Isolation, Identification and characterization of Jute Genomic LTR retrotransposon sequences.

A DISSERTATION SUBMITTED TO BRAC UNIVERSITY IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE DEGREE OF MASTERS IN BIOTECHNOLOGY

SADAF SAAZ SIDDIQI

DEPARTMENT OF MATHEMATICS AND NATURAL SCIENCES,
BRAC UNIVERSITY,
DHAKA, BANGLADESH
JANUARY 2012

SUBMITTED BY
ID: 07176003
SESSION:

To whom it may concern

This is to certify that the research work embodying the results reported in this thesis entitled " Isolation, Identification and characterization of Jute Genomic LTR retrotransposon sequences" submitted by **Sadaf Saaz Siddiqi**, has been carried out under co-supervision at the Department of Biochemistry and Molecular Biology, University of Dhaka, Dhaka, Bangladesh. It is further certified that the research work presented here is original and suitable for submission for the partial fulfillment of the degree of Master of Science in Biotechnology, BRAC University, Dhaka.

Professor Haseena Khan
Supervisor
Department of Biochemistry
and Molecular Biology,
University of Dhaka

Professor Naiyyum Choudhury
Supervisor
Biotechnology Program
Department of MNS
BRAC University

ABSTRACT

Long Terminal Repeat (LTR) retrotransposons make up a significant part of eukaryotic genomes and play an important role in plant genome evolution. Jute is an important fiber crop, and the Jute genome, consisting of 1,250 Mbps, is largely unexplored. In this study an attempt was made to identify and characterize the LTR retrotransposons of the Jute genome *Chorchoru solitorius* in order to understand the genome better. The Reverse Trancriptase domain of Ty1-*copia* and Ty3-*gypsy* LTR retrotransposons of Jute were amplified by degenerate primers. The sequences were analyzed and clustered into groups. Reverse transcriptase PCR was used to analyze any transcriptional activity. Sequence analysis showed a higher heterogeneity among Ty1-*copia* retrotransposons than Ty3-*gypsy* retrotransposons. One of the groups of retrotransposon Ty1-*copia* was found to be transcriptionally active. As the LTR retrotransposons constitute a large portion of the Jute genome, the findings of this study suggest the importance of these elements in Jute genome evolution.

TABLE OF CONTENTS

9. APPENDIX

1. Introduction:

1.1 General overview of Jute

Jute is a long soft shiny vegetable fibre, produced from the *Corchorus* plant. The fibre is spun into coarse, strong threads and used for a variety of commercial purposes. The jute fibre is composed of cellulose and lignin, so it is a partially textile fibre and partially wood. It is known as a bast fibre, as the fibre originates from the bast or skin of the plant. The fibre is an off white to brown colour, and range from to 3-12 feet long.

There are two main types of Jute, White Jute (*Corchorus capsularis*) and Tossa Jute (*Corchorus olitorius*). White Jute has been mentioned historically as being used to make clothes for poor villagers using handlooms (Ain-e-Akbari, Abul Fazal, 1590) as well as being used in Bengal and other parts of India for ropes and twines. Tossa Jute, the Afro-Arabian variety, has been mentioned in the Hebrew Bible. Silkier, softer and stronger than white jute, it grows well in the humid climate and alluvial soil of the Gangetic Deltaic regions.

1.2 Properties of Jute

Jute is one of the strongest as well as one the cheapest of all natural fibres, and is the second most important vegetable fibre after cotton in terms of usage global consumption, production and availability. It is also the most versatile of natural fibres, being historically used for packaging, textiles, non-textiles, construction and agricultural sectors.The properties of its high tensile strength, low extensibility, low thermal conductivity, as well as its biodegradable, recyclable and environmentally friendly properties mean that it has potential for a number of uses. It has good acoustic insulating properties, manufactures with no skin irritations, and has the flexibility to blend with range of other synthetic and natural fibres, and to accept cellulose dyes like natural, basic, vat, sulfur reactive and pigment dyes.

1.3 History of Jute

Historically Jute has been an important part in the culture and economy of southwest Bangladesh and parts of West Bengal. In the 19th and early 20th century during the time of the British Raj, raw jute was sent to the United Kingdom where it was processed in mills in Dundee. Margaret Donnelly, who was a jute mill landowner, set up the first jute mills in Bengal in the 1800s. Before the use of nylon and polythene, jute was used widely world-wide. The export of jute from the then East Pakistan, was one of the primary sources of foreign exchange for Pakistan. The jute industry faced a decline due to introduction of a variety of synthetic materials.

1.4 Jute and Bangladesh

The Jute industry is the second largest employer in Bangladesh; it directly and indirectly provides livelihood to 35 million people. It is the third largest source of foreign exchange for Bangladesh. Bangladesh is the largest cultivator of Jute in the world, and India is the largest exporter of jute goods.

1.5 Jute and the Global scenario

Recently there are signs of jute regaining its economic importance. With the current consumer demand for reduction of the hazardous health and environmental effects of synthetics, there is increasing demand for the use of traditional jute goods, as well as jute in other diversified forms. Natural jute fibres are being substituted increasingly in a range of industries, including paper, celluloid products, non-woven textiles, composites (pseudo-wood), and geotextiles. Geotextiles for example are lightly woven fabrics used to prevent soil erosion, for seed protection, weed control and other agricultural and landscaping purposes. The biodegradable nature of jute is very well suited to this. It is also used to produce climate neutral bags; bags which are committed to reduce Co2. The cellulose in the jute plant produced from the inner woody core or parenchyma of the jute stem could also be used as an effective wood substitute. The recent development of the use of jute in car manufacturing in the automobile industry is example of the kind of huge potential future opportunities there are for use of jute (www.jute.org).

1.6 Molecular biology of Jute

With the growing importance and re-emergence of Jute as an economically promising product, there is a need to understand the Jute plant at the molecular level in order to best avail the considerable economic potential of jute. With climate change there is a need to find ways to enable jute to grow in the increasing challenging conditions of land availability, temperature change and increased salinity, in order to maintain and increase its yield, and to enable its diversification for a variety of commercial uses. This study aims to contribute to the understanding of jute at the molecular level by looking at the role of retrotransposons in Jute, which take up about 19% of the Jute genome.

2. Literature Review:

Transposons or mobile genetic elements are an important component of eukaryotic and plant genomes. Retrotransposons are the largest type of such transposable elements. Even though they are ubiquitous in plants, their presence varies considerably. For example retrotransposons are only 5.5% of the genome of *Arabidopsis thaliana* but constitute 50% of the genomic content of *Zea mays*.

There are various classes of transposons. Retrotransposons belong to class I element of transposons. This classification is based on their method of transposition and propagation. Retrotransposons are one of two groups of transposons that multiply using an RNA intermediate. They are transcribed into RNA, are reverse transcribed and then are reintegrated into the genome. Retrotransposons are divided into further subclasses; one of which are Long Terminal Repeat (LTR) retrotransposons. These LTR transposons contain long terminal repeats at both ends of their sequences. The LTR transposons are further sub-divided into two groups: Ty1-*copia* and Ty3-*gypsy* both of which have the *pol* gene which consists of four domains: protease, integrase, reverse transcriptase and ribonuclease H (RNase H). The LTR groups Ty1 and Ty3 are classified depending on the order of these domains within the *pol* gene.

The important role of retrotransposons in determining the size of plant genomes, and their evolution due to the transposition or proliferation of retrotransposons via reverse transcriptase, is generally acknowledged (Wessler et al, 1995). In fact the maize genome, consisting of 2400 mega base pairs includes at least 70% retrotransposons belonging to Ty1-*copia*, Ty3-*gypsy* and LINEs groups, with variable copy number (SanMiguel et al, 1996; Ma and Bennetzen 2004). It has also been suggested that retrotransposons in the course of their coevolution with host genomes, have acquired various functional roles (Kidwell and Lisch 1997).

Ty1-*copia* retrotransposons have been isolated and characterized in many monocotyledons (Hirochika *et al* 1992, 1996) and dicotyledons (Flavell *et al*, 1992b; Pearce et al. 1996a; Yanez *et al* 1998; Asi'ns *et al* 1999; Villordon *et al* 2000); they have been studied in rice, maize,

Arabidopsis, wheat, rye, soybean, barley, tobacco, tomato, potato, citrus, strawberry among others. There has also been characterization of Ty3-*gypsy* retotransposons in plants like rice, maize, citrus and cotton (Su and Brown 1997; Friesen *et al* 2001; Bernet and Ass'ns 2003; Zaki and Abdel Ghany 2004).

Retrotransposons are important factors in the evolution of plant genomes, due to their role in chromosomal rearrangements, which result in alteration of gene regulation and function. Despite the fact that a large portion of plant retrotransposons are thought to be transcriptionally inactive, there are studies which have shown their differential expression under various stages of development and under certain biotic and abiotic stress conditions. In fact, it seems that the expression of retrotransposons in plants and other eukaryotes is regulated (Kumar and Bennetzen, 1999). During normal development retrotransposons seem to be silent in many plants, possibly a mechanism developed to minimize the possible deleterious effects of retrotransposons on host genome. However under biotic and abiotic stress conditions many plant retrotransposons are seen to be transcriptionally active (Tahara *et al,* 2004; Muthukumar and Bennetzen 2004; Hirochika *et al* 1996).

2.1 Why Jute?

Jute (*Corchorus* spp.) is an important source of a natural fibre, well known for its high tensile strength, biodegradability and resistance to heat. Jute is second only to cotton in the world's production of natural fibres (Roy A., 2006). Even though it has significance due to being an important cash crop, molecular research on jute is minimal. Only 1,210 sequences have been deposited in GenBank.

2.2 Overview of Jute

Jute fibre essentially is produced from two species; white Jute (*Corchorus capsularis*) and Tossa Jute (*Corchorus olitorius*). These two species have distinctly separate growth habitats, branching methods and characteristics; such as leaf, flower, fruit, seed, best fiber and photosensitivity (Basu A. *et al,* 2004).

2.2.1 History

White Jute is thought to originate from Indo-Burma or South China, while Tossa is thought to originate from Africa. The word Jute may come from the word Jhuta or jota, from Orrisa. Interestingly the use of '*Jutta potta*' cloth has been mentioned both in the Monushanghita-Mahabharat and the Bible, which indicates the use of jute in ancient times. There is also mention of the trading of jute cloth in the 16th century where Ain-I-Akbari (1590) mentions that jute sack cloth originated from Bengal.

2.2.2 Physical properties:

An annual shrub, the jute plant reaches a height of about 9-10 feet under normal conditions, and is sparsely branched. It bears light green arrow-shaped leaves and small yellow flowers which bloom singly or in clusters. Both species *C.capsularis* and *C. olitorius* are mostly self-pollinating; with seeds that are small and numerous (Janik *et al*, 1974).

2.2.3 Structure of Jute: The Jute and Jute allied fiber (JAF) stems are made up of the epidermis, cortex, large phloem, cambium, wide xylem or wood, and central pith tissues. The phloem is the most important part of the plant, as it is the thickened phloem tissue fibres which are extracted after disintegration of the other tissues through retting for commercial use. Jute is known as a best fiber, whereby the fiber is collected from the bast or skin of the plant. Other such bast fibers are kenaf, industrial hemp, ramie and banana fibers (Janik *et al*, 1974).

2.2.3.1 Taxonomy

Jute has fourteen diploid chromosomes, hence seven pairs of chromosomes (Hossain M.B. *et al,* 2003). Jute is a soft, long, shiny plant fiber composed largely of cellulose and lignin (Hossain MB. *et al,* 2002). The Kewensis mentions over 170 *Corchorus* species names (Edmonds JM.1980).

Taxonomical classification:

Domain: Eukaryota

Kingdom: Plantae

Division: Magnoliophyta

Class: Magnoliopsida
Order: Malvales
Family: Malvaceae
Genus: Corchorus

The world's leading producers of Jute are India, Bangladesh, China and Thailand, with Southwest Asia and Brazil also produces Jute. It is used in packaging, agriculture, quality industrial yarn, fabric, net and sacks (http://banglapedia.net).

2.3 Molecular biology research on Jute

There are only a few studies available on jute at the molecular level as below:

2.3.1 Analysis of Genetic Diversity

Molecular marker based techniques have been used to characterize the genetic diversity of jute, although there is potential for much more work to be done in this area. By using Random Amplified Polymorphic DNA (RAPD) marker, the genotypes of 9 different varieties and 12 accessions of jute cultivars from both species, *Corchorus olitorius* and *Corchorus capsularis* were explored, using the germplasm collection at the Bangladesh Jute Research Institute (BJRI) (Hossain MB *et al,* 2002). Twenty nine arbitrary oligonucleotide primers were screened, whereby polymorphisms were detected by 7 primers within the varieties and 6 within the accessions. Using RAPD and AFLP (amplified fragment length polymorphism), fingerprints to generate polymorphisms between cold sensitive and cold-tolerant accessions have also been carried out in Bangladesh, whereby 5 out of 30 arbitrary oligonucleotide primers were found to give polymorphism between the varieties. Eight out of 25 combinations of selective-amplification primers gave the best results with 93 polymorphic fragments, and it was possible to discriminate between the two cold sensitive and four cold tolerant varieties (Belayet *et al* 2003).

Other studies have evaluated genetic diversity using Simple Sequence Repeat (SSR) marker loci and an Amplified Fragment Length Polymorphism (AFLP) PCR assay (Basu *et al.,* 2004). One cultivar and 21 wild accessions of *C. capsularis*, and 2 cultivars and 25 wild accessions of *C. olitorius* were investigated using this method. AFLP analysis, using 10 pairs of primers (EcoRI

and MseI), detected 305 polymorphic variants from 49 genotypes from the two jute species.SSR markers are now being widely used in genetic diversity analysis studiesdue to their abundance, multi-allelic and co-dominant characteristics, high level of reproducibility, cross-species transferability etc. (Gupta *et al*2004). SSR markers were used for genetic diversity analysis of 45 genotypes of *C. olitorius* and 36 genotypes of *C. capsularis* (Mir et al, 2008), whereby 7 out of 8 pairs of primers designed to amplify the SSR loci generated polymorphic products 34 accessions studied at 7 SSR loci, 8 alleles were found.

Sequence Tagged microsatellite Site (STMS), Inter Simple Sequence Repeat (ISSR) and RAPDS markers have been used for genetic diversity analysis of 20 exotic germplasm lines and 20 commercial varieties of the two cultivated species (*Corchorus olitorius* and *C. capsularis*) and 2 wild relatives of jute (*Corchorus aestuans* and *Corchorus trilocularis* (Roy *et al,* 2006). Interspecofic polymorphism in this study was found to be low. The four ISSR and 22 RAPD primers showed 98.44% and 100% polymorphism respectively across all the species. In a subsequent study, a total of 106 alleles among 10 cultivars from *C. olitorius* and *C. capsularis* were detected using 23 primer pairs (Akter et al, 2008).

Interspecific genetic variability could be used to develop genotypes which have the desirable properties of both species. Tossa jute (*C. olitorious*) is relatively more disease and pest resistant than white jute (*C.capsularis*), whereas the latter is relatively more resistant to floods and drought. There has been some success in obtaining interspecific hybrids between these two supposedly cross-incompatible species, suggesting future possibilities in this area (Islam and Rashid 1960; Swaminathan et al, 1961; Islam 1964; Sinha *et al* 2004).

2.3.2 Sequencing of Jute Genome

Recently the entire jute genome has been sequenced, though the results have not been published. On 16 June 2010, Prime Minister Sheikh Hasina declared that Bangladesh successfully completed the draft genome of jute. A consortium of researchers from University of Dhaka, Bangladesh Jute Research Institute (BJRI) and private software firm Data Soft Systems Bangladesh Ltd. in collaboration with Centre for Chemical Biology, University of Science,

Malaysia and University of Hawaii, were involved in this project, using Next Generation Sequencing techniques (internet: M.Alam *et al*, jutegenome.org June 2010).

2.3.3 Primer based gene walking and gene sequencing

Gene walking is a basic molecular biology technique which is used to determine uncharacterized DNA sequences flanking a known sequence region (Guo *et al*, 1997). Previously the technique of gene walking from regions of known sequence into unknown flanking DNA sequences involved the successive probing of libraries with clones obtained from prior screening. However this method was relatively time consuming and required the need of radioactive probes. Advanced PCR techniques have enabled reduction of time required as well as avoiding radioactive probes (Brown *et al* 1997).

The Universal gene walking system helps researchers make uncloned libraries for walking by PCR for any genomic DNA Primer based gene walking is fairly quick and reliable. This method of sequencing or cloning the DNA adjacent to a known region is using a combination of a single targeted sequence specific PCR primer with a second nonspecific 'walking' primer. This can replace conventional cloning and screening methods with a single step PCR protocol, which substantially reduces the time required to isolate sequences either upstream or downstream from known sequences (Chawla *et al*, 2003).

