SYNOPSIS

A brief summary of work on repeated DNA sequences in rice

Rice is the most important food crop of the developing world and a staple food for more than half the world's population. It belongs to the family Gramineae, and genus Oryza, which includes 20 wild species and two cultigens O. sativa and O. glaberrima which have the same genome AA, but were independently domesticated (1). While O. sativa is the major cultivated rice on the Asian continent; O. glaberrima is cultivated in Western Africa. O. sativa has three dominant sub species namely indica (origin:India), japonica/sinica (origin:China), javanaica/bulu (origin:Indonesia). Rice genome has been classified into six types : AA, BB, CC, DD, EE and FF (2). It has twelve chromosomes (n=12), containing only 5.8×10^5 kbp per haploid genome, and has 52% of repetitive DNA as determined by Cot analysis (3,4).

The organization of repeated DNA sequences in rice O. sativa has been studied by Gupta et al. (5) and Dhar et al. (6). Recently, a satellite and a moderately repeated DNA sequence from O. sativa have been used as hybridization probes to study interrelationship of cultivated rice species with the O. perennis complex (7). In O. sativa, T.Wu and R.Wu (8) have identified a novel transcribed repetitive DNA sequence that shows

homology to both 5S and tRNA genes and also resembles 5S RNA gene in organization . DNA sequences specific to 4 genome types of rice namely AA, CC, EE and FF have been isolated and characterized (9). Attempts are also being made to use these genome specific sequences as molecular markers to study the origin and evolutionary relationships among various genomes and species of rice. Higher eukaryotic ribosomal genes are organized in tandem repeats. Variation of the intergenic spacer region of the ribosomal DNA is observed in cultivated and wild rice species (10). Recently, restriction fragment length polymorphism of rDNA spacer has been studied in Oryza using a cloned rice rDNA probe. While African rice of the species O. glaberrima do not display any rDNA size variation, wild rice species show extensive variation (11).

In the present work, an in-depth analysis of a rice dispersed repeat has been carried out with an aim of gaining more information about its organization, structure, abundance, methylation status at three different stages in rice and its potential use as a molecular marker for studying genomic/genetic variability among rice germplasm.

Thesis plan

The thesis is organized as follows

Chapter 1: Introduction

Here, I have compiled most of the available recent literature on rice repeated DNA sequences under the title: **Novel features of repeated DNA sequences in the rice genome.**

Chapter 2: Materials and Methods

During my Ph.D. work, I have handled an array of molecular techniques starting from DNA extractions up to DNA sequencing. The detailed protocols of all these experimental procedures are included in this section.

Chapter 3: Results

This section is divided into following three parts.

SECTION I : Construction of a partial genomic libbary of rice.

SECTION II : Characterization of a dispersed repeat sequence pOSM1C-2 in rice genome.

SECTION III : Assessment of genomic/genetic variability among 18 cultivars of rice using dispersed repeat sequence pOSM1C-2.

Chapter 4: Discussion

Here,I have made an attempt to discuss the results which I have described in three sections of chapter 3. Mainly I have discussed the role of repeat sequence

pOSM1C-2 in genome organization and its potential role in analysis of genomic variability among selected rice varieties.

Summary of work

Construction of a partial genomic library of rice

To start with, I constructed a partial genomic library of rice O. sativa cv. Malkolam in a plasmid vector pUC18. Malkolam (an indica rice) was selected because it is locally cultivated and is a land race. It has a conserved gene pool which is quite different from the modern cultivars and is likely to contain genes of commercial value. It has a few elite characters like early maturity, medium slender (MS) type of grain and good cooking qualities. The vector pUC18 was of choice because of its easy screening procedures and its direct use in sequencing and expression studies. After selecting the recombinants, I screened the library for repetitive/low copy clones by colony hybridization using P-32-labelled genomic DNA against the colony filters. Since my interest was mainly in repeated DNA sequences, I specifically selected those colonies which showed intense signals on the autoradiogram. I checked the insert sizes in a few of these random genomic clones by digestion with PstI and the size ranged from 0.2 to 2 kbp. The library

represented 55% low copy clones and 45% repeat sequences and served as a source of required types of probes for further studies.

