

ISOLATION AND CHARACTERIZATION OF LOW TEMPERATURE RESPONSIVE EXPRESSED SEQUENCE TAGS (ESTs) OF *CARAGANA JUBATA* (PALL.) POIR.

INTRODUCTION

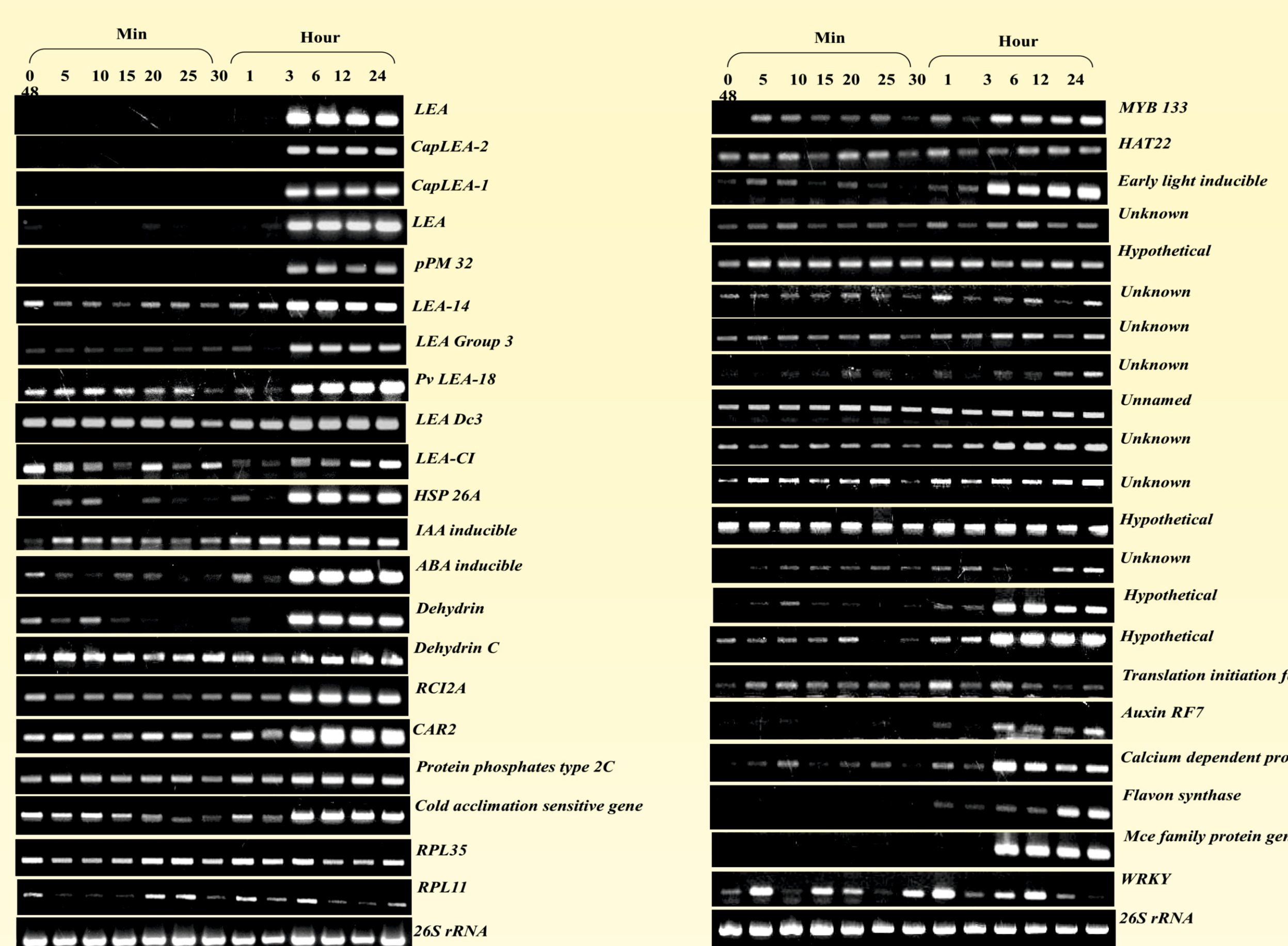
- Caragana jubata* (Pall.) Poir is a perennial leguminous shrub of temperate zone of the world. In India, the plant is uniquely distributed in high altitude cold deserts of western Himalayas (32° 21' 11 "N, 78° 00' 52" E; altitude 4200 m).
- A well developed snow cover persists from the month of October onwards which blankets the vegetation in Spiti valley of Himachal Pradesh. Some of the shrubs such as *Caragana* are spotted growing under snow at the time when snow starts melting.
- The plant experience extremes variations in temperature ranging between 5 to 37°C during the day and 2 to -10°C during night throughout the growing period.
- The plant is expected to possess inherent mechanism (s) to tolerate low temperature and hence should be the candid source of genes for tolerance to low temperature and drought.
- The plant is also used in the treatment of boils, swellings, coughs, headaches and rheumatic arthritis. A fiber obtained from the bark is used for making cordages, gunny bags or used as fodder.

