Genomic approaches to understand Floral Transition at the Shoot Apical Meristem of Soybean

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Areas of research:
- Male Gamete formation and Epigenetic programming of sperm cells
- Floral transition in soybean
- Abiotic and Biotic stress tolerant crop plants
- Pollen allergens

Web: www.unimelbplantbiotech.com
Why Soybean

- a major legume crop used for human and animal feed
- 55% of the worldwide oilseed production
- rich source of protein
- Maturity groups

availability of individual genotypes that show variability in the

photoperiod (and/or temperature)
- vernalization-unresponsive, SD species

- floral meristem can revert to leaf production when environmental
  growth conditions are switched from SD to LDs
- has a flower development system in which more than one types of
  organ is initiated at the same time

Arabidopsis

- diverged from a common ancestor 92 million years ago

- unique vegetative and floral development complexities
Plant Meristems

SAM

Stem Cells
Organizing Centre
Peripheral Zone

RAM

Stem Cells
Quiescent Centre
Gene expression profiles of Soybean shoot apical meristem using affymetrix GeneChip® soybean array

Transcript profiling of SAM undergoing floral transition
Out of 1101 genes 245 genes seems to be specifically expressed in SAM.
**In situ Hybridization Analysis on Selected Candidate Genes**

**AINTEGUMENTA (ANT)**
- A transcription factor associated with ovule development in Arabidopsis
- Signals detected in the outer peripheral zone of the SAM and the adaxial side of leaf primordia
- Expression pattern consistent with the observation in Arabidopsis

**ANGUSITFOLIA3 (AN3)**
- A putative transcription co-activator
- Signals seemed to be excluded from the stem cell region
- Roles in leaf cell expansion in Arabidopsis but likely to have additional roles in coordinating activity in the SAM

**PINHEAD / ZWILLE (PNH)**
- Member of the ARGONAUTE family of proteins
- Expression associated with the provascular region, adaxial side of leaf primordia

**SHOOTMERISTEMLESS (STM)**
- Signals detected throughout the meristem as well as in the inter-primodia region
- Likely conserved function as in Arabidopsis in the meristem maintenance
Structural Organisation and Functional Domains

Cellular organization in Arabidopsis

Spatial expression of CLAVATA3 in the shoot apical meristem suggests it is not a stem cell marker in soybean
Glycine max cv. Bragg

Small RNA Sequencing and Expression Profiling
- 32 conserved miRNAs families
  (16 TF Arabidopsis, rice, maize)
- 10 novel miRNAs
  several miRNAs*
Novel 107: ATAGCTTGGGCTGGCACACCCCT
(Predicted targets: carboxylesterase, LIGHT SENSITIVE HYPOCOTYL, ABC transporter, RNA lariat debranching enzyme)

Zoomed in at the SAM region

J Exp Bot 2011
Molecular basis of floral transition in soybean
Development of Soybean SAM under Short Days
No of transcript displayed Significant changes = 331 Transcripts

Clustered into 10 clusters according to their temporal changes

Increased trend= cluster 2-8 (237 transcript; cluster 4 highest fold changes in gene expression level

Decreased gene expression=cluster 1,9,10
In situ Hybridization Analysis on Selected Candidate Genes

AP1 floral identity gene
Gm WUS during Floral initiation
Cluster 3 & 5 contain transcripts with gene expression induced prior to floral identity genes i.e between 0-2 days

Cluster 3 (3 transcripts)
- IAA-amino acid hydrolase 6 (Gma.3543.1.S1_at),
- beta-amylase (GmaAffx.89961.1.S1_at),
- shikimate dehydrogenase (Gma.6184.1.S1_at).

Cluster 5 contains 29 transcripts
Phytohormones- auxin, ABA, Jasmonic acid, unknown
ABA and Floral initiation
NCED catalyse rate limiting step
Computational approach

- Vernalisation & ambient-temperature pathways

- Each Arabidopsis flowering gene has 2-3 orthologous copies in soybean
The genome mapping of the soybean flowering genes showed that these genes are scattered randomly across the genome.
A paralogue comparison indicated that the soybean genes comprising the largest orthologue group are clustered in a 1.4 Mb region on chromosome 16 of soybean.
Comparative genomic analysis:
Undomesticated soybean (Glycine soja)

- the light-signalling and ambient temperature pathways
- hundreds of SNPs, structural variants of putative soybean flowering genes

RNA/Deep sequencing during flower transition
RNA Seq

7.9% (3829) SAM specific transcripts
4.8% (2328) Leaf
SAM-Higher diversity;
30% transcript

The expression level of transcripts in reads per kb per million (RPKM) for each library

SAM- 0, 1, 2, 3, 4; Leaf- 0, 1, 2, 3
90bp high quality read,
200 millions (26 million/library);
Illumina HiSeqTM 2000 platform
Differential expression of genes during short-day treatment

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LEAF

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The floral initiation process in soybean

Red- up
Green-down
Orange-both up & down
Model-The floral initiation process in soybean

-Major reprogramming in leaves & SAM
-GA & cytokinin
Acknowledgements

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☐ Agricultural Biotechnology;
  ☐ abiotic and biotic stress tolerance in crops
  ☐ crop improvement for nutrition and biomaterials
  ☐ germplasm sources for adaptability to climate extremes
  ☐ disease resistance.
  ☐ biofuels.

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