Characterization of a dispersed repeat sequence pOSM1C-2

In my initial analysis of the PstI probes, I identified one dispersed repeat sequence in Malkolam rice genome. During the characterization of repeat, I digested the genomic DNA with different units of PstI (ie. setting up partial and complete digestions of genomic DNA), and challenged these digests with P-32-labelled pOSM1C-2. I observed that the repeat hybridized giving prominent bands superimposed on a background smear. This pattern is expected for a repeat sequence that is interspersed with either single copy or repeated sequences. Interestingly thus, the repeat appeared to be dispersed or non-tandem in nature. Since very little was known with respect to dispersed repeats in rice, I decided to concentrate on this particular repeat sequence and study its nature, organization, abundance and distribution in the rice genome. This repeat, as I named it pOSM1C-2, gave a moderately intense signal on the colony autoradiogram and had an inserted DNA fragment of 1.55 kbp.

After confirming that pOSM1C-2 is a dispersed repeat, its organization was studied in the rice

genome. For this purpose, genomic DNA was digested with 15 different restriction endonucleases and probed with pOSM1C-2 repeat sequence. In the case of BamHI, SacI, SalI and BstEII digests, the repeat hybridized to high molecular weight relic regions in the genomic digests, while distinct hybridizing bands of different molecu lar weights with a background smear were observed in case of EcoRI, BglII, HaeIII, BglI, PvuII, ScaI and HindIII digests indicating that the former set of enzymes has rare sites close to pOSM1C-2 repeat while the latter set has sites in the vicinity of region homologous to pOSM1C-2 sequence in the rice genome.

The next criterion of characterisation was to quantitate the abundance of the repeat in the genome. A filter containing dilution series of this repeat and three different concentrations of genomic DNAs was hybridized with the P-32-labelled pOSM1C-2 insert. When the autoradiogram was scanned with a densitometric scanner, the repeat showed the presence of approximately 150 copies in the rice haploid genome.

The restriction mapping work revealed the occurrence of a single site for enzymes like EcoRI, ScaI, HincII, AccI and SmaI in the repeat while absence of sites for BamHI, HindIII, ClaI, EcoRV, PvuII, BglI, BglII and SacI.

To investigate homologies within the repeat

sequence, smaller AccI-PstI fragment (0.450 kbp) was used as probe against the double digests of pOSM1C-2 with PstI-AccI, PstI-EcoRI and PstI-SmaI. The probe showed intense hybridization to itself and very low homology in the other part of the repeat i.e. PstI-AccI larger fragment (1.1 kbp). Further studies revealed that these two subdivisions of the repeat namely AccI-PstI (0.450 kbp) and PstI-AccI (1.1 kbp) showed differential organization and abundance in the genome.

Assessment of genomic variability among 18 cultivars of rice using the dispersed repeat sequence pOSM1C-2

The main aim of this study was to explore the potential role of pOSM1C-2 as a RFLP marker for distinguishing rice cultivars. It is now well known that RFLP technology has opened a door to detect and measure genetic variation in plants, in a manner not previously possible (12). Genomic DNAs from 19 elite cultivars were digested with 6 restriction enzymes namely EcoRI, EcoRV, DraI, ScaI, XbaI and HindIII and probed with P-32-labelled pOSM1C-2 insert. The probe was informative in revealing polymorphism with three enzymes namely EcoRV, DraI, and ScaI and deciphered different number of alleles with each enzyme. The similarity indices between the cultivars were calculated according to Nei's F statistic (13) using computer software developed in our laboratory. A dendro-

gram was then constructed by the same software using the unweighted pair group method with arithmetic mean (UPGMA) for the variability analysis. For the construction of the dendrogram, polymorphic probe-enzyme combinations of only 18 cultivars were selected. One cultivar namely Pusa-33 was excluded all the time since the autoradiography picture with this cultivar was inconclusive. From the dendrogram, it was possible to group the cultivars into two major clusters, with smaller sub-clusters within them. Interestingly enough, this grouping agreed with the pedigrees of these cultivars. These interrelationships deciphered amongst closely related indica cultivars will prove useful in the planning of future rice breeding programs in Maharashtra state and India.

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