Designing Drought Resistant Transgenic rice

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GENETECH

Biosafety workshop
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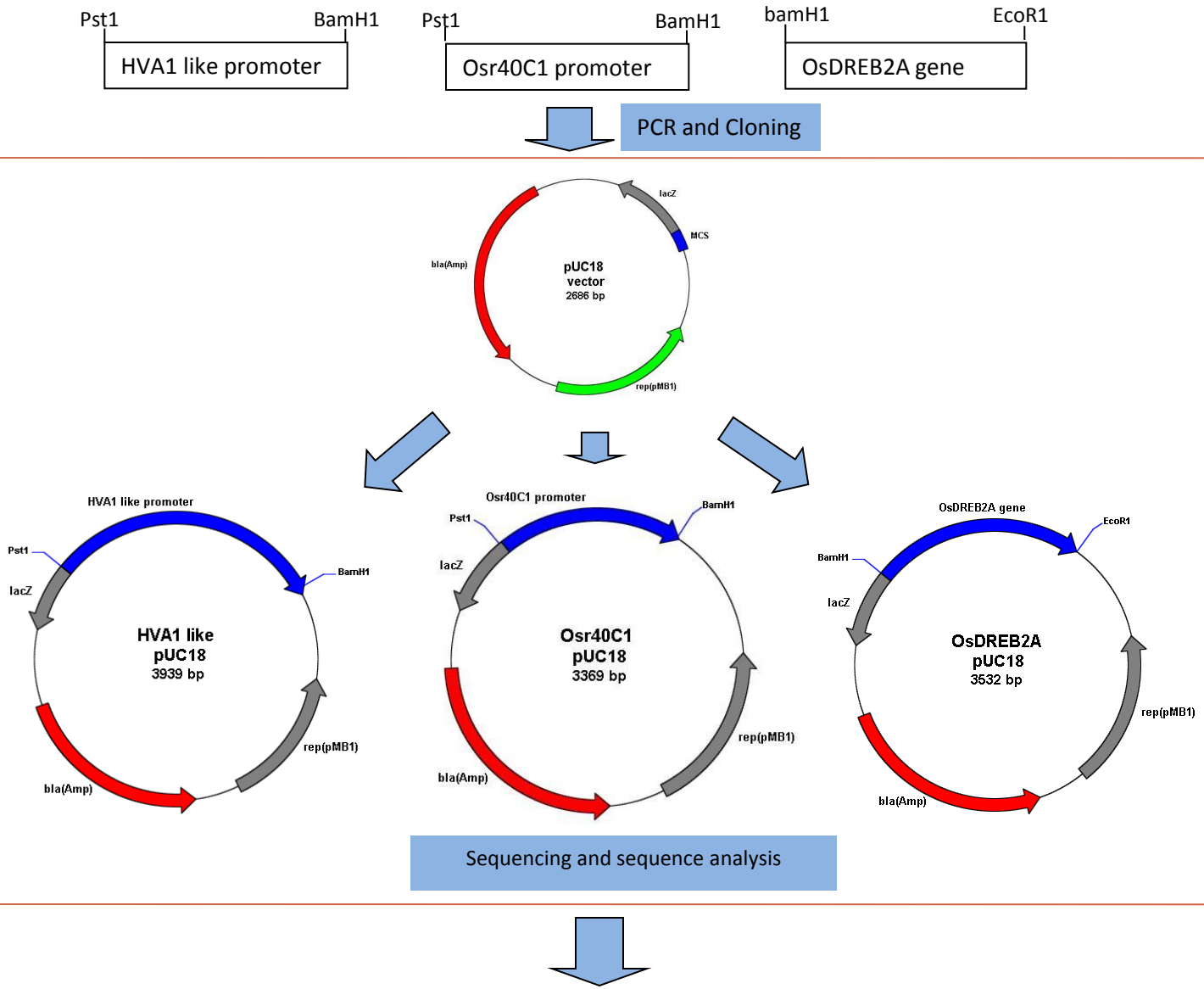
Objectives of the research

- Overall objective is to develop drought resistant transgenic rice from local rice varieties

In order to achieve the overall objective following short term goals..

- Cloning and characterization of HVA1 Like and Osr40C1 promoters and OsDREB2A coding region
- Analyze the promoter activity of HVA1 like promoter and Osr40C1 promoter
- Study the expression of OsDREB2A gene under the control of three different promoters
- Study the stress inducible expression of Indica OsDREB2A against drought stress


Pst1
BamH1
HVA1 like promoter
Pst1
BamH1
Osr40C1 promoter
bamH1
EcoR1
OsDREB2A gene
PCR and Cloning
lacZ
MCS
bla(Amp)
pUC18
vector
2686 bp
rep(pMB1)
HVA1 like promoter
Pst1
BamH1
lacZ
HVA1 like
pUC18
3939 bp
rep(pMB1)
bla(Amp)
Osr40C1 promoter
Pst1
BamH1
lacZ
Osr40C1
pUC18
3369 bp
rep(pMB1)
bla(Amp)
OsDREB2A gene
EcoR1
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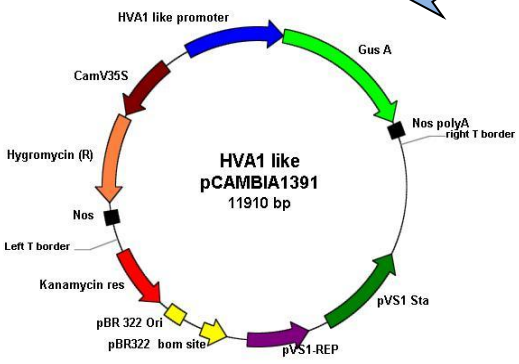
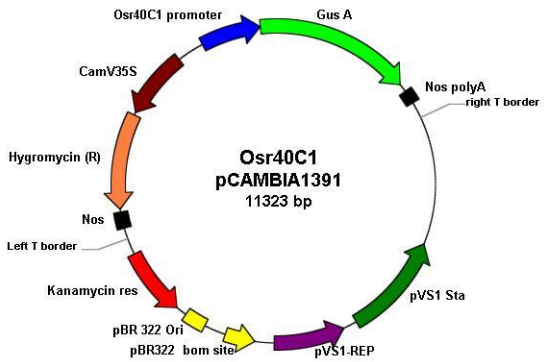
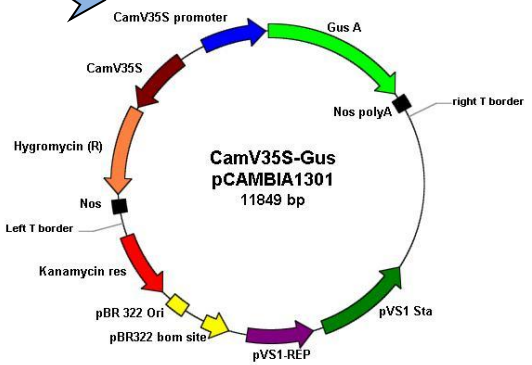
Sequencing and sequence analysis

