“Using Jute to Meet the Challenges of Climate Change

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JUTE: A Symbol of National Identity

- For Bangladeshis, jute is not just a plant that produces fibre; it is rather a national icon, linked to the adage: Sonar Bangla

- It is also linked to our quest for economic emancipation.

- In mythical golden Bengal, around which much of our national lore is constructed, we had undulating rice fields together with a field of fibre, golden in colour.

Introduction

- Jute is a diploid (2n=14) dicot annual shrub and belongs to the plant family *Malvaceae.*

- The fiber is phloem tissue obtained from the bark of two cultivated species C. *capsularis* and *C. olitorius*.

- The yield and quality of *olitorius* is better than *capsularis*.

- It is a summer season crop grown from March to July.

- Natural genetic variability is narrow due to self pollination.

Jute: The cash crop of Bangladesh

- Two species of jute, *capsularis* and *olitorius* are widely cultivated in our country.
- It is our principal cash crop.
- It contributes to about **6% of the total export earning and employs around 30 million people** in different events involving jute.
- On an average Bangladesh produces **0.794 million tons of raw jute on about 0.392 million hectares of land**.
- Jute offers cash earnings to **5 million farm families**.

Fibre Retting

Fibre Extraction

Washing Fibre

Drying Fibre

Transporting the Fibres

2009: The Year of Natural fibres

- United Nation declared

  "2009 The International Year of Natural fibres"

- According to FAO this will contribute to further developing the efficiency and sustainability of jute agricultural industries that employ millions of people in some of the world's poorest countries.

- This is encouraging for the improvement or jute production and processing sector, as these fibres are natural fibres.

Jute: The Soil Enricher

- The root system is capable of breaking the plough pan.
- Enrich microbial population and improve drainage condition.
- It also provides 1.92 tons/ha dry leaves.
- It **contributes 36% of the dry weight as organic matter** through leaves, roots and about half of the bark immediately as retting waste.
- Jute **contributes 10 times more organic matter than it receives** as nutrient from the soil.
- For **breaking disease and pest cycle** and also for balancing the soil nutrients, jute is a good candidate for inclusion in the cropping system.

Some Unique Properties of Jute Compared to Synthetic Fibers

- **Biodegradable** and environmentally safe.
- As **geo jute**, has tremendous promise for river embankments to check soil erosion.
- Green jute can be used as a **source of paper pulp**.
- Jute sticks are now extensively used for making partex and **softwood in furniture.**
- As a Biofuel option to substantially mitigate climate change.

Say Yes to Jute Bags!!

- Increasing demand of pulp and paper,
- Growing consciousness on environmental issues
- Restriction on deforestation and preservation of forest resources in many developed and developing countries
- **All these justify the use of non-wood fibrous materials for pulp and paper processing.**
- A number of such non-wood fibrous raw materials have been tested and then found out that jute are technically suitable.

- Use of non-wood fibrous jute as raw materials in the paper industry, **if it can be produced round the year.**

- Forests can be preserved and more CO_2 can be absorbed by these preserved forests.

A Need to Grow Jute Round the Year

* To be used as a source of pulp in the paper industry

* Possible use as a source of biofuel

Why Low Temperature Tolerant Jute Variety Is Economically Important?

•The development of a high-yielding and environmental-stress tolerant jute variety would be beneficial for the agro-economy of Bangladesh.

• This requires the identification of defined markers linked to the suitable and desired traits.

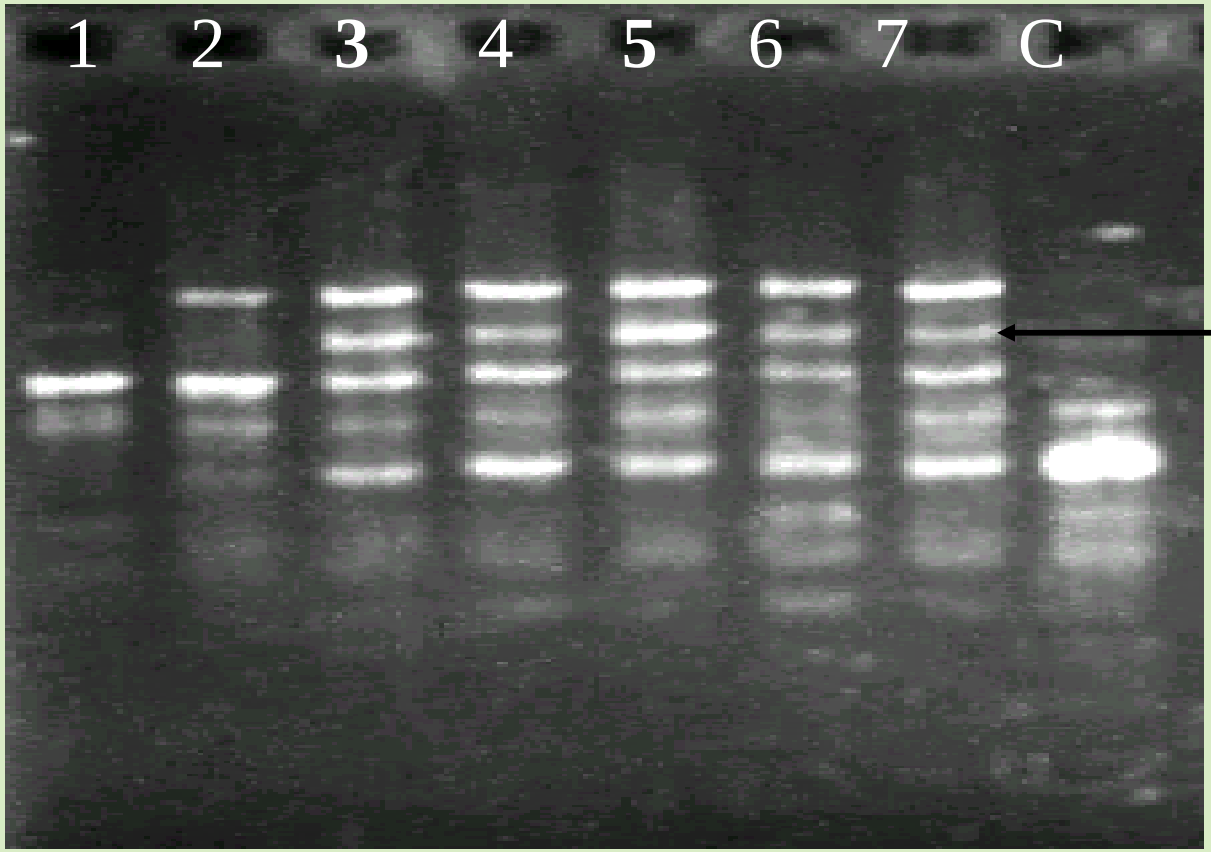
• Jute metabolism is strongly influenced by temperature.

• Jute could be grown more profitably in Bangladesh if an intense cropping pattern could be developed, where jute would be cultivated from late February to early March.

• This may meet the increasing local demand of jute all through the year. For this purpose, new varieties that are tolerant to low temperatures will be required.

