CLONING OF RICE DNA AND IDENTIFICATION OF *t*RNA GENE CLONES

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ABSTRACT

DNA from 48 hr germinated rice embryos was cut with restriction endonuclease *Bam* H1 and cloned to the *Bam* H1 site on plasmid pBR 322. The clones containing recombinant DNA were selected by their sensitivity to tetracycline and resistance to ampicillin. Using ^{32}P-labelled rice embryo *t*RNA as a probe two clones were identified to contain *t*RNA genes by colony hybridization.

INTRODUCTION

THE rapid development of recombinant DNA technology has brought forth a revolution in biology[1,2]. It aids us to have a closer look at the way genes are organized, especially in the complex eucaryotic genomes[3–6]. Although many animal and yeast genes have been studied in detail using recombinant DNA technology, plant genes have seldom been targets for such studies. Germination is an ideal process to study gene expression because it effects a shift in the metabolic status of seeds from a state of dormancy to an active one. An understanding of gene organization and regulation during germination can be accomplished by molecular cloning of DNA from seeds like rice. To study the status of histone, *r*RNA, *t*RNA and other genes in the rice genome, a general method was developed to clone eucaryotic DNA in a plasmid vector pBR 322. This essentially involves the following steps. The rice embryo and plasmid pBR 322 DNAs were cut with restriction endonuclease *Bam* H1 to generate sticky ends. The plasmid DNA was phosphatased, the DNAs were annealed and joined by T4 phage DNA ligase. The recombinant DNA molecules thus produced were transferred into *E. coli* and colonies containing them were selected by their sensitivity to tetracycline and resistance to ampicillin. Two clones were identified as having *t*RNA genes by hybridization of the DNA in the clones with ^{32}P-labelled rice *t*RNAs.

MATERIALS AND METHODS

The two strains of *E. coli* Hb 101 (one that harbours the plasmid pBR 322 and the other that does not) as well as enzymes *Bam* H1 and T4 phage DNA ligase were supplied by Dr. E. S. Srivatsan, University of California at San Diego, La Jolla. Rice DNA was provided by Dr. Elizabeth Zachariah of this laboratory. Trizma base, 2-mercaptoethanol, polyvinylpyrrolidone (PVP) m.w. 360,000, ampicillin, tetracycline, ATP, dithiothreitol (DTT), alkaline phosphatase and sodium dodecylsulphate (SDS) from Sigma Chemical Co., St. Louis; oligo(dT)-cellulose from Collaborative Research, Waltham, Massachusetts; bovine serum albumin (BSA) from Calbiochem, La Jolla; lysine-sepharose 4B and Ficoll, m.w. 400,000, from Pharmacia Fine Chemicals, Uppsala; BA 85 nitrocellulose filters from Schleicher and Schuell, Keene; formamide from Fischer Scientific Co., New York; and bactotryptone and bactoyeast extract from Difco Laboratories, Detroit were used. ^{32}P-Ortho phosphoric acid was from Bhabha Atomic Research Centre, Bombay. All other reagents were of analytical grade.

Luria broth contained 10 g of bactotryptone, 5 g of bactoyeast extract, 0·5 g of NaCl and 2 g of glucose per 1. Luria agar contained 1·4% agar in Luria broth.

Preparation of labelled rice tRNA

Rice seeds were germinated for 18 hr at 30° C in the dark in the presence of ^{32}P-orthophosphoric acid (0·5 mCi/100 seeds) under sterile conditions[7]. Total RNA was prepared according to the method of Palmiter[8] and purified by the method of Bellamy and Ralph[9]. Poly (A$^-$) RNA was separated by using an oligo (dT)-cellulose column[10]. The poly (A$^-$) RNAs were further separated into *t*RNA, *r*RNA and others on a lysine-sepharose 4B column[11].

Cloning

Cleaving with Bam *H*1 : DNA (2 μg) obtained from 48 hr germinated rice embryos was treated with 8 units of *Bam* H1 in a reaction buffer containing 20 mM tris-HCl, pH 7·0, 100 mM NaCl, 7 mM $MgCl_2$ and 2 mM 2-mercaptoethanol, in a total volume of 10 μl at 37° C for 90 min. Plasmid pBR 322 DNA (2·5 μg) was similarly cut with *Bam* H1.

Alkaline phosphatase treatment : The *Bam* H1 treated pBR 322 DNA was deproteinized first by phenol saturated with 10 mM Tris-HCl, pH 7·4, 1 mM EDTA (TE buffer) and then by chloroform. The DNA was ethanol precipitated, dried under vacuum and dissolved in 10 μl of TE buffer. This DNA was treated with 1 unit of *E. coli* alkaline phosphatase

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PHYSIOLOGICAL STUDIES IN THE REGENERATING ROOT CUTTINGS OF *CLERODENDRUM VISCOSUM* VENT.

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ABSTRACT

The data on the regeneration of root cuttings of *Clerodendrum viscosum* revealed that old and lengthy roots have yielded higher percentage regeneration than the young and short cuttings. The cuttings with ring-cut brought about physical separation of the cutting into two portions on either side of the cut, resulting in the establishment of physiologically apical and basal regions at the cut faces and as a consequence shoots developed at the apical region of the lower half and roots at the basal region of the upper half of the root cutting.

THE regenerative potentiality of root cuttings depends on the factors such as season, age, length and volume of cuttings[1,2] as well as hormonal balance[3,4]. Though the polarity in the regenerating roots is reported by many workers, the physiological separation of a single cutting into two halves by ring-cut is not reported for root cuttings. The development of adventitious buds is well studied[5–6]. The present paper deals with the variables such as length and age in the regeneration of stem and root cuttings which are made with ring-cuts and half-cuts.

The tap root cuttings of 2, 4, 8 and 12 cm length from 3, 6 and 9 months old plants of *Clerodendrum viscosum* were made with ring-cuts (Fig. 1A) at their middle parts in such a way that only xylem remained interconnected between two parts of the cutting. The cuttings of 12 cm were made with half-cuts at two parts of the cutting (Fig. 1B) in such a way that half of the root tissue is removed from the half-cut. The stem cuttings of 10, 15 and 25 cm length from 6th and 9th months old plants were also made ring-cuts as well as half-cuts as it was done for root cuttings. All the root and stem cuttings were planted horizontally in the pots containing garden soil. Twenty cuttings for each group were taken for each experiment. The data has been collected after 20 days of plantation.