Cloning of transgene constructs for analysis of promoter activity

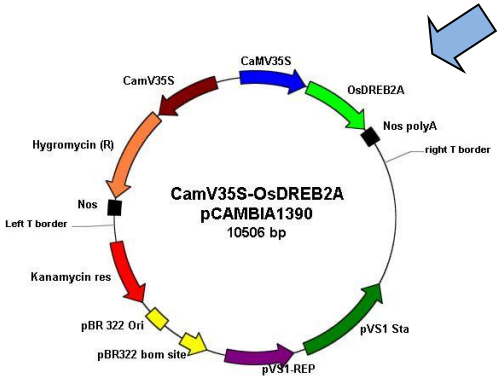


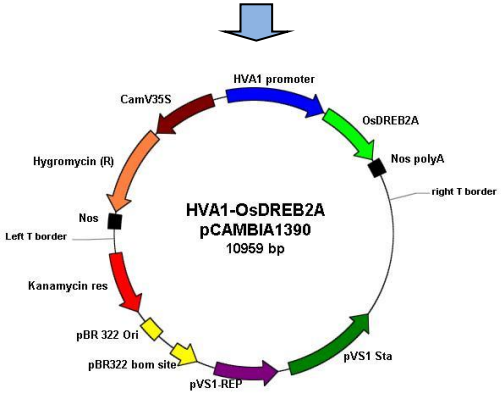


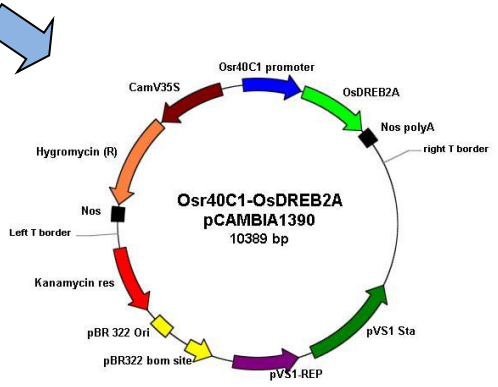


Transgene constructs designed for analysis of promoter activity

Cloning of transgene constructs in pCAMBIA1390 vectors







Transgene vectors designed for transformation of OsDREB2A in to rice

Rice

- Rice (*Oryza sativa*) is grown on 157 million ha world wide and is globally one of the most important food crops
- more than 3 billions people includes 700 millions malnourished
- In molecular level, rice is a model because has a smaller genome compared to other cereals

Drought global and Sri Lankan perspectives

- Water limitation permanently affect 28% of the world's soil
- Nearly 50% intermittently affected due to
  - Poor water holding capacity
  - Shallowness of soil
  - Fluctuation of ground water table
- Nearly 40% yield drop in Yala season
- 64 % of the island fall in to dry zone where rainfall is below 1800mm

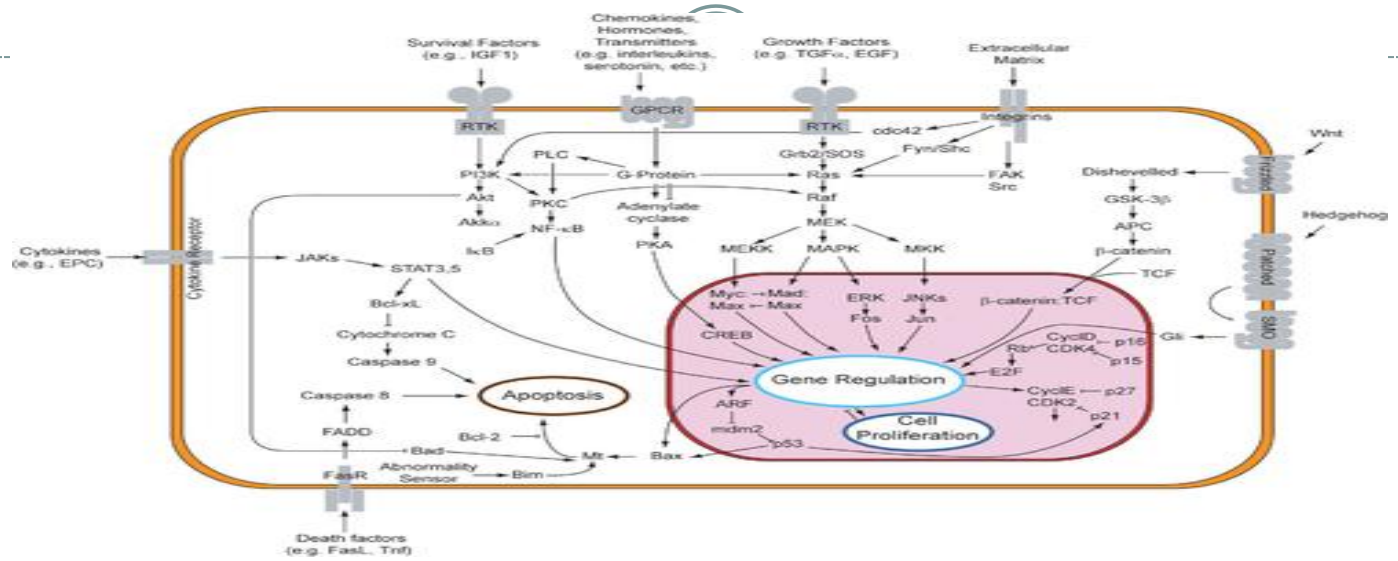
Drought

- Determine the productivity of the crop
- Demand for food increase the demand for water for agriculture
- Crops have to be grown with same or less water presently available while maintaining high crop productivity
- This drives the requirement for increasing the water use efficiency in crops
- Crop water use efficiency has to be considered when designing approaches to increase the crop productivity

Plant defense mechanisms against drought

- Drought escape- escape from the drought period by quick completion of life cycle
- Drought avoidance- deep rooting and more hairy roots, searching for water, minimum stomatal activity and minimum transpiration.
- Drought tolerance- adjusting and withstanding the plants molecular, cellular environment for drought and changing physiological and biochemical conditions of the plant to tolerate unfavorable conditions

