 0.6kb) with the primers set (EF1 + EF2 and T1 + T2) from gDNA of *Fusarium* associated with malformation led to the confirmation of *Fusarium* as a *F. mangifeare*. The sodium dodecyl sulfate-PAGE of proteins has been exploited comprehensively for identification and *Fusarium* at the strain and species level (Snider,1973). Distinctive fingerprint profiles generated by the random amplified polymorphic DNA techniques can be exploited for race identification purposes (Abd-Elsalam et al.,2004). In this study, genetic variability among the isolates of *F. mangiferae* from mango plants of the eastern part was detected, which indicates more than one strain of *F. mangiferae* could be associated with mango malformation.

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