A recent PCR protocol to amplify gene flanking regions with the use of partially degenerate primers (hybrid consensus degenerate primers) and a single specific primer which requires only a small amount of DNA to start, has been reported (Garcia *et al*,2005). One disadvantage of this technique is the resultant non specific PCR products due to the partial degenerate primers. To suppress these PCR artifacts, and enable specific genome walking for a wide range of genomes, an improved method which uses a nested PCR with a set of nested specific primers and a partially degenerate primer has been developed. Such degenerate primers have proven to be an effective molecular biology tool and they have been used in the identification of jute genes such as LDLP, LRRK and Ribosomal protein S8. The procedure requires the product from the first round of PCR to serve as a template for the second round of PCR, the product of which in turn serves as a template for the third round of PCR. It is carried out through a hemi-nested design

with a set of three nested locus-specific primers and a common partially degenerate primer (walker primer) for use in successive rounds of PCR. Four universal walker primers were designed whereby one can test which walker primer can produce the best result by carrying out four series of reactions in parallel (Salim *et al*, 2009).

Hence even though limited molecular biology research on jute has been done, some work is being conducted using primer based gene walking techniques, non- radioactive differential display and other molecular techniques.

In this study, the degenerate primer based gene walking technique was used to sequence the LTR retrotransposons of Jute.

2.4 Tissue culture and transformation studies on Jute

Varietal development and intra specific hybridization have been historically used to bring about improvements in cultivated jute, using multidirectional crossing followed by selection (Ghosh, 1999). However introduction of specific genes for specific purposes is not possible due to the limited genetic variability in the two cultivated jute species. As mentioned earlier, these two cultivated jute species also do not normally cross-fertilize, which may be due to the presence of a strong sexual incompatibility between them (Patel and Datta, 1960; Swaminathan *et al*, 1961). Attempts at sexual hybridization between these two species have shown varied results; some researchers have been unsuccessful when attempting hybridization between the two species (Finlow, 1917,1921,1923, 1924; Datta *et al.* 1960) while others are reported to have succeeded in producing hybrids (Islam and Rashid, 1960; Swaminathan *et al.* 1961); However the putative hybrids produced showed dominance of the female parent in F1 and F2 generations. It was also found that the regeneration protocols were difficult to reproduce, which was further confirmed by isoenzyme profiles (Khatun, 2007).

Jute is generally recalcitrant to regeneration and transformation, unlike other crops which are easily regenerated from tissues given the appropriate stimuli from growth regulators in the culture medium. Regeneration percentage in Jute is therefore seen to be low and sporadic. Somatic hybridization between the two cultivated species has been attempted, whereby protoplasts were

isolated, and callus production and somatic embryogenesis was induced from protoplasts. The putative hybrid exhibited strong growth and green pigmentation which was absent in the parent strains. Prominent hybrid bands, including both parental bands, were present in hybrid cell lines in isoenzyme profiles (Khatun, 2007). Jute transformation of *Corchorus capsularis* var. JRC 321 has been achieved by biolistic particle delivery system (Ghosh et al, 2004), using the apical meristemmatic region of a germinating seedling as the target for transformation. The transmission of the trait was achieved to T1 generation, though the whole procedure was expensive and laborious. Successful transformation without using tissue culture has also been achieved for *C. olitorius* (Sajib *et al* 2008). Dividing cells in the meristematic region were transformed by *Agrobacterium tumefaciens*; some of the cells retained their capacity for cell division, and thus the transgene was transferred to the progeny cells. Some of these cells differentiated to form floral buds, and the seeds from these buds inherited the transgene in the next generation.

2.5 Bioinformatics

Bioinformatics is the application of computer science and allied technologies to the field of biology. It involves the analysis and interpretation of biological data. Hence the main aim of bioinformatics is to increase the understanding of biological processes, with a focus on developing and applying computationally intensive techniques in order to do so. Bioinformatics therefore uses algorithms, database and information systems, web technologies, soft computing and artificial intelligence, signal processing, data mining, image processing, modeling and simulation, discrete mathematics, control and system theory, statistics, circuit theory and software engineering to achieve this. Applications to molecular biology include analyzing DNA sequence data to locate genes, analyzing RNA data to predict protein structure, analyzing protein sequence data to predict their cellular location or function, analyzing gene expression patterns and helping to identify new drug molecules. Specific bioinformatics software or bioinformatics tools have emerged and are readily available to aid bioinformatics analysis (Nair, 2007).

Bioinformatics Tools

The bioinformatics tools that are currently available for molecular biology and biotechnology are described as below (Gupta, 2004):

2.5.1 Alignment Tools

These allow us to use the database to find out the location and identity of a query (input) sequence. The following programmes can be used for this:

Basic Local Alignment Search Tool (BLAST) (Altschul *et al*, 1990) with its orthologous sequence information stored in its database gives an idea about the candidate sequence. The following are specific BLAST tools:

- BLASTp compares an amino acid query sequence against a protein sequence database.
- BLASTn searches a nucleotide database using a nucleotide query.
- BLASTx compares a nucleotide query sequence translated in all reading frames against a protein sequence database.
- tBLASTn searches a translated nucleotide database using a protein query.
- tBLASTx searches a translated nucleotide database using a translated nucleotide query.
- BLASTz compares long stretches of nucleotides >2kb.
- Specialized BLAST (later version 2.1).

2.5.2 VecScreen is a system, which can quickly identify segments of nucleic acid sequences that may be of vector origin. VecScreen searches for a query for segments with any sequence, in a specialized non redundant vector database (UniVec) including sequences of plasmids, phages, cosmids, BACs, PACs and YACs.

2.5.3 Multiple Sequence Alignment Tool:

Multiple sequence alignment technique is used to study a group of related genes or proteins to see if there is an evolutionary relationship between the genes, and to discover patterns which indicate functionally or structurally related sequences. Some tools in multiple sequence alignments are CLUSTALW, PHYLIP etc. CLUSTALW is also used for constructing a phylogenetic tree.

ORF finder finds the coding region, or the open reading frame, in a given nucleotide sequence.

2.5.4 Translating tools:

This tool translates a given nucleotide sequence into a protein sequence. Some of these tools are EBI, EXPASY Translate tool etc. (Internet: NCBI)

2.6 Phylogenetic tree:

A phylogenetic tree shows evolutionary relationships between various biological species or other entities which have a common ancestor. The evolution or historical development of plants or animals can be seen through this.

2.7 Rationale of the present study

Retrotransposons seem to play an important role in plant genome evolution in general. As Jute genome retrotransposons seem to have played an important role in Jute evolution, it is of importance to understand their role in Jute. This would lead to a better understanding of the molecular biology of the Jute genome. Results from this research can contribute to the following:

- Identification of full length sequences of retrotransposons.
- The correlation of retrotransposons with abiotic and biotic stress responses.
- Tagging genes of agronomic importance.
- Developing retrotransposon based marker system which is more specific than the SSR marker system.

2.8 Overall Objective:

To have a better understanding of the jute genome at the molecular level.

2.9 Specific objectives of this study:

- To isolate the RT domains of LTR retrotransposons from jute (*Corchorus olitorius*).
- To characterize these LTR retrotransposons using different bioinformatic tools.
- To identify transcriptionally active LTR retrotransposons.

3. Materials and Methods:

3.1 Isolation and confirmation of Ty1-*copia* and Ty3-*gypsy* retrotransposons from Jute genome:

3.1.1 Plant materials

Young Mature leaves of Jute (*C. olitorius*) variety O-9897 was used in this study obtained from the Bangladesh Jute Research Institute (BJRI).

Properties of Jute (*C.olitorius*) O-9897:

- Low temperature Sensitive
- Common variety
- High yielding
- Good fiber quality
- Good water logging capacity

The leaves were stored at 4C for 30-40 minutes before isolation of DNA.

3.1.2 Isolation of DNA from jute seedling:

Materials:

All solutions were made up with deionized, sterile water and autoclaves, except phenol

- Liquid Nitrogen
- 1M of Tris. HCl (stock solution) (MW. 121.14)
- 1M Tris. HCL was prepared by dissolving 12.11g of Tris in 80ml of dd water with the help of magnetic a stirrer; pH was adjusted to 8.0 by concentrated HCl. The final volume was made to 100ml and sterilized by autoclaving.
- 0.5M EDTA (stock solution) (MW. 372.2). To prepare 0.5M a stock solution of EDTA 40ml of dd water was taken and 18.61g of EDTA was dissolved with the help of a

magnetic stirrer, pH was adjusted to 8.0 by an NaOH pellet. The volume was then adjusted to 50ml and sterilized by autoclaving.

- Buffer saturated phenol. This was stored at 4^0C in a dark bottle
- Chloroform : Isoamylalcohol (24:1, v/v)
- TE buffer
  i. 10mM Tris HCl (pH 8,0)
  ii. 1mM EDTA (pH 8.0)
- 3M Na-acetate (pH 5.2)

40.81g of sodium acetate was dissolved in 40ml of water. The pH was adjusted to 5.2 with glacial acetic acid. Final volume was made to 100ml and sterilized by filtration.

- RNase solution (DNase free) (stock 10mg/ml)

- CTAB isolation buffer (100ml)
  i. CTAB 2.0g
  ii. 5M NaCl 28.0ml
  iii. 1M Tris.HCL (pH 8.0) 10.0ml
  iv. 0.5M EDTA (pH 8.0) 4.0ml
  v. 2-mercaptoethnol 0.2ml.

  All the materials were added to a conical flask and heated to 60^0Cin a water bath until all the CTAB melted. Then the volume was adjusted to 100ml with autoclaved water, and stored and room temperature.

- Ethanol
- Isopropanol
- 5M NaCl (MW. 58.44). 29.22g of NaCl was dissolved in 40ml of dd water. The volume was then made to 100 ml by dd water and the solution was autoclaved.

3.1.2.1 Procedure of DNA isolation:

It is difficult to isolate high molecular weight plant DNA, but it is suitable for digestion with restriction endonucleases. The CTAB method (Murray et al, 1980) is a relatively less expensive procedure that results in high yields of DNA from a small amount of tissue (Doyle and Doyle, 1987). This method was used as follows:

- 5 to 7.5 ml of CTAB isolation buffer was preheated in a 60^0Cwater bath in a screw capped tube.
- 1g of fresh tissue (leaves) were ground to a powder in a mortar containing liquid nitrogen and paste.
- The powder was directly put into the preheated buffer and mixed gently by swirling.
- The sample was incubated at 60^0C for 30 minutes with occasional gentle swirling.
- The mixture was extracted with an equal volume of phenol: chloroform: isoamylalcohol (25:24:1) mixed thoroughly and centrifuged at 4000 rpm for 10 minutes at room temperature.
- The aqueous phase was removed with a pipette and transferred to a clean screw capped tube.
- A two-thirds volume of ice-cold isopropanol was added and gently mixed to precipitate the DNA (when required it was kept overnight at -20^0C).
- This solution was then centrifuged at 140000 rmp for 10 minutes, the supernatant discarded, and the precipitate was washed twice with 70% ice-cold ethanol.
- The Pellet was allowed to dry and re suspended in a minimal amount of TE buffer (in an eppendorf tube).
- DNase free RNase was added to a final concentration of 10 µg/ml and incubated at 37^0C for 2-5 minutes.
- An equal volume of phenol: chloroform: IAA (25:24:1) [2x] was added.
- This was then centrifuged at 14000 rpm for 10 minutes and the supernatant collected
- Then one-tenth 3M Na-acetate and a double volume of 99% ice-cold ethanol was added and kept on ice for 30-60 minutes.
- This was centrifuged at 14000 rpm for 10 minutes and the supernatant removed.
- The DNA was washed by vigorous rinsing [x2] with 70% ethanol.

- The supernatant was removed and the pellet allowed to dry completely.
- The pellet was dissolved in a minimal amount of TE and stored at -20^{0}C.

3.1.3 Amplification of RT domain of jute LTR retrotransposons by Polymerase Chain Reaction:

3.1.3.1 Primer Design

Degenerate primers to amplify the RT – domains of two retrotransposon groups (Ty1-*copia* & Ty3-*gypsy*) were taken (Kumekawa et al, 1999 and Kumar et al, 1997) as given below;

Primer name	Group name	Direction	Sequence	Length (BP)	Tm (C)
Ty1RT	Ty1-*Copia*	Forward	5'-ACNGCNTTYYTNCAYGG-3'	17	57.2
Ty1RT	Ty1-*Copia*	Reverse	5"-ARCATRTCRTCNACRTA-3'	17	51.2
Ty3Da	Ty3-*gypsy*	Forward	5'-TAYCCNHTNCCNCGNATHGA-3"	20	60.7
Ty3Da	Ty3-*Gypsy*	Reverse	5'-ARCATRTCRTCNACRTA-3'	17	51.2

3.1.3.2 Amplification of Ty1-*copia* and Ty3-*gypsy* RT domains by Polymerase Chain reaction

Materials

The following were used for the polymerase chain reaction procedure

- 10X PCR Reaction Buffer containing:
  - 500mM Kcl
  - 100mM Tris.Hcl (pH 8.3)
  - 0.1% gelatin
- MgCl2 (25mM) in water for PCR
- Sodium salt of deoxy-thymidine-5'-triphosphate (10mM aq. solution, pH7.35)
- Sodium salt of deoxy-guanosine-5'-triphosphate (10mM aq. solution, pH7.35)
- Sodium salt of deoxy-cytidine-5'triphosphate (10mM aq. solution pH7.35)
- GeNei™ Taq DNA Polymerase
- Jute genome DNA (the DNA template) collected from Jute leaves earlier
- Ty1RT and Ty3Da primers
- Autoclaved ultra-pure water
- TE solution for the dilution of the template DNA
  10mM Tris. HCl (pH 8.0)
  0.1mM EDTA (pH 8.0)

3.1.3.3 Preparation of dNTPs mixture

20 µl of each dATP, dGTP, dCTP, dTTP (at a concentration of 100mM each) were mixed in an autoclaved eppendorf tube, and the final volume was made up to 1000 µl by adding PCR water, and stored at -20°C. Hence the concentration of nucleotides in the mixture was 2.0mM.

3.1.3.4 Dilution of the DNA template

As the isolated genomic DNA was unsuitable for using as sample DNA for PCR at a high concentration, it was diluted with TE solution (10mM Tris-HCl, 0.1mM EDTA, pH 8.0) before using PCR. The concentration of the template DNA was made up to 60ng/µl.
10ng/µl of lamda (λ) was used for reference to adjust the concentration of the genomic DNA sample.

3.1.3.5 Dilution of primer:

To dilute the primer for use in the PCR reaction, 10 µl of each of the primers from the stock solution (100 µM) was added to 90 µl of TE buffer (pH 8.0). Hence the final concentration of the primers was 10 µM/µl.

3.1.3.6 Preparation of the PCR Master Mix

PCR Reagent mixture

Reagents	Volume
10 x PCR buffer	2.5 µl
50 mM Mg Cl_2	2.0 µl
2 mM dNTPs	1.5 µl
Forward primer	1.5 µl
Reverse primer	1.5 µl
Taq polymerase	0.2 µl
Template DNA	1.0 µl
PCR-H_20	14.8 µl

3.1.3.7 Thermal Cycling Profile used in PCR

The thermal cycling profile used in the PCR procedure for 35 cycles, to amplify Ty1-*copia* RT domain of jute, is given below:

Steps	Temperature	Time	No. of cycle
Initial denaturation	95°C	5 minutes	1 (First)
Denaturation	95°C	45 minutes	35
Annealing	48.5°C	50 seconds	35
Elongation	72°C	1 minute	35
Final elongation	72 °C	5 minutes	1 (last)
Hold temperature	4°C	∞	

The thermal cycling profile used in the PCR procedure for 35 cycles, to amplify Ty3-*gypsy* RT domain of jute, is given below:

Steps	Temperature	Time	No. of cycle
Initial denaturation	95°C	5 minutes	1 (First)
Denaturation	95°C	45 minutes	35
Annealing	49°C	50 seconds	35
Elongation	72°C	1 minute	35
Final elongation	72 °C	5 minutes	1 (last)
Hold temperature	4°C	∞	

3.1.3.8 Amplified products after PCR:

The amplified products after PCR were separated on 1.0% Agarose gel following staining with ethidium bromide solution. The gel was then seen under UV light staining and photographed.

Materials:

- Ultra pure agarose
- TAE buffer
- Gel loading dye
- Gel electrophoresis kit

Preparation of stock solution (1 liter) of 50X TAE buffer:

- Tris base 242.0g
- Glacial acetic acid 57.1ml
- 0.5M EDTA (pH 8.0) 100ml

This solution was made up to 1000ml with dd H_20.

Preparation of 1% agarose gel (100ml)

1. In order to prepare 25ml of 1% agarose gel, 0.25g of agarose powder was weighed in a conical flask.
2. 0.5ml of 50X TAE buffer was put in a measuring cylinder and the volume was made up to 25ml with dd H_20.
3. The mixture of 50X TAE and dd H_20 was put in a conical flask containing the agarose and then melted in a microwave oven at 60°C for 2-3 minutes.

