Differtiation of Setaria digitata and Setaria labiatopapillosa using the 5s ribosomal RNA intergenic region and Random Amplified Polymorphic RNA (RAPD) Analysis

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Abstract:

The 5S ribosomal RNA intergenic regions of S. digitata, S.labiatopapillosa and S. cervi were amplified using the forward and reverse primers, 5SF1 and 5SR1.These primers were selected from a study carried out for filarial parasites and were custom synthesized. A ladder pattern was obtained for the PCR mplication for the 3 species studied, but a distince difference in size of the fragments could be seen. A 385 bp fragment (Sd4) and a 397 bp fragment (Sd6) of two separate S. digitata worms, a 582 bp fragment (Slp9) and a 747 bp fragment (Slp2) of S.labiatopapillosa and the 428 bp fragment (Scl) of S.cervi, were cloned and sequenced completely. While all 5 sequenced fragments showed AT-rich sequences (60 percent-70 percent), they all contained internal repeat sequences.When the cloned sequences were aligned with each other, a region of approximately 100 bp showed a high degree of homology. This homologous region is the leader sequence that is involved in the trans-splicing of the 22 nucleotide spliced leader wequence to certain other mRNAs. The 22 nucleotide spliced leader wequence could be seen in all the cloned sequences. The intron-exon splice junction at the 3' end of this spliced leader sequence was seebn in all the sequences studied. The two S. digitata clones Sd4 and Sd6, showed 97 percent homology. The clones derived from S.labiatopapillosa , Slp9 and Slp2, showed 68 percent homology. Sd6 showed 64 percent homology to Slp9, while Scl showed 75 percent homology to Sd6 and 65 percent homology to Slp9. The phylogenetic tree drawn for these results showed that the relationship between S. digitata and S.cervi is closer than that between S. digitata and S. labiatopapillosa. RADP analysis of S. digitata and S. labiatopapillosa was carried out for 40 primers for the pooled samples of the two species. Ten primers that showed the highest degree of polymorphism were selected to study seven individual samples each of the two species. The bands obtained for these ten primers were scored as being present or absent and this data was used to construct a dendrogram. The dendrogram showed the individuals of S. digitata and S. labiatopapillosa as two distinct groups

Key Words : DNA, Helminth-analysis / Setaria Nematode- isolation purification / Setaria Nematode / Setariasis-parasitology / Setaria Nematode-genetics