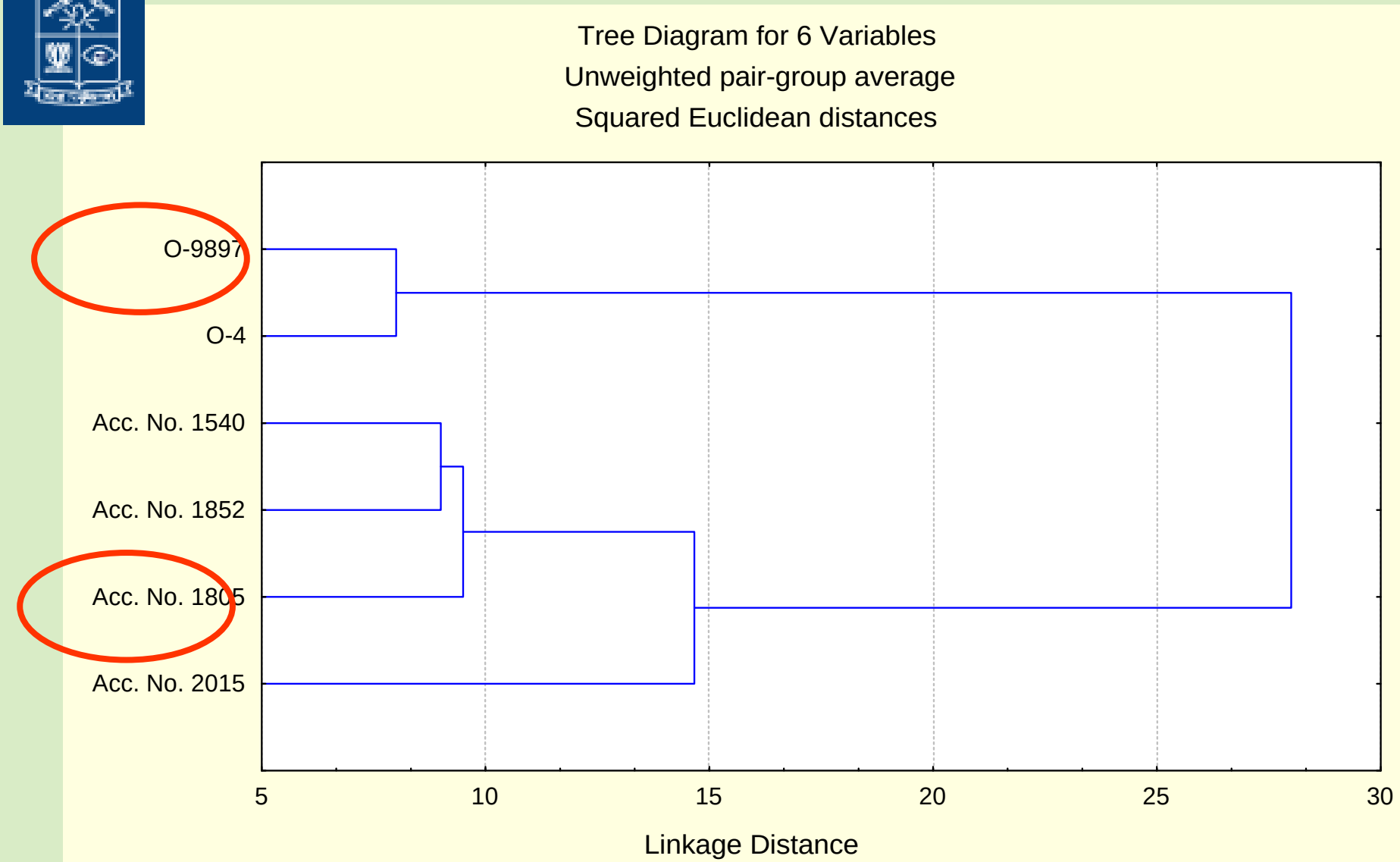
Showing germination and growth of selected jute accessions

in growth chamber at 16°C temperature (at 35 days)

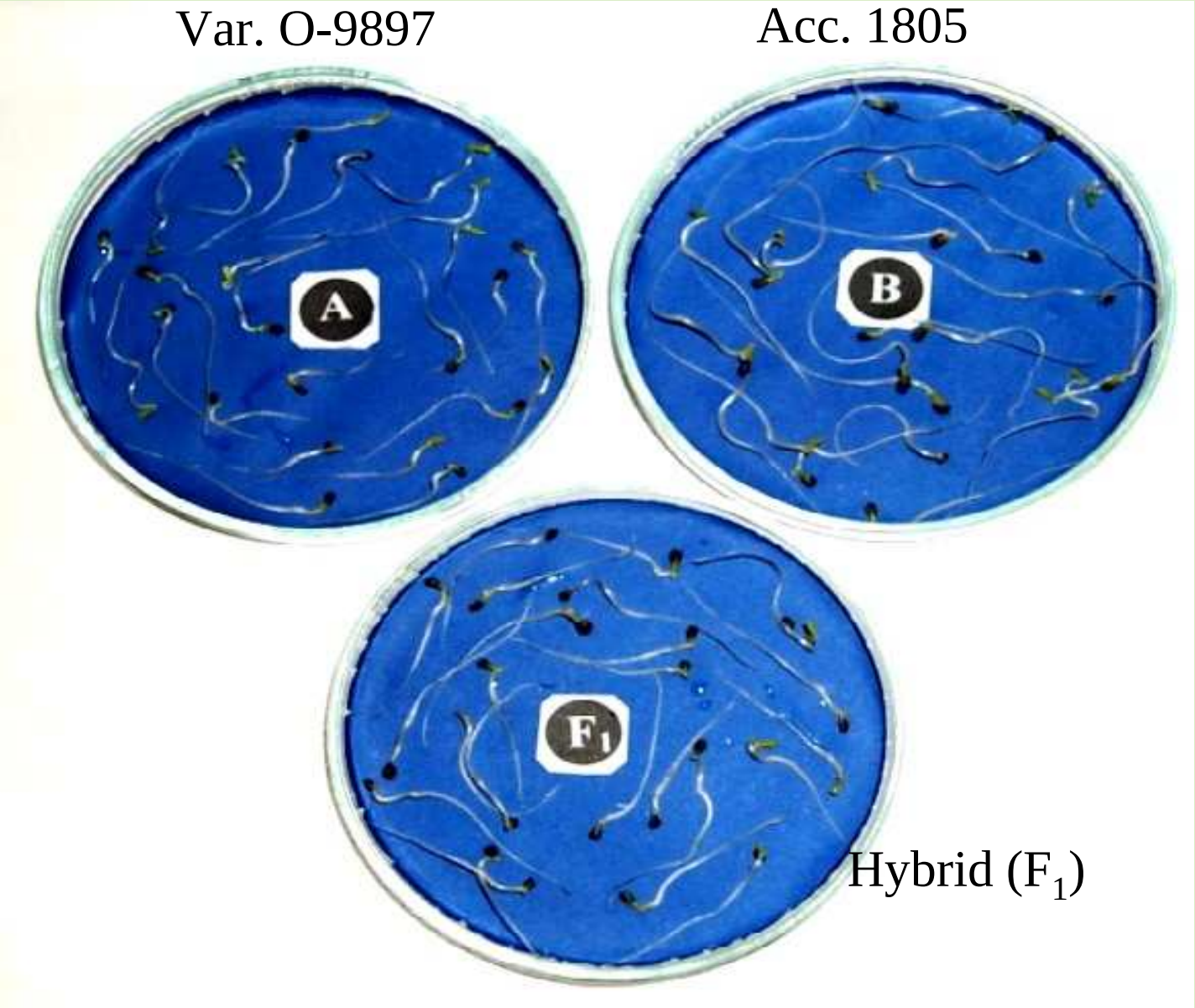


Showing Polymorphic band in low temperature tolerant accessions in RAPD with primer OPG-05

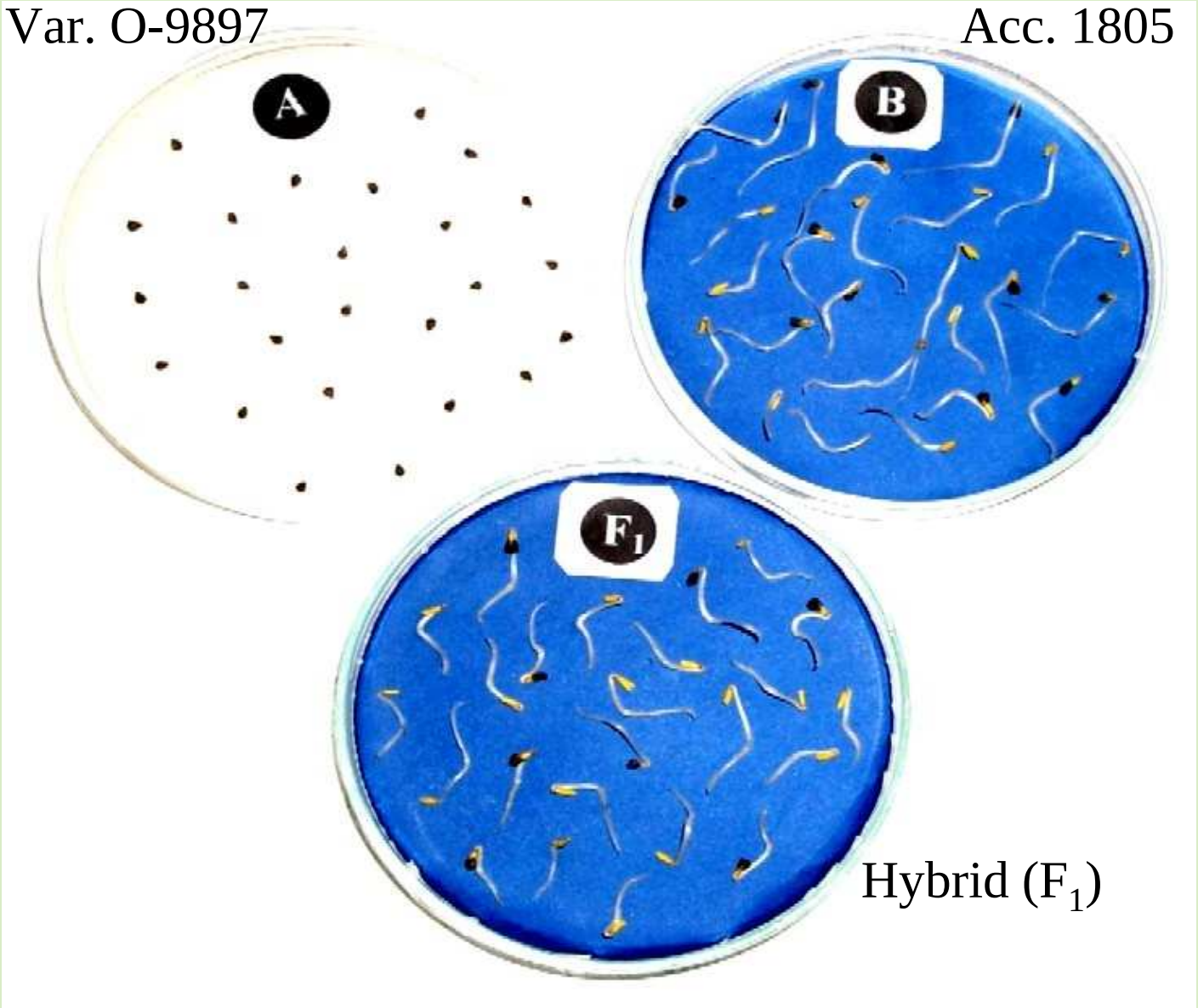
Legend: 1-Var. O-4, 2-Var. O-9897, 3-Acc.1805, 4-Acc.1540, 5-Acc.1805, 6-Acc.1852, 7-Acc.2015 & 8-Control