Composition of the DNA dye

- Xylene cyanol : 0.25%
- Bromophenol blue : 0.25%
- Glycerol : 30%

Preparation of 1X TAE buffer used in gel electrophoresis

250ml of TAE buffer to use for gel electrophoresis was prepared by mixing 5ml of 50X TAE buffer with 245 ml of dd H_20.

Procedure for gel electrophoresis:

The gel was poured in the gel case (in which the comb was assembled for wells) and allowed to solidify. The DNA samples were loaded in the wells and the electrophoresis was carried out using 80 volts. The DNA bands were then visualized under ultraviolet trans-illuminator (Kodak EDAS, Japan).

3.1.4 Cloning of PCR products using CR 2.1 and transformation into competent *E.Coli*

3.1.4.1 Preparation of Competent *E.Coli*

Materials:

- $CaCl_2$ solution (60mM $CaCl_2$, 15% glycerol, 10mM PIPES pH 7.0)
- LB (luria-Bertani) medium (5g Yeast extract, 10g Peptone, 10g NaCl per liter pH 7.0)
- *E.Coli* cell DH5a strain

Procedure:

- A single colony of *E.coli* cell was incubated into 5 ml LB medium overnight at 37^0C with shaking at 250rpm.
- 1 ml of this culture was put into 100ml LB medium in a 250 ml conical flask. This was then incubated at 37^0C with shaking at 250 rpm, until the optical density had reached 0.375 at 590nm.
- The culture was then put into 2 pre-chilled sterile tubes, placed on ice for 5-10 minutes, and then centrifuged for 7 minutes at 1600g at 4^0C.
- Each pellet was re-suspended in 10 ml of ice-cold $CaCl_2$ solution. This was then centrifuged at 1100g for 5 minutes at 4^0C.

- The pellets were again re-suspended in 10 ml ice-cold $CaCl_2$ solution and kept on ice for 30 minutes, after which it was again centrifuged at 1100g for 5 minutes at 4^0C.
- Each pellet was then re-suspended in 2ml of ice-cold $CaCl_2$ solution completely. 250 µl of this solution was put into each sterile pre-chilled micro-centrifuge tube and stored at -80^0C for subsequent use in the transformation procedure.

3.1.4.2 Preparation of the Ligation reaction

The ligation reaction was prepared as per the protocol given below, modified from Promega, Technical Manual.

Materials

- pCR 2.1 Vector
- 10X Rapid ligation buffer,
- PCR products of both Ty1 and Ty3
- T4 DNA ligase
- Water

Procedure

- The pCR 2.1 Vector was centrifuged briefly.
- The ligation reaction was set up, in 200 ul thin wall PCR tubes or 1.5 ml eppendorf tubes, as given below:

PCR product		Xul (1-3ul is the working range)
10x Ligation Buffer		1ul
PCR2.1 vector (25ng/µl)		2ul
Sterile water	to a total volume of	9ul
T4 DNA Ligase (4.0 Weiss units)		1ul
Final Volume		10ul

- The reaction was mixed by pipetting.
- The reaction was then incubated at room temperature for 1 hour.

Alternatively, for a maximum number of transformants, the reactions were incubated overnight at 16^0C.

3.1.4.3 Method for Transformation

Transformation was done as follows, modified from the protocol from Promega, Technical Manual.

Materials

- IPTG stock solution (0.1M)

1.2g of IPTG was dissolved in 50ml of ultra pure water. This solution was then sterilized by filtration and stored at 4^0C.

- X-Gal (2ml)

100mg of X-Gal (5-bromo-4-chloro-3-indolyl-β-galactoside) was dissolved in 2ml N,N$^{/}$ - dimethyl-formamide. The sample was covered with aluminum foil and stored at -20^0C.

- Ampicillin stock solution (50mg/ml)
- LB Media
- LB plates with ampicillin

1.5g agar was added to 100ml of LB medium and then autoclaved. The medium was cooled to 50^0C before adding ampicillin to a final concentration of 100μg/ml. 25ml of medium was poured into 85mm petri dishes. After the agar hardened, it was stored at 4^0C.

- LB plates with ampicillin/IPTG/X-Gal.

The LB plates were made with ampicillin as above; then supplemented with 0.5mM IPTG and 80μg/ml X-Gal poured over the plates.

- 2M Mg^{2+} stock
  i. $MgCl_2.6H_2O$
  ii. $MgSO_4.7H_2O$

10.165g of $MgCl_2.6H_2O$ and 12.325g of $MgSO_4.7H_2O$ were dissolved in 40ml ultra pure water. The final volume was made up to 50ml and sterilized by filtration.

- 2M glucose stock

36g of glucose was dissolved in 80ml ultra pure water. The final volume was made up to 100ml, and then sterilized by filtration.

- SOC medium (50ml)

i. Peptone	1.0g
ii. Yeast extract	0.25g
iii. 1M NaCl	0.5ml
iv. 1M KCL	0.125ml
v. 2M Mg^{2+} stock	0.5ml
vi. 2M glucose	0.5ml

Peptone, yeast extract, NaCl and KCL were added to 47ml distilled water. The sample was autoclaved, and then allowed to cool to room temperature. Subsequently 2M Mg^{2+} stock and 2M glucose were added, each to a final concentration of 20mM. The final volume was made up to 50ml by adding sterile, distilled water. The pH was adjusted to 7.0.

- High Efficiency Competent Cells (DH5α)

Procedure

1 2 LB/ampicillin/IPTG/X-Gal plates were prepared for each ligation reaction.

2 The tubes containing the ligation reactions were centrifuged. The contents collected at the bottom of the tubes.

3 5µl from each ligation reaction was added to a sterile 1.5ml micro-centrifuge tube on ice.

4 Tubes of frozen DH5α High Efficiency Competent Cells were removed from storage at -70^0C storage, and placed in an ice bath until just thawed (which took about 5 minutes). The cells were mixed by gently flicking the tubes.100µl of cells were carefully transferred into each of the tubes prepared in step 3.

5 The tubes were flicked gently to mix and placed on ice for 20 minutes.

6 The cells were heat-shocked for 45-50 seconds in a water bath at exactly 42^0C.

7 The tubes were immediately put on ice again for 2 minutes.

8 500µl room temperature SOC medium was added to the tubes containing cells transformed with ligation reactions and 900µl to the tube containing cells transformed without ligation reactions for transformation control.

9 The samples were incubated for 1.5 hours at 37^0C with shaking (~200rpm).100µl of each transformation culture was plated onto duplicate LB/ ampicillin/ IPTG/X-Gal plates.

10 The plates were incubated overnight (16-24 hours) at 37^0C.

3.1.4.4 Screening Transformants for Inserts

Recombinant clones were identified by color screening of the indicator plates, as successful cloning of an insert in the pCR2.1 Vector interrupted the coding sequence of galactosidase. However, the characteristics of PCR products cloned into the pCR2.1 significantly affected the ratio of blue: white colonies obtained following transformation of competent cells. Clones that contained the PCR products produced white colonies. Blue colonies resulted from PCR fragments that were cloned in-frame with the *lacZ* gene. DNA fragments up to 2kb have been cloned in-frame and produced blue colonies (Promega, Technical Manual).

3.1.5 Isolation of plasmid DNA

Materials

Table 1: Solutions for Plasmid DNA isolation

Solution I (200 ml)		Solution II (5 ml)		Solution III (5 ml)	
Glucose	1.8 g	10% SDS	0.5 ml	5M KOAc	3.00 ml
1M Tris(pH 8.0)	5.0 ml	5N NaOH	0.2 ml	Glacial acetic acid	1.25 ml
0.5 M EDTA	4.0 ml	H_2O	4.3 ml	H_2O	0.75 ml

Others

- Isopropanol
- 70% Ethyl alcohol
- RNase solution (10µg/ml)
- TE buffer
- Tris.HCl (pH 8.0) 10mM
- EDTA (pH 8.0) 1mM

All the solutions apart from solution II were autoclaved and stored at 4^0C. Solution II was prepared just before the isolation of plasmid and stored at room temperature

Method

1. Transformants were picked and then grown in LB media overnight at 37°C with vigorous shaking (spectinomycin and hygromycin were in LB media at a concentration of 100µg/ml each).
2. 10ml of culture was transferred to each sterile falcon tube and centrifuged at 4000rpm for 10 minutes.
3. Supernatant was discarded and the pellet was completely dried in the eppendorf Concentrator 5301vacuum dryer.
4. 200µl of solution I (ice-cold temperature) was added and mixed by vortexing.
5. The suspension was transferred to an eppendorf and 400µl of solution II was added to each eppendorf tube, the contents were mixed by inverting the tubes rapidly five times. They were then kept on ice for exactly 5 minutes.
6. 300µl of solution III (ice-cold temperature) was added and mixed until a clot formed. This was then put on ice for 5-10 minutes.
7. Centrifugation at 14000rpm was carried out for 10 minutes at 4^0C.
8. The Supernatant was transferred into another eppendorf tube and extracted with equal volume of phenol: chloroform: IAA (25:24:1).
9. Centrifugation at 14000rpm was carried out again for 10 minutes at 4^0C.
10. The Aqueous phase was then transferred into another eppendorf tube.
11. The DNA was precipitated with a double volume of isopropanol (ice-low temperature). This was kept at room temperature for 2-4 minutes and the centrifuged at 14000rpm again for 10 minutes at 4^0C.
12. The Supernatant was discarded and the pellet was washed with 70% ice-low temperature ethanol.

13. If no precipitate was observed, then 500µl of absolute (99%) ethanol (ice-low temperature) and 10µl of 3M Na-acetate was added.

14. The Pellet was completely dried and suspended in 10-30µl of TE buffer.

15. 0.2µl of RNase at a concentration of 10µg/ml was added and incubated at 37°C for 30 minutes.

16. The Isolated plasmid was stored at -20^{0}C.

3.1.6 Confirmation of successful transformation by PCR:

The materials and methods used in this step was same as previously except the primer set and Thermal Cycling Profilechanged as follows:

Primer pairs used for PCR were as follow:

Name	Direction	Sequence	bps	Tm °C
M13	Froward	5'-GTAAAACGACGGCCAGT-3'	17	58
M13	Reverse	5'-AACAGCTATGACCATG-3'	16	58

Table 3.4: Thermal Cycling Profile

Steps	Temperature	Time	No. of Cycles
Initial denaturation	95°C	5 minutes	1 (First)
Denaturation	95°C	45 seconds	35
Annealing	57°C	45 seconds	35
Elongation	72°C	1 minute	35
Final elongation	72°C	5 minutes	1 (last)
Hold temperature	4°C	∞	

Gel extraction and sequencing

The QIAGEN MinElute Gel Extraction Kit was used. This is a protocol designed to extract and purify DNA of 70 bp to 4 kb from standard or Low-melt agarose gels in TAE or TBE buffer resulting in high end-concentrations of DNA. Up to 400 mg agarose can be processed per MinElute column

Materials

- Agarose gel
- Electrophoresis buffer (1X Tris-Glycine buffer)
- 1kb plus Ladder
- Loading dye
- PCR products
- Ethidium bromide containing staining solution

Method

- PCR-products (50 µl of PCR products + 4 µl of dye) and ladder (3µl) were run on the 1.5% agarose gel. These were stained with an ethidium bromide containing solution and the desired band was excised under UV light from the multiple non-specific bands from the agarose gel with a clean, sharp scalpel. The size of the gel slice was minimized by removing extra agarose.
- Then the gel slice was weighed in a colorless tube. 3 volumes of Buffer QG was added to 1 volume of gel (100 mg or approximately 100 µl).
- The tube was incubated at 50°C for 10 min until the gel slice has completely dissolved. To help dissolve gel, it was mixed by vortexing the tube every 2–3 min during the incubation.
- After the gel slice was completely dissolved, the color of the mixture was checked whether it was yellow (similar to Buffer QG without dissolved agarose).
- 1 gel volume of isopropanol was added to the sample and mixed by inverting the tube several times.
- A MinElute column was placed in a provided 2 ml collection tube in a suitable rack.
- To bind DNA, the sample was applied to the MinElute column, and centrifuged for 1 min at 10,000 x g.
- The flow-through was discarded and the MinElute column was placed back in the same collection tube
- 500 µl of Buffer QG was added to the spin column and centrifuged for 1 min
- The flow-through was discarded again and the MinElute column was placed back in the same collection tube

- 750 µl of Buffer PE was added to the MinElute column and centrifuge for 1 min, for washing. The column was left to stand 2–5 min after addition of Buffer PE, before centrifuging.

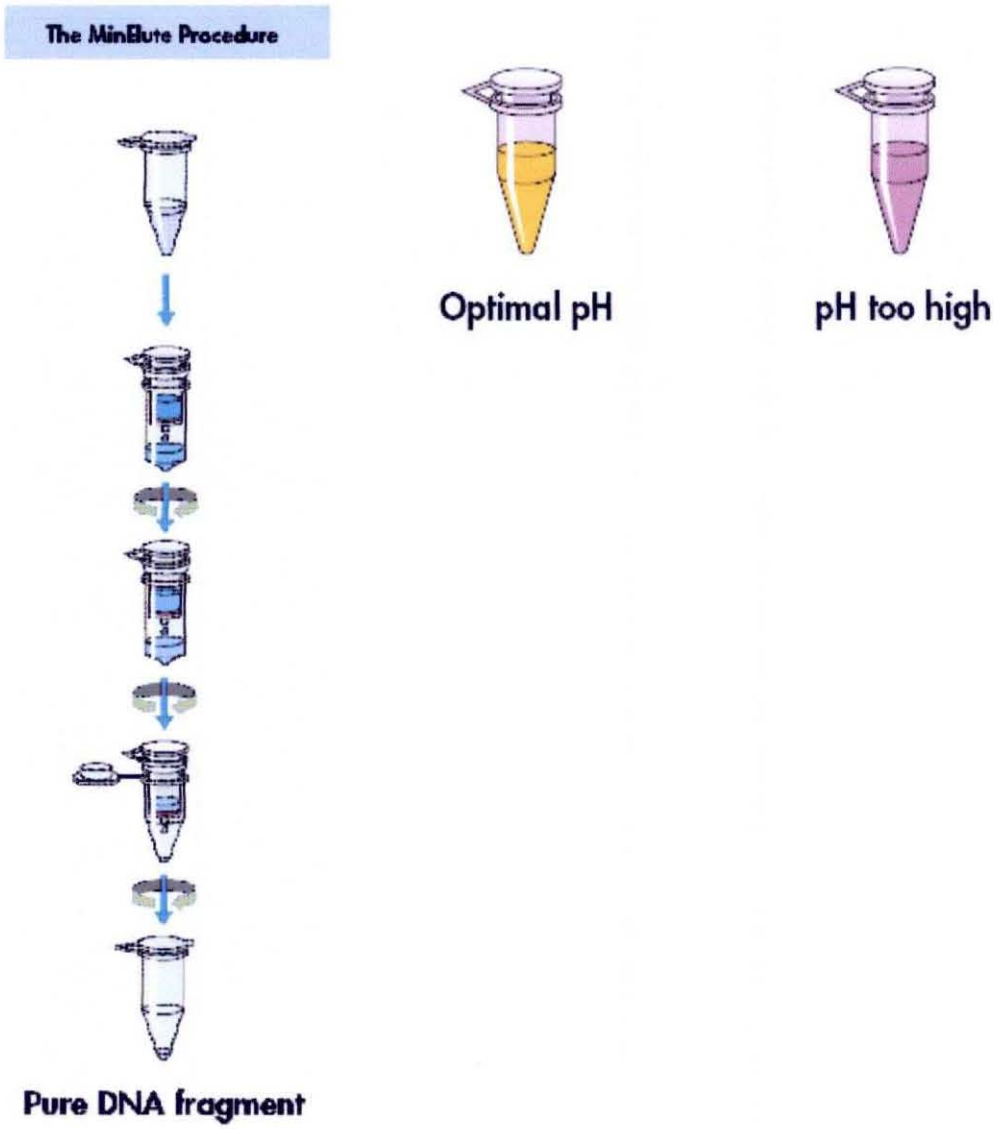


Figure 1: Gel extraction process

- The flow-through was discarded and the MinElute column was centrifuged for an additional1 min at 10,000 x g.
- The MinElute column was then placed into a clean 1.5 ml micro centrifuge tube

- To elute DNA, 20 µl of Buffer EB (10 mM Tris·Cl, pH 8.5) was added to the center of the membrane, the column was left to stand for 1 min, and then centrifuged for 1 min. The elution buffer was then dispensed directly onto the center of the membrane for complete elution of bound DNA. Elution efficiency is dependent on pH. The maximum elution efficiency is achieved between pH 7.0 and 8.5.