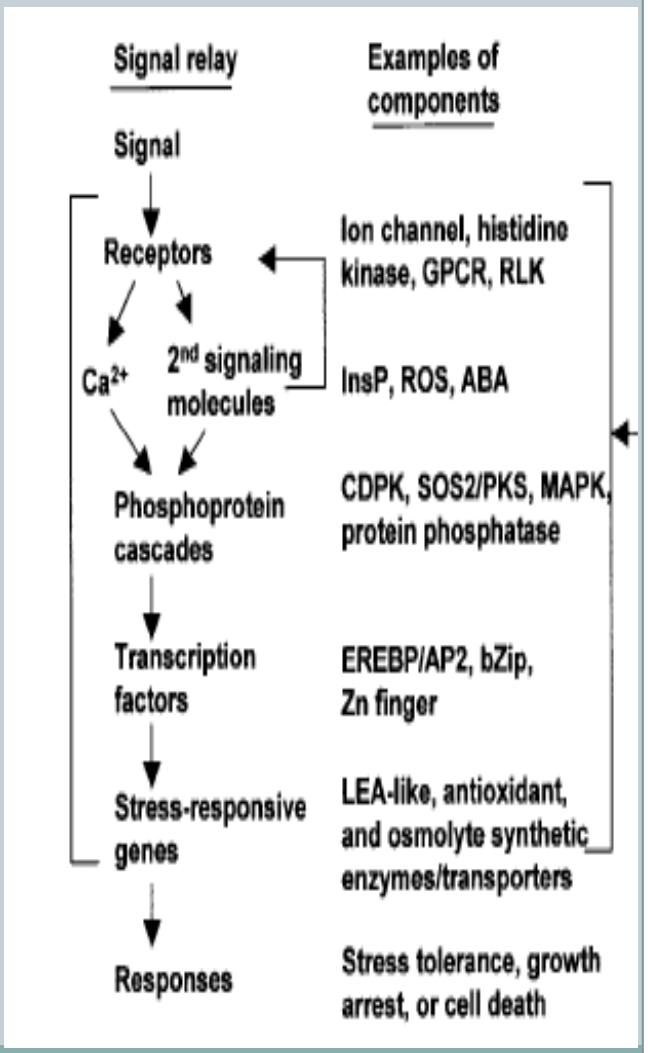
Molecular mechanism for drought tolerance in plants



- Drought, salt, freezing conditions generate physiological responses arise from cellular gene expression profiles
- These gene products can be divided in to two main categories
  - Protect against drought directly- Osmoprotectants, LEA, anti freezing, chaperons, detoxification enzymes
  - Regulate the expression of other genes and facilitate signal transduction- Transcription Factors, PKCs, metabolic enzymes

Genes for drought

- Genome wide analysis and Micro-array analysis generated several important genes against drought, cold, salt stresses in *Arabidopsis*
- Transcription factors have been identified as key molecules which respond to abiotic stresses including drought
- It is believed transcription factors are the entry point of the massive network of signal transduction and manipulation of the entrance can regulate stress inducible gene expression



Transcription factors

Sequence-specific DNA-binding proteins that are capable of activating and/or repressing transcription

- A number of transcription factor families have been identified in response to stress
- More than 1500 TFs in *Arabidopsis* genome
- Some important TF families induced by abiotic stress
  - ERF/AP2
  - bZIP
  - NAC
  - bHLH
  - C2C2 zinc finger
  - C2H2 zinc finger
  - WRKY

*TFs belonging to ERF/AP2 family which bind to DRE/CRT domain have been identified and named as DREB genes

DREBs and OsDREBs

- Are TFs belong to ERF/AP2 familly
- Have cis-acting DRE element with Core sequence A/GCCGAC
- Two types of DREBs in *Arabidopsis* as DREB1 and DREB2 was first isolated in yeast one hybrid screening
- Homologs in rice for DREB were identified and named as OsDREBs
- OsDREB1 type response for cold, high salt while OsDREB2 type for drought and salt

* Over expression of DREBs and OsDREBs in rice and *Arbidopsis* has shown improved resistance to abiotic stresses

OsDREB genes

- Main two types of OsDREBs as OsDREB1s and OsDREB2s
- Seven OsDREB1 genes as 1A, 1B,1C, 1D, 1E, 1F and 1H broadly response for cold and salt stresses and share similar features at N-terminal and C-terminal ends
- Seven OsDREB2 genes 2A, 2B, 2C, 2D, 2E, 2F and OsAB14 broadly responds to drought and salinity stresses and share similar features at Amino acid level
- OsDREB2A shows higher response to drought and salinity and contains introns less coding region and exhibit single copy in the genome (Matsukura et al., 2010)
- Genomic DNA of OsDREB2A coding region used for the study

OsDREB2A Indica homolog

- Homology search using OsDREB2A japonica as the reference sequence

ATGCTGTTTCGATTTGTGTCTTGCAATGTTCAGCTTTGTGGAATTATTGAGTTACCTCATTGG
GTCAGGAAGAAGAGAACGCGAAGGAAAAGCGATGGCCCTGATTCAATCGCTGAAACCATCAA
GTGGTGGAAGGAGAAAACCAGAAGCTCCAGGAGGAGAATAGCTCCAGGAAAGCGCCAGCCA
AGGGGTCCAAGAAAGGGTGCAGGCTGGGAAAGGAGGTCCGGAAAATTCAAATTGTGCTTAC
CGCGGTGTCAGGCAACGGACATGGGGTAATGGGTGGCTGAGATCCGTGAACCAAACCGTGG
AAGGCGCCTATGGCTAGGATCATTTCCTACTGCGCTGAGGCTGCGCATGCATACGATGAGGC
GGCAAGGGCAATGTATGGTCCCACAGCACGTGTCAATTTTGCAGATAATTCCACAGATGCCA
ACTCTGGCTGCACATCAGCACCTTCATTGATGATGTCTAATGGGCCGGCCACTATACCTTCTG
ATGAGAAGGATGAGCTGGAATCTCCTCCTTTCATCGTGGCTAATGGGCCAGCTGTGTTGTAT
CAGCCTGATAAGAAGGATGTGTTGGAACGTGTAGTCCCTGAGGTGCAGGATGTTAAAACAGA
AGGGACAATGGCTTGAAACGTGTTTGTCAGGAGCGGAAGACTATGGAGGTATGTGAATCAG
AAGGGATCGTTTTCACAAAGAAGTGAACATAAGTTATGATTATTTCAATGTCCATGAAGTTGT
TGAGATGATAATTGTTGAATTAAGTGCTGATCAGAAAACGGAAGTACATGAAGAGTACCAAG
AGGGAGATGATGGGTTTAGCCTTTTCTCCTATTAG

Source Genbank Accession No HM807364.1

OsDREB2A Indica homolog

- Putative amino acid sequence analysis

MLFRFVSCNVQLCGIIELPHWVRKKRTRRKSDGPDSIAETIKWWKEQ
NQKLQEENSSRKAPAKGSKKGCMAGKGGPENSN*CAYRGVRQRTWG*
KWVAEIREPNRGRRLWLGSFPTALEAAHAYDEAARAMYGPTARVNF
*ADN*STDANSGCTSAPSLMMSNGPATIPSDEKDELESPPFIVANGPAVL
YQPDKKDVLERVVPEVQDVKTEGSNGLKRVCQERKTMEVCESEGIVL
HKEVNISYDYFNVHEVVEMIIVELSADQKTEVHEEYQEGDDGFSLFSY

N terminal change in the sequence : from 1-22 Amino Acids
NLS region: 25-64 Amino Acids
Ap2/ERF domain: 81-141 Amino Acids