MATERIALS AND METHODS

- Plants of *Caragana* were grown in the pots and maintained in greenhouse at normal growth condition. The twelve week old plants were housed in plant growth chambers maintained at 25±3°C and 4±3°C. Samples were harvested, immediately frozen in liquid nitrogen and stored at -80°C till further use.
- A forward and reverse suppression subtractive (SSH) cDNA library was constructed to identify low temperature responsive genes.
- Reverse northern analysis was performed to characterize differentially expressed cDNAs.
- Semi-quantitative RT-PCR was used to study the gene expression kinetics in response to low temperature (4°C).
- Full length cDNAs of some novel genes were cloned by (Rapid Amplification of cDNA Ends) RACE.
- Promoter of a novel gene was cloned by Genome Walking.

RESULTS

Expression pattern of genes in response to LT (4°C)



In-silico analysis of promoter

CGACGGCCCGGGCTGGTCTTAGTCTTTAAAGGTGTTGTTGATGTTATCCTCA
CCAAGAGTCCAAGACATATCCCCTCTTGATCTACTCATGTGCTTACACGTTTC
ATGGTAAACATTATTAGATAAAAAAAGATAATAATAAACAACAACTG
GCTACCACTTGCTTCAATACCTGAGTAAATCCAACTATGAGCTGGGTATGG
AACTTGAAATGACATTGAGGCTTTCATCTCGGCGAGCAACAGAATATACA
AAGTAAATGAAACCACTTTATGATTTGATTTGAAATCAACCAATTAATCATTTCTTA
AGAATTGTGCACAACTCAATAGAACACTAAAACGAGATAGAGACAGAGG.....

GATTATGAGATAAATTATACGAATGGATGACAAACAAGGATTGAGTCTAGCGGA
ARR-box WRKY-box
AGGAGTTAGTCTCGCAGCGGTAACTTTCTGACCTGCTCGAACAGGATTACC
TTGATTTGAGTGAACCTGACGCGGTAGTTGAACCCACCCAGCCCTTGTGGGG
TATGAGCCAATCCATACGAGCTGAGCGGCTGAGCGGAGCTGCTGTGCGACAT
CBF (CRT/DRE)
GATCTATATGTAATAAAGTAAACCAATCATAGTCAAGTCACATGTATGTGTC
HSE-box BIND1
ATGCAACTTTTGGCCGCAACAACTAATTTGAAGTCTAGGAAACCAACATAC
MYB-box
TACGGGAACTTATCCTCAATACCTATCTAGTATCATAGTATACACTACCTCA
TCTATAAATACCCCATATAGCATTATACCTAAGCATATTTCTACCAAACTA
TATA-box
CTAATAGAGCTCTAGGTTCACAAAAAATGCTTCCATCAGTTTCTGGTCACTC
ATTTTACCACCTGTTGACTTCAATTTTCAAGTGTGAGGGGGCAAGGAAT
CTGCAACATCAATTTGCAAAAGTACATGAAGTTTCTAAAATGATTTGCCTC
CAGTTGCAGGACTACCAATCTTCCATGCGCACTTCCAAACGAGATGCGCAC
CACTGCCAATGTTCACCTCGGTGTGCTGT