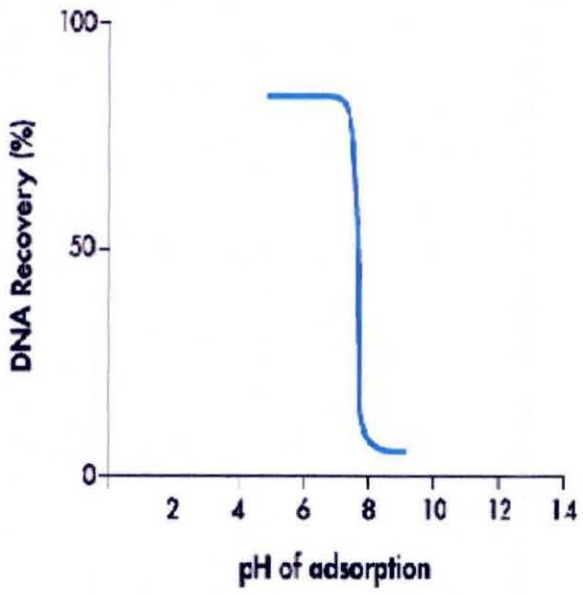


Figure 2: DNA Recovery chart

- The purified DNA was analyzed on the gel, 3 volumes of Loading Dye was added to1 volume of purified DNA and the solution was mixed by pipetting up and down before loading the gel. 2 ul of lambda DNA was also run with the sample for comparing the concentration of purified DNA with the known lambda DNA concentration.

The sample was then sent to 1st Base in Malaysia for sequencing.

3.1.8 Bioinformatics analysis of the sequences

3.1.8.1 Vecscreen analysis

VecScreen is used to identify whetherthe sequences from the genomic clones contain any vector sequences or not. VecScreen gives output in a graphical format along with the sequence alignment with the vector sequence. The VecScreen tool was used from NCBI web portal. The web link is:

http://www.ncbi.nlm.nih.gov/VecScreen/VecScreen.html

The procedure was as follows:

1. DNA sequence of the clone was copied
2. This was then pasted in the appropriate box
3. "Run VecScreen" was clicked.

3.1.8.2 Cap3

Cap3 is a program which assembles a set of contiguous sequences (contigs). It was used to form contigs from forward and reverse sequences. The weblink is:

http://pbil.univ-lyon1.fr/cap3.php

The procedure was as follows:

1. Both forward and reverse sequences were copied.
2. These were pasted in the appropriate box.
3. "Submit" was clicked.

3.1.8.3 Verification by BLAST analysis

For local alignment of the sequences, both BLASTn and BLASTx were used. BLASTn searches a nucleotide database using a nucleotide query whereas BLASTx search protein database using a translated nucleotide query. The weblink is:

http://www.ncbi.nlm.nih.gov/blast/Blast.cgi?CMD=Web&PAGE_TYPE=BlastHome

3.1.8.4 Transeq

Transeq is a programme which translates nucleic acid sequences to the corresponding peptide sequence. It can translate any of the 3 forward or three reverse sense frames, or in all three forward or reverse frames, or in all six frames. The weblink is:
http://www.ebi.ac.uk/Tools/emboss/transeq/index.html

3.1.8.5 ClustalW

ClustalW2 is a general purpose multiple sequence alignment programme for DNA or proteins. It produces biologically meaningful multiple sequence alignments of divergent RT sequences. The best match for the selected sequences are revealed, and lined them up such that the identities, similarities and differences are seen. Evolutionary relationships were observed via viewing Cladograms or Phylograms. The weblink is:
http://www.ebi.ac.uk/Tools/clustalw2/index.html

The procedure was as follows:

1. All the Ty1/Ty3 sequences were copied.
2. These were pasted in the appropriate box.
3. "Submit" was clicked.

3.2 Transcriptional analysis

Plant materials

The plant materials were same as the materials described previously.

3.2.1 Isolation of RNA by using TRIZOL Reagent (GibcoBRL):

TRIZOL is a ready-to-use reagent for the isolation of total RNA from cells and tissues. It is a mono-phasic solution of phenol and guanidine isothiocyanate, which maintains the integrity of the RNA, while disrupting cells and dissolving cell components.

Materials

- Jute seedlings
- Liquid nitrogen
- Mortar and pestle: This was cleaned with soap and rinsed with deionized water and 100% ethanol, and then allowed to dry. RNAse *AWAY*™ was applied and wiped dry with kimwipes.
- RNase-free microcentrifuge tubes: To make microcentrifuge tubes RNAse free they were treated with DEPC.
- Spatulas: Autoclaved and cleaned with RNAse *AWAY*™.

Solutions

- TRIZOL reagent®
- 5M NaCl (MW. 58.5): This was prepared by dissolving 29.25g of NaCl in 100ml of dd water, then treated with DEPC for ~4-5 hours and sterilized by autoclaving.
- Chloroform.
- Isopropyl alcohol.
- 75% ethanol.
- RNAse *AWAY*™.
- DEPC-treated water

Procedure

1.0 g of frozen seedlings (from four samples of both sensitive and tolerant species) were ground to powder in liquid nitrogen using mortar and pestle.

- 1 ml TRIZOL reagent was added to 1.0g of the grounded tissue, homogenized thoroughly and incubated at room temperature for 10 minutes.
- This mixture was then centrifuged at 12,000x g for 10 minutes at 4°C.

- The supernatant (aqueous phase) was transferred to a new tube, 0.2 ml chloroform was added and shaken vigorously by hand for 15 seconds. This was then incubated at room temperature for 3 minutes.
- The sample was again centrifuged at 12,000x g for 15 minutes at 4°C and supernatant was transferred (aqueous phase) to a new tube. 0.25 ml of isopropanol and 0.25ml of 2M NaCl was then added to this supernatant.
- This mixture was mixed by inverting the tubes, and incubated for 10 minutes at room temperature.
- It was then centrifuged at 12,000xg for 10 minutes at 4°C and the supernatant (aqueous phase) was removed and the pellet was washed with 75% (v/v) Ethanol (2 times).
- The pellet was air-dried and re-suspended in 10 - 30 µl DEPC-treated water.
- The isolated RNA was stored at -80°C.

3.2.1.1 Precautions for isolating total RNA

Some precautions were carefully followed to avoid contamination with RNases and thus subsequent damage of RNA

- Disposable gloves were used and changed frequently during the isolation process
- All equipment and non-disposable items were carefully wiped with RNase *AWAY*™ to remove RNase contamination.
- DEPC treateddoubly autoclaved water was used for all solution preparations and dilutions. .
- RNA was isolated inside the laminar flow hood.

Preparation of 0.1% DEPC treated water

0.1% (v/v) solution of Diethyl Pyrocarbonate (DEPC) in distilled water was prepared,
Shaken vigorously, and allowed to stand overnight at room temperature. The solution was autoclaved twice in order to totally remove the DEPC.

3.2.1.2 Quality check of isolated RNA

The quality of purified RNA was checked by subjecting it to electrophoresis in a 1.3% agarose gel.

Materials

- Ultra pure agarose
- MOPS buffer
- RNA gel loading dye
- Gel electrophoresis kit

3.2.1.2 i Preparation of stock solution (1 liter) of 10X MOPS buffer

- The following reagents were mixed in the given amounts:

MOPS (Mw=209.27)	41.86g
Na-Acetate(50 mM)	6.8 g
10 mM EDTA (pH 8.0)	20.0 ml

- The pH was adjusted to 7.0 using 1N NaOH prepared with DEPC treated water.
- The solution was then made up to 1000ml with DEPC treated water.

3.2.1.2 ii Preparation of 1.3% agarose gel (50ml)

Materials

- 1×MOPS
- Formaldehyde
- Agarose
- DEPC-treated water

Method:

- 50 ml of 1.3% gel was prepared by mixing 0.65g of agarose powder with 5.0 ml of 10x3-Morpholinopropane sulfonic acid buffer and 42.5 ml of DEPC treated water in a conical flask.
- The mixture was melted in a microwave oven at 60^0C for 2 minutes.
- As the mixture was cooled to 40-45°C temperature, 2.5 ml formaldehyde was added and then the solution was mixed by gentle swirling.
- The melted solution was poured on a gel-case to solidify the gel.

3.2.1.2 iii Sample preparation and gel electrophoresis

Method

- Thermal cycler
- RNA-loading buffer (dye)
- RNA

Composition of RNA loading buffer:

10×MOPS	40µl
Formamide	200µl
37% Formaldehyde	70µl
Glycerol	20µl
DEPC-treated Water	20µl
0.5 M EDTA (pH 8.0)	0.5µl
10 mg/ml ethidium bromide	2.0µl
A few drops of bromophenol blue	

Procedure

- 1.0 µl of RNA sample was added to 2.5µl of dye. The samples were then denatured in a thermal cycler at 65^0C for 20 min.

- 1 g of 0.1% sodium dodecyl sulfate and 4 g of 0.1 N NaOH were weighed, and 1 lit DEPC water was added and mixed. The gel tank was treated with this solution half an hour before the running the gel.
- The gel was set for electrophoresis and samples were applied into the wells. Electrophoresis was carried out at 45V for 4 hours.
- The gel was then rinsed with water and photographed in a trans-illuminator under UV light.

3.2.1.3 Understanding RNA quality:

Intact total RNA run on a denaturing gel will have sharp 28S and 18S rRNA bands (eukaryotic samples). The 28S rRNA band should be approximately twice as intense as the 18S rRNA band (Figure 1, lane 3). This 2:1 ratio (28S:18S) is a good indication that the RNA is intact. Partially degraded RNA will have a smeared appearance, will lack the sharp rRNA bands, or will not exhibit a 2:1 ratio. Completely degraded RNA will appear as a very low molecular weight smear (Figure 1, lane 2). Inclusion of RNA size markers on the gel therefore allowed the size of any bands or smears to be determined and also was a good control to ensure the gel was run properly (Figure 1, lane 1).

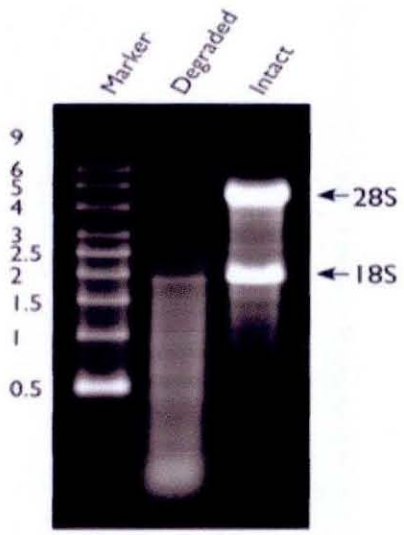


Figure 3: Intact vs. Degraded RNA. Two µg of degraded total RNA and intact total RNA were run beside RNA Markers on a 1.3% denaturing agarose gel. The 18S and 28S ribosomal RNA

bands are clearly visible in the intact RNA sample. The degraded RNA appears as a lower molecular weight smear.

3.2.1.3 i Quantification of RNA

Concentration of the isolated RNA was quantified by using Nanodrop (Nanodrop 100, Spectophotometer). The ratio between readings of 260nm and 280 nm (OD_{260}/OD_{280}) was also measured directly by this machine.

3.2.1.3 ii Purity of RNA

The ratio of the absorbance at 260 nm and 280 nm (*A*260/*A*280) provides an estimate of the purity of RNA with respect to contaminants that absorb in the UV, such as protein. Pure RNA has an *A*260/*A*280 ratio between 1.9 and 2.0.

3.2.2 Reverse Transcriptase-PCR

3.2.2.1 i First strand cDNA synthesis

Reagents

- Degenerate forward primer Ty1RT and Ty3Da were used.
- 5X First Strand Buffer
- 0.1M DTT
- 2mM dNTP Set
- RNaseOUT Ribonuclease Inhibitor
- SuperScript II1 Reverse Transcriptase (200 units/ul)
- *E. coli* RNase H

The specific primers used here is GSP – 7rt

3.2.2.1 ii Dilution of primer

To dilute the primer, 10µl of the primer from stock solution (100 µM) was added to 90µl of TE buffer (pH 8.0), resulting in a final concentration of 10µM.

3.2.2.1 iii Preparation of dNTPs mixtures

100µl of each stock dATP, dGTP, dCTP, dTTP (100µM) were mixed in fresh, autoclaved eppendorf tube and the final volume was made up to 1000µl by adding 600µl of PCR-water. This was then stored at –20°C. The concentration of each of the nucleotide in the above mixture was about 10.0mM.

Method

- The following items were added to a nuclease free thin walled tube:

  1 µl of specific primers (2µM)
  3 µg of total or mRNA sample
  1 µl of 10.0 mM dNTPs mixture
  DEPC treated PCR water upto 13 µl
- The mixture was heated to 65°C for 5 minutes and then incubated on ice for at least 1 minute.
- After brief centrifugation, the following components were added to the above tubes and mixed by pipetting gently up and down:

  4 µl 5×RT-buffer
  1 µl 0.1 M DTT
  1 µl RNaseOUT™
  1 µl Super Script™ III RT (200 units/µl)
  - The mixture was incubated at 25°C for 5 minutes followed by at 50°C for 60 minutes.
  - Super Script™ III RT activity was inhibited by heating at 70°C for 15 minutes.
  - In order to remove the RNA complementary to the cDNA, 0.5 µl of *E. coli* RNaseH was added and incubated at 37°C for 20 minutes.

3.2.2.2 PCR for confirming cDNA synthesis

PCR was performed using the same degenerate primers and cycling conditions as described earlier except that cDNA was used as template rather than genomic DNA. The PCR product was used in cloning.

3.2.2.3 pCR 2.1 Vector mediated transformation

Methods and materials are as described earlier

Isolation of plasmid DNA

Methods and materials are as described earlier.

Confirmation of successful transformation clone by PCR

Methods and materials are as described earlier.

Gel extraction and sequencing

Methods and materials are as described earlier.

3.2.2.4 Bioinformatics analysis of the sequences

Methods and materials are as described earlier.

4. RESULTS:

4.1 Isolation and confirmation of Ty1-*copia* and Ty3-*gypsy* retrotransposons from Jute genome:

After checking correct isolation of genomic DNA using gel electrophoresis, the reverse transcriptase domains of the Ty1-*copia* and Ty2-*gypsy* like retrotransposons of jute genome were amplified using degenerate primers, and length of PCR products obtained were 280bp and 290 bp for Ty1-*copia*and Ty2-*gypsy* respectively. After cloning of the PCR products, 25 colonies of Ty1-*copia* and Ty2-*gypsy* were selected and plasmids were isolated followed by sequencing. Homology based searching (BLASTx and BLASTn) indicated that the Ty1-*copia* and Ty2-*gypsy*sequences isolated were similar to these Ty1-*copia* and Ty3-*gypsy* retrotransposons of other plant species (see sections given below).

4.1.1. Gel electrophoresis of isolated genomic DNA:

The purity and integrity of DNA isolated from O-9897 leaf, by liquid nitrogen and CTAB isolation buffer, were checked by 1% agarose gel electrophoresis and ethidium bromide staining. The high molecular weight genomic DNA was seen as a large sized band on the top part of the agarose gel.

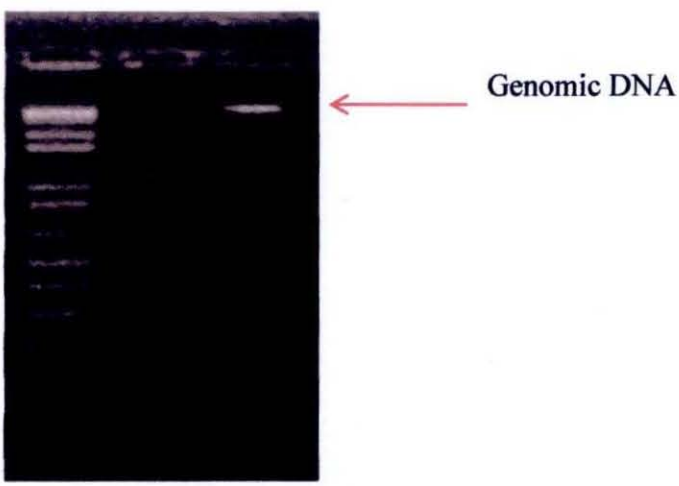


Figure 4: Gel electrophoresis of the isolated genomic DNA from O-9897 leaf.

4.1.2. Gel electrophoresis of PCR product amplified degenerate primers

The amplified reverse transcriptase domains of the Ty1-*copia* and the Ty3–*gypsy* retrotransposons were amplified using degenerate primers.

4.1.2.1 Gel electrophoresis of PCR product amplified by Ty1-RT degenerate primers

When O-9897 leaf genomic DNA underwent PCR using degenerate primer set Ty1-RT forward and Ty1-RT reverse, a 280 bp band was produced (see figure 4.1). These products were then cloned.

Figure 4.1: Gel electrophoresis of Ty1-*copia* PCR product

4.1.2.2 Gel electrophoresis of PCR product amplified by Ty3-Da degenerate primers

PCR from O-9897 genomic DNA using degenerate primer set Ty3-Da forward and Ty3-Da reverse produced a desired 290 bp band (see figure 4.2). This PCR product was also used in cloning.