HVA1 like promoter

- Promoter region of drought inducible OsLEA3 gene

AAGGGCCTCCATAACCTACGCCTAGCCCTAGCACGATGGATGGCACAGTGCGTGCCCATCCTGCATCTGC
ATGGGTTAGTGCGTGCTACGCTGCGACGGCGACGATCGATGTAGCCTAGCCGGTGTGTGCAGTGCAGTGC
AGGTCAGGATTGCCACTATGACCAAAGGATGCTTGTGTGCGATCAATAATGGCCGCTCAATGTGTCATCG
TACGGTGACACACCACTCATCCTTTGTTGATCTGTGGTGATCGACTTGAGTTAATCGGCAAGGCCCAGCC
CATGGTTTGAGGTCAGGGCCAGGCTGAATTTGGCCCAGTAATTTTGGTTTGAGAAGCCCACTTCGTCACA
GCGTCAGGCCGAATTACTGGCCCATGGTGAGCCCATGGCATCCATTCCCCATGATTGACCTTGTCTTTCT
CTTTTTCTCTCGATCTCGAAAAGATGAGCAGATACTCGTAATTAAACCGCAAACATCTGCCACCCATGTA
ATGATAACAATCGTTAACAATGCCATGCATCTCCCGAAGCTTCTGTGCCTACTCATTTGAGTGCGAGACC
TTCCTAACATGTGTCCCCTTAACATTGTTTACTCCCTTTGCCGCCAAAGTGGTTACTACACACTCCAAAC
TTTTGTGGCAGAAGTACACTCAAAAGCGAAAGGTAGCAGAACACATCAGGCATCCAAATTAACAACAACA
CCATTTACAATCAGACCTGAACACGTTGATCGGCGACATCAGGCGCCGCACATGGCAACGACACCCGATC
GATCACCAAGTGTAAAAACTAAAGCCGCATCCAACTTGTACTCGCCAAACAGCCACCGATCGATCGACGT
TTCGATCGCCTGTATCGACACACTGATCGATCTGATCATGATCAGTTTCAACTCGCTGTGCCCACGTGTC
GAGAGATCGGCACGTGCCTGAGCTCTCAGCCGCTCATAAATACACTTGTTTAGTAGCAACAGTATACTAT
AGTAGTCCTCTCCTGTTTGGCTTTTAGCTTGCATCGATGGATGGATGGATGGATCGCATGAGAGGGCTTC
GCGAAGGTACGGAACCTTACACAACGCGTGTCCTTTCTACGTGGCCATCGTGTAGGCGTCTCGCCATGCT
ACGTGTCCCGGAGGATGTCTCGATGCCAACCCTTATAAATACTGTTCCATTCCAATCCCATCGCCACAGC
CAGTGCAAATCTGATCGATCAAGATATCGAGCAAAATCCATCAGAGTTCTAACATTCGCGCGT

Source: Genbank Accession No DQ837728.1

Osr40C1 promoter

- Promoter region of the salinity inducible Osr40C1 gene

ACGGCCGGTT TATGCTCTGC CTTTTTCTTT ATTTTTTCCT CACACGGTGC
TTTACTAGCG TGAAGCCAAC GCGAACCGAT TACCGGTACT CCAAATGACC
TCACTGCATT TCTTTTCATT TGAAAAAATG TTGTCGTTGG CAAAACAGAA
CACACTCTCG AACAAATACC CTTCACCCGT TGGGTTGATG GGTTCCAAGT
GAAAAATCTC GTCCCAGTAA AAGCTGGGCA TACTGCAAAC GACAAATTCT
GCATTACCTT TAGGAAGGGT TAGATTTTTC TTCGACAGCA GGTGAGGCAG
TAATCAGGTC AAGCGTGTGA GGCGTGAGCC ATCACTTGGT ACAGATGGGT
TCCATTTAAC CAACCGATCG TCGCTAATCA ACACAGCTCA TCCGTAAT**CA**
CGTAATCACG CCCAGCATCC AGAACGTTCC TCCGGTCTGG ACCCCACACG
CCATGGACAC GAGGCACCCC ACACCACACC ACGCGTTTCG TCTCACCCGG
GATTTTTTTT TTCCTTCACT CCAAGCAGTA AATCAGTAAC AAATGGGCAG
TAAATCCCTT CTATAAATGG GGACCCCCAA GGAGGCGCAG CTTCTCATCG
CCTCGTCT**CA** **CGT**TTGATCC ATCACACTCA CACAACACAA CCCTCCCCAA
AACCTCCCGA AGCGAGCAAG ATGTTCGGCT TCG

Source:Genbank Accession No C126222.2

Plant materials

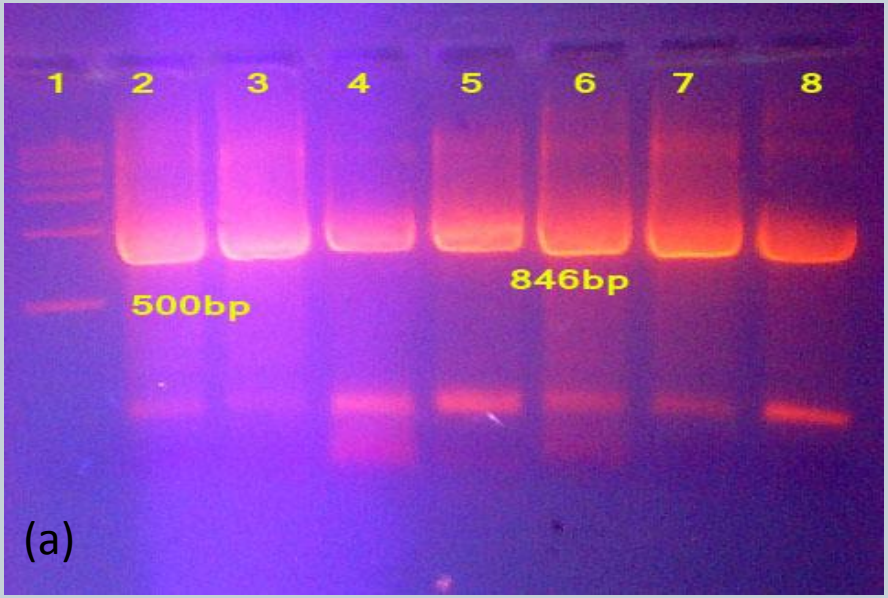
- Drought resistant INGER46 for OsDREB2A gene and HVA1 like promoter
- 10 rice varieties were used to amplify the OsDREB2A coding region, same amplification results
- No amplification with OsDREB2A japonica sequence specific primers
- Salinity resistant Pokkali variety for Osr40C1 promoter
- Osr40C1 gave amplifications only with Pokkali and Nonabokra varieties (not with other indica varieties ie: Bg300, Bg360, INGER46 and INGER36)

