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Investigations of Biotic Agents Associated with Dieback Disease of *Dalbergia sissoo* Roxb. in Bangladesh

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Abstract

The dieback of *sissoo* (*Dalbergia sissoo* Roxb.) is a devastating disease occurring in Bangladesh as well as in India, Nepal, Pakistan and Afghanistan. The dieback symptom complex is characterised by wilting and subsequent loss of side branches leading to stagheadedness, constantly accompanied by gummosis on the trunk. Trees die within short time after the first appearance of symptoms. Fungi, bacteria and insects were reported to be associated with the dieback syndrome, but the causal agent(s) were not yet unequivocally identified. Our studies are focused on the molecular detection and characterisation of putative pathogens in leaf, wood and bark specimens from dieback affected *sissoo* trees, which had been collected at various sites in Bangladesh.

In a first approach we isolated bacteria from dieback-affected and unaffected specimens and started characterisation by sequence analyses including 16S rDNA and typical genes (RNA polymerase, RNase P, gyrase, among others). The sequence data indicated the association with the dieback syndrome of still unassigned bacteria belonging to the genus *Pseudomonas*. Hypersensitivity assays on indicator plants (*Chenopodium quinoa*, *Nicotiana tabacum*) revealed the phytopathogenic potential of several isolates.

On the other hand, the fungal pathogen *Fusarium solani*, which was supposed to be one of the major causes of *sissoo* dieback, was hardly detectable by means of molecular characterisation in our specimens, whereas *F. oxysporum* and in particular *Lasiodiplodia theobromae*, a well known pathogen associated with dieback syndromes of various tropical plants, could be clearly identified.

To make the situation even more complicated, electron microscopic inspection of leaf homogenates revealed the presence of virus-like particles of 60–130 nm in diameter. Preparation and gel electrophoretic analysis of double stranded RNA (dsRNA) allowed cloning and sequencing of cDNA fragments with similarity to viral replicases. Therefore, viral infections are also likely to contribute to the dieback disease.

In conclusion, our data provide clear evidence for a diverse aetiology of the dieback syndrome of *D. sissoo*, and strongly argue for intensive future efforts to the understanding and possibly controlling of this disastrous disease.

Keywords: *Dalbergia sissoo*, dieback, DOP-PCR, *Fusarium oxysporum*, *Lasiodiplodia theobromae*, *Pseudomonas*, Viral dsRNA

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