Genetics of tassel and ear development in maize

Erik Vollbrecht
Department of Genetics, Development and Cell Biology
Iowa State University
Shoot Architecture

Principal elements: Branch (shoot) axes

Branching variables:
• Mode of branching
  – Frequency
  – Placement
• Branch angle
• Continuous, intermittent or transient growth
  – Long or short shoots
• Persistence of branch

Architecture is an end product
Shoot architecture reflects the position and behavior of shoot apical meristems (SAMs)
SAM Properties

- Iterative meristem functions
  - Pattern formation
  - Organogenesis
  - Self renewal

Basic meristem structure
(example: *knotted1* gene in maize)
The *knotted1 (kn1)* gene maintains SAM function

- *kn1* encodes a homeobox transcription factor expressed in all shoot meristems
- Loss of function *kn1* mutants display shoot meristem failure

*kn1* mRNA expression pattern

Jackson *et al.* 1994

Vollbrecht *et al.* 2000
SAM Properties

• Iterative meristem functions
  – Pattern formation
  – Organogenesis
  – Self renewal

  → Basic meristem structure

• Meristem characteristics (meristem identity)
  – Primordium type
  – Determinacy: capacity for continued iterative growth

  → Contextual meristem behavior
Maize Inflorescences

Maize

Tassel

= Spikelet

Ear
Outline

• Maize inflorescence architecture
  – Inflorescence development
  – Developmental, molecular genetics of three *ramosa* mutants

• *ramosa1* and inflorescence natural diversity
  – A branch number QTL in maize
  – Maize domestication

• Genes that integrate whole shoot and inflorescence architecture
Maize Inflorescences

Tassel

Ear
The terminal SAM generates the tassel

Vegetative phase

Reproductive phase
Maize inflorescence development

Ear and tassel spike, meristems:
1° = Inflorescence (IM)
2° = Spikelet pair (SPM)
3° = Spikelet (SM)
4° = Floral (FM)

Tassel base, meristems:
2° = Branch (BM)
3° - 4° - 5° = SPM - SM - FM
Genetics of sequential meristem identities

Malcomber et al. 2006
ramosa1 mutations alter branch architecture

ra1-A619 Normal inbred
ra1-R Strong ra1 mutant
ra1-RS Weak ra1 mutant
The *ra1* gene imposes determinacy on 2° meristems

Normal inbred

*ra1-R* mutant

Bar = 250 um
Mutant *ramosa* tassels: novel 2° meristem determinacies
Novel 2° meristem determinacy

Tassel length

- B73
  - Normal
  - Strong allele
- ra1-R
  - Strong allele
- ra1-RS
  - Weak allele
- ra2-R
  - Strong allele

Legend:
- SPM
- IDSPM
- BM/IDSPM
- BM
Mutations in 3 different *ramosa* genes

- **ra1-RS**: Weak *ra1* mutant (chromosome 7, bin 2)
- **ra2-R**: Strong *ra2* mutant (chromosome 3)
- **ra3-R**: Strong *ra3* mutant (chromosome 7, bin 4)
**ramosa single mutants in B73 - ears**

- Normal B73
- *ra1-R* strong
- *ra1-RS* weak
- *ra2-R* strong
- *ra3-R* strong
ramosa double mutants in B73 - ears

ra1-RS; ra3-R  
ra1-RS; ra2-R  
ra3-R; ra2-R  
ra3-R; ra1-R
The *ramosa* genes

- *ra1* EPF zinc finger
  (Vollbrecht *et al.* 2005)
- *ra2* LOB domain
  (Bortiri *et al.* 2006)
- *ra3* trehalose 6-phosphate phosphatase homolog
  (Satoh *et al.* 2006)

*ra1* and *ra2* are expressed only in inflorescences.
ra2 regulates ra1

Vollbrecht et al. 2005, Bortiri et al. 2006
ramosa1 expression:
junction of determinate 2°s with main axis
*ramosa1* expression domain marks a boundary region, not the meristem

B73 inbred  
*ra1-R* point mutant  
*ra2-R* mutant
*ramosa2* expression: in the anlage and base, of all 2° and 3° meristems

A Transition inflorescence meristem  
B Developing inflorescence  
C  
D *ra1-R* mutant

*Bortiri et al. 2006*
*ra3* expression: discrete domains below all inflorescence axillary meristems