Cloning of gene constructs

- pUC18, pCAMBIA1301, pCAMBIA1391, pCAMBIA1390 and PBI221 as vectors
- Gene and promoter sequence cloning was done using pUC18
- Fermentas® double digestion protocols for plasmids and gene constructs digestions
- pCAMBIA vectors as binary vectors
- pCAMBIA 1391 for promoter analysis and pCAMBIA1390 for promoter-gene constructs transfer

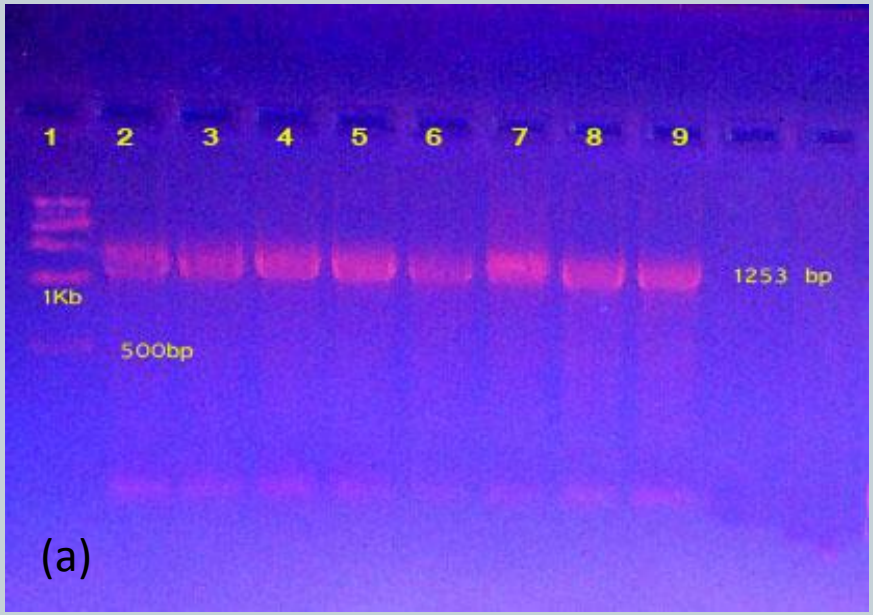
PCR amplification and cloning of OsDREB2A

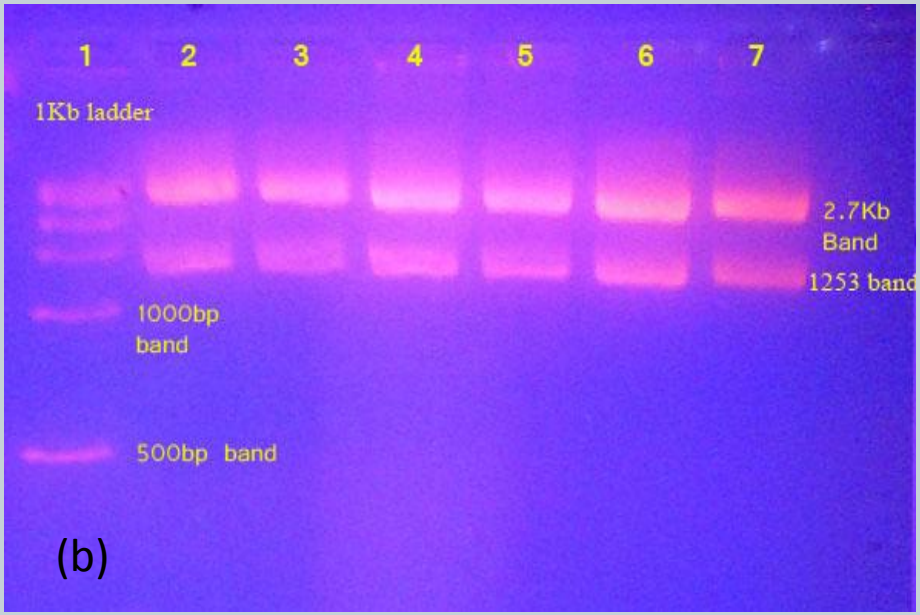
•pUC18 (2.7Kb) as the cloning vector



(a) PCR amplified 846bp sequence of the OsDREB2A coding region
(b) Re-digestion of recombinant pUC18 containing OsDREB2A gene