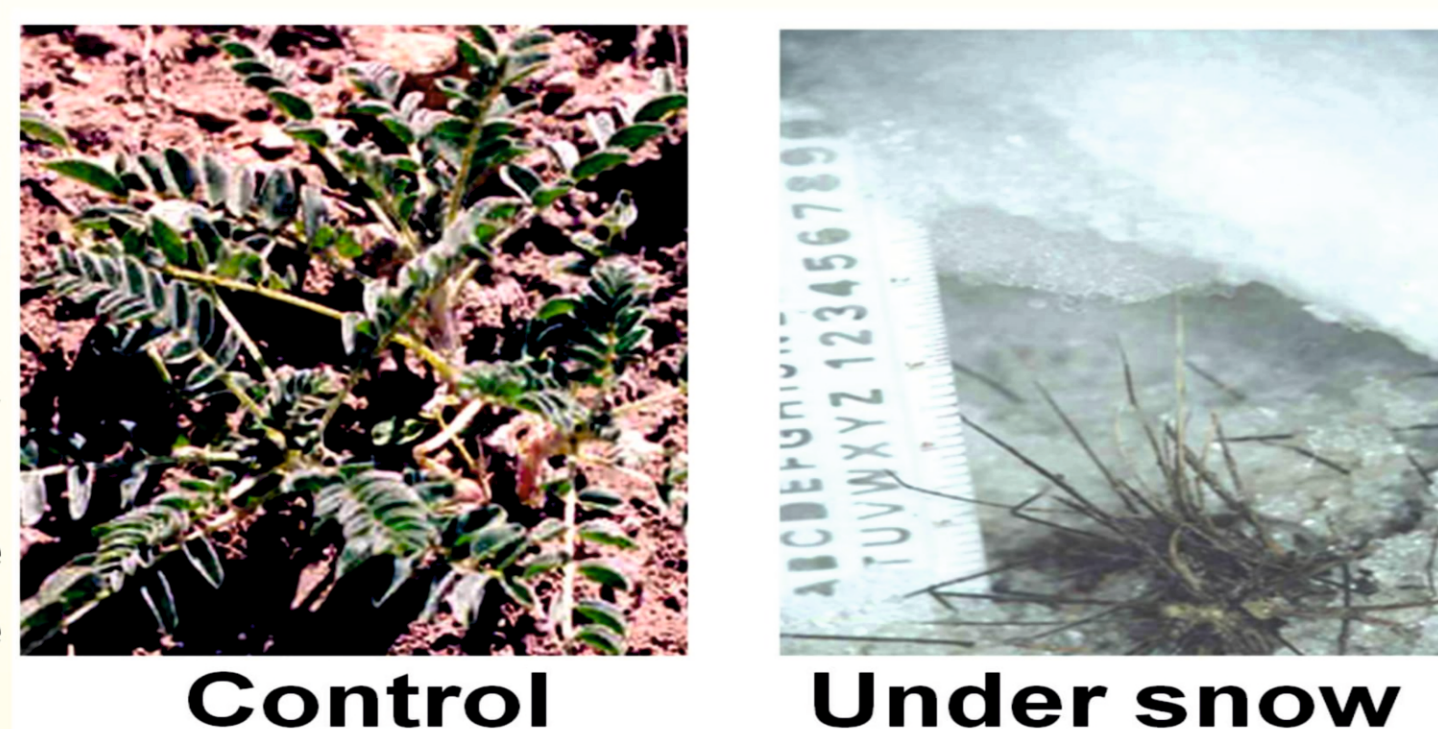
CONCLUSIONS AND FUTURE PROSPECTS

- The EST data and analysis presented here is an overview of the transcripts that are differentially expressed in *Caragana jubata* during low temperature stress. Expression analysis has shown numerous novel genes and transcriptional activators which responded within 10-20 min of exposure to low temperature and influence the expression of cascades of genes associated with stress response.
- Identified novel genes are potential candidates for modulating low temperature stress.
- Full length cDNAs and promoters has implications in raising transgenic plants tolerant to low temperature.

