Differentiation of *Setaria digitata* and *Setaria labiatopapillosa* using Molecular Markers

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SUMMARY

5S rRNA intergenic regions of *Setaria digitata* and *Setaria labiatopapillosa* were PCR amplified with primers designed from the 5S rRNA gene of *Brugia malayi*. The ladder-like banding patterns obtained for the amplifications were distinctly different for the two species. Four amplified products were cloned into the pBS vector and completely sequenced. DNA clones from two individual samples of *S. digitata*, Sd4 and Sd6, showed 97% sequence homology to each other.

All sequenced clones showed the presence of the spliced leader (SL) RNA gene with a 22 nucleotide spliced leader sequence. The phylogenetic tree constructed using these data and the 5S rRNA intergenic regions of several other filarial nematodes showed the *Setaria* species sharing a branch with *Dirofilaria*. RAPD-PCR analyses identified 107 bands of which 86 were polymorphic (80%). A dendrogram constructed for *S. digitata* and *S. labiatopapillosa* separated the two species into two distinct clusters. The polymorphic loci identified by the RAPD-PCR analyses can be studied further to develop species-specific probes/PCR primers for the identification of each species.

KEYWORDS: *Setaria digitata*; *Setaria labiatopapillosa*; 5S rRNA; spliced leader; RAPD-PCR.

INTRODUCTION

Setaria species are parasitic nematodes, found in the peritoneal cavity of ungulates such as cattle and buffalo and are non-pathogenic in these natural hosts. The infective larvae can be transmitted accidentally to aberrant hosts such as sheep, goats, and horses through mosquito vectors. In these aberrant hosts, the larvae may migrate along the central nervous system and cause mechanical damage and inflammatory reactions, leading to cerebrospinal nematodiasis (CSN) (Innes *et al.*, 1952).

About 43 species of *Setaria* have been identified (Wijesundera, 2001). *Setaria digitata* and *Setaria labiatopapillosa* are two species predominantly found in Sri Lanka, the former being the more common. In the past, there has been some confusion in the allocation of species of *Setaria*. While Innes *et al.* (1952) suggested that both *S. digitata* and *S. labiatopapillosa* are identical and synonymous, Yeh-Liang Sheng (1959), on the basis of observation of distinct morphological features, suggested that the two species should be retained. This view was supported by Shoho and Uni (1977), who studied the morphological characteristics of several *Setaria* species including *S. digitata* and *S. labiatopapillosa*, using scanning electron microscopy.

Our study focused on the differentiation of the above two species at a molecular level through analyses of the 5S rRNA intergenic region and by the use of random amplified polymorphic DNA-PCR (RAPD-PCR technique). Variation between species measured at a DNA level, as opposed to morphological findings, offers the advantages that it can be quantified and that it is not subject to environmental effects (Kazan *et al.*, 1993).

MATERIALS AND METHODS

All chemicals used were of molecular biological grade and unless otherwise specified were pur-

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0169-4758/03/ $ - see front matter