

Figure 3. TMI SST images on (a) 30 October and (b) 31 October 1999.

is quite common in the Atlantic Ocean and Gulf of Mexico, following the passage of a hurricane⁵.

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Y. SADHURAM

*National Institute of Oceanography,
Regional Centre,
176, Lawsons Bay Colony,
Visakhapatnam 530 017, India
e-mail: sadhuram@kadali.nio.org*

Interlocus homogenization of ribosomal DNA repeat units in barley

Eukaryotic ribosomal RNA genes that encode 18S, 5.8S and 26S ribosomal RNAs (rRNAs), are found as parts of repeat units that are organized as tandem arrays, located at the chromosomal sites known as nucleolar organizing regions (NORs). These ribosomal repeats at one or more loci constitute what is described as ribosomal DNA (rDNA). The ribosomal repeat units at an individual NOR are present in hundreds to thousands of copies¹. Each rDNA repeat unit consists of a highly conserved coding region (for 18S, 5.8S and 26S rRNAs) and a variable non-coding intergenic spacer (IGS) region. Each IGS in its turn contains a set of subrepeats which range in size from about 100 bp to about 4000 bp in different plant species². Generally, variation in the length of rDNA repeat units occurs due to variation in the size of the IGS region that itself depends on the variation in the number of intergenic spacer subrepeats. This variation in the number of subrepeats alters the length of the whole spacer region, leading to the occurrence of spacer-length-variants (slvs). These slvs at a locus are described as rDNA alleles and can be identified by restriction enzyme digestion coupled with Southern hybridization^{3–9}.

Barley has two major rDNA loci (*Rrn1* on chromosome 6 or 6H and *Rrn2* on chromosome 7 or 5H). Each barley rDNA repeat unit has two restriction sites for *SacI*, one in the 18S and the other in the 26S region. Consequently, on digestion with *SacI*, each ribosomal repeat will produce two DNA fragments; one of them containing the coding region would be constant in size, and the other containing full IGS and a part of the coding region would be variable in size. Such a feature is easily resolved as two bands that are visualized on Southern blots after hybridization with a ribosomal DNA probe. If there are two loci, as in barley, they will each produce a constant band and a variable band, so that one denser constant band is observed along with two lighter variable bands, which represent two slvs. Sometimes, three to five slvs are also observed in barley accessions either due to presence of complex loci or due to heterozygosity, but a solitary slv associated with two loci is only rarely observed.

For a study of ribosomal DNA polymorphism, we used 42 wild barley accessions collected from four eco-geographically contrasting microniches (sun-deep soil, sun-shallow soil, shade-deep soil and

shade-shallow soil) of Newe Ya'ar microsite (3182 m²) in Israel and supplied to us by E. Nevo from Haifa (Israel). We digested appropriate amounts of DNA of each of these 42 wild barley accessions with *SacI* restriction enzyme. Digested DNA was fractionated by electrophoresis on 1% agarose gel for 16 h at 4 V/cm, blotted to nylon membrane (Hybond N+) and subsequently hybridized with $\alpha^{32}\text{P}$ -labelled wheat rDNA probe pTA71. Membranes were exposed to X-ray films for

Table 1. Frequencies of different rDNA slv phenotypes in 42 accessions of wild barley (*H. spontaneum*) from Newe Ya'ar, Israel

slv phenotype	No. of accessions	Frequency
105	2	0.0476
106	2	0.0476
107	11	0.2619
108a	4	0.0952
108	4	0.0952
109	12	0.2857
110	2	0.0476
108a, 110	3	0.0714
108, 113	1	0.0238
109, 113	1	0.0238
Total	42	1.00