Acknowledgements:

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Caragana jubata growing at Kibber (HP)



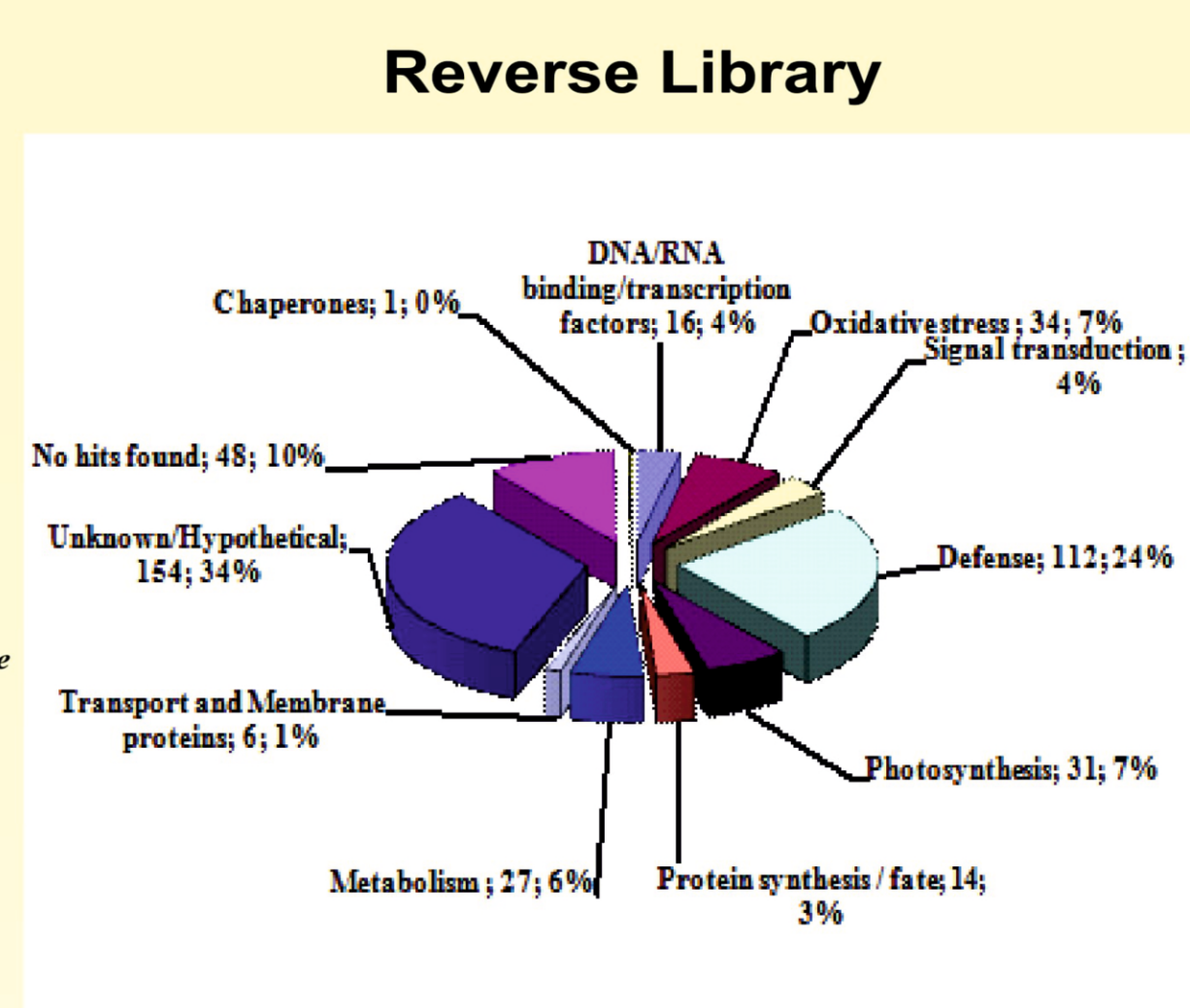
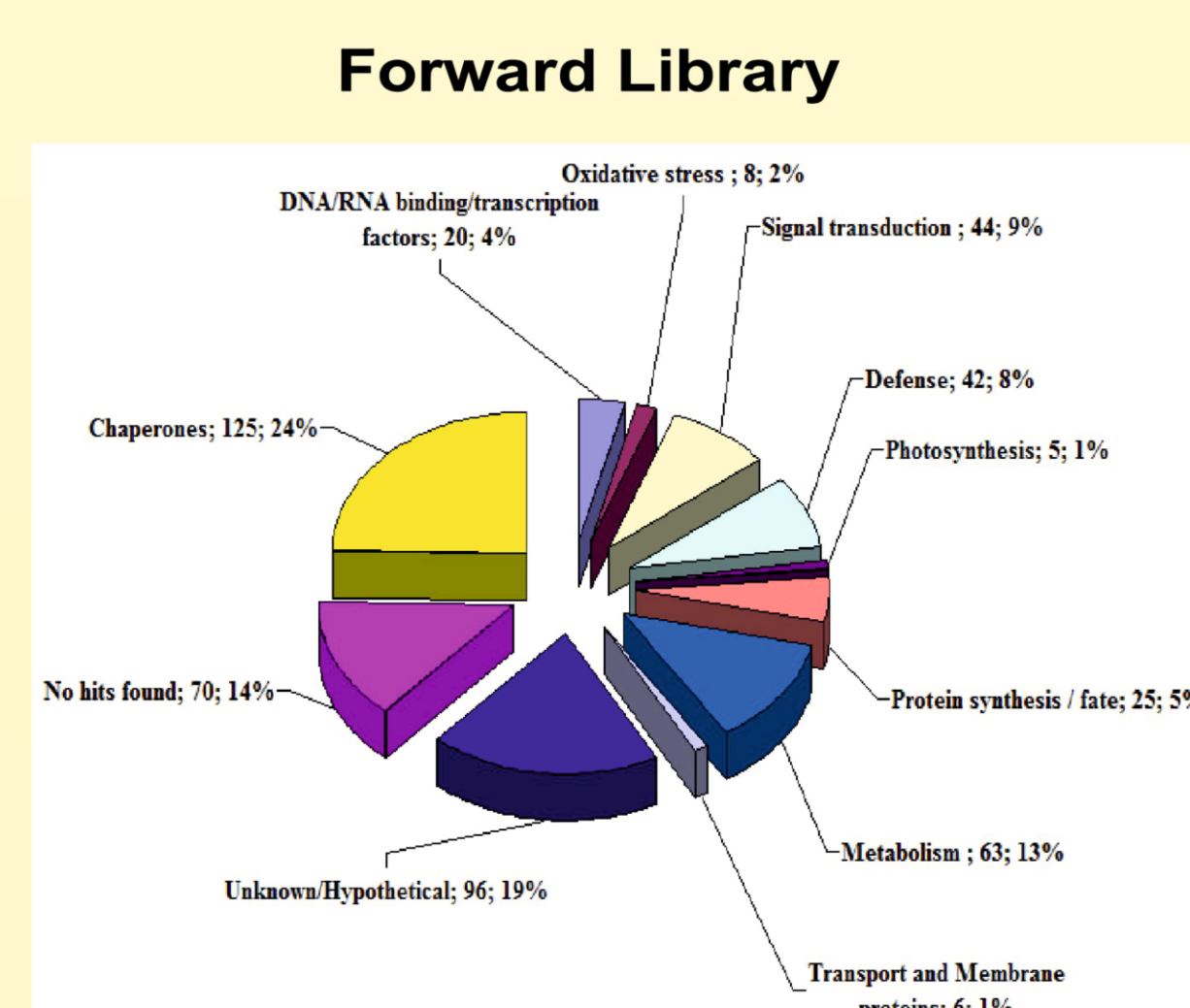
Control