Dendrogram of jute genotypes obtained from the molecular marker data

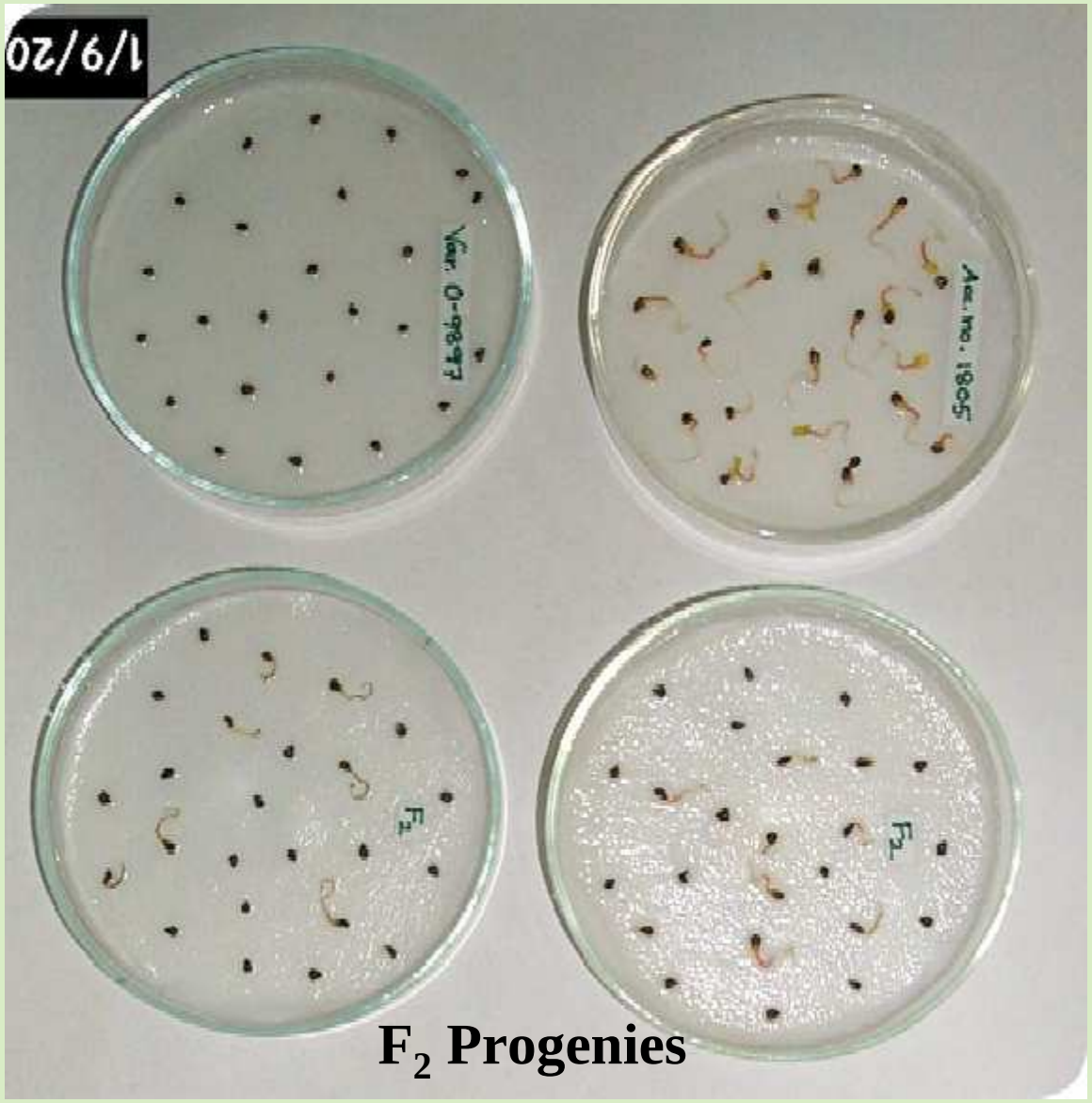


3 day germination of parents and F_1 seeds at 30°C



3 day germination of parents and F_1 seeds at 16ºC

Var. O-9897 **Acc. 1805**

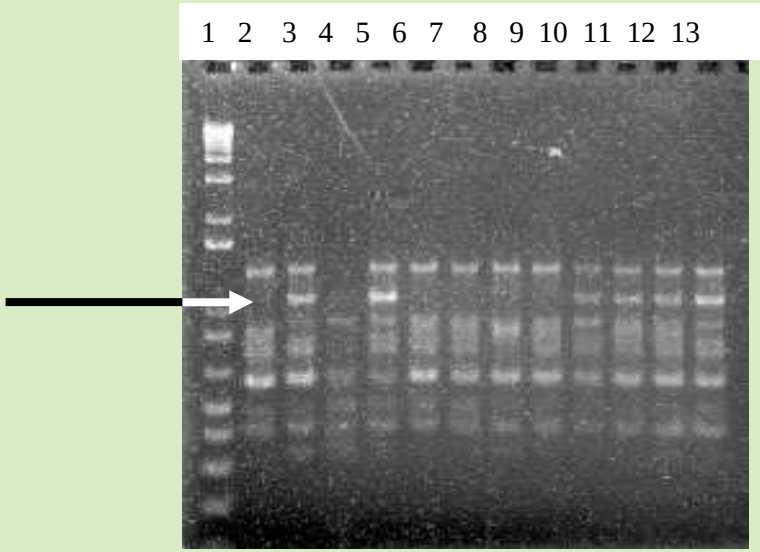


F_2 Progenies

3 day germination of parents & F_2 seeds at 16ºC

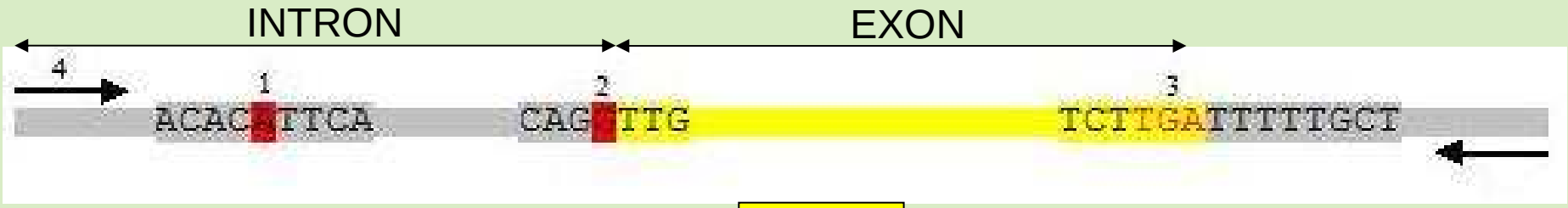
Amplified fragment closely linked to low temperature tolerance: Sequence Analysis

- Amplification with arbitrary decamer has identified a fragment that shows strong co-relation with the low temperature resistance trait in jute.
- The fragment is isolated from the gel and cloned in a plasmid.
- The fragment is sequenced and analyzed with Bioinformatic Tools



Lanes 2, 4, 6-9 are from susceptible plants, and Lanes 3, 5, 10-13 are from tolerant plants

Amplified Fragment closely linked to low temperature tolerance: Sequence Analysis



1. Branch Point “A”
2. Acceptor Site “G”
3. Stop Codon
4. Amplification Primer
5. Coding Region
6. Non-Coding Region

Jute Sequence: (translated sequence)

ESTLKLGSILTDGQVGIFKDRSAAAMSTFGDILPVQAGGLLSSFTTTRSDS-

ESTLKLGSILTDGQVGIFKDRSAAAMSTFGDILPAQAAGLLSSFTNTRSE-

Arabidopsis Sequence: (sequence match from data base)

Out of 51 Amino Acid: 46 Identical + 3 Highly Conserved + 1 Addition + 1 Substitution.