Satoh *et al.* 2006
A *ramosa* pathway

\[ ra2 \xrightarrow{\text{LOB}} ra1 \xrightarrow{C_2H_2} 2^\circ \text{ meristem determinacy (short } 2^\circ \text{ branches)} \]

ra3
T6PP
Boundary domains and 2° meristems in inflorescence architecture

Gene

$ba1 = barren\ stalk1$
$ra2 = ramosa2$
$ra1 = ramosa1$
$ra3 = ramosa3$
$bd1 = branched\ silkless1$

Gene product (txn factor)
(bHLH)
(LOB domain)
($C_2H_2$-EPF zinc finger)
(phosphatase)
(ERF-AP2 domain)

ramosa1 and natural diversity

- QTL study in maize
- Maize domestication
A large-effect QTL for branch number in IHO maize

IHO maize

\[ \text{ra1-IHO} \]
\[ \text{ra1-IHO} \]

\[ \times \]

\[ \text{ra1-B73} \]
\[ \text{ra1-B73} \]

QTL population
Branch number QTL maps v. near \text{ra1}

Berke and Rocheford 1999, Upadyayula et al. 2006a, b
Introgression lines for IHO genetics

IHO maize $\times$ B73

\[
\begin{array}{c}
ra1-IHO \\
ra1-B73
\end{array}
\]

$ra1-IHO > B73$ (BC1)

\[
\begin{array}{c}
ra1-IHO \\
ra1-B73
\end{array}
\]

$ra1-IHO > B73$ (BC6)

\[
\begin{array}{c}
ra1-R > B73 \\
ra1-T232 > B73
\end{array}
\]

$ra1-R > B73$ (BC6)

\[
\begin{array}{c}
ra1-R > IHO \\
ra1-T232 > IHO
\end{array}
\]

$ra1-R > IHO$ (BC7)
**ra1-IHO does not complement ra1-R**

B73 inbred line introgressions:

- **ra1-IHO**
  - **ra1-B73**
  - **ra1-IHO**
  - **ra1-R**
- **ra1-B73**
  - **ra1-R**
  - **ra1-R**
  - **ra1-R mutant**
**ra1 gene expression:**

3 4 5

- **ra1-IHO**
- **ra1-B73**

**RT-PCR**

**IHO tassel Development:**

stage 2 3 4 5
The *ra1-IHO* allele is a common haplotype

e.g., *ra1-IHO* = *ra1-T232*:

![Diagram showing genetic markers and plant introgressions](image-url)

**B73 introgressions:**

- **ra1-T232**
- **ra1-IHO**
- **ra1-R**
Map-based clone the *ra1-IHO* modifier

---

**Diagram:**

- **Genetic Locations:**
  - *ra1-IHO*
  - *ra1-R*
  - *ra1-recomb*

- **Frequency:**
  - Frequency ?

---

**Images:**

- *ra1-IHO* × *ra1-R*
- *ra1-recomb*
A *ramosa* pathway

$ra2 \rightarrow LOB \rightarrow ra1 \rightarrow C_2H_2 \rightarrow ra3 \rightarrow T6PP \rightarrow$ Limited outgrowth of 2° meristems (short branches)

- *ra1* quantitatively regulates endogenous, mobile indeterminacy signal
- Signal strength is modulated by *ra1* activity level:
  - RA1 protein function (weak alleles)
  - level of wild type *ra1* transcript (*ra2* mutants)
  - timing of expression
    - of wild type transcript (*ra1-IHO*)
DNA gel blot
probe = maize ra1

ra1 in closely related grasses

Sorghum
T. floridanum
T. dactyloides
Z. perennis
Z. diploperennis
Z. luxurians
Z. mays huehue...
Z. mays mexicana
Z. mays parviglumis
Z. mays mays
B73

Other teosintes
Tripsacum spp

Maize
Sister teosintes

Sorghum spp
ra1 in closely related grasses
Domestication of maize from teosinte
Domestication reduces genetic diversity

HKA test compares divergence:diversity among genes

Yamasaki et al. 2005
Nucleotide diversity is low at *ramosa1*

![Nucleotide diversity graph](image)

- **ra1, 12 teosintes**
- **Average gene, *parviglumis***
- **ra1, 30 maize inbreds**
- **Average gene, inbreds**

**HKA test region**

Inbreds vs. *Tripsacum*: $p < 0.0001$
Questions about selection

In HKA tests that use *Tripsacum* as an outgroup, the *ramosa1* gene carries a signature of selection (Vollbrecht et al. 2005).

