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Comparative analysis of the 5S ribosomal RNA intergenic region of three common species of *Setaria*

The comparison of three *Setaria* species, *S. digitata*, *S. labiata papillosa* and *S. cervi* was accomplished by studying the 5S rRNA intergenic region. This study involved the PCR amplification of genomic DNA using primers flanking the intergenic region, which was followed by cloning and sequencing.

The PCR amplification products showed a ladder pattern. The fastest moving fragment of each species was chosen for cloning and subsequent sequencing. The two *S. digitata* clones Sd 4 (385bp) and Sd6 (397bp), showed 97% homology, showing that there could be variation between individuals of a species.

The clone derived from *S. labiata papillosa*, Slp9 (582bp) was 64% homologous to Sd6 and 75% homologous to Scl (428bp), the clone derived from *S. cervi*. Sequences of all 4 clones were AT-rich (60-70%). The individual clones contained internal repeat sequences, some of which were found in all 4 clones.

When the sequences of these 4 clones were aligned with each other, a region of approximately 100bp showed a high degree of homology. A 22 nucleotide spliced leader sequence was found within this region and was identical in all the species analysed. This 100bp region could be the leader sequence that is involved in the trans-splicing of the 22 nucleotide spliced leader sequence to certain other mRNAs.

It can be inferred that *S. digitata* is more closely related to *S. cervi* than it is to *S. labiata papillosa*. The results indicate distinguishable differences in the fragment sizes and in the sequences of the three species. This confirms that *S. digitata*, *S. labiata papillosa* and *S. cervi* are three distinct species.