Abstract

Rice is one of the most widely cultivated cereals in the world and more than half of the world's population consumes rice as their staple food. Thus it is only natural that it should become a crop of great importance from a commercial as well as fundamental point of view. Developmentally regulated genes are prime candidates for the study of basic plant mechanisms. Seed storage protein genes are expressed in a tissuespecific fashion with different levels of expression. Rice is one of the unique cereals which has glutelins as their major storage proteins and prolamins form only a minor fraction. Copy number estimation of these genes has revealed the occurrence of 8 - 10 copies of glutelin and 80 - 100 copies of prolamin genes per haploid genome of rice. Information regarding the mechanism of regulation of prolamin genes in rice is very limited. In fact, isolation of genomic clones of prolamin has been very sparse. Moreover, the disparity between high copy number and low expression levels of prolamin genes makes it an interesting system for investigation. In an attempt to attain this objective, molecular as well as tissue culture approaches were utilized. In the molecular approach, an attempt was also made to understand the regulation of storage protein genes using a theoretical approach. The first step towards isolation of prolamin genes from an elite indica cultivar of -rice namely, Basmati-370 was the construction of a genomic library of rice in a replacement vector namely, Charon 4A. One of the critical steps in genomic library construction was preparation of a packaging extract. Though commercial preparations were available, packaging extracts lose their viability when stored for a long time. Hence I prepared the packaging extract from an E. coli SMR-10 culture. The library was screened for prolamin clones with the help of a heterologous probe namely, kafirin, a prolamin gene from S. bicolor. Kafirin was used as heterologous probe since homologous rice prolamin probes were not yet available at the time of initiating this work. This probe enabled me to identify three putative clones of prolamin. Another advantage of construction of this library is that it can also be used to isolate any other genes of interest from rice. Parallel to the above work, I undertook a study of the 5' upstream regions of storage protein genes in order to identify regions that could be playing an important role in their expression. Free energy of these regions will indicate their ability to easily "open up" for transcription or translation. A lower free energy implicates a lesser tendency to form secondary structures. Interestingly, free energy profiles of monocot and dicot storage protein genes were distinctly different. An additional peak exhibited in the free energy profile of monocot genes could probably indicate that monocot and dicot storage protein genes have different regulatory mechanisms. But these studies need to be explored further, both theoretically and experimentally, in order to reach any definitive conclusion. The consensus sequences of monocot and dicot genes also differ significantly. The number of conserved nucleotides in monocots is higher than in dicots which could probably be related to a more evolved nature of monocots as compared to dicots. There is a higher number of A and T nucleotides in the region preceding the translation start site which also proves that this region has a lesser tendency to form secondary structures as combinations of A and T dinucleotides have a lower free energy value. This is shown by the common unstable peak present -35 bp upstream of the translation start site in monocot and dicot storage protein genes. An indepth analysis of monocot and dicot upstream regions could also provide a clue towards their differential levels of expression. These studies could also help in identifying regions that may be influencing the regulation of storage protein genes. Once the seed storage protein genes were isolated, it was necessary to have a system in which its expression could be monitored. Since the prolamin genes were isolated from an indica variety of rice namely, Basmati-370, a gene expression system of an indica variety had to be optimised. The regeneration of japonica

rice protoplasts is well established and is now possible using routine procedures. Such routine procedures are not yet available for indica varieties. Hence it was necessary to optimise conditions for callus initiation and protoplast isolation from indica cultivars. During optimisation, differences in isolation conditions for the three indica cultivars were noted. In order to assess their ability to express introduced DNA, protoplasts were transformed with control DNA. A plasmid containing the reporter genes gus and nptll was used for transformation of these protoplasts. The PEG method of transformation was preferred since it caused minimal injury to protoplasts. Though the protoplasts of all the three varieties did express GUS activity, they had different levels of background GUS-like activity. Now further studies can be carried out in both molecular and tissue cultural aspects. Firstly, the isolated putative prolamin clones can be further subcloned into smaller fragments in order to identify 5' upstream regions. These regions can then be fused to reporter genes like gus or nptll and their transient gene expression can be monitored in indica rice protoplasts. Secondly, conditions for regeneration of indica protoplasts can be optimised.