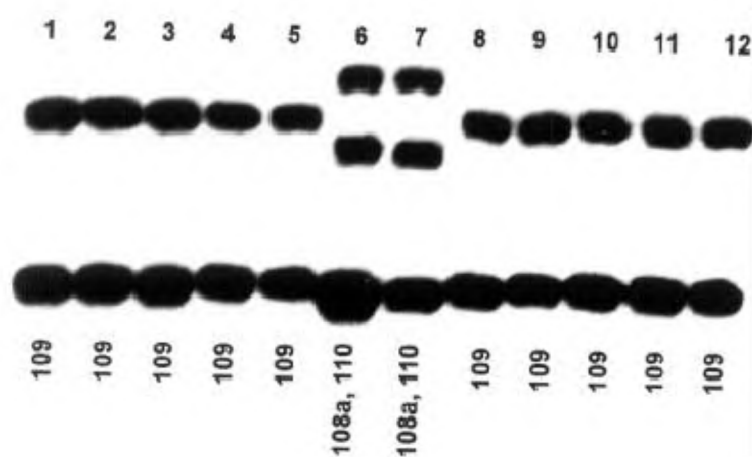


Figure 1. Autoradiogram showing two spacer-length-variant (slv) phenotypes in 12 accessions (marked 1–12) of barley. slv phenotypes are indicated by allelic compositions at the bottom (note that 10 out of 12 accessions have a solitary slv).

Table 2. Frequencies of single rDNA slv phenotypes in different studies on barley

Plant species	Number of accessions studied	Frequency of individuals showing single slv phenotype	Reference
<i>Hordeum spontaneum</i>	25	0 (0%)	3
<i>Hordeum vulgare</i>	100	6 (6.0%)	3
Composite cross-population of barley	377	53 (14.0%)	3
<i>H. spontaneum</i>	16	0 (0%)	4
<i>H. vulgare</i>	2	0 (0%)	4
<i>H. spontaneum</i>	267	0 (0%)	5
<i>H. vulgare</i>	92	7 (7.6%)	5
<i>H. vulgare</i>	305	2 (0.6%)	6
<i>H. spontaneum</i>	16	1 (6.3%)	6
<i>H. spontaneum</i>	21	5 (23.8%)	7
<i>H. vulgare</i>	27	5 (18.5%)	7
<i>H. spontaneum</i>	63	19 (30.1%)	8
<i>H. vulgare</i>	7	1 (14.2%)	8
<i>H. spontaneum</i>	285	19 (6.6%)	Our unpublished results

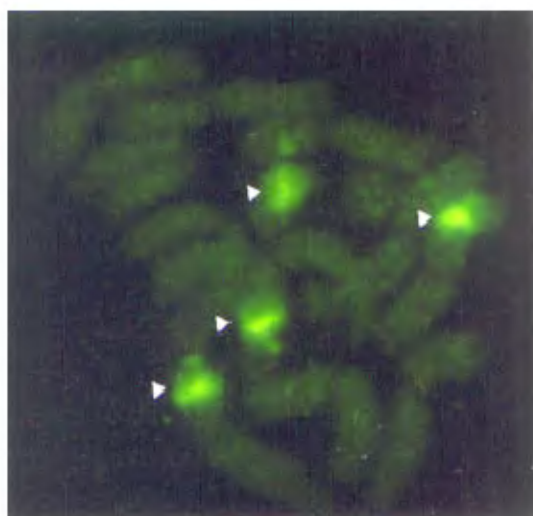


Figure 2. Somatic pro-metaphase chromosomes of root-tip cells of *H. spontaneum* ($2n = 2x = 14$) showing results of fluorescence *in situ* hybridization with biotin-labelled wheat rDNA probe, pTA71. Arrowheads indicate strong hybridization sites for rDNA on two pairs of chromosomes.

appropriate time and then developed to obtain corresponding autoradiograms. Lengths of DNA fragments in each band were worked out using the software SEQAID. Eight slvs constituting 10 different rDNA phenotypes were observed, a phenotype being represented by all the slvs available in an accession (Table 1). To our surprise and deviating from expected results, 88% of wild barley accessions showed the unexpected single slv phenotype, which was rather intriguing, but was an important observation (Figure 1). The results were confirmed by repeating the experiments with increase of electrophoresis time from 16 to 24 h at 3 V/cm for proper separation of digested fragments. The observed results, therefore, could not be an artifact of the technique.

We consider the above results interesting, because single slv in such a high frequency has never been reported in barley^{3–8}. In all earlier studies, phenotypes with two slvs each were predominant, and single slv phenotypes were only rare (Table 2). Since single slv could also result from loss of any one of the two rDNA loci, we conducted another experiment to examine if this could be the case. Fluorescence *in situ* hybridization (FISH) on somatic metaphase chromosomes (derived from fixed root tips) of barley accessions with solitary slv was conducted using biotin-14-dCTP-labelled ribosomal DNA probe (pTA71). Four hybridization signals on two pairs of homologous chromosomes were observed in each case, thus ruling out the possibility of loss of one of the two loci (Figure 2). It suggested, therefore, that the presence of the same slv at both the loci in barley is real and may have adaptive significance in the barley populations examined by us.