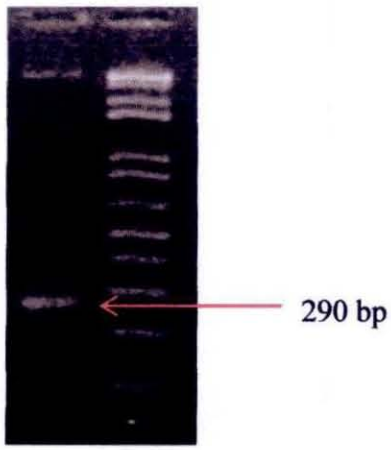


Figure 4.2: Gel electrophoresis of the Ty3-*gypsy* PCR product

4.1.2 Cloning of the PCR product:

4.1.2.1 Transformation using pCR 2.1 Vector

Heat shock method was then used to transform the ligated PCR product into *E. coli* chemical competent cells. The transformed and non-transformed cells could be identified by the white and blue colony screening respectively on the agar plates. Only white colonies were selected for further analyses for Ty1 and Ty3. 25 colonies for Ty1-*copia* and 25 colonies for Ty3 were randomly selected and plasmids were isolated.

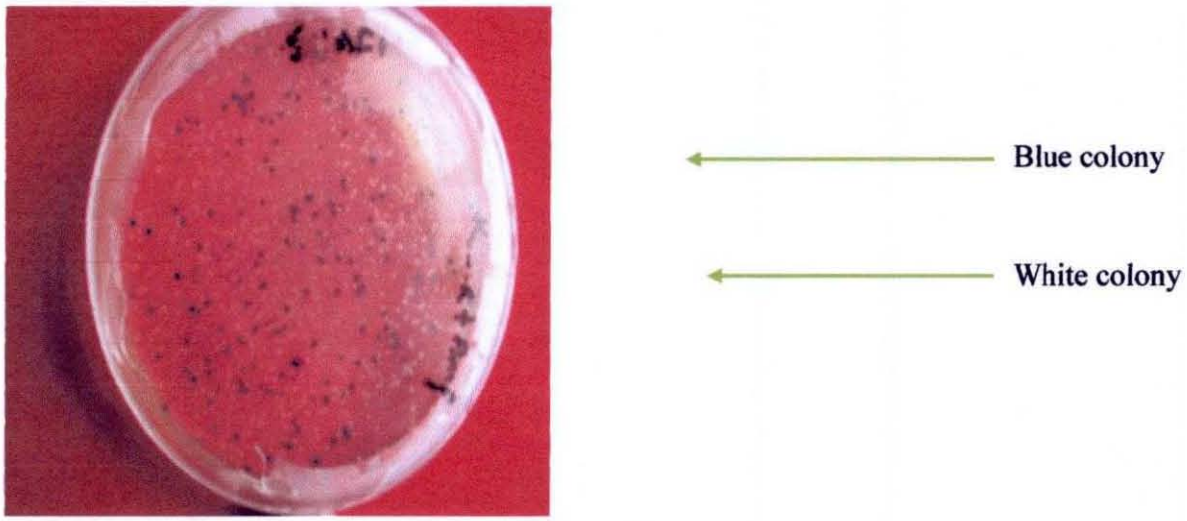


Figure 4.3: Blue-white screening of the cloned LTR retrotransposons.

4.1.3 Plasmid DNA isolation from transformed *E.coli*

Plasmid DNA were isolated from individual liquid cultures of transformed *E.coli* cells. These were then run in agarose gel and seen under UV light. Distinct bands for plasmid DNA near 4000bp in size were observed. As the size of the PCR product was 280 bp and 290 bp, and the pCR 2.1 Vector harboring the insert was over 3900bp, it could be inferred that the band visualized was characteristic of the required plasmid DNA.

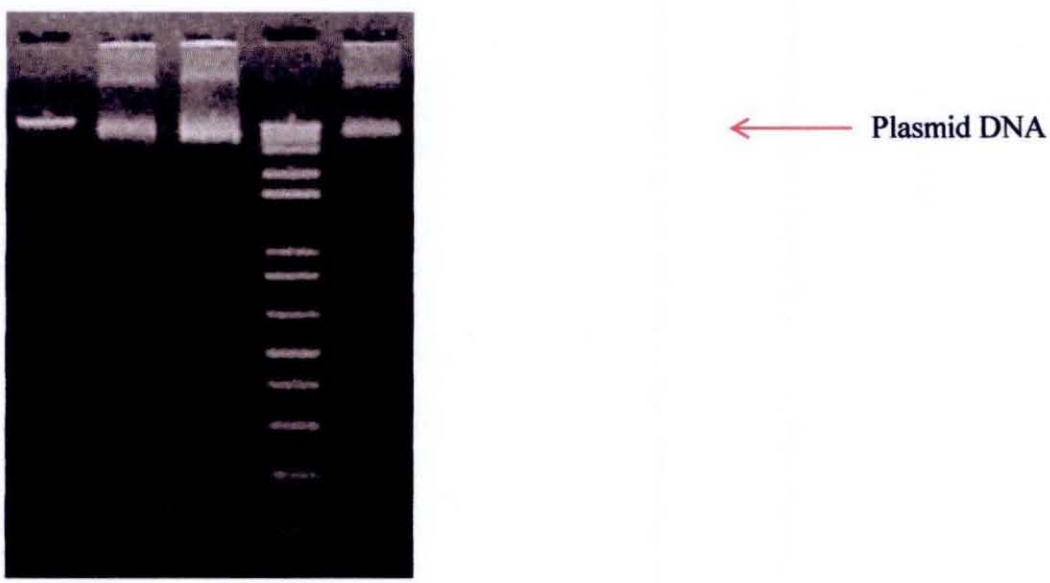


Figure 4.4: Gel electrophoresis of Isolated plasmid DNA

4.1.4 Confirmation of successful transformation by PCR:

To confirm the transformation, PCR products of the thermal cycling were seen in an agarose gel. As the size of the PCR product was 280 bp and in plasmid, M13 primer binding sites are 200 bp apart, the expected product size was around 480 bp. After gel electrophoresis and staining, the expected bands were found.

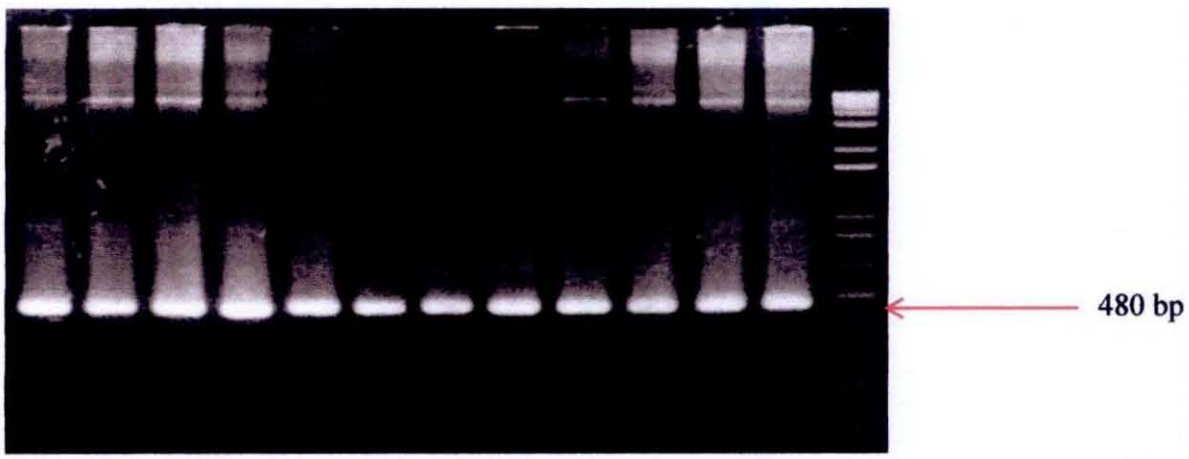


Figure 4.5: Gel electrophoresis of PCR products by using M13 primer set and isolated plasmid as template DNA

4.1.5 Sequencing result of the gel purified product:

The nucleotide sequences of gel purified PCR products were then sent to a commercial laboratory 1st Base in Malaysia for sequencing. The excellent chromatogram results obtained for all sequences indicated a high quality of sequence purification.

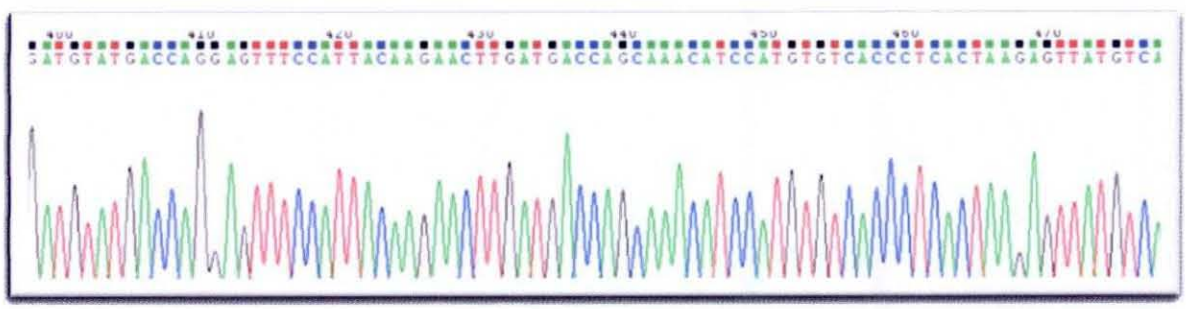


Figure 4.6: Chromas of the sequenced DNA

4.1.6 Bioinformatics analysis

4.1.6.1 VecScreen analysis

VecScreen analysis revealed vector contamination at both ends of all 50 sequences. This outcome was anticipated, as the primers used in the PCR (M13) resided in the pCR 2.1 Vector. The vector contaminated sequences were therefore deleted from both ends to filter out the original Ty-1 and Ty-3 sequences. An example of vector contaminated Ty-1 clone is given below:

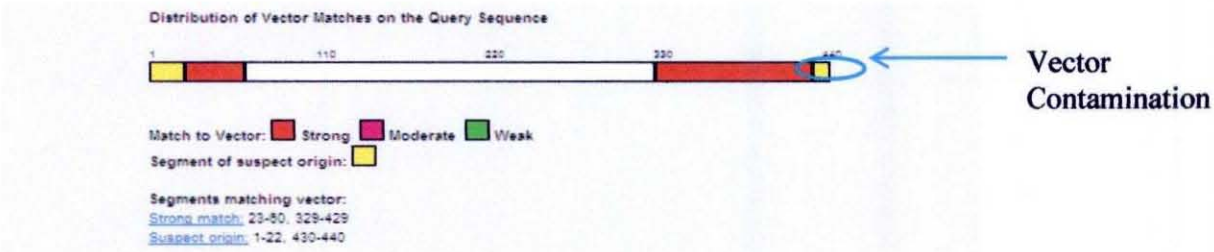


Figure 4.7: A graphical view of the VecScreen result

4.1.6.2 Cap3

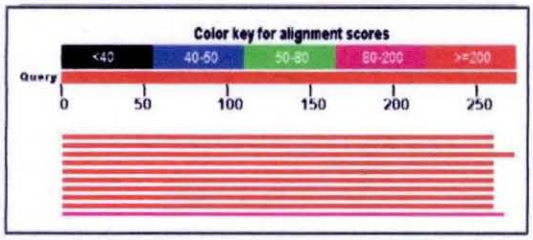
On the basis of given forward and reverse sequences for each clone, Cap3 came up with 25 Ty1-*copia* and 25 Ty3-*gypsy* contigs. The Ty1-*copia* and Ty3-*gypsy* contigs were named as JTEC and JTEG respectively. The contigs are given in the appendix as unique Ty1-*copia* and Ty3-*gypsy* sequences.

4.1.6.3 Verification by BLAST analysis

Homology based searches using by BLASTn and BLASTx revealed that all Ty1 sequences had close similarity to those in other plant species.

Within these 25 Ty1-*copia* sequences, based on the Blastx and Blastn results along with Blast2seq results, it was found that among 25 sequences of Ty1-*copia* 14 sequences were unique and among 25 sequences of Ty3-*gypsy*, 12 were unique.

An example of Blastn result is given below



criptions

nd for links to other resources: U UniGene E GEO G Gene S Structure M Map Viewer

Sequences producing significant alignments:
(Click headers to sort columns)

Accession	Description	Max score	Total score	Query coverage
AM478174.2	Vitis vinifera contig VV78X245374.2, whole genome shotgun sequence	248	248	94%
AM454721.1	Vitis vinifera contig VV78X190657.4, whole genome shotgun sequence	248	248	94%
AM444267.2	Vitis vinifera contig VV78X168278.1, whole genome shotgun sequence	246	246	99%
AM446323.2	Vitis vinifera contig VV78X151180.11, whole genome shotgun sequenc	244	244	94%
XM_002268311.1	PREDICTED: Vitis vinifera hypothetical protein LOC100262113 (LOC10	239	239	94%
XM_002271312.1	PREDICTED: Vitis vinifera hypothetical protein LOC100263944 (LOC10	239	239	94%
AM456269.2	Vitis vinifera contig VV78X008391.9, whole genome shotgun sequence	239	239	94%
AM459683.2	Vitis vinifera contig VV78X278253.5, whole genome shotgun sequence	235	235	94%
XM_002274814.1	PREDICTED: Vitis vinifera hypothetical protein LOC100267893 (LOC10	233	233	94%
AF398198.1	Zea mays clone Tzm13 Ty1-copia type retrotransposon, partial sequer	192	192	96%

A Ty1 clone showing match with a *Zea mays* Ty1-copia sequence

Figure 4.7: A graphical view of the BLAST result

4.1.6.4 Transeq

Transeq was used to convert the nucleotide sequences into their corresponding amino acid sequences. The result showed that among 14 Ty1 unique sequences 4 contained stop codon and among 13 Ty1 unique sequences 3 contained stop codon.

4.1.6.5 ClustalW analysis

For multiple sequence alignment

As there was a large variability in the nucleotide sequences, multiple sequence alignments were carried out on the amino acid sequences using ClustalW. Multiple sequence alignment using ClustalW on 14 Ty1-*copia* amino acid sequences showed that these sequences were heterogeneous in *Corchorus olitorius*. The result indicated highly conserved TAFLMG and FLVYVDD domains at the ends and YGLKQA domain in middle of the Ty1-*copia* RT sequences. The ClustalW results along with the domains are shown in the following figure:

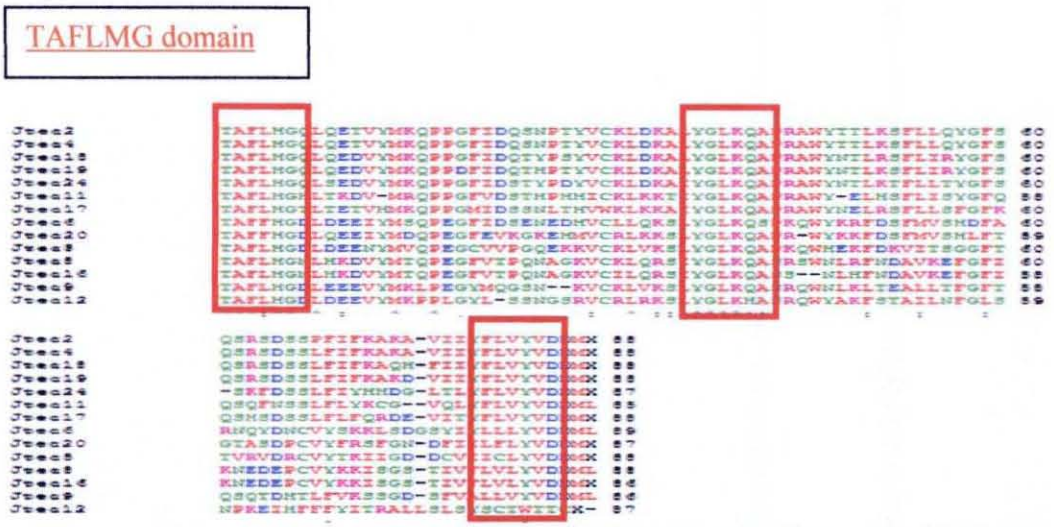


Figure 4.8: Graphical view of multiple sequence alignment of Ty1-*copia* sequences

The same alignment with 13 Ty3-*gypsy* amino acid sequences showed that these sequences are less heterogeneous in *Corchorus olitorius*. The result verified the presence of highly conserved LVDN, LFSFMDGFSGY, APED, FCYKVMPF, QRAMV, LFMD, EVYV domains in the Ty3-*gypsy* RT sequences.

