MOLECULAR MARKER ASSISTED SCREENING FOR DISEASE SUSCEPTIBILITY IN SHISHAM SEEDS AND PATHOGENIC VARIABILITY IN *FUSARIUM SOLANI*

ABSTRACT

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JAMIA MILLIA ISLAMIA, NEW DELHI

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By

Mohammad Arif

DEPARTMENT OF BIOSCIENCES
JAMIA MILLIA ISLAMIA
NEW DELHI – 110 025

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ABSTRACT

Dalbergia decline or shisham wilt is one of the most serious plant diseases of present times. This disease has already caused and still causing enormous losses and has attained status of a disease of national importance in India. There are a lot of gaps in our knowledge about this malady, which are proving to be major hindrance in developing an effective disease management strategy to combat it. The present investigation was undertaken with the primary objective of establishing the etiology of the disease beyond doubt and investigating the genetic diversity in the pathogen and host by using conventional, molecular and bioinformatics tools. Other objectives were to develop molecular markers for the identification of the pathogen at species and/or strain level and to develop selective primers for the rapid identification of wilt resistant seed sources/ plants. The samples of infected plant parts (stem, root and stem ooze) were collected from different shisham wilt affected areas of the country. Attempts were made to isolate both bacteria and fungi from infected samples. No bacterium was ever isolated from any of the samples. Almost all the samples from infected tissues and ooze yielded fungi. Thirty-eight fungal isolates were collected from different plant parts and purified by single spore isolation. They were identified as *Fusarium* spp. based on morphological characteristics, particularly presence of macro and micro conidia studied. After initial identification, molecular marker based identification was conducted in which two sets of specific primers for *Fusarium* genus and *F. solani* were developed. Specificity of markers was verified by using 11 *Fusarium* spp. obtained from IMTECH, Chandigarh. This study indicated that all 38 isolates were *Fusarium* spp. Twenty-two isolates from stem and root samples and two (out of 14) isolates from stem ooze were *F. solani* while rest of the isolates from stem ooze were identified as *Fusarium* spp. These specific markers gave 495bp and 355bp fragments with *Fusarium* genus and *F. solani*, respectively. Molecular marker based identification was further confirmed by using BLAST study in which bands of all isolates obtained from *Fusarium* genus specific markers were sequenced which includes 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal

RNA gene, partial sequence. In pathogenicity test, when inoculated through roots and soil, 18 isolates (all *F. solani* from root and stem) out of 38, induced wilting in 2.5 months old seedlings. None of the isolates from stem ooze were pathogenic. Pathogen was re-isolated from infected root and also from the stem of the wilted seedlings. Using molecular markers it was conclusively proved that strain of the *F. solani* isolated from stem of the inoculated plant was the same as the one isolated from rotted roots from same plant. Both these isolates were the same as the one inoculated through roots and soil. Root rotting was found to be invariably associated with the wilt in shisham both in naturally wilted plant and also in inoculated seedlings. Root rotting was partial in partially wilted plans. In fully wilted plants most of the roots were rotted. These observations conclusively proved, for the first time, that *F. solani* was the causal agent of the disease. It not only caused root rotting but also enters the vascular system. Wilt symptoms seems to be net result of both root rotting as well as colonization of vascular system of the plant by the fungus. Out of 18 pathogenic isolates, nine were further inoculated on 1 and 15 moth old seedlings. They showed the similar results.

In order to identify sources of resistance 22 shisham genotypes (seed sources), collected from different places (Table 3.3) were inoculated with two virulent strains (F1 and F3) of the *F. solani*. Only one genotype *viz*. Ds-18 (disease index 0%) was found to be resistant to both the isolates with 0% disease index. Genotype Ds-1 was susceptible to one isolate and it was rated as moderately resistant to shisham wilt. A total of four molecular markers namely AFLP, RAPD, ISSR and SSR were used for comparative genetic analysis of host (shisham) genotypes. A total of 128 primers were selected for analysis. The matrices generated by different molecular markers were compared and two-way Mantel-test was conducted using MXCOMP programme of NTSYS pc for the validation of the result. Maximum polymorphism (69.1%) was revealed by SSR marker followed by AFLP (64.11%), RAPD (60.89%) and ISSR (53.41%). In case of RAPD, similarity indices varied from 0.606 to 0.932. Maximum similarity coefficient between Ds-3 and Ds-7 (0.932) and also between Ds-6 and Ds-7 (0.932) indicated closeness of these genotypes.