In wheat also, concerted evolution has rarely been observed between NOR loci on different chromosomes within a genome. For instance, in a study on rDNA in wild wheat (*Triticum dicoccoides*) which also harbours two NORs like barley, 112 individuals representing as many as 12 populations were studied. Sixteen individual plants (with no exception) from one specific population out of 12 showed a single slv phenotype each associated with two NORs that could be detected by FISH¹⁰. The extensive homogeneity of repeat lengths in this study was attributed to selection for the same particular variant at two loci. It seems, therefore that natural selection, driven by micro-ecological conditions, while directing dif-

ferentiation at rDNA loci, may rarely also involve homogenization of repeat unit lengths at the two rDNA loci.

In wheat, transfer of rDNA nucleotide sequences was suggested to be responsible for interlocus homogenization of rDNA between NOR loci on different chromosomes^{10,11}. Sequence comparison of spacer region and thermal dissociation technique have proved high homology between the spacer sequences which led to interlocus homogenization in wheat. Although such a transfer would lead to sequence homogenization, if whole repeat unit is thus transferred, this may also lead to homogenization of spacer length. In barley, though no direct evidence on the mechanism of interlocus homogenization is available, the mechanism of transfer of rDNA nucleotide sequences (including transfer of whole repeat units) between the loci, as evidenced in the case of wheat, is the most plausible explanation. High frequency of single slv phenotype must have resulted due to an adaptive value of this phenotype.

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S. SHARMA
S. KUMAR
H. S. BALYAN
P. K. GUPTA*

*Molecular Biology Laboratory,
Department of Agricultural Botany,
Ch. Charan Singh University,
Meerut 250 004, India*

**For correspondence.*

e-mail: pkgupta36@vsnl.com

Comparative RAPD analysis of causative agent of rhinosporidiosis and *Microcystis* isolated from pond waters

Rhinosporidiosis is endemic in several states of South India. The disease does not respond to any form of treatment, and after surgical excision of polyps, recurrences are common. Polypoidal masses of rhinosporidiosis contain microscopic round bodies which are diagnostic for this disease, and are believed to be the causative agent. The identity of a round body filled with numerous daughter cells, has been controversial for many decades^{1,2}. Investigations on samples of polyps have led earlier authors to suggest that the round body could be a protozoan^{1,3}, a fungus named *Rhinosporidium seeberi*⁴, alga with precursors of chlorophyll⁵, an organism similar to that found in plant crown gall tumours⁶, and an agent showing phylogenetic relationship with protist parasites of fish referred to as DRIP clade⁷. Most authors however, consider the round body as the sporangium of a fungus *R.*

seeberi filled with spores, but fungal etiology could not be proved. Since patients have a history of bathing in ponds, water samples were collected from exact locations in ponds where patients had been dipping, from four endemic states of India, and analysed in the laboratory. A cyanobacterium *Microcystis* could be isolated from all water samples and the same cyanobacterium was demonstrated microscopically in round bodies *in vivo*⁸. Daughter cells in round bodies, which had not been cultured successfully in the laboratory so far⁹, could now be grown on media used for cyanobacteria¹⁰. These studies suggested that daughter cells in round bodies could be cells of prokaryotic *Microcystis* instead of fungal spores, as pointed out by some authors⁹. This study was undertaken to find out whether or not a pathogenic strain of *Microcystis* exists in ponds in which patients had been

bathing, by comparing the RAPD profile of DNA extracted from round bodies in clinical samples with that of *Microcystis* in pond-water samples.

RAPD analysis has been considered valuable for determining genetic diversity and relatedness among diverse isolates of pathogenic organisms, and is widely accepted as a taxonomic tool, including strain identification. A modified low-stringency PCR technique using short arbitrary primers which anneal with mismatches in the template DNA to produce polymorphism profiles can be analysed and compared to distinguish organisms at subspecies and strain levels¹². Since previous genomic sequence information is not required, a universal set of primers of arbitrary nucleotide sequence and simple amplification protocol using small amount of DNA allows quick detection of several polymorphic DNA markers in a few