PCR amplification and cloning of HVA1 like promoter

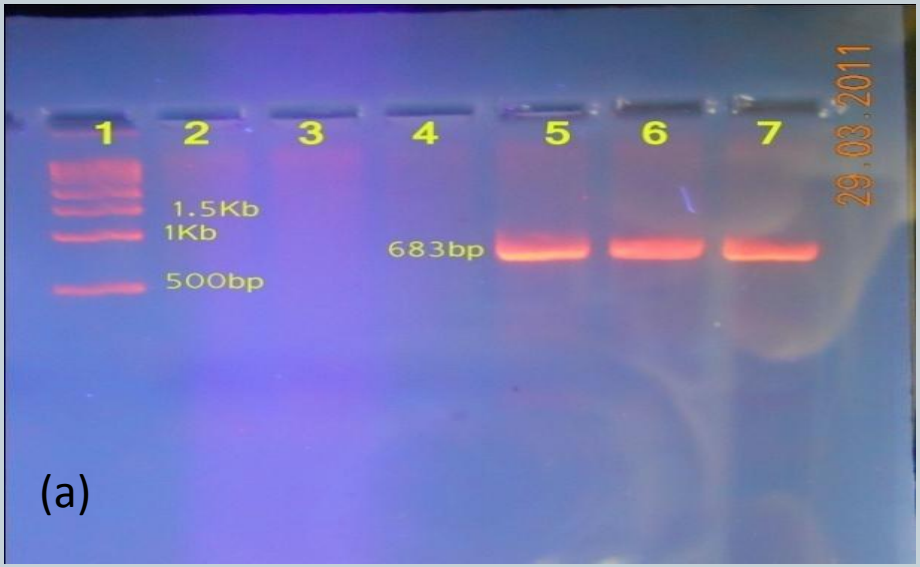


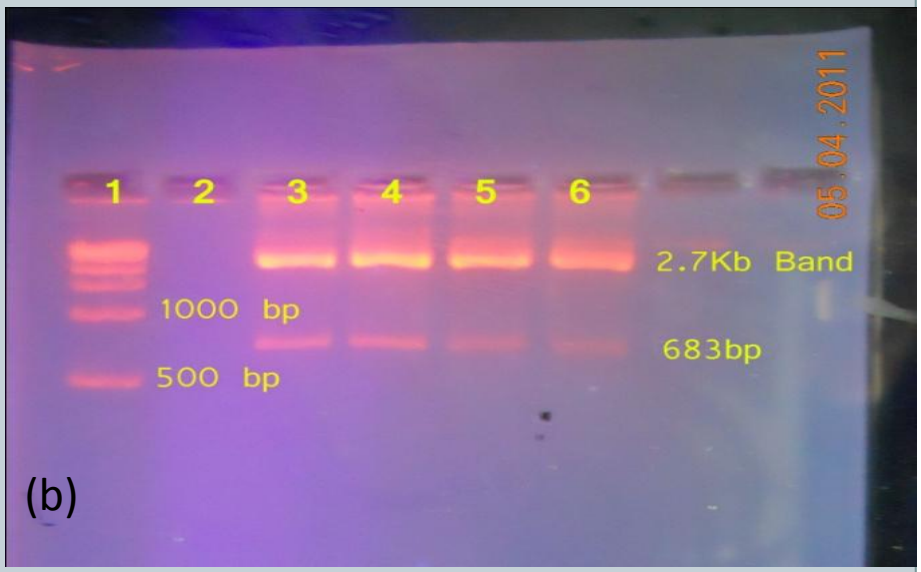


(a) PCR amplified 1253bp region of the HVA1 like promoter

(b) Re-digestion of recombinant pUC18 containing HVA1 like promoter

PCR amplification and cloning of Osr40C1 promoter



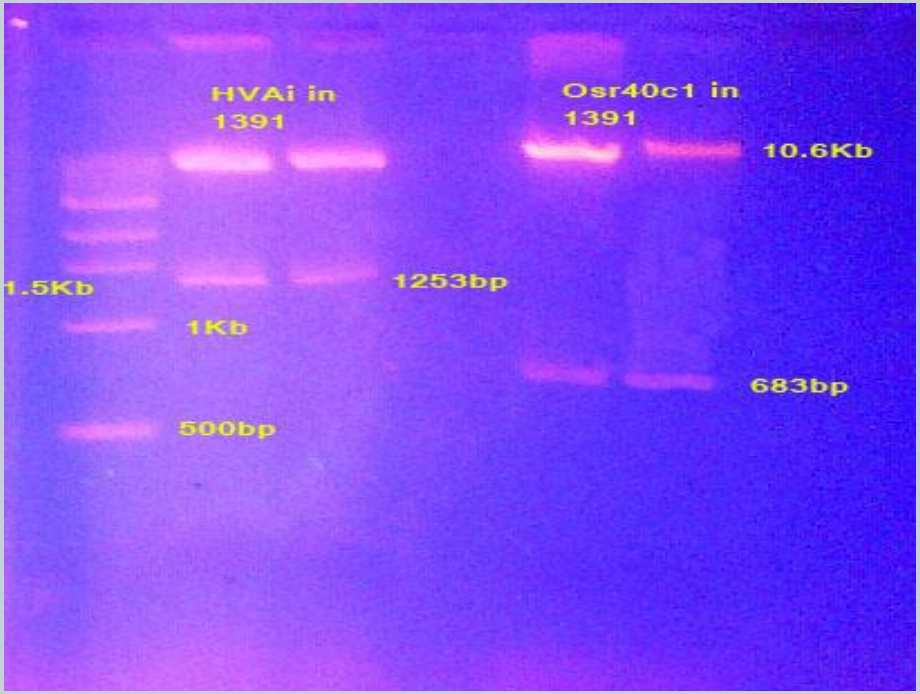


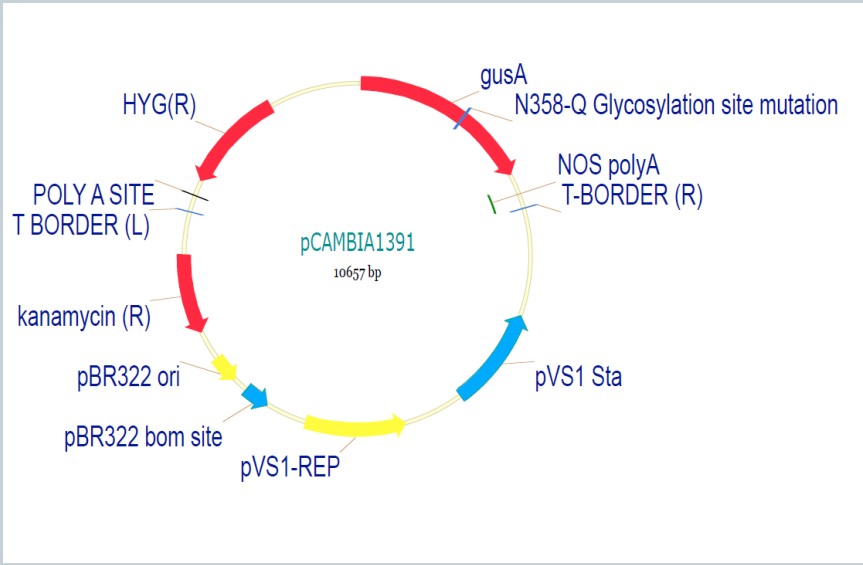
(a) PCR amplified 683bp region of the Osr40C1promoter

(b) Re-digestion of recombinant pUC18 containing Osr40C1 promoter

Designing of promoter-GUS gene constructs

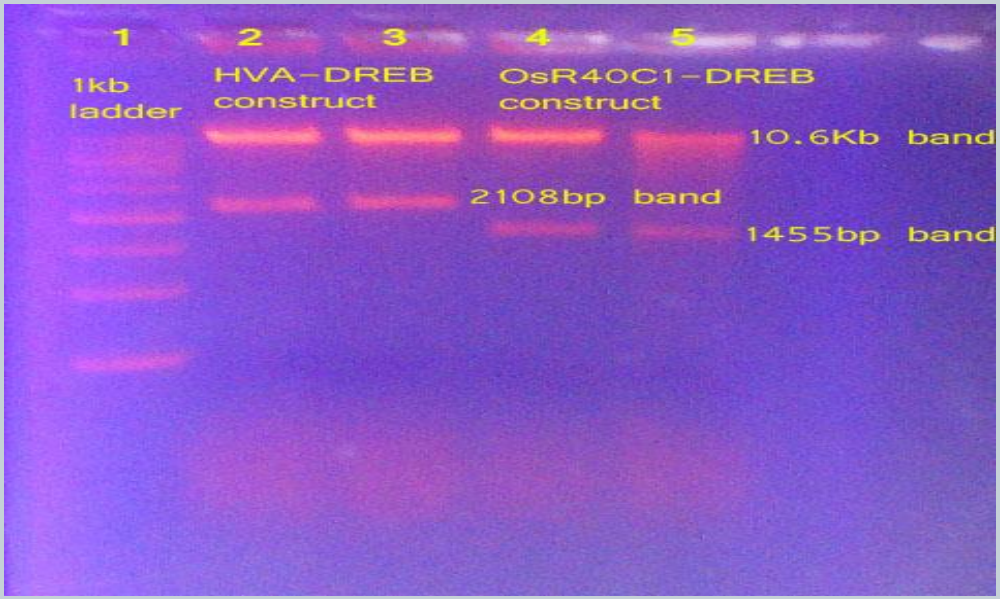
- HVA1 like promoter, Osr40C1 promoter were cloned in front of GUS gene in pCAMBIA1391 binary vector for promoter construct analysis