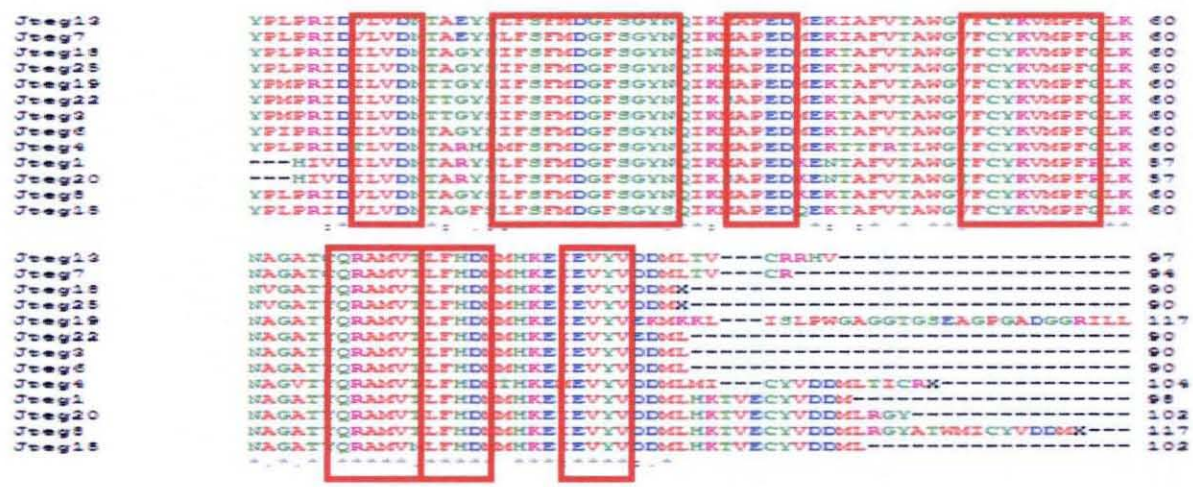


Figure 4.9: Graphical view of multiple sequence alignment of Ty3-*gypsy* sequences

Overall even though the CLustalW analysis showed heterogeneity in a few sequences, the homology based BLASTn and BLASTx searches indicated these as sequences of the respective groups Ty1 and Ty3.

4.1.7 A Phylogenetic tree construction:

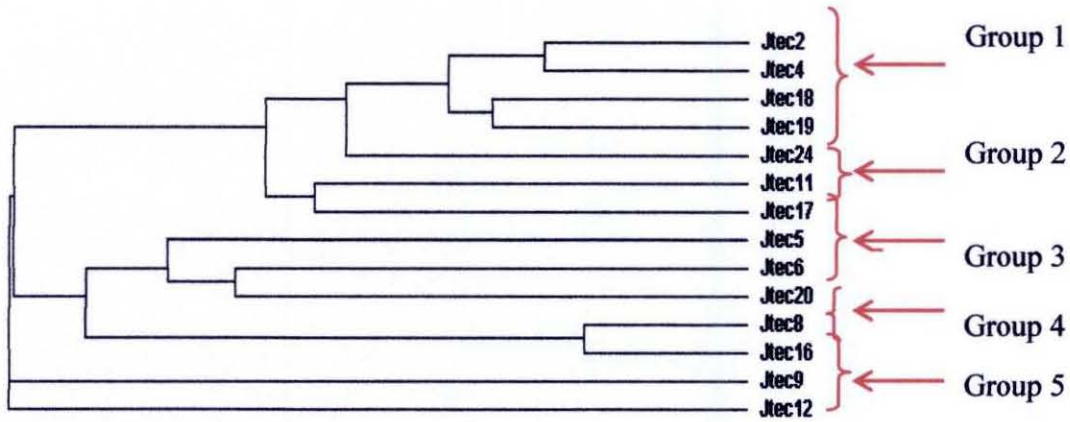


Figure 5: Neighbor joining tree of Ty1 generated by ClustalW

A phylogenetic tree of the Ty1 and Ty2 sequences wasmade based on the ClustalW results, using the Neighbour Joining (NJ) method. Ty1 sequences were grouped into five subgroups and Ty3 sequences were grouped into 4 sub-groups. Figure 5.1 and 5.2 below show the phylogenetic relationships or Ty1 and Ty3 respective.

A similar tree generated for Ty3 using ClustalW classified the RT sequences in 4 groups.

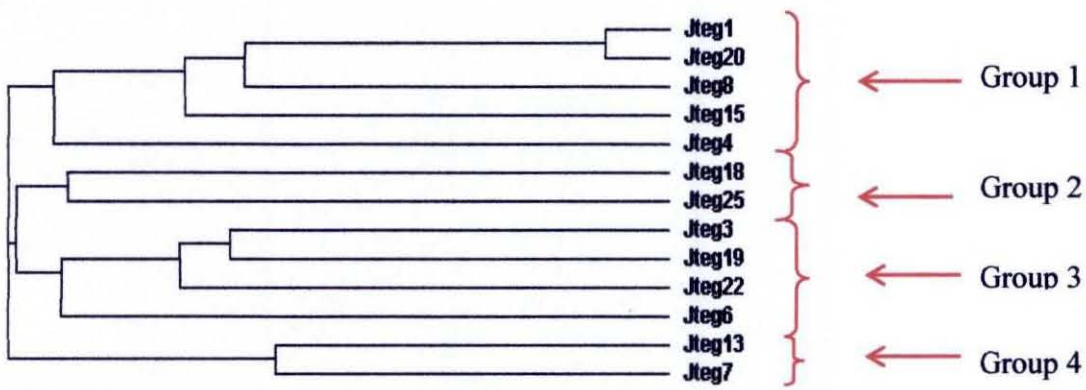


Figure 5.1: Neighbour joining tree of Ty3 generated by ClustalW

Table 2: Retrotransposon groups and their constitutive sequences

Retrotransposon type	Sub-Groups	Sequences
Ty1-*copia*	Group 1	Jtec2, Jtec4, Jtec18, Jtec19, Jtec24
	Group 2	Jtec11, Jtec17
	Group 3	Jtec5, Jtec6, Jtec20
	Group 4	Jtec8, Jtec16
	Group 5	Jtec9, Jtec12
Ty3-*gypsy*	Group 1	Jteg1, Jteg20, Jteg8, Jteg15, Jteg4
	Group 2	Jteg18, Jteg25
	Group 3	Jteg3,Jteg19, Jteg22
	Group 4	Jteg13, Jteg7

4.2 Transcriptional analysis

4.2.1 Isolation of RNA

Gel electrophoresis of the isolated RNA

Isolation of RNA from leaves of O-9897 was carried out. Denaturing agarose gel electrophoresis and ethidium bromide staining was used to check the purity and integrity of the RNA. Ribosomal bands were visualized as bands on the stained gel. 28S ribosomal RNA bands appeared to have an intensity approximately twice that of the 18S rRNA band (see figure 5.2)

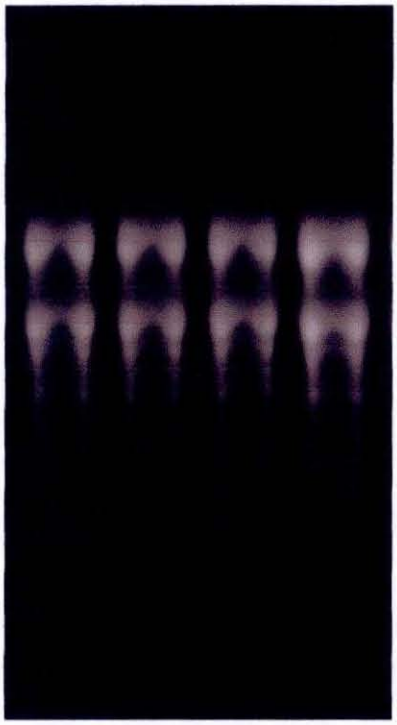


Figure 5.2 : Total RNA isolated from O-9897

4.2.1.1 Results of quantification and purity of RNA

The concentration and spectrophotometric ratio at 260 nm and 280 nm of isolated RNA was measured by nano drop, as given in the following table,

Table 3: Spectrophotometric ratio and concentration of isolated RNA

Genotype	Ratio of $OD_{260\ nm}$ to $OD_{280\ nm}$	Concentration of RNA (ng/µl)
O – 9897	1.93	3603.7

4.2.2 Confirmation of successful RT-PCR

The RT-PCR process was confirmed by subjecting the products to gel electrophoresis. Only a band of 280 bp for Ty1-*copia* was seen, shown in Lane 1 of the gel image below (figure 5.3). There was no band of DNA for Ty3 (Lane 3 of the figure 3.15). This result strongly indicates that Ty1-*copia* LTR retrotransposons are transcriptionally active in *Corchorus olitorius* genome whileTy3-*gypsy* retrotransposons appear to be transcriptionally inactive.

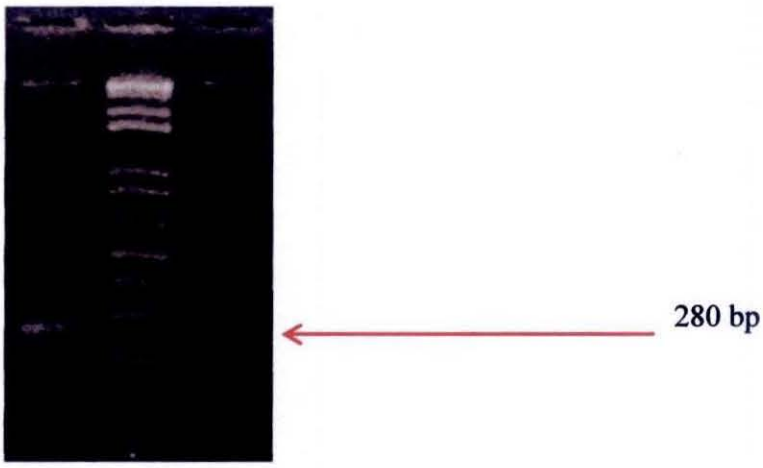


Figure 5.3: Gel electrophoresis of RT-PCR product for both Ty1 (Lane-1) and Ty3 (Lane-3). The Lane-2 is 1Kb+ ladder.

4.2.4 Cloning and Transformation with pCR 2.1 Vector

The RT-PCR product of Ty1-*copia* was successfully cloned into pCR 2.1 Vector.

Transformation of vectorinto *E. coli* chemical competent cell by a process of heat-shock was carried out successfully. As the transformed cells were put on LB agar plate containing ampicillin and kanamycin for selection, and IPTG and X-gal was used for blue white screening, only white colonies were selected for further screening. 15 colonies for Ty1-*copia* were selected.

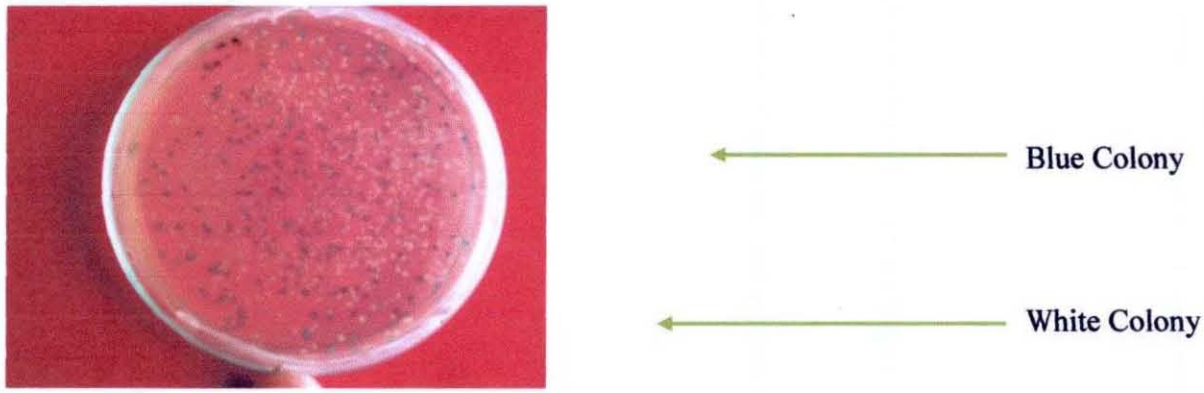


Figure 5.4: Blue-white screening of the cloned LTR retrotransposons

Plasmid DNA isolation from transformed *E.coli*

Plasmid DNAs isolated from individual cultures of transformed *E.coli* cells were run in agarose gel and visualized by UV light. Distinct bands for plasmid DNA near 4000bp in size were obtained. As the size of the PCR product was 280 bp and the pCR 2.1 Vector harboring the insert was over 3900bp, the band obtained was characteristic of desired plasmid DNA.

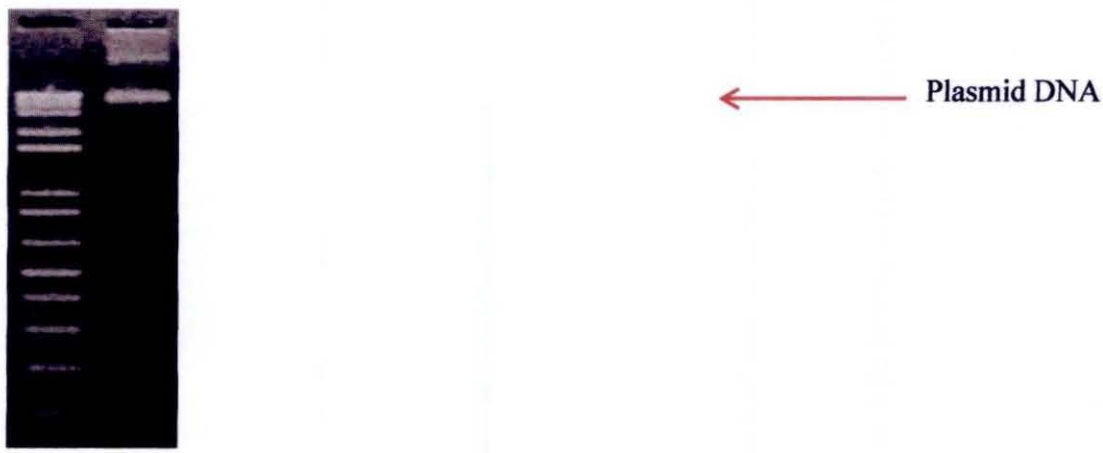


Figure 5.5: Gel electrophoresis Isolated plasmid DNA

4.2.4.1 Confirmation of successfully transformed clone by PCR

PCR obtained from isolated plasmids using M13 primer set was confirmed by agarose gel electrophoresis. As the size of the PCR product was 280 bp and in the plasmid, M13 primer

binding sites are 200 bp apart, it was estimated that the expected product size would be approximately 480 bp. After gel electrophoresis and staining, the expected bands were found.

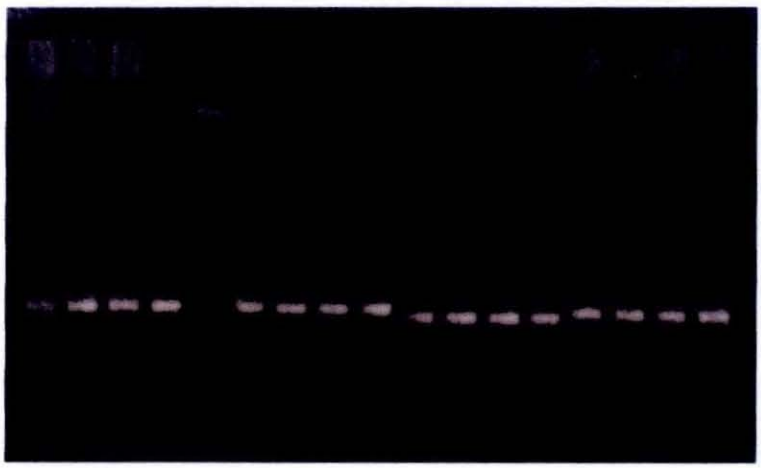


Figure 5.6: Gel electrophoresis of PCR products by using M13

4.3 Result of sequencing of the gel purified product:

The nucleotide sequences of gel purified PCR products were sent for sequencing to 1stBase in Malaysia. The excellent chromatograms results of the sequences was a validation of the good quality of sequences isolated, and showed that Ty1-*copia* retrotransposons are transciptionally active in the jute *Corchorus olitorius* genome.

Bioinformatic analysis with BLAST and ClustalW suggested that the 15 RT_PCR positive sequences could be divided into 2 groups; JTEC-RT-1 and JTEC-RT-2.

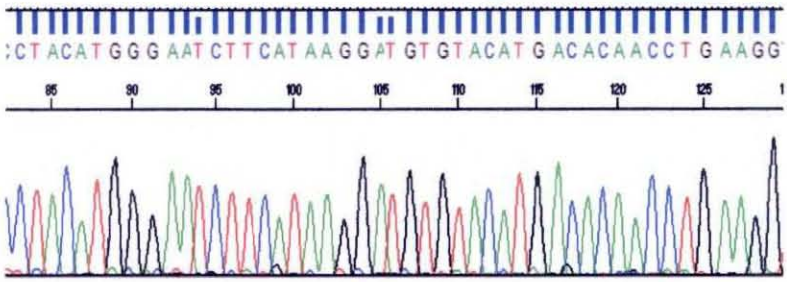


Figure 5.7: Chromas of the sequenced DNA

4.3.1 Identification of the transcriptionally active group by ClustalW

To identify the transcriptionally active groups from the previously constructed groups of Ty1 retrotransposons of jute genome, two representative sequences (JTEC RT-1a, JTEC RT-2a) were selected and a phylogenetic tree was constructed based on the NJ principle. Both of these two representative sequences were found to fit in the cluster of previously constructed Group 3 of Ty1 sequences. So it can be said that, the sequences of Group 3 of Ty1 LTR retrotransposons of jute genome are transcriptionally active. This result is shown in the phylogenetic tree give below (Figure 5.8).