5' walking gave a new sequence of ~ 65 bp. 2 SNPs were observed in the RAPD binding site between the low temperature tolerant (Acc-1805) and low temperature sensitive (O-9897).

In case of 3' walking a 365 bp sequence was amplified. No SNP at the 3´ RAPD binding site.

A SNP is present in the upstream RAPD binding site of the CT3 fragment which leads to differential banding pattern between O-9897 and Acc.no. 1805.

SNPs found at one of the two RAPD binding site after primer walking

CT3 Fragment of Acc. 1805 (Cold tolerant):

TGCCATCCCCTATGATAATGG– O-9897(Cold Sensitive)

AGTCGTCCCCTATGATAATGGAGTAGAAGTATAAACCCCAAGGGTGTTTCCTGTTTTGGTGCAAAGGGCAAAAAGAAACATAGATCTAC

OPG05 SCR CT3 Hi F → ← INV-CT3H-R

AAAATCATGAAAACGGAAAATCACAAGGGGTATTAATATGATTTACCCTGAGAAAACAATTTTGAATTCAGAGAACTTACAAAACCATA

TATAATCTTACTGAAAATTGTAAAGACTAGCTAGTTAATAGATAAAAGACAGAAGGTAGGTTTACACAACTTTATTTCAGTGTAAAAAG

TATGCAGTGAGGATTAACATGTTAAAGAAATTCGTTTTAGTGGGTTTGGCCAAAAAAAGGGTTTTAGTGGTCCCATTAAGTTAAGGGGA

AGGGCAAACACAATTCCCATTAATGCTGACTAGCATCTGACGTCCTTGTTGGAAAAAGGCAATCCTAAACTGGAGGAAGCCAGCTAAGA

ATTTGGACTTGTTACATAAGAAGGTCGGATTGTTTTACTCATCAACAAGGAAGTCCTGATAATCATGTCAACCTTAAGTAGAGCTGTGT

CTGGCTTTTCACTAAATCTACTTTCTCACATATTTAAGAGACTCAAATGAGCATGAAAGGCATGCTAAAATACCCCTTCAGTGTTGTCG

GAAATGTTTGATCTTATAATGGACAAAGTAGAAAATTTTGATCTTGAAATGAGATCGTATGAAGGTTTGAACCTTCCTTGGGAATTAGC

CTTATGTAAAGCAGCTAATTTGCAAGTTATGAATATTTACTGCAATTGTAAAGTGGATTAGATGTTTTGCTATCTGTTTGTAACACATT

CAAGGTTTTGATATTGATTTCCTTTGGACTGGCCAGGTTGGAAGTAAATTCGGAGAGAGCACTTTAAAGTTGGGATCTATACTAACGGA

TGGGCAAGTGGGCATATTCAAGGATAGATCAGCAGCTGCCATGTCAACATTTGGTGACATTTTACCTGTACAAGCTGGGGGATTTCTTT

CTTCATTTACCACCACCAGATCAGATTCTTGATTTTTGCTTGGTCCTTAGCTCTTTGTCCTCCCACCAAATTTAGATGCATGCTTTGTT

TCCATTGGATAGGGGTGCATATACTTCCAGCTTGTACGTAAATGATGAGGGGACGGCA

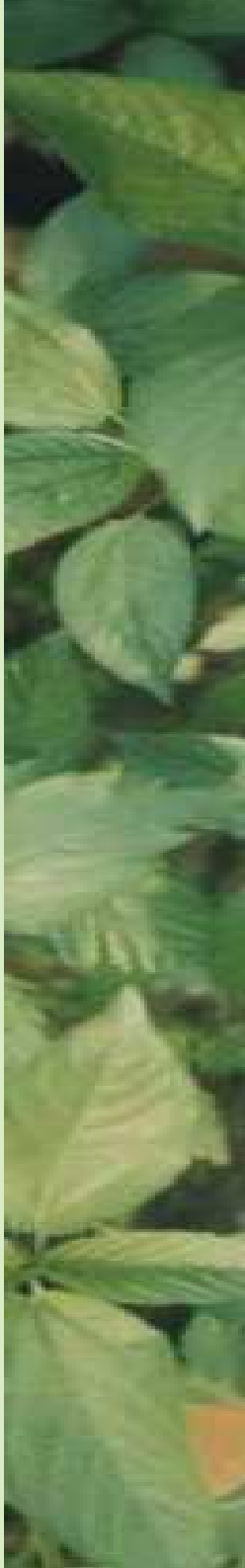
→ INV-CT3H-F

← SCR CT3Hi R OPG05

TAAATGATGAGGGGACGGCA ← O-9897(Cold Sensitive)

cDNA of putative LDLP gene sequence obtained so far (2250bp)