Under snow

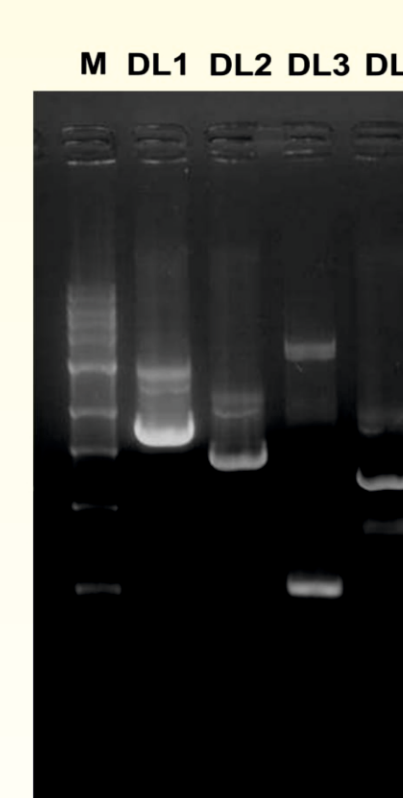
Caragana jubata growing at Palampur (HP)



Summary of LT responsive ESTs of Caragana jubata



Cloning of promoter



Differential Screening of ESTs

To identify differentially expressed genes during low temperature stress, a forward and reverse SSH library was constructed. Based on reverse northern analysis, cDNAs were identified and characterized.