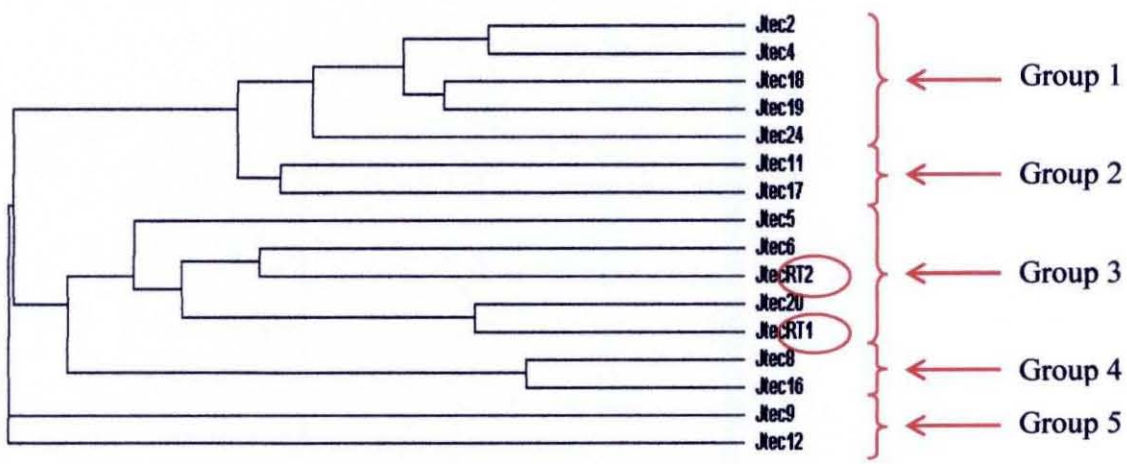


Figure 5.8: Phylogenetic tree to identify the transcriptionally active Ty1 LTR retrotransposons of jute.

Phylogenetic relationships:

Sequence similarity within and between groups of a phylogenetic tree are generally indicated on a threshold of 80% sequence similarity. From the multiple sequence alignment it is apparent that the partial Ty1 and Ty3 reverse transcriptase domains are highly heterogeneous and diverse. The similarity is even less when it comes to looking at the nucleotide sequences. As there were stop codons in some of these sequences, we were unable to use amino acid sequences to aid in constructing the phylogenetic tree.

5. Discussion:

5.1 Isolation and Characterization of LTR retrotransposons

The partial reverse transcriptase domains of Ty1-*copia* and Ty3 *gypsy* retrotransposons of the Jute genome were amplified using degenerate primers, which have been successfully used in many higher plants earlier (www.le.ac.uk/bl/phh4/retros.htm). The successful amplification of these sequences was confirmed by analyzing the amplified sequences, which were shown to be reverse transcriptase domains of jute LTR retrotransposons. It was observed that the Ty1-*copia* reverse transcriptase sequences from jute were highly heterogeneous, though bioinformatics analysis did demonstrate despite their heterogeneity there was enough similarity to be identified as being from the Ty1-*copia* group. This is in line with similar findings of heterogeneity in Ty1-*copia* in other species, like conifers and sorghum. However the RT sequences of Ty3–*gypsy* retrotransposons had a low level of heterogeneity as also seen in other species like tomato (*Avramov Z et al*, 1995, *Su* and *Brown* 1997, *Freisenet et al*, 2001, Muthukumar B and Bennetzen Jl, 2004, Dixir*et et al,* 2006).

These highly heterogeneous sequences may have resulted for several reasons as follows;

(1)The replication mechanism may not always be accurate, due to an error-prone retrovirus type process which lacks fidelity and efficient proof-reading of the reverse transcriptase (Domingo E and Holland J, 1994).

(2) There could be a number of both active and defective populations of retrotransposons (Kumar A, Bennetzen JL, 1999).

(3) The divergence of a retrostansposon in any population seems to be proportionate to the copy number hence, pressure of copy number (Flavell AJ *et al,* 1992, Charlesworth B, 1986).

(4) Homologous recombination (Heslop-Harrison JS *et al,* 1997, Liu B *et al,* 1999).

(5) Horizontal and/or vertical transmission (Kumar A, Bennetzen JL, 1999).

Also, interestingly the substitution pattern analysis results show that dN:dS (the ratio of the number of non-synonymous nucleotide substitutions to the number of synonymous nucleotide substitutions) is higher in the Ty1-*copia* group, which could also be a reason for heterogeneous sequences.

Some of the Ty1 and Ty3 elements in this study contained stop codons, despite their low frequency in the genome, compared to the other elements undergoing strong positive selection. This could have happened when weakly selected amino acid sites saturate rapidly when non synonymous substitution rates reach a maximum. Contrary to this, synonymous substitution or divergence increases (Springer MS. et al, 1991, Springer MS. *et al* 1995). However there is nothing to indicate in this study that there are more weakly selected amino acid sites in Ty1 and Ty3-*gyspy*. Newly transposed retroelements are rarely seen to act as a source of new elements (McAllister and Warren, 1997)), and they are seen to acquire mutations like pseudogenes. Moreover transcription may not mean active transposition, so for Ty1-*copia* retrotransposons, this 'pseudogene effect' could be the reason for their low abundance and the reason why mutations are accumulated. Hence, natural selection may only act at the genomic level, hiding the tendency to collect mutations to become non functional, but conserving the retroelements.

5.2 Transcriptional analysis of LTR retrotransposons

In other studies, the LTR subclass of retrotransposons, like Tnt1A, BARE-1 and Tto1 have been seen to demonstrate the most transcriptional activity (Todorovska E, 2007). However during the developmental stages plant retrotransposons are usually transcriptionally inactive to lessen the detrimental effect on the host, and seem to be often activated when subjected to certain biotic and abiotic stresses. Retrotransposons and the host genomes have co-existed throughout their evolutionary history, and have had a profound effect on genome activity. Strategies like methylation and RNAi based silencing have been used by the host genome to regulate its expression and activity. Recombination by the host genome is also another way to control copy number and expression patterns. Plant retrotransposons have been found to be transcriptionally active in roots, tassels and leaves (SanMiguel P. *et al,* 1996, Avramov Z *et al,* 1995, Suoniemi A. *et al,* 1996, Grandbastien MA *et al,* 1997, Tahara M. *et al,* 2004).

In this study two distinct groups of transcriptionally active sequences were detected, and 2 representative sequences from these two groups were analyzed, and found to fit into group 3 of the Ty1-*copia* phylogenetic tree, constructed based on the ClustalW results after characterizing the isolated and amplified retrotransposons from Ty1-*copia*. These transcriptionally active sequences from group 3 of Ty1-*copia* retrotransposons were found to display transcriptional

activity in leaf tissue, under normal conditions. This is an surprising finding, as previously most transcriptionally active LTR retrotransposons studied have shown transcriptional activity under various biotic and abiotic stresses, and not under normal conditions (Tahara *et al*, 2004, Muthukumar and Bennetzen 2004, Kumar and Bennetsen, 1999, Hirochika *et al* 1996). As one of the first studies on LTR retrotransposons of the jute genome, it is therefore interesting to note that transcriptional activity of LTR retrotransposons in normal conditions could be demonstrated. The other unusual finding was that the expression of retrotransposons has often been detected in protoplasts, cultured cells and callus (Hirochika and Hirochika 1993, Pearce *et al*, 1996, Tahara *et al*, 2004), but rarely in leaves (SanMiguel *et al*, 1996), as for this study. Mature leaves of the common variety *C. olitorius* O-9897 were used in the case of this study as experiments like this have not been done on jute previously, in order to a standard to build on for future reference. Having identified the presence of a transcriptionally active group of retrotransposons under normal conditions, there is a good possibility that different stresses to the plant may result in more transcriptional activity, either by producing a higher transcript level from the existing group, or by transcriptional activity from other groups, as has been demonstrated in other plants. Biotic and abiotic stresses like chilling, infection, mechanical damage, in vitro regeneration, hybridization, generation of doubled haploids, tissue culture, exposure to hydrolases, have shown to increase transcriptional activity LTR retrotransposons, in a variety of species including rice and tobacco(Pouteau *et al*, 1991, Hirochika, 1995 & 1996, Grandbastien *et al*, 2005, Salazar *et al*, 2007). Hence there remains scope for future studies to identify the transcriptional activity of retrotransposons responding to stress.

6. Conclusion:

6.1 Summary

This study started out to isolate and characterize the reverse transcriptase domains of the Jute LTR retrotransposons. Results indicate the heterogeneous nature of Ty1 and more homogenous nature of Ty3 are similar to those in other species. The interesting finding was the identification of transcriptionally active retrotransposons in Jute, especially under normal conditions.

6.1 Future Directions

The following would be interesting areas of research for the next stage. Such studies may lead to a greater understanding of the way the jute genome functions;

- Identification and tagging of Jute genes of agronomic importance.
- Develop retrotransposon based marker systems
- Identification of the full length sequences of transcriptionally active elements.
- Study of promoters of transcriptionally active retrotransposons
- Find the relative position of retrotransposons in the chromosome
- Analyze retrotransposon transcription expression patterns and their response to various abiotic and biotic stress conditions, as well as function under normal conditions.
- Analyze how other jute genes are affected by transcriptional expression patterns of retrotransposons.

Understanding of how the jute genome functions and how expression in the genome is regulated is important. Jute is an important cash crop with a chance to re-emerge as an even more important crop given the environmentally conducive properties of jute products, and the emerging range of diverse uses of Jute. Understanding of the Jute genome will enable identification and tagging of genes of agronomic importance. The effects of external stresses on Jute, like those of climate change, which may affect its growth and commercial value, may be tackled if we are able to understand the mechanism behind transcriptional activation under normal conditions and various stress conditions.

7. REFERENCES:

1. **Akter J, Islam MS, Sajib AA, Ashraf N, Haque S and Khan H**(2008) Microsatellite markers for determining genetic identities and genetic diversity among jute cultivars. Aus. J. Crop Sci. 1: 97-107
2. **Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ** (1990) Basic local alignment search tool. J. Mol. Biol. 215: 403-410
3. **Asi'ns MJ, Monforte AJ, Mestre PF, Carbonell EA** (1999) Citrus and Prunus *copia*-like retrotransposons. Theor Appi Genet 99:503—5 10
4. **Avramova Z, SanMiguel P, Georgieva E, Bennetzen JL**(1995) Matrix Attachmenr Regions and Transcribed Sequences within a Long Chromosomal Continuum Containing Maize Adh1. Plant Cell 1995;7:1667-80
5. **Basu A, Ghosh M, Meyer R., Powell W, Basak SL, and Sen SK**(2004) Analysis of Genetic Diversity in Cultivated Jute Determined by Means SSR Markers and AFLP Profiling, Crop Science, 44, 2004, p 678—685.
6. **Belayat MH, Awal A, Rahman MA, Haque S and Khan H**(2003) Distinction between Cold-sensitive and -tolerant Jute by DNA Polymorphisms
7. **Beguiristain T, Grandbastien MA, Puigdomenech P, Casacuberta JM** (2001) Three Tntl subfamilies show different stress-associated patterns of expression in tobacco. Consequences for retrotransposon control and evolution in plants. Plant Physiol 127:212—221
8. **Bernet GP, Asi'ns MJ** (2003) Identification and genomic distribution of *gypsy* like retrotransposons in Citrus and Poncfrus. Theor Appl Genet 108:121—130
9. **Betenvi M, Gichuki T, Schmidt, J, Burg K**(2002) Ty 1-*copia* retrotransposon-based S-SAP (sequence-specific amplified polymorphism) for genetic analysis of sweet potato theor Appl Gencr 2002; 105:862-9.
10. **Cavalier-Smith T** (1978) Nuclear volume control by nucleoskeletal DNA, selection for cell volume and cell growth rate, and the solution of the DNA C-value paradox J cell Sci 1978;34:247-78

11. **Chadha S, Gopalkrishna T** (2007) Comparative assessment of REMAP and ISSR marker assays for genetic polymorphism studies in *Magnaparthe grisea*, Current Science 2007; 93:688-92

12. **Charlesworth B** (1986) Genetic divergence between transposable elements. Genet Res 48:111— 118

13. **Chawla R, DeMason D** (2003) Genome walking in pea: an approach to clone unknown flanking sequences. Botany and Plant Sci. VOL 35 Univ. of California, Riverside, CA 92507, USA.

14. **Cheng C, Daigen M, Hirochika H** (2006) Epigenetic regulation of the rice retrotransposon Tosl7. MolGenet Genomic 276(4): 378-390, 2006.

15. **Datta RM and Patel** (1960) Interspecific hybridization between *Corchorus olitorius* and *C. capsularis* and the cytogenetical basis of incompatibility between them. Euphytica 9: 89—110.

16. **Dellaporta SL, Chomet PS, Mottinger JP, Wood JA, Yu SM, Hicks JB**(1984) Endogenous transposable element associated with virus infection in maize. Cold Spring Harbor *Symp QuantBlot*. 49: 321-328, 1984

17. **Dixir A, Ma KH, Yu JW, Cho EG, Park YJ**(2006) Reverse transcriptase domain sequences from Mungbean (*Vigna radiate*) LTR retrotransposons; sequence characterization and phylogenetic analysis. Plant cell Rep 2006; 25:100-11

18. **Domingo E, Holland J**(1994) Mutation rates and rapid evolution of RNA viruses. In Morse SS, ed. The Evolution Biology of Virus. New York: Raven Press, 1994;161-4

19. **Doyle JJ and Doyle JL** (1987) Isolation of plant DNA from fresh tissue Focus. 12(1): 13- 15

20. **Edmonds, JM**(1980) Systematic and eco-geographic studies on crop gene pools. Herbarium Survey of African *Corchorus L.* species. IBPGR / IJO, Rome, Italy., 1990

21. **Flavell AJ** (1999) Long terminal repeat retrotransposons jump between species. Proc Natl Acad Sci USA 96:12211—12212

22. **Flavell AJ,Dunbar E, Anderson R, Pearce SR, Hartley R, Kumar A**(1992) Tyl-*copia* group retrotransposons are ubiquitous and heterogeneous in higher plants. Nucleic Acids Res 20:3639— 3644

23. **Flavell AJ,Smith DB, Kumar A** (1992) Extreme heterogeneity of Tyl-*copia* group retrotransposons in plants. Mol Gen Genet 231:233—242

24. **Friesen N, Brandes A, Heslop-Harnson** (2001) Diversity, origin and distribution of retrotransposons (*gypsy* and *copia*) in conifers. Mol Biol Evol 18:1176—1188

25. **Gabriel A, Willems M, Mules EH, Boeke JD** (1996) Replication infidelity during a single cycle of Tyl retrotransposon. Proc Nati Acad Sd USA 93:7767—7771

26. **Gupta, PK** (2004). *Biotechnology and Genomics*. First Edition. Rastogi Publications, Meerut.

27. **Guo A, Cheng X** (1997) Isolation and characterization of total DNA from several best fibre crops and their allies. China's Fibre crops. 4:p 9-14.

28. **Grandbastien MA** (1998) Activation of plant rertotransposons under stress conditions. Trends in Plant science 1998; 3:148-57.

29. **Grandbastien MA, Audeon CE, Bonnivard JM, Casacuberta B, Chaihoub APP, Costa QH, Lea D, Melayah M, Petit C, Poncet SM, Tam MA, Van Sluys C and Mhiria**(2005) Stress activation and genomic impact of Tnt 1 retrotransposons in *Solanaceae*. *Cytogenet Genome Res*. 110: 229-241, 2005.

30. **Garcia J. Levano, Verjovski-Almeida, S. da Silva, ACR**(2005) Mapping transposon insertion sites by touchdown PCR and hybrid degenerate primers. Biotechniques 38, 225—229

31. **Grandbastien MA Lucas H. Morel JB, Mhiri C, Vembettes S, Casacuberra JM**(1997) The expression of the tobacco Tntl retrotranspson is linked to plant defense responses. Genctica 1997:100:241-52.

32. **Haque S, Asraf N, Awal A, Sarker R, Begum S, Khan H** (2004)Method for Quality DNA Isolation from Different Parts of Jute Plant: *Corchorus capsularis I*, and *C, olistorius I.*Plant Tissue Cult 2004;14:143-8.