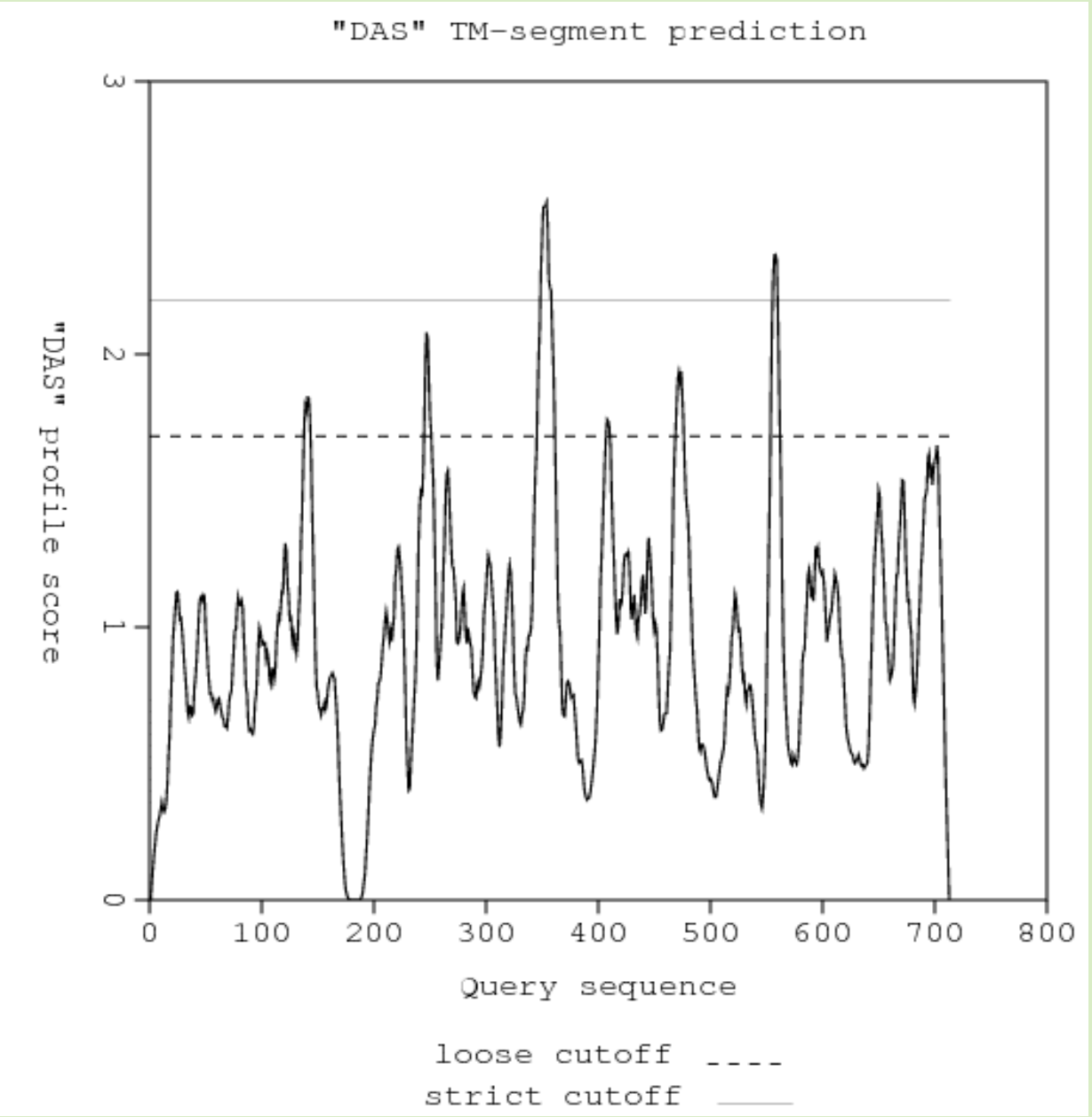
AAACTTTCATTTCGAGCACTTGTTGGAATTGGTCGCTGTATTGCGGAGAACAGATTGGCAATAAGATTAATGGG
AGGTATCTCGTTGATGCCATACCAAGTGGCCAAGAACTTGCAACTTCTGAGAAGTTGATAAGGCACACAATAG
AAAGCAAGGAGGTTTTGGAAGGGAGTTTGGAATGGCTTAAAAGTGTTTTTGGGTCTGAGATCGAGATGCCATG
GGATAGGATTAGAGAACTTGTTCTGGAAGGTGATTTGGATCTTTGGGATGAGATATTTGAAGATGCTTTCGTTA
GGAGGATGAAAGTAATTATCGACTTACGATTTGAAGATCTGACGAGATCTGTCAATGTACCAGATGCAGTCCG
TACTATTGTGGTCACAGCTGGTGAGAAGATGGATTTCCAGGCATATTTGAATAGGCCTTCTAGGGGTGGGGGG
ATTTGGTTCACAGAACCTAATAATGTTAAGAAGCCTGTTCCACTATTGGGAAGTAAAGCATTAACTGAAGAAGA
TAATTTCCAAAGTTGTCTCAATGCCTACTTTGGTCCTGAAGTGAGTCGAATTAGGGATATAGTAGACAGCTGCT
GCAAAAGCATTCTTGAGGATCTATTGAGTTTCTTAGAATCTGCCAAGGCATCTCTGAGGTTGAAGGATCTAGTT
CCATATCTGCAGAATAAATGTTATGAAACTAGTTCCATATCTGCAGAAATAAAATGTTATGAAAGCATGTCAGC
CATATTGAATGAACTAAAAACTGAGCTTGATATTTTATACACGTCCATCGGAAGTGAACATAAGGAAGGTGATT
CTGTGCCTCCTCCTATAATTGTTGAGAGATCCCTATTTATTGGCCGACTCATGTTTGCATTTGAGAAATACTCTA
AACACATTCCTTTGATTCTTGGTTCTCCACGGTTCTGGGTGAAATACACATCCACTGCAGTTTTTGAGAAGTTAC
CTTCCCTGTGGCAGTCTAAAGTTGCCACCGATTCTCCTCTCTCTAACGGCCTTGGAATACAAATGTTCAGTGGC
TCCCAGAGGCAAAGTTCGTCTACTACTTCCGCATTGCTTGGAGCAAATGAAAGTGCAAGCCCTAAACTTGACGA
ACTTGTTAAGATTACGCGAGAGCTCTGCATCAGAGCTTACAGCTTGTGGATATTATGGCTTTATGATGGGCTTT
CAGTAATTCTCTCTCAGGAGCTTGGACAAGATGATGGATTATCTGCAACATCTCCCTTAAGGGGTTGGGAAGA
GACAGTTGTTAAGCAAGAACAGACCGATGAGGGGTCATCAGAGATGAAAATATCACTACCGTCAATGCCTTCT
CTTTATGTCATCTCCTCCTATGCCGAGCATGCAGTTCCGCACTGTATTGGAGGCCATGTTCTTGATAAATCCATT
GTGAAAAAGTTTGCATCAAGCCTCACCGAAAAGGTCATTTCTGTCTACGAAAATTTTCTCTCTAGTAAAGAAGC
CTGTGGAGCTCAAGTGTCAGAGAAAGGAATTTTGCAGGTCTTGTTAGACATAAGATTTGCTACTGATATTCTTT
CAGGTGGTGATTTCAATGTGAATGAAGAGTTATCTAGCACATCAAAGACAAAATCATCATTTAGAAGGAAGCA
GGATCAAATTCAGACAAAGTCTTTTATTAGAGAACGTGTTGATGGGTTAATCTATCGTCTTTCGCAAAAATTAG
ATCCCATTGATTGGCTCACGTATGAGCCATACTTATGGGAGAATGAAAGGCAAAAGTACCTCCGGCATGCTGT
CCTCTTTGGGTTCTTTGTTCAACTTAATCGAATGTACACAGATACAATGCAAAAACTGCCTACAAATTCAGAGTC
AAATATAATGAGATGTTCTGTGGTTCCACGGTTCAAATATCTTCCAATAAGTGCTCCAGCATTGTCTTCTAGAG
GGACTACTGGGGCATCTATTACAGCTGCCTCAAATGATATTGCTTCAAGAAGTTCCTGGAGAGCTTATACAGAT
GGAGAGATTTCCCGGAAAGTTGATATGGATGACCAACAGAGTTTTGGTGTTGCAACGCCATTCCTAAAGTCTTT
CATGCAGGTTGGAAGTAAATTCGGAGAGAGCACTTTAAAGTTGGGATCTATACTAACGGATGGGCAAGTGGGC
ATATTCAAGGATAGATCAGCAGCTGCCATGTCAACATTTGGTGACATTTTACCTGTACAAGCTGGGGGATTTCT
TTCTTCATTTACCACCACCAGATCAGATTCTTGA



LDLP Homologs in other plants

Protein type	organism	Score	E	Identity	similarity
unknown protein	Arabidopsis thaliana	197	2e-49	68%	78%
unknown protein	Arabidopsis thaliana	196	5e-49	68%	77%
low density lipoprotein B-like protein	Arabidopsis thaliana	196	5e-49	68%	77%
putative low density lipoprotein B	Oryza sativa	177	3e-43	58%	71%
Low density lipoprotein B-like protein	Ostreococcs tauri	80.1	4e-14	54%	63%

Transmembrane Segment Prediction



Potential transmembrane segments

Start	Stop	Length	~	Cutoff
138	143	6	~	1.7
245	251	7	~	1.7
346	361	16	~	1.7
349	358	10	~	2.2
407	410	4	~	1.7
469	476	8	~	1.7
554	562	9	~	1.7
556	559	4	~	2.2

Predicted domain in LDLP

As LDLP is an uncharacterized protein no domains found in domain database.

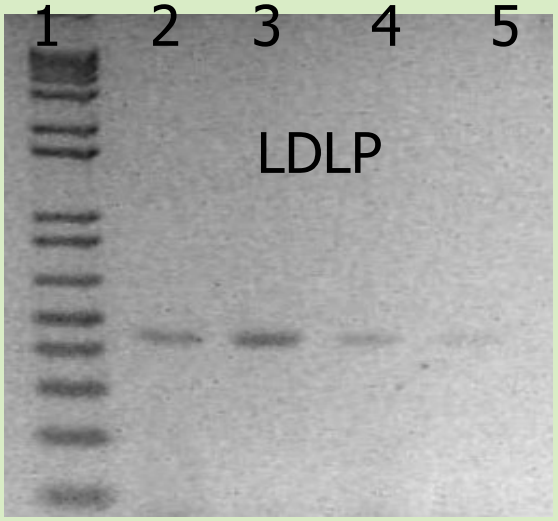
However DomSSEA, a domain prediction software that predicts domain by comparing secondary structure, predicts a number of domains in the putative LDLP gene having SCOP code of a.126.1.1

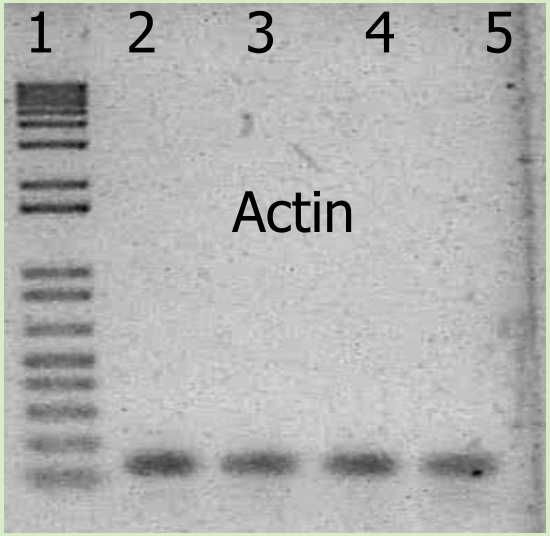
Function of the predicted domain: Obtained from SCOP database

