We report the gene structure of a nuclear Gly tRNA from a member of the higher plants, commonly called ragi (Eleucine coracana). This is an economically important cereal grown in arid and semi-arid regions of the world. The characteristic genetic traits of drought tolerance and high nutritional value make it a particularly attractive system to study gene expression from the standpoint of understanding osmoregulation and storage proteins. Identification of tRNA genes could serve as a starting point to map genes of interest, characterize their gene products, and further understand the regulatory mechanisms.

The general organization of nuclear-encoded tRNA genes in plants is to be dispersed as individual genes in the genome or to occur in clusters of tRNA genes (Bawnik et al., 1983; Waldron et al., 1985). From a λgt10 genomic library of ragi nuclear DNA, several clones containing ragi tRNA genes were identified using [32P]tRNA as a probe. A clone carrying a 647-bp DNA insert was further characterized by subcloning and the complete nucleotide sequence was determined. A section of this DNA sequence could be folded into a typical secondary structure of tRNA when subjected to a tRNA search program developed by Staden (1980). From the anticodon (GCC) it was deduced that this gene codes for tRNAGly (GCC) (Table I). The nucleotide sequence of the nuclear-encoded ragi tRNA Gly (GCC) shares 100% sequence identity with rice (Reddy and Padayatty, 1988) and the deduced RNA sequence is similar to the major species of Gly tRNA from wheat germ (Marcu et al., 1977). The gene does not encode conserved motifs like the internal split promoters, the 5' internal control region GGTCTAGTGG and 3' internal control region sequence GTTCGATTC, have been identified. A computer analysis was carried out to determine the frequency of occurrence of the particular nucleotide at every position of the polymerase III internal split promoter. To accomplish this, a sample of 100 eukaryotic tRNA gene sequences from various data bases (GenBank, EMBL, NBRF, etc.) were analyzed. The consensus sequence of the “A” box has been determined to be TGGCNNAGTTG and that of the "B" box is GGTTCGANTCC. The nucleotides A and G in the "A" box and nucleotides G, T, and C in the "B" box are conserved to an extent of 100%. The conserved nature of these nucleotides not only reflects their implied role in stabilizing the tertiary structure of tRNA (Kim et al., 1974), but it is also suggested that they play a critical role in gene transcription (Galli et al., 1981). The high degree of conservation of the "A" box and "B" box promoter element in tRNA genes was confirmed by dot-blot hybridization analysis. The copy number of tRNA Gly (GCC) in ragi was determined by dot-blot hybridization analysis (Kafatos et al., 1979).

It is estimated that there are six Gly tRNA gene copies per haploid genome. In the 3' flanking region the direct repeat motifs 5'-TTCCGTGT-3', 5'-TTATTCCTCT-3', and 5'-TCTCTGAAGAA-3' can be readily recognized. Such direct repeat motifs at the 3' terminus have been identified to be part of mobile genetic elements that have often been located in the proximity of tRNA genes in Saccharomyces cerevisiae (Clark et al., 1988) and Dicyostelium discoideum (Marschalek et al., 1989). Interestingly, a 6-bp region upstream to the coding sequence shows a region with a perfect dyad sym-
metry. The existence of such median symmetry elements has been reported in the upstream region of nuclear-encoded plant tRNA genes (Akama and Tanifugi, 1989); however, its significance remains unknown.

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