Functional categorization of ESTs

The putative function was assigned to each of the ESTs by sequence comparisons at NCBI database. The identified ESTs were grouped into the following categories: 1. Oxidative stress 2. Cellular defense 3. Metabolism 4. Signal transduction 5. Photosynthesis 6. Protein synthesis and fate 7. Cellular transport 8. Energy generation 9. Transcription and post-transcription 10. DNA processing 11. Proteins with unknown functions.

Expression analysis of LT responsive cDNAs

Expression level of selected genes was studied in response to low temperature stress.

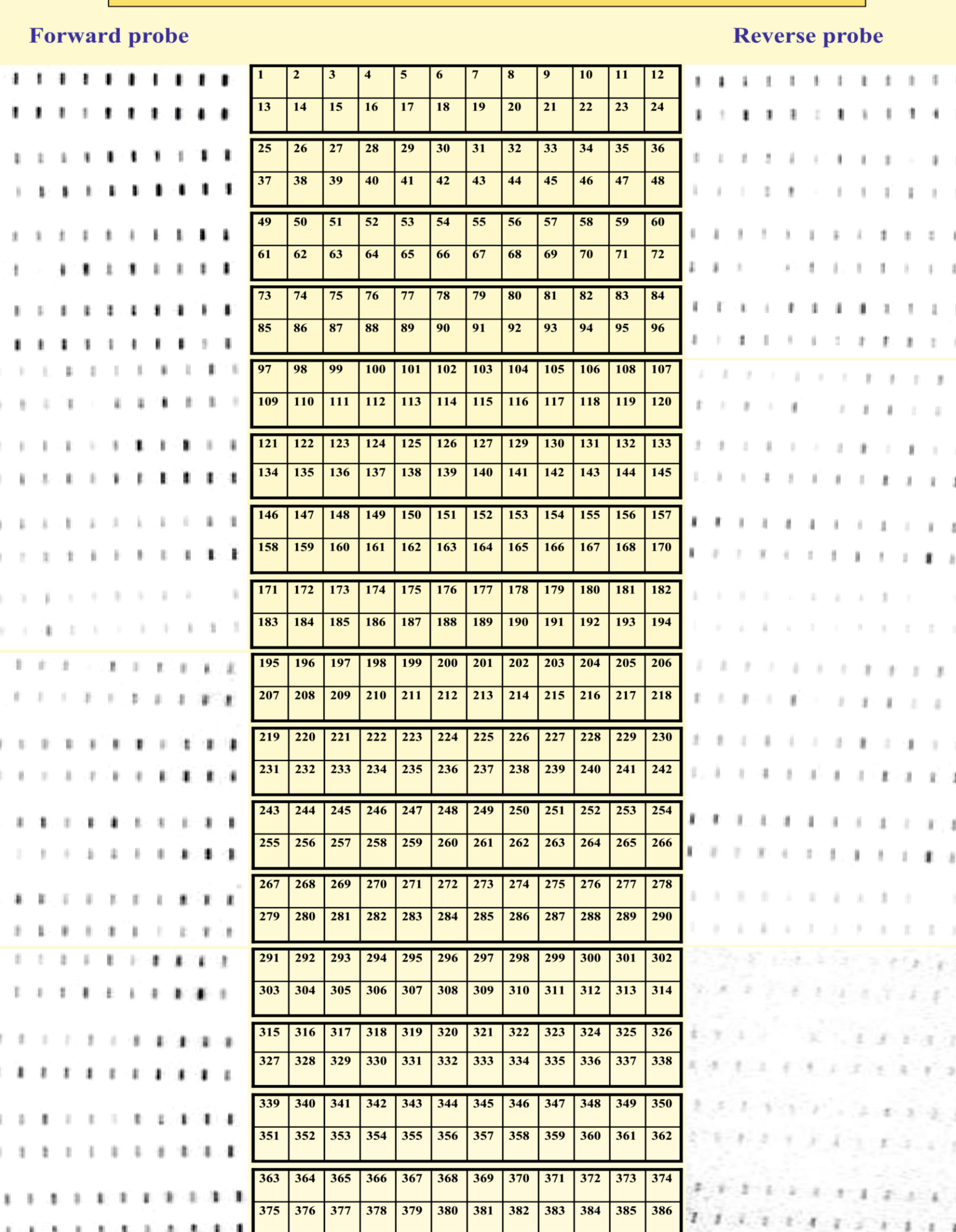
Some of the genes responded within 10-20 min of exposure to low temperature, while a few genes took a longer time period.