- Molecular Function: carrier activity | transporter activity | lipid binding |
- Physiological function: transport | water homeostasis | body fluid osmoregulation |

Study of expression by Semi quantitative PCR

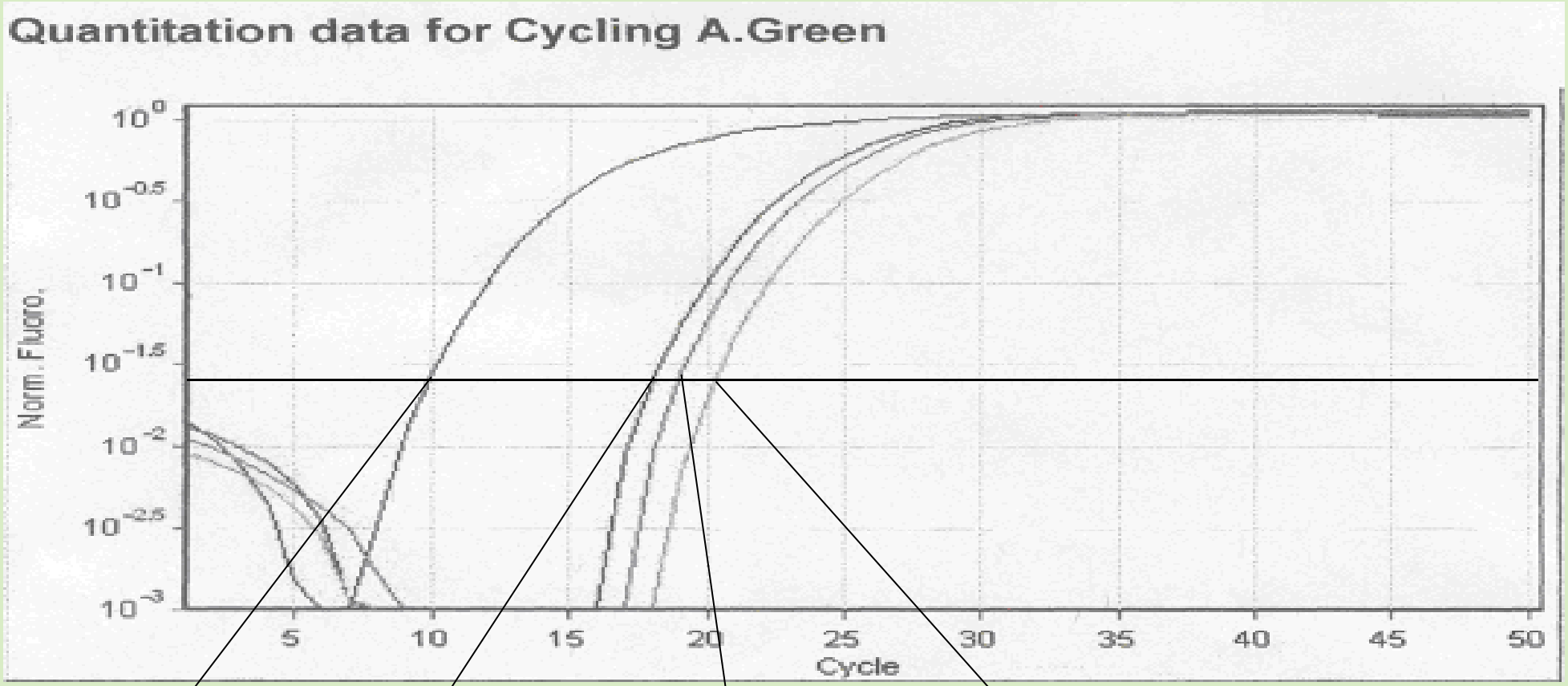
- Different expression pattern of the gene was observed in cold sensitive (9897) and tolerant (SDLT) jute variety.
- Expression level of LDLP is increased in cold Tolerant variety under cold stress and decreased in sensitive variety.
- Expression levels of Actin is used as internal control, which indicate the LDLP may play a role in low temperature tolerance in jute





(1) DNA Ladder (2) 1805 normal (3) 1805 cold (4) 9897 normal (5) 9897 cold

Real Time PCR showing similar result as Semi quantitative PCR



1805-Cold 1805-normal 9897-normal 9897-Cold

Differential Display of Low Temperature Tolerance trait in Jute

- Jute seed germinates at a base temperature of 20^0C.
- Few strains of jute are known to germinate at a lower temperature of 16^0C.
- No relevant gene responsible for low temperature tolerance in the Malvaceae family is known.