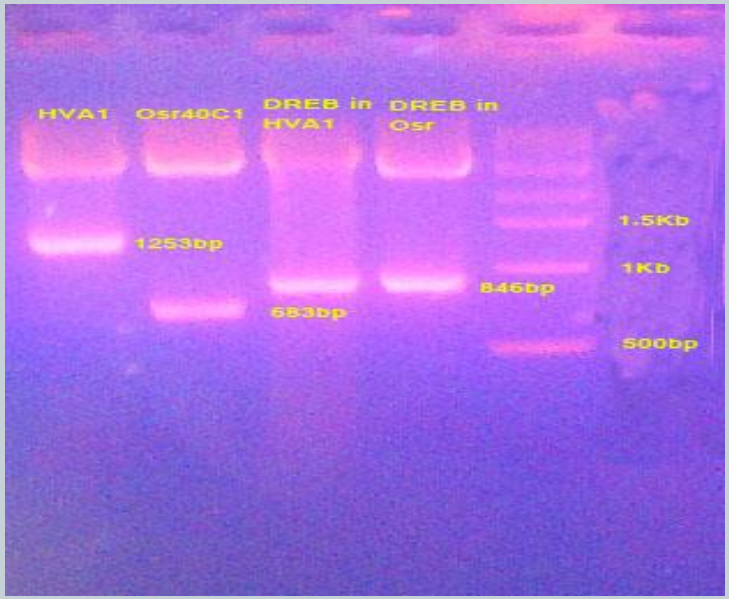




Designing of Promoter-OsDREB2A constructs

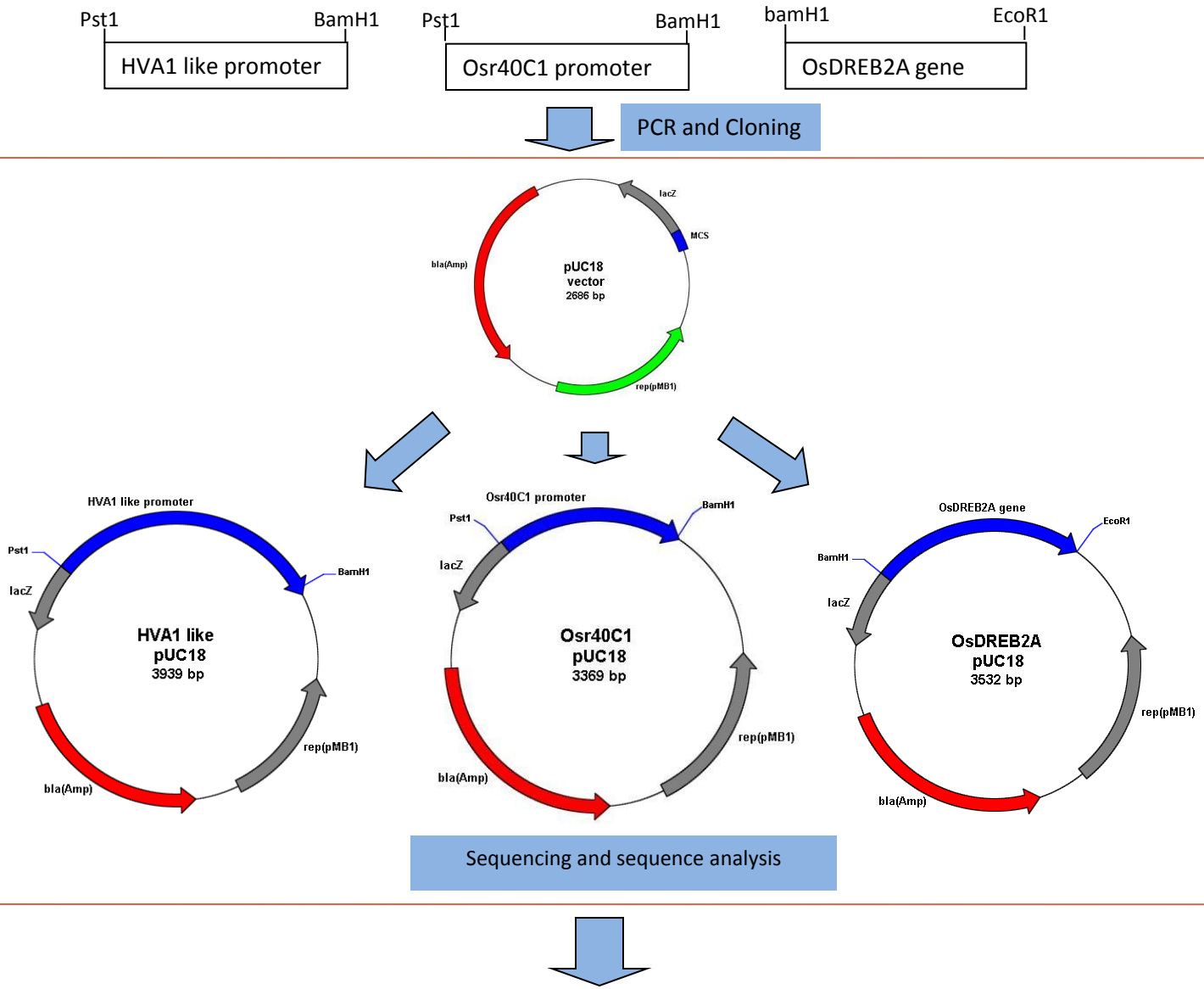
- HVA1 like-OsDREB2A and Osr40C1-OsDREB2A constructs were cloned in front of NoS terminator of pCAMBIA1390





(a) Re-digestion recombinant pCAMBIA1390 containing HVA1-DREB and Osr40C1-DREB gene constructs

(b) Re-digestion of recombinant pCAMBIA1390 to cleave promoters and coding regions respectively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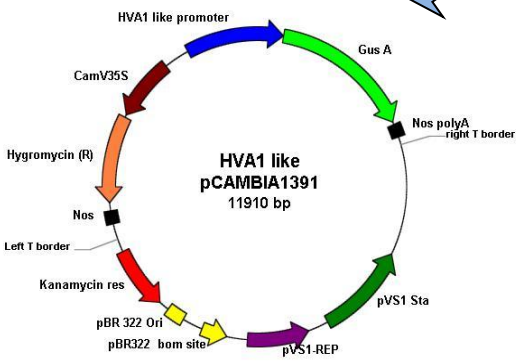