• Where in the Tripsacum-maize lineage, did selection occur?

• What is the genomic extent of the selected region?

• What was the selected character?
Teosintes:

Z. m. parviglumis: 12 haplotypes

Z. m. mexicana: 4 haplotypes

Maize Inbreds:

3 haplotypes

Survey of Nucleotide Diversity

Landraces:

Colombia: Pira Naranja
Magdalena 399
Huila 305
Boyaca 476
Cariaco
Magdalena 469
Chococeno Negrito

Peru: Puno 19
Puno 26
Huanuco 63
Lambayeque 5
Cajamarca 15
Cuzco 63

Argentina: Arg 538
Arg 2334

Brazil: White Soft Corn, Lenha

Mexico: Veracruz 85
Conico
Celaya
Onaveno
Michoacan 286
Pepitilla
Jala
Zapalote Grande
Benz 543
Conico Norte
Nal-tel

Cuba: Cuba 94

U.S: Cherokee Flour Corn
Onaveno
Cooks Early Yellow Dent
Sallu-yah
Bear Island Chippewa

Canada: Gehu
### HKA test results

<table>
<thead>
<tr>
<th>Population</th>
<th>Gene</th>
<th>pi</th>
<th>theta</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Landrace</td>
<td>adh1</td>
<td>0.01978</td>
<td>0.02005</td>
<td>Not sign.</td>
</tr>
<tr>
<td>Landrace</td>
<td>bz2</td>
<td>0.01664</td>
<td>0.01664</td>
<td>Not sign.</td>
</tr>
<tr>
<td>Landrace</td>
<td>glb1</td>
<td>0.03774</td>
<td>n/a</td>
<td>Not sign.</td>
</tr>
<tr>
<td>Landrace</td>
<td>ra1</td>
<td>0.00099</td>
<td>0.00172</td>
<td>p=0.00017</td>
</tr>
<tr>
<td>Landrace</td>
<td>ra1; noncoding</td>
<td>0.00028</td>
<td>0.00076</td>
<td>p=0.00013</td>
</tr>
<tr>
<td>Landrace</td>
<td>ra1; coding</td>
<td>0.00318</td>
<td>0.00472</td>
<td>p=0.00616</td>
</tr>
<tr>
<td>Teosinte</td>
<td>ra1</td>
<td>0.00578</td>
<td>n/a</td>
<td>p=0.16593</td>
</tr>
</tbody>
</table>

**Neutral genes**

*T. dactyloides* was used as the outgroup.
You get what you select for but … what was it?
Straight rows
Flowering time

Flowering time effects, \textit{lg2-}

\begin{itemize}
  \item day 3.55 \textit{lg2-R}: day 3.56
\end{itemize}

\begin{itemize}
  \item p < 0.005
\end{itemize}

Flowering time effects, \textit{ra1-}

\begin{itemize}
  \item day 5.2 \textit{ra1-R}: day 6.5
\end{itemize}
Delayed flowering in *ramosa* mutants

**Flowering time in segregating, introgression lines**

<table>
<thead>
<tr>
<th>Genetic Background</th>
<th>Segregating Mutation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>Mutant</td>
</tr>
<tr>
<td>B73</td>
<td></td>
</tr>
<tr>
<td>(\lg 2-R)</td>
<td>(\mbox{ra1-R}^{***})</td>
</tr>
<tr>
<td>(\mbox{ra2-R})</td>
<td>(\mbox{ra2-R})</td>
</tr>
<tr>
<td>(\mbox{ra1-RS})</td>
<td>(\mbox{ra3-R})</td>
</tr>
</tbody>
</table>

Day of flowering:

- \(\lg 2-R\)
- \(\mbox{ra1-R}^{***}\)
- \(\mbox{ra2-R}\)
- \(\mbox{ra2-R}\), B73
- \(\mbox{ra3-R}\), B73
- \(\mbox{ra1-RS}\)

Note the delayed flowering in mutants compared to the normal genetic background.
tassels replace upper ears1 = tru1
*tru1* mutants: gradient of change in successive axillaries

= region of staminate (male) spikelets
Typical maize

ra1 mutant

tb1 mutant

tru1 mutant
Summary

• The *ramosa* pathway regulates meristem determinacy; *ra1* is downstream of *ra2* and *ra3*