Genomic resources and gene/QTL discovery in cereals

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Outline

• Genomics approaches
• Available genetic resources
• Examples of target traits
• Perspectives
QTL mapping and cloning strategies

Linkage mapping (biparental)
- QTL coarse mapping
- Near isogenic lines (NILs)
- Positional cloning

Genetic resolution
- 10-20 cM

Association mapping (set of accessions)
- Genome-wide (high LD panel)
- Candidate gene (low LD panel)

Candidate gene validation
- 1-100 kb
### Genetic resources for gene/QTL discovery in cereals

<table>
<thead>
<tr>
<th></th>
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<th>Introgression libraries</th>
<th>AB-QTL populations</th>
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With TILLING, a library of DNA samples from thousands of individuals is screened for artificially-induced or naturally-occurring (EcoTILLING) single-nucleotide polymorphisms (SNPs).

TILLING recovers a range of mutation types

Barley, maize, sorghum, wheat

- Proper
- Altered
- Truncated
- Nonsense

improper RNA splicing

\[
\ldots \text{AG}_{\text{GT}} \ldots - \ldots \text{AA}_{\text{GT}} \ldots \\
\text{Intron} \_\_ \text{Exon}
\]

\[
\text{CGA} \rightarrow \text{TGA} \\
\text{R} \rightarrow \text{STOP}
\]

Courtesy of L. Comai, modified
ROOT MUTANTS – Reverse analysis

TILLING with TILLMore in barley

Wild-type  12/G5  37/C7

Photos: courtesy of Iwona Szarejko
Cloning genes and QTLs

... looking for a needle in a haystack ...
To clone or not to clone QTLs?

QTL cloning as an essential step towards:

• More effective marker-assisted selection

• Understanding the functional basis of traits

• Genetic engineering of quantitative traits

• Unlocking the allelic richness at target loci via direct sequencing of germplasm

## Summary of QTL cloning for abiotic stress tolerance in cereals

<table>
<thead>
<tr>
<th>Stress</th>
<th>Barley</th>
<th>Maize</th>
<th>Rice</th>
<th>Sorghum</th>
<th>Wheat</th>
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<td><strong>in progress</strong></td>
<td><strong>in progress</strong></td>
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<td><strong>in progress</strong></td>
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<tr>
<td>Salinity</td>
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<td>-</td>
<td><strong>yes</strong></td>
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<tr>
<td>Low N</td>
<td><strong>in progress</strong></td>
<td><strong>yes</strong></td>
<td><strong>in progress</strong></td>
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<td><strong>in progress</strong></td>
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<td>Low P</td>
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<td><strong>in progress</strong></td>
<td><strong>in progress</strong></td>
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<td>Aluminum</td>
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<td>Boron</td>
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<td>-</td>
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<tr>
<td>Low temps</td>
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<td>High temps</td>
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<td>-</td>
<td>-</td>
<td><strong>in progress</strong></td>
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Perspectives in gene/QTL mapping and cloning
Maize Diversity Project Team
The Nested Association Mapping (NAM) project

Courtesy of Mike McMullen
Linkage mapping
- Recent recombination
- High power
- Low resolution
- Analysis of two alleles
- Moderate marker density

Association mapping
- Historic recombination
- Low power
- High resolution
- Analysis of many alleles
- High marker density

Nested Association Mapping
- Recent and ancient recombination
- High power
- High resolution
- Analysis of many alleles
- Moderate genetic marker density
- High projected marker density
Conserved Orthologous Set (COS) markers
(J. Salse & coworkers, INRA, France)

- Set of ca. 170,000 gene sequences available for cereals
- Rice, maize, sorghum, *Brachypodium* genomes have been sequenced

COS markers have been derived from orthologous Expressed Sequence Tags (EST) that have been conserved across evolutionary related species.

Advantages
- Transferable among species
- Mostly codominant
- Gene-based markers

Disadvantages
- Need expertised inference of synteny
- Low level of polymorphism
Salse & co-workers (INRA, France)
9,138 COS markers for the monocots

Bolot et al. (2009)
COPB, 12: 11-15
NUE
Starch
Tiller
Viscosity
Size
Hardiness

Bolot et al. (2009)
COPB, 12: 11-15
Future challenges/opportunities for unlocking valuable natural variation for target traits

- High-throughput genotyping (SNPs) & phenotyping
- Resequencing and managing the data
- *In silico* analysis, annotation and comparative genomics
- Tissue-specific expression profiles
- Functional maps (ESTs, FLcDNAs, eQTL)
- QTL cloning
Evolution of QTL mapping and cloning strategies

Phenotyping is the major limiting factor

- Integration of linkage mapping and association (LD) mapping
- Linkage mapping
- Biparental crosses
- Association
- Association at candidate genes
- Multiparental crosses
- Integration of linkage mapping and association (LD) mapping
- Association
- Association at candidate genes

Genomics tool box

- Genomics tool box
- Little or no constraints in mapping information and resolution
- Genome-wide genotyping (SNP, DArT haplotypes)
- Genome sequencing (Solexa, Solid, etc.)
- Next-generation sequencing
- Genomics tool box
- Little or no constraints in mapping information and resolution
Past

Future

Genotyping

Phenotyping

Genotyping

Phenotyping
Conclusions

• Plenty of genetic resources are available in cereals for gene/QTL discovery.

• Devising the right phenotyping screening and choosing the right materials is key to the discovery of loci relevant for the desired trait in the target environment.

• The cost of molecular profiling and sequencing will continue to decrease, making genomics approaches more affordable.

• Association mapping will play an increasingly important role for relatively simple traits; it is more questionable to what extent it will work with physiologically complex traits.

• The contribution of wild germplasm to gene discovery will increase.
Thanks for your attention!