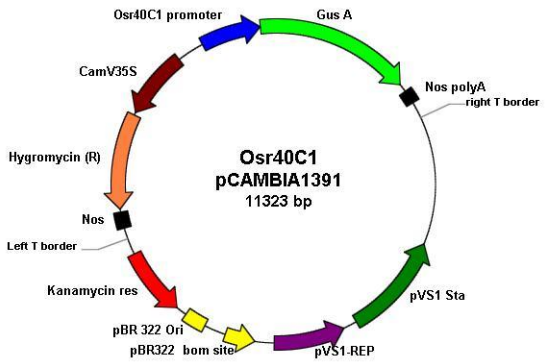

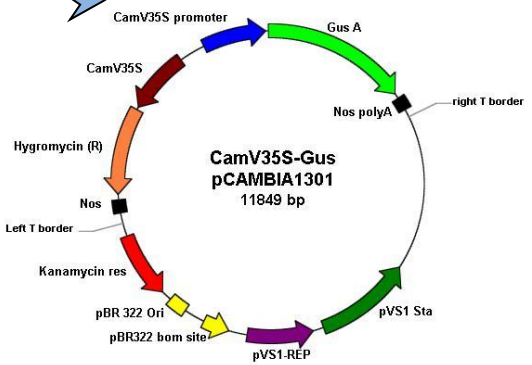

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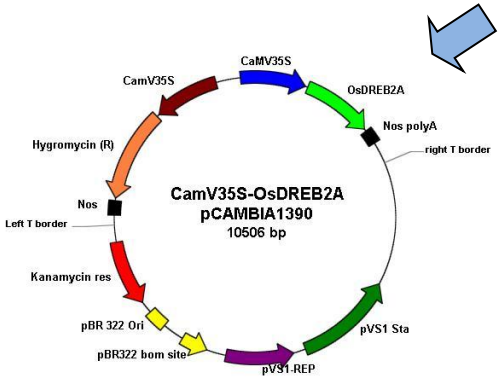
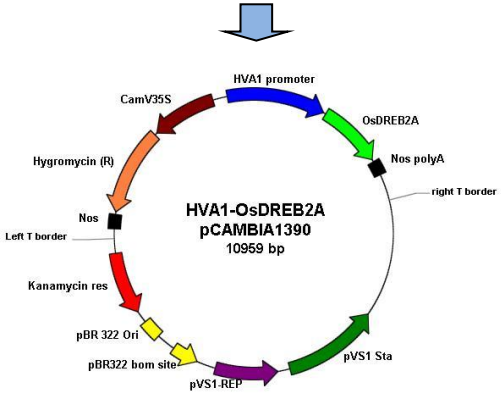
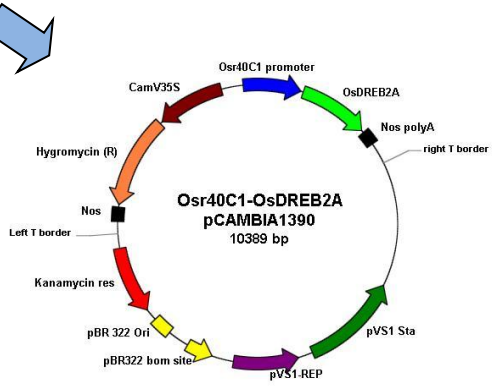


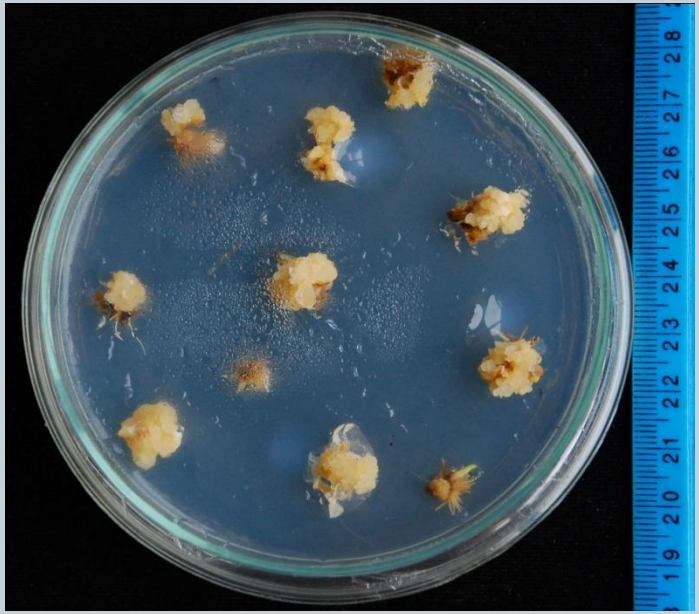




Transgene vectors designed for transformation of OsDREB2A in to rice

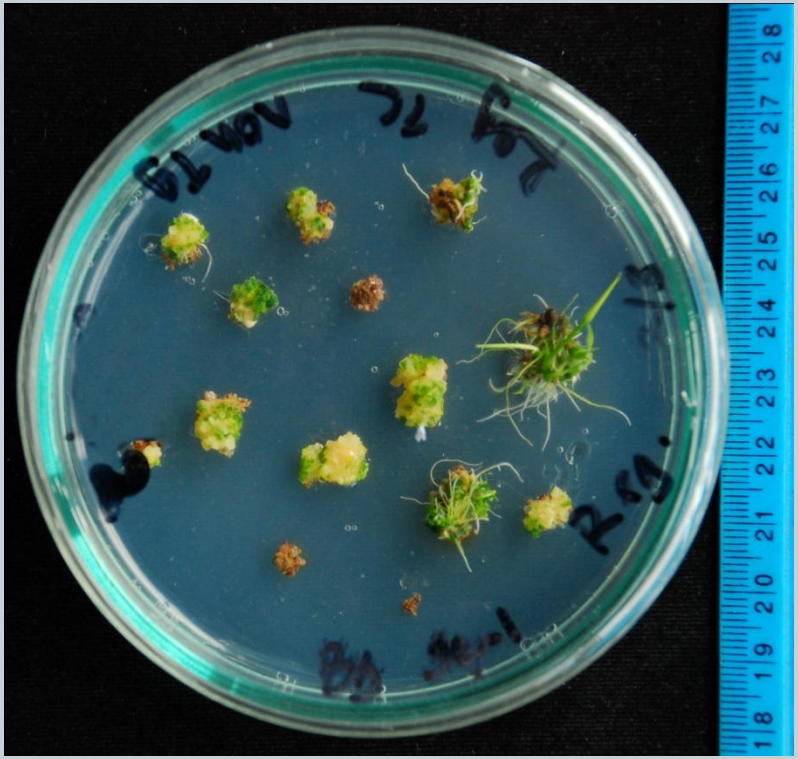
Rice tissue culture

- Rice Scutellum derived calli will be used for plant transformation
- Bg94-1 was used to see the callus induction and regeneration
- Callus induction was observed during 3 weeks time in the callus induction medium



Shoot regeneration

- After subculturing for two days shoot regeneration was observed with Bg94-1 in regeneration medium within three weeks time



Research need to be done

- Analyze the promoter activity of HVA1 like and Osr40C1 by expression study(callus stage and seedling stage)
- Rice calli transformation with gene cassettes and Intact plant regeneration to study the activity of OsDREB2A with a selected promoter
- Transformant analysis and phenotype evaluation

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