Differential Display Amplification of the reverse transcribed mRNA

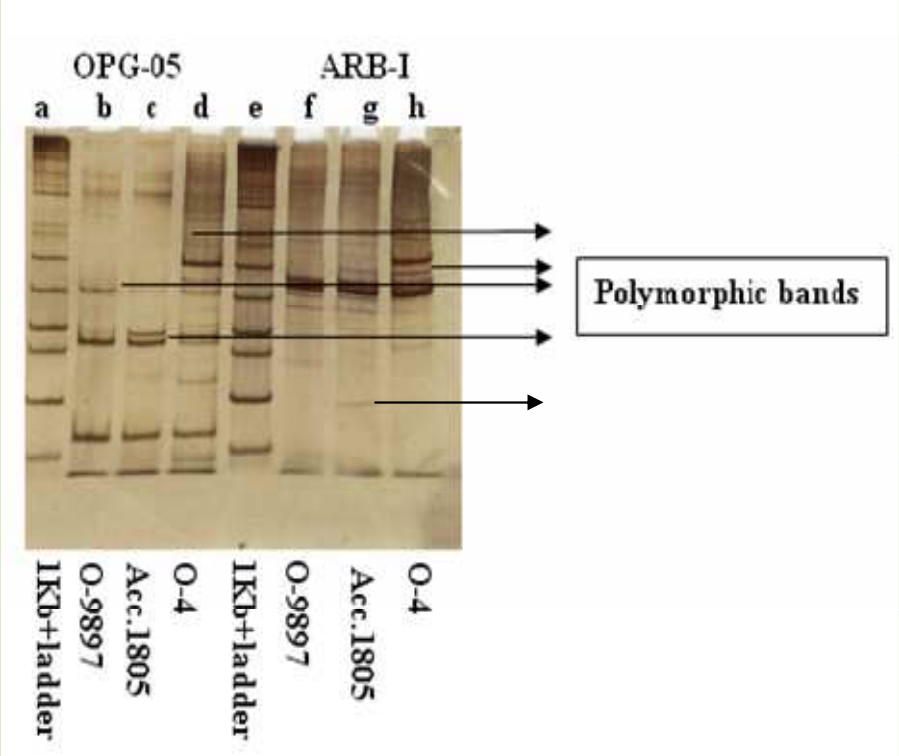


Fig: Jute transcriptome analysis by $T_{12}AG$ and OPG-05/ARB-I primers

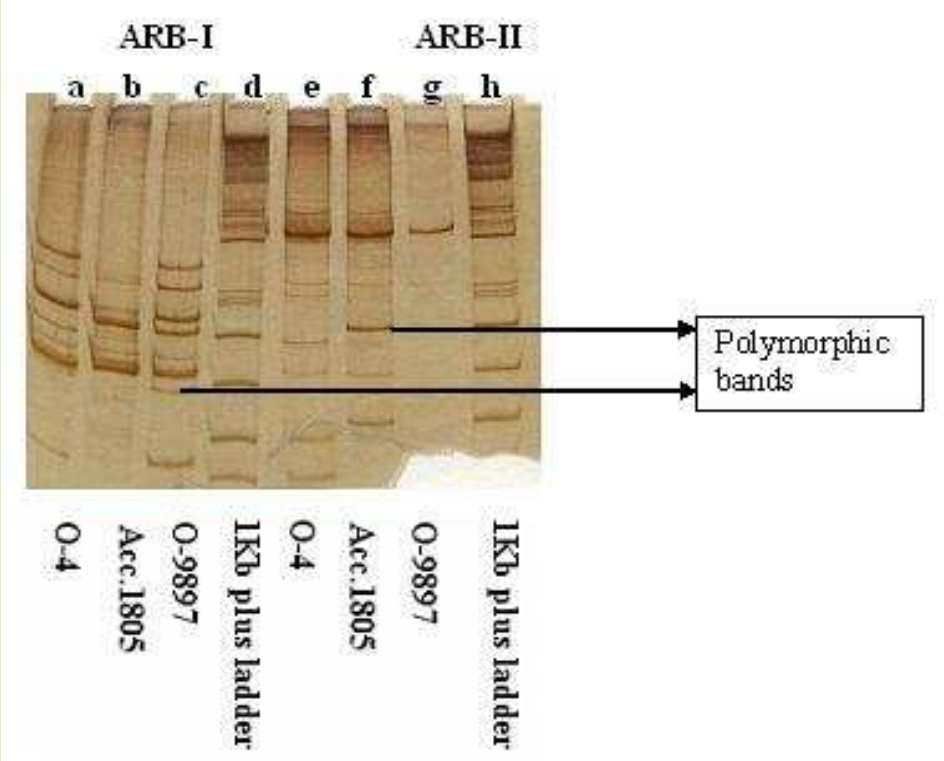


Fig: Jute transcriptome analysis by $T_{12}G$ and ARB-I/ARB-II primers

Distribution of tBLASTx Hits on the Query Sequence

Query sequence	Nucleotide Sequences producing significant Alignments with Query Sequence		Threshold Level		Features
	Accession no.	Definition	Score Bit	E value	
CT1	>gi|24413764|emb|AL939112.1|SCO939112 >gi|89949249|gb|CP000282	*Streptomyces coelicolor* A3 *Saccharophagus degradans*	61.11 45	6e-10 2e-32	Putative secreted arabinase Arabinan endo-1,5-alpha-L-arabinosidase
CT2	>gi|71553748|gb|CP000058.1 >gi|17428522|emb|AL646065	*Pseudomonas syringae* pv. phaseolicola 1448A *Ralstonia solanacearum* GMI1000 chromosome	102 107	1e-19 1e-26	GGDEF domain/EAL domain protein Probable transmembrane protein
CS1	>gi|56160984|gb|CP000002.2 >gi|51854827|dbj|AP006840.1	*Bacillus licheniformis* ATCC 14580, complete genome *Symbiobacterium thermophilum* IAM 14863 DNA	97.78 6.3	9e-18 3e-14	GCN5-related N-acetyl-transferase Putative acetyl-transferase

Quantitative PCR

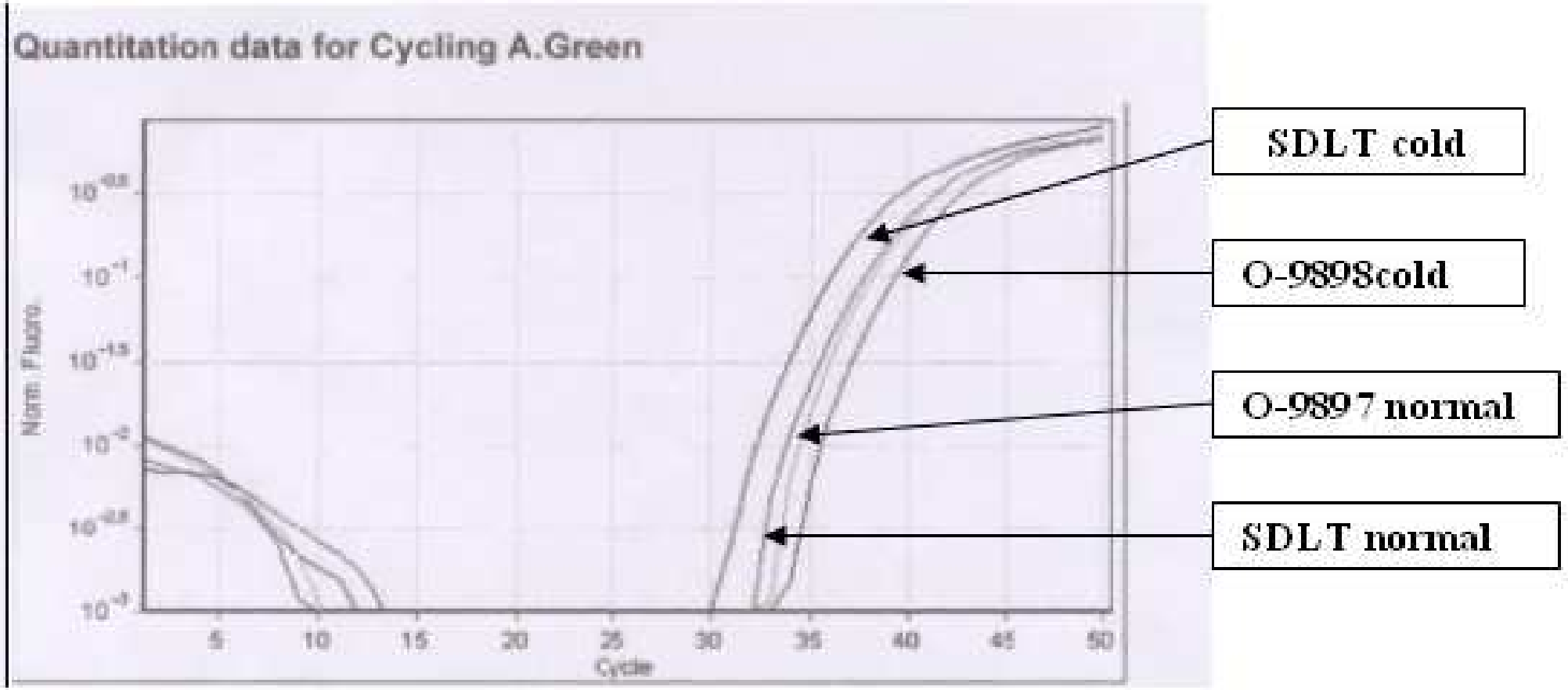


Figure: Quantitation curve for real time PCR

Functions of EAL/GGDEF Domains

- **Biological Processes**
- regulation of transcription, DNA-dependent Inferred from electronic annotation. Source: InterPro
- signal transduction Inferred from electronic annotation. Source: InterPro
- **Molecular function**
- signal transducer activity Inferred from electronic annotation. Source: InterPro

Development of a protocol for efficient genetic transformation of Jute species by *Agrobacterium tumefaciens*.

Why the need for genetic transformation?

Two cultivated jute species, *Corchorus capsularis* L. and *C. olitorius* L. contain very limited genetic variability with respect to-

(i) adaptability to different agronomic environments,
(ii) fiber quality
(iii) fiber yield, and
(iv) susceptibility to diseases and pests, etc.

These two species do not cross successfully with each other.

Therefore jute requires modern biotechnological approach for improvement.

- For going into modern biotechnological approach, we need an easy and efficient transformation protocol.
- Transformation protocols requiring extensive periods of tissue culture have several drawbacks, including time (typically many months), space and, importantly, the tendency to induce somaclonal variation
- Moreover, whole plant regeneration following tissue culture and transformation was not very successful with jute due to its recalcitrance in tissue culture.

- Only one established transformation protocol for jute involving *Corchorus capsularis* described by Ghosh *et al*. (2002) which involved particle bombardment. But this technique is expensive involved laborious tissue culture steps.
- Though regeneration following tissue culture is hard to achieve with *Corchorus capsularis,* it is near to impossible, if not completely, with *C. olitorius*.
- To avoid problems associated with jute tissue culture and regeneration, we have developed tissue culture independent transformation techniques for *Corchorus olitorius*.

- Early young jute plants (30 - 45 cm in height) were injured at shoot tips
- After one hour injured shoot tips were infected with drops of *A. tumefaciens* suspension.
- Then again after another hour injured shoot tips were infected with drops of *A. tumefaciens* suspension.

- Efficiency of transformation process was determined following selection of plants on kanamycin containing medium.

