Functional mechanisms of drought tolerance in maize

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Importance of Maize in India

Area: 8.55 m ha
Production: 21.74 m t
Productivity: 2.54 t/ha

Maize Utilization Pattern in India

- Food: 49%
- Animal Feed: 25%
- Starch: 12%
- Brewery: 12%
- Seed: 1%
- Poultry Feed: 1%

How to improve productivity?
- Better cultivars
- Better cultural practices
- Stress tolerance

Rosegrant et al 2009
The Palmer Drought Severity Index

IPCC weather report for 2030-2039
Yield loss - Water stress

- % yield reduction
- Emergence
- Pollination
- Critical stage
- Tasseling
- Silking
- Blister stage
- Milk stage
- Soft dough
- Hard dough
- Maturity
Phenotyping for Drought

Drought nursery, Summer 2011, 2012
- 88 genotypes/maturity group = 264 genotypes
- Alpha lattice design

Phenotyping
- Well-irrigated control
- Flowering stage stress

Locations
1. IARI, New Delhi
2. ANGRAU, Hyderabad
3. Maize Research Station, Karimnagar
Genotyping

240 genotypes was genotyped by genome-wide SNPs using Illumina Infinium assay.

~56,000 SNPs covering entire maize genome.

240 x 56000 = 13.4 million SNPs

LD Heatmaps

Chromosome 3

Chromosome 8

Cured SNPs

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**Per se LD pattern**

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<td><strong>Mean</strong></td>
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- **LD decay**
  - $r^2 \leq 0.1$
  - $r^2 \leq 0.2$
  - $> 5$ MAF
  - $> 10$ MAF

- **Per se LD pattern**

- **Colors**
  - Highly associated ($r^2 > 0.8$)
  - Intermediate ($0.4 < r^2 < 0.8$)
  - Low ($r^2 < 0.4$)
Population stratification

Cross validation

Principal components
Model effect - ASI, Hyderabad

Mixed model for BLUPs:
3 location × 2 year × 2 treatment × 3 maturity group

R: GenAbel
1. G + P + Kinship + MDS + FDR
2. G + P + Kinship + MDS + Pop structure + FDR

R: GAPIT
3. G + P + Kinship + PCA + FDR
4. G + P + Kinship + PCA + Pop Structure + FDR

Anthesis- Silking Interval, Across data
### P value heat map of significant SNPs

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<th>SNP</th>
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*Note: The table and heatmap represent the results of a genome-wide association study (GWAS), showing the association between single nucleotide polymorphisms (SNPs) and various agronomic traits. The heat map visualizes the p-values for each SNP across different traits, with darker colors indicating lower p-values, which suggests a stronger association between the SNP and the trait.*
**Gene model**

- Drought responsive genes
- Other genes

**Chromosome 5**

- MYB transcription factor
- ERF - Ethylene responsive factor
- SBP - Transcription Factor

**Grain Yield**

- IARI
- Hyderabad
- Karimnagar
- Mean
- M1
- M2
- M3
- M4
Functional roles of candidate SNPs associated with drought tolerance

Drought-tolerant genes
- WRKY, BZIP, CAMTA, BHHL, MYB, C2H2, ZF-HD, NCED, NAC, SBP, ERF, NIF-YA

Molecular mechanisms
- Auxin Biosynthesis & Transport
- Phospholipid Hydrolysis
- tpa: Phospholipase D family partial
- GST, GP, MAPK
- MYB, BHHL

Trait expression
- NAC, flavin monoxygenase, V-type PPase H⁺ pump
- ABA Dependent Pathway
- Root Development
- Detoxification Signaling
- Epigenetic Mechanism
- DNA Methylation
- DCM
- V-type ATPase & PPase H⁺ pump
- Stomatal Closure
- Ion Homeostasis
- Reduced water potential
- Flowering
- ROS Homeostasis
- ABA Dependent Pathway
- Molybdenum cofactor sulfurase
Expression of Transcriptomes

HKI1532 (Tolerant)  PC3 (Sensitive)

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<th>SUBJECT</th>
<th>FEATURES</th>
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<td>Cultivars</td>
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<td>Representation</td>
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<td>Genes in UniGene clusters</td>
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174 drought-related genes
75 unique to HKI1532

- HSP-coding gene was co-expressed with 19 DEGs
- MYB coexpressed with 50 DEGs
- ERF coexpressed 84 DEGs
Drought-responsive genes of HKI1532

- **Cluster 1:** Stomatal closure (*NAC, WRKY, ERF, AP2, MYB, SBP, C2H2, and NF-YB)*
- **Cluster 2:** Signalling and phosphoprotein cascade genes
- **Cluster 3:** Photosynthesis

**Cluster 1 Details:**
- Stomatal closure genes include *NAC, WRKY, ERF, AP2, MYB, SBP, C2H2, and NF-YB*.

**Cluster 2 Details:**
- Signalling and phosphoprotein cascade genes.

**Cluster 3 Details:**
- Photosynthesis-related genes.

**Legend:**
- **Up-regulated DEGs:**
  - 0-10 Fold Change
  - >10-100 Fold Change
  - >100-1000 Fold Change
  - >1000 Fold Change
- **Down-regulated DEGs:**
  - 0-10 Fold Change
  - >10-100 Fold Change
  - >100-1000 Fold Change
  - >1000 Fold Change
Functional relationship of genes

A. Maintenance of photosynthesis and other metabolic pathways

B. Maintenance of water balance

C. ROS scavenging
Comparative co-expression maps

Maize
544 samples, 1810 genes

Rice
1531 samples, 2539 genes

Wheat
901 samples, 3438 genes
**bHLH**
- Regulate ABA, signalling, jasmonate pathway

**C2H2**
- Stomatal closure, ABA pathway, regulation of transcription

**NAC**
- Stomatal closure, embryo/flower/root development, cell wall

**ERF**
- Transcription regulator

**WRKY**
- ABA signalling, jasmonate, embryogenesis, signalling

### Comparative gene regulation

**Maize**
- 17 families
- 287 genes

**Rice**
- 17 families
- 494 genes

**Wheat**
- 17 families
- 449 genes
Role of miRNAs in gene regulation

- 42 target mRNAs expressed differentially under drought were regulated by 13 miRNAs families.
- Single miRNA was controlling multiple mRNA targets.

Expression of miRNAs and their targets

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<tr>
<th>MiRNA</th>
<th>Target mRNA</th>
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<th>PC3 (Sensitive) miRNA</th>
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Summary

Genes/SNPs responsible for important biological functions
- Photosynthesis
- Osmoregulation
- ROS scavenging
- Metabolic processes

Selected founder lines
- Shuffling/combining the alleles
- Selection of genotypes with better allele/gene compositions
Acknowledgements

**Donors**
National Agricultural Innovation Project
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**Collaborators**
Firoz Hossain
Sumalini
Shobarani
Sreelatha

**Research Fellows/Technicians**
Rajesh Kumar
Kaliyugam
Rinku Sharma
Kanika
Sweta
Swati
Rita Kumari
Namratha
THANK YOU