33. **Hill P, Burford D, Martin DMA, Flavell AJ** (2005) Retrotransposon populations of *Vicia* species with varying genome size. Mol Genet Genomics 273:371—381

34. **Heslop-Harrison JS, Brandes A, Taketa S, Schmidt T Vershinin Av. Alkhinmove EG,*et al*** (1997) The chromosomal distributions of Ty-I *copia* group retrotransposable elements in higher plants and their implications for genome evolution. Genetica 1997;100:197-204

35. **Hirochika H, Fukuchi A, Kikuchi F**(1992) Retrotransposon families in rice. Mol Gen Genet 233:209—216

36. **Hirochika H, Hirochika R**(1993) Tyl-*copia* group retrotransposons as ubiquitous components of plant genomes Jrn J Gener 68:35-46

37. **Hirochika H, and H Otsuki, (1995)** Extrachromosomal circular formsof the tobacco retrotranaposon Tto l. Gene 165;229-232

38. **Hirochika H, Otsuki H, Yoshikawa M, Otsuki Y, Sugimoto K, Takeda S** (1996) Autonomous transposition of the tobacco retrotransposon Tto 1 in rice. Plant Cell 8:725—734

39. **Hossain MB, Haque S, Khan H** (2002) DNA fingerprinting of jute germplasm by RAPD. J. Biochem. Mol Biol. 35: 414-419

40. **Hossain MB, Awal A, Rahman MA, Haque S, Khan H** (2003) Distinction between cold-sensitive and cold-tolerant jute by DNA polymorphisms. J. Biochem. Mol. Biol. 36 : 427-432

41. **Hossain MB, Awal A, Rahman MA, Haque S, Islam MM, Khan H** (2007) Frequency of occurrences of pathogenic fungi in jute seeds *Int. J. Sustain. AgrilTech.* 3(1), 1-6

42. **Islam AS, Rashid A**(1960) First successful hybridization between the two jute yielding species. Nature, 185: 258-259.

43. **Islam AS** (1964) A rare hybrid combination through application of hormone and embryo culture. Nature, 210: 320-320.

44. **Jensen S, Gassama MP, Heidmann T**(1999) Taming of transposable elements byhomology dependent gene silencing. *Nat Genet*. 21: 209- 2 12

45. **Jordan IK, Matyunina LV, McDonald JF** (1999) Evidence for the recent horizontal transfer of long terminal repeat retrotransposon. Proc Natl Acad Sci USA 96:12621—1 2625.

46. **Jurka J, Kapitonov VV, Kohany O and Jurka MV** (2007) Repetitive Sequences in Complex Genomes: Structure and Evolution. *Annu. Rev. Genomics Hum. Genet*. 8:241-259

47. **Janik JR, Schery W, Woods FW and Ruttar VW** (1974) Plant Source:An Introduction to world crops 2nd edition page 206, WH Freeman Co. San Francisco, California, USA.

48. **Kalendar R, Tanskanen J, Immonen S, Nevo E and Schulman AH**(2000) Genome evolution of wild barley (*Hordeum spontaneum*) by BARE-1 retrotransposon dynamics in response to sharp microclimatic divergence. PNAS. 97 (12): 6603-6607

49. **Khatun A** (2007) Recent agricultural developments in Jute, Kenaf and Mesta through Traditional and Biotechnological approaches, paper presented in a seminar in Myanmar co-organized by IJSG, www.jute.org/news.htm.

50. **Kidwell MG, Lisch D** (1997) Transposable elements as sources of variation in animals and plants. Proc Natl Acad Sci USA 94:7704—7711

51. **Kidwell MG, Lisch DR** (2000) Transposable elements and host genome evolution. *Trends Ecol Evol*. 15: 95-99

52. **Koundal KR, Mohapatra T**(2006) Evaluation of genetic diversity in jute (*Corchorus species*) using STMS, ISSR and RAPD markers, Plant Breeding, 125, p292—297.

53. **Kumar A, Bennetzen JL** (1999) Plant retrotransposons. *Annu Rev Genet* 33:479—532

54. **Kumekawa N, Ohtsubo H, Horiuchi T, and Ohtsubo E** (1999)Identification and characterization of novel retrotransposons of *gypsy* type in rice, Mol. Gen. Genet. 260; 593-602.

55. **Liu B, PIao H, Zhao F, Fhao J, Liu Z, Huang B** (1999) DNA Methylation changes in Rice Induced by *Zizania Latifolia* (griseb) DNA inrtogression Here*ditas* 1999 131:75-8

56. **Ma J, Bennetzen JL** (2004) Rapid recent growth and divergence of rice nuclear genomes. Proc Natl Acad Sci USA 101:12404—12410

57. **Ma, Y, Sun H, Zhao G, Dai H, Gao X, Li Het al**(2008) Isolation and characterization of genomic retrotransposon sequences from octoploid strawberry (*fragaria X ananassa Duch*) Plant cell Rep; 27:449-507

58. **McAllister BF; Werren JH** (1997) Phylogenctic analysis of a retrotransposon with implications for strong evolution any constraints on reverse transcriptase. Mol Biol Evol 1997;14:69-80

59. **Meyers BC, Tingey SV, Morgance M**(2002) Abundance, distribution and transcriptional activity of repetitive elements in the maize genomes. Proc Natl Acad Sci USA 2002:11:1660-76

60. **Mir, Reyazul R, Rustgi S, Sharma S, Singh R, Goyal A, Kumar J, Gaur A, Akhilesh K, Tyagi, Khan H, Sinha MK, Balyan HS and Gupta PK** (2008) A preliminary genetic analysis of fibre traits and the use of new genomic SSRs for genetic diversity in jute; Euphytica, 2008 161:413-427

61. **Murray MG, Thompson WF**(1980) Rapid isolation of high molecular weight plant DNA. Nucleic Acids Res.8:4321-4325

62. **Muthukumar B, Bennetzen JL** (2004) Isolation and characterization of genomic and transcribed retrotransposon sequences from sorghum. Mol Genet Genomics 271:308—316

63. **Nair AS** (2007) Computational Biology and Bioinformatics – A general overview

64. **Navarro Quezada A, Schoen DJ**(2002)Sequence evolution and copy number of Ty1-*copia* retrotransposons in diverse plant genomes. Proc Natl Acad Sci USA 2002; 99:268-73

65. **Okamoto H and Hirohiko H**(2001) Silencing of transposable elements in plants. *Trends in Plant Science*. 6(11): 527-534, 2001.

66. **Parker JD, Rabinovitch PS, Burmer GC** (1999) Targeted gene walking polymerase chain reaction. Nucleic Acids Res. 19, 3055—3060.

67. **Patel GI and Datta RM** (1960) Interspecific hybridization between *Corchorus olitorius* and *C. capsulris* and the cytogenetical basis of incompatibility between them Euphytica 9: 89—110.

68. **Pearce SR, Harrison G, Li D, Heslop-Harrison JS, Kumar A, Flavell AJ** (1996) The Tyl-*copia* group retrotransposons in *Vicia* species: copy number, sequence heterogeneity and chromosomal localisation. Mol Gen Genet 250:305—3 15

69. **Pouteau S, Huttner E, Grandbastien MA and Caboche M**(1991) Specific expression of the tobacco Tntl retrotransposon in protoplasts.*EMBO J.* 10:1911-1918

70. **Purugganan Md. Wessler S.R** (1994) Molecular evolurion of magellan a maze Ty3/*gypsy*-like retrotransposon. Proc Natl Acad Sci USA 1994; 91:11674-8

71. **Roy A, Bandyopadhyay A, Mahapatra AK, Ghosh SK, Singh NK, Bansal KC, Koundal KR and Mohapatra T** (2006) Evaluation of genetic diversity in Jute (*Corchorus* species) using STMS, ISSR and RAPD markers. Plant Breed. 125: 292-297.

72. **Roy A., Bandyopadhyay A. , Mahapatra A. K., Ghosh S. K, Singh N. K , Bansal K. C, Ricao- Cabnas L, Mareinez-lzquierdo J, GIREL**(2007) A novel transcriptionally active Tyl-*copia* retrotansposon from *Citrus sine sis*. Mol Genct Genomics 2007; 277:365-77

73. **Saha T, Ghosh M,Sen SK** (1999) Plant regeneration from cotyledonery explants of jute *Corchorus capsularisL.* Plant Cell Rep 18:544—548.

74. **Sajib AA, Islam MS, Reza MS, Bhowmik A, Fatema L, Khan H** (2008) Tissue culture independent transformation for *Corchorus olitorius*; plant cell tiss and organ cult, DOl 10.10007/s 11240-008-9447-0

75. **Salazar M, Gonzalez E, Casaretto JA, Casacuberta JM and Ruiz-Lara S**(2007) The promoter of the TLC1.1 retrotransposon from *Solanum chilense* is activated by multiple stress-related signaling molecules. *Plant Cell Rep.* 26(10): 1861-1868

76. **Salim Ahmed, Md. Maksudul Alam, Md. Sazzadul Islam, Md. Shafiuddin , Md. Mosharrof Hossain Mondal, Md. Amzad Hossain and Haseena Khan** (2009) Identification and Characterization of Ribosomal Protein S8 Gene of Jute Plant Tissue Cult. & Biotech. 19(2): 207-215

77. **SanMiguel P, Tikhonov A, Jin YK, Motchoulskaia N, Zakharov D, Melake-Berhan A, Springer PS, Edwards KJ, Lee M, Avramova Z, Bennetzen JL** (1996) Nested retrotransposons in the intergenic regions of the maize genome. Science 273:765—769

78. **Sant VJ, Sainani MN, Sami-subbu R, Ranjekar PK, Gupta VS** (2000) Tyl-*copia* retrotransposon-like elements in chickpea genome: their identification, distribution and use for diversity analysis. Gene 257:157—1 66

79. **Santini S, Cavallini A, Natali L, Minelli S, Maggini F, Cionini PG** (2002) Ty1-*copia*-like and Ty3-*gypsy*-like DNA sequence in Helianthus species. Chromosoma 111:192—200

80. **Silva JC, Loreto EL, Clark Jonathan BC** (2004) Factors that affect the horizontal transfer of transposable elements. Curr Issues Mol Biol 6:57—72

81. **Sinha MK, Mitra S, Nandy S, Sengupta D, Dutta P, Das F, Chakrabarty SC** (2004) Interspecific hybrid between two jute (*Corchorus*) species for textile quality fibre. Ind. J. Genet. 64: 310-316.

82. **Sinha MK, Karmakar PG, Hazra SK, Ramasubramanian T, Mandal RK, Sen HS**(2008) Jute and allied fibre updates, Central Research Institute for Jute and Allied Fibres, India.

83. **Springer MS, Davidson EH, Britten RJ** (1991) Retroviral-like element in a marine invertebrate. Proc Natl Aca Sci USA 1991;88:8401-4

84. **Springer MS, Tusneem NA, Davidson Eh, Britten RJ** (1995) Phylogeny rates of evolution and patterns of codon usage among sea urchin retroviral-like elements with implication for the recognition of horizontal transfer. Mol Biol Evol 1995;12:219-30

85. **Su PY, Brown TA** (1997) Ty3-*gypsy*-like retrotransposon sequencesin tomato. Plasmid 38:148— 157

86. **Suoniemi A, Narvanto A, Schulman AH**(1996)The BARE-1 retrotransposons is transcribed in barley from an LTR promoter active in transient assays. Plant Mol Biol 1996;31:295-306

87. **Swaminathan, MS, Iyer RD and Subbha K** (1961) Morphology, cytology and breeding behaviour of hybrides between *Corchorus olitorius*and *Corchorus capsularis*. Curr. Sci., 30: 67-68.

88. **Tahara M, Aoki T, Suzuka S, Yamashita H, Tanaka M, Matsunaga S, Kokumal S** (2004) Isolation of an active element from a high copy number family of retrotransposons in the sweet potato genome.Mol Gen Genomics 272:116—127

89. **Takeda S, Sugimoto K, Kakutani T and Hirochika H** (2001) Linear DNA intermediates of the Tto1 retrotransposon in *Gag* particles accumulated in stressed tobacco and *Arabidopsis thaliana*. PlantJ. 28(3): 307-17

90. **Tang YM, Ma YZ, Li LC, Ye XG** (2005) Identification and Characterization of Reverse Transcriptase Doming of transcriptionally Active Retrotransposons in wheat Genome . J Integer Plant Biol 2005, 47:604-12.

91. **Tao NG, Xu J, Cheng YJ, Hong L, Gua W-W, Yi HL*et al***(2005): Isolation and Characterization of *Copia*-like Retrotransposons from 12 Sweet Orange (*Citrus sinensis Osbeck*) Cultivars, J Integr Plant Biol 2005;47:1507-15

92. **Todorovska E** (2007)Retrotransposon and their role in plant genome evolution, Biotechnol & Biotechol Eq2007;21:294-305

93. **Vance V, Vancheret H**(2002) RNA silencing in plants defense and counterdefense. Science. 292: 2277-2280

94. **Vicient CM, Suoniemi A, Anamthawat-Jo'nsson K, Tanskanen J, Beharav A, Nevo E and Schulman AH**(1999) Retrotransposon BARE-1 and its role in genome evolution in the genus *Hordeum.Plant Cell*. 11: 1769-1780, 1999.

95. **Villordon AQ, Jarret RL, Labonte DR** (2000) Detection of Ty1-*copia*likereverse transcriptase sequences in *Ipomoea batatas* (L.) Pow. Plant Cell Rep 19:12 19—1225

96. **Voyras DF, Cummings MP, Koniczny A**(1992) Ausubel FM, Rodermel SR. *copia*-like retrotransposons are ubiquitous among plants. Proc Natl Acad Sci USA 1992;89:7124-8

97. **Waugh R, McLean K, Flavell AJ, Pearce SR, Kumar A, Thomas BBT, Powell W** (1997) Genetic distribution of Bare-1-like retrotransposable elements in the barley genome revealed by sequence-specific amplification polymorphisms (S-SAP). Mol Gen Genet 253:687—694.

98. **Wessler SR, Bureau TE, White SE** (1995) LTR- retrotransposons and MITEs: important players in the evolution of plant genomes. Cuff Opin Genet Dev 5:814—821

99. **Wilbelm M. Wilhelm FX**(2001)Reverse transcription of retroviruses and LTR retrotransposons. Cell mol Life sci 2001; 58: 1246-62

100. **Winstanley C, Rapley R**(2000) The Nucleic Acid Protocols Handbook; Extraction and Purificaiton of Plasmid DNA. Totowal Humana Press Inc.

101. **Witte C, Panaud O** (2005)LTR retrotransposons and flowering plant genome size emergence of the increase decrease model. Cytogenet Genome Res 2005; 110: 91 107.

102. **Yanez M, Verdugo 1, Rodriguez M, Prat S, Ruiz-Lara S** (1998) Highly heterogeneous families of Ty1-*copia* retrotransposons in the *Lycopersicon chilense genome.* Gene 222:223-228

103. **Zaki EA, Abdel Ghany AA** (2004) Ty3-*gypsy* Retro-Transposons in Egyptian cotton (*barbadense*). J Cott Sci 8:179-185

8. APPENDIX

LIST OF ABBREVIATIONS

BJRI	Bangladesh Jute Research Institute
bp	Base pair
C	Celsius
cDNA	Complementary DNA
Conc.	Concentration
CTAB	Cetryl trimethyl ammonium bromide
dd water	Distilled deionized water
DMSO	Dimethyl sulphoxide
DTT	Dithiothritol
dNTP	Deoxyribonucleotide triphosphate
dATP	Deoxy adenosine triphosphate
dTTP	Deoxy thymidine triphosphate
dCTP	Deoxy cytidine triphosphate
dGTP	Deoxy guanosine triphosphate
dUTP	Deoxy uridine triphosphate
DNA	Deoxy ribonucleic acid
DNase	Deoxy ribonuclease
EDTA	Ethylene diamine tetra acetic acid
e.g.	For example
et al.	With others
etc.	Etceteras
g/gm	Gram
Fig.	Figure
IJO	International Jute Organization
ISSR	Inter simple sequence repeat
SSR	Simple sequence repeat
kb	Kilobase
L	Liter

M	Molar
MOPS	3-(N-morpholino)propanesulfonic acid
mg	Milligram
min.	Minute
ml	Milliliter
mM	Millimolar
Mol. Wt. /MW	Molecular weight
NaCl	Sodium chloride
ng	Nanogram
nm	Nano meter
NaOH	Sodium hydroxide
No./no.	Number
O.D.	Optical density
PCR	Polymerase chain reaction
p^H	Negative logarithm of hydrogen ion
RAPD	Random amplified of polymofphic DNA
RFLP	Restriction Fragment Length Polymorphism
Rnase	Ribonuclease
rpm	Rotation per minute
RT	Room temperature
SDS	Sodium dodecyl sulphate
sec.	Srcond
TAE	Tris acetate EDTA
TE	Tris EDTA
TEMED	N,N,N',N', tetramethyl ethylene diamine
UV	Ultra violet
vol.	Volume
µl	Micro liter