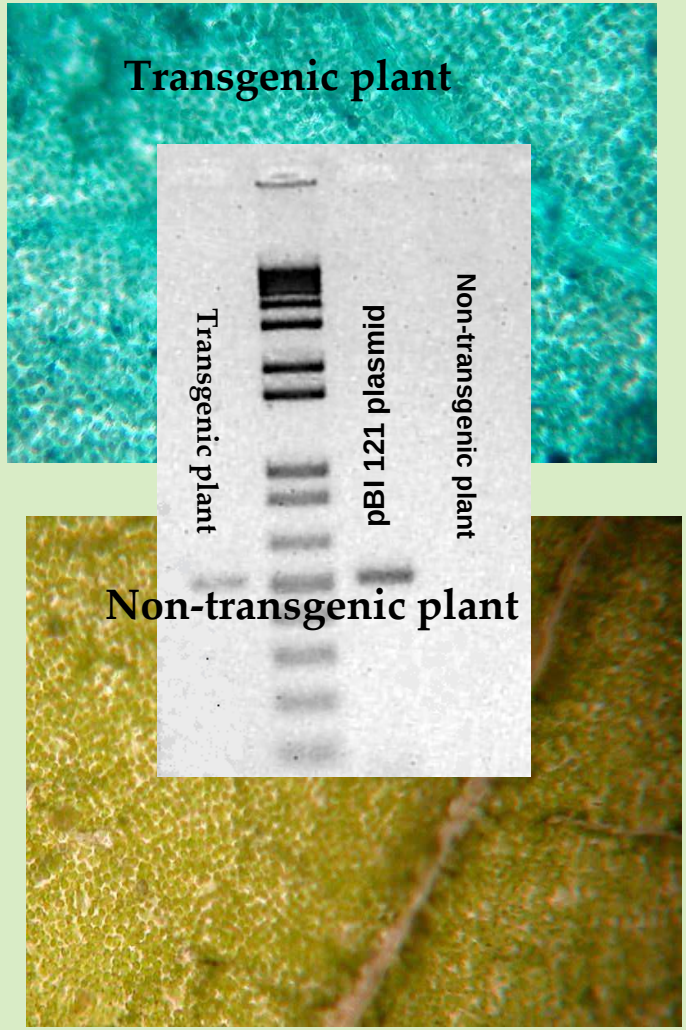
- Efficiency varied between 13.11% and 48% for different transformation events with an average of 27.01± 7.01.

- None of the seeds from non-transgenic control plants showed germination and growth in selection medium.

- For both transgenic and non-transgenic plants similar growth rate was observed in non-selection medium which eliminated any doubt about the vigour of non-transgenic seeds for germination.

T_1 Plants

- Seeds from mature plants were collected and germinated.
- Leaves from these new generation plants were tested for GUS activity.
- PCR was also performed with DNA isolated from the leaves of these plants using *gus* specific primers.



T_2 Plants

- Plants with positive results were identified and next generation seeds were collected from these plants.

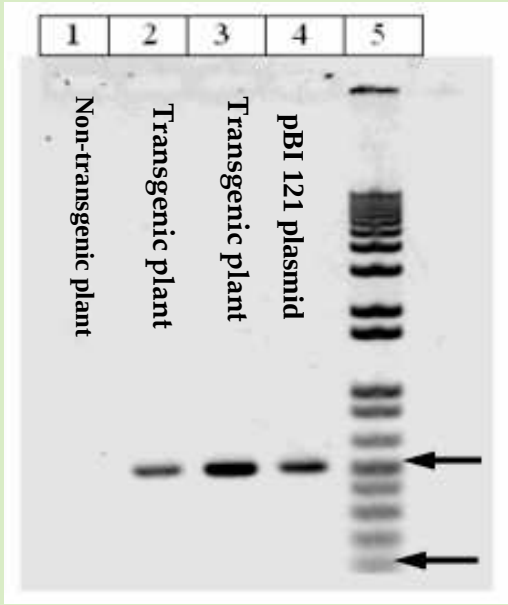
- Transgenic seedlings were confirmed by three lines of evidences -

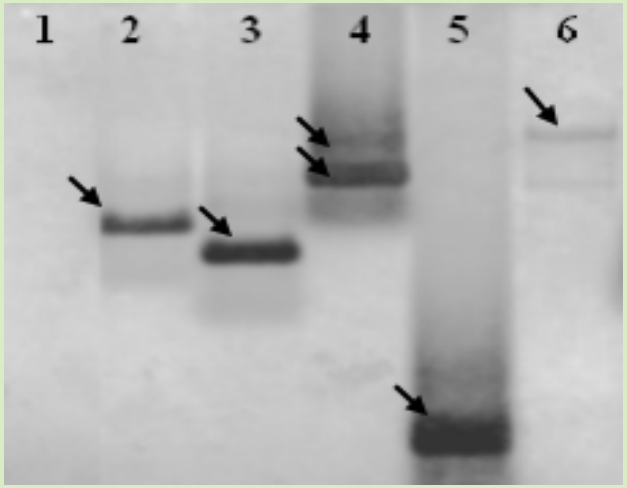
1. Genomic DNA level
2. RNA level and
3. Protein level

1. Presence of *gus* gene(s) in the genome of these plants was confirmed by-

   A. PCR.

   B. Southern hybridization





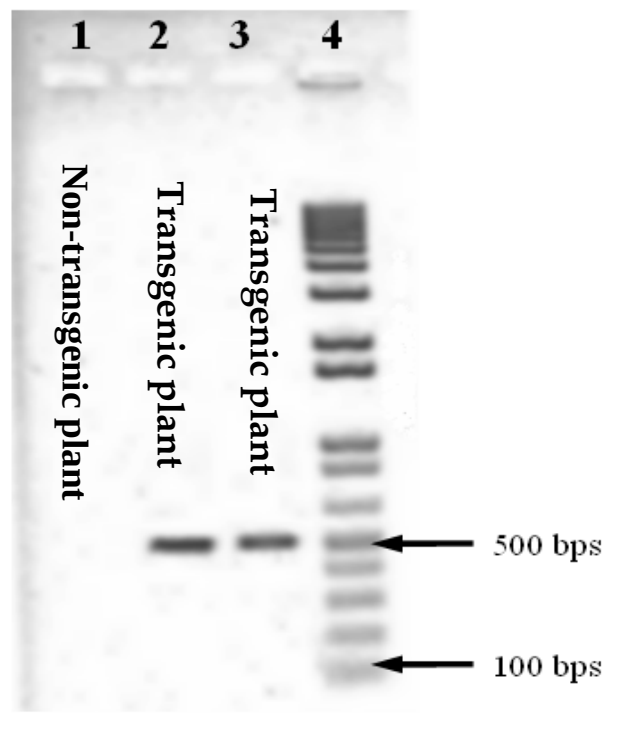
Lane 1: Lane 1: non-transformed jute;

Lane 2, 3 and 4: *EcoRI* digested transformed (T2) jute genomic DNA;

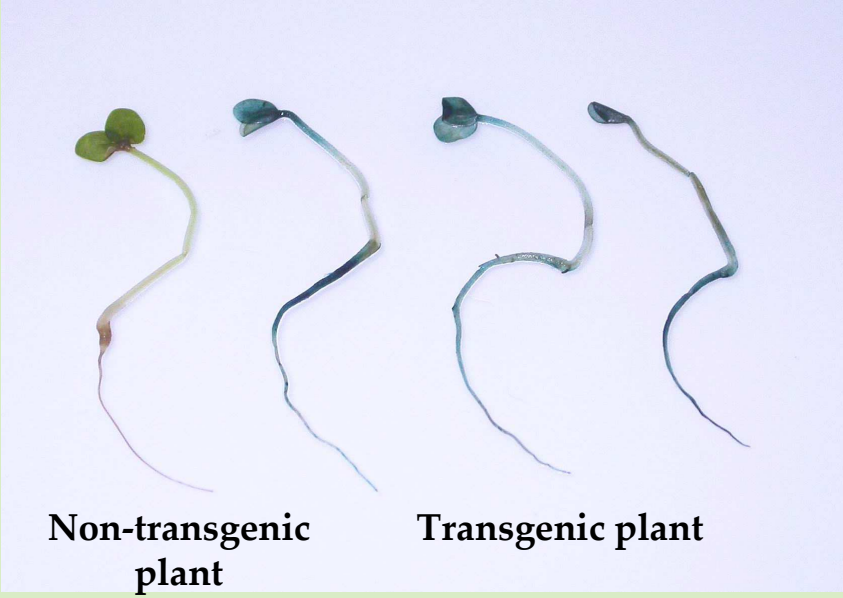
Lane 5: PCR product from transgenic plant using *gus* specific primers;

Lane 6: *EcoRI* digested pBI121 plasmid containing *gus* gene, used as positive control.

2. *gus* gene expression was confirmed by RT-PCR,



3. Activity of *gus* gene product was confirmed by GUS assay,



Plant Cell Tiss Organ Cult
DOI 10.1007/s11240-008-9447-0

ORIGINAL PAPER

Tissue culture independent transformation for *Corchorus olitorius*

Abu Ashfaqur Sajib · Md. Shahidul Islam · Md. Shamim Reza · Arpita Bhowmik · Layla Fatema · Haseena Khan

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Abstract In vitro regeneration is difficult for species of *Corchorus* and several transformation attempts based on tissue culture have failed. We describe a successful transformation protocol for *C. olitorius* using a technique independent of tissue culture. generation. In this study young jute plants were transformed at shoot apical meristematic region using *Agrobacterium tumefaciens*. Heritable transmission of the transgene to progeny from genetically modified plants was confirmed by *gus* gene expression by

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