Cloning of full length cDNAs and promoters

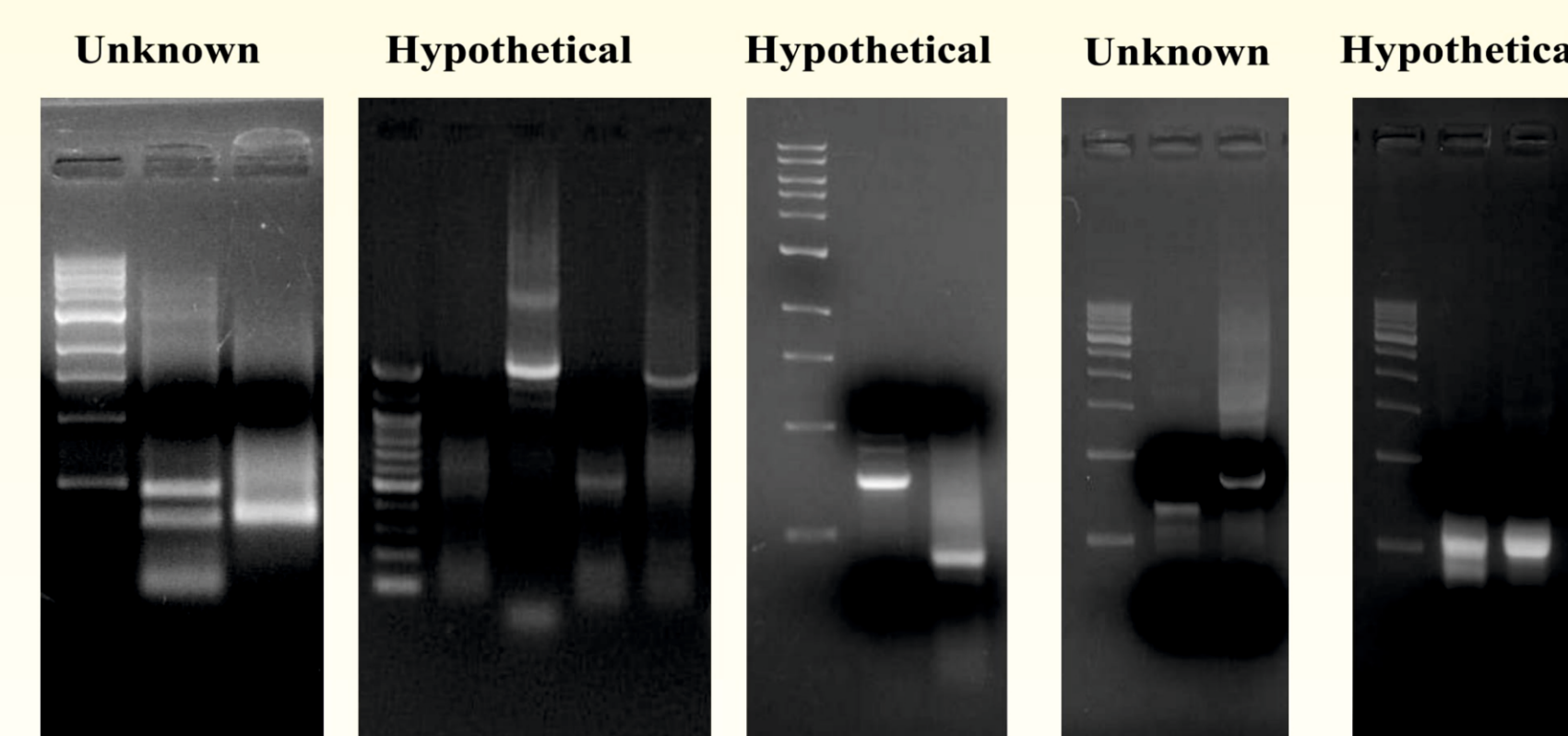
Full length cloning of some novel low temperature responsive cDNAs was performed.

Promoter analysis of a novel gene shows characteristic domains of low temperature tolerance.

Reverse northern analysis



Cloning of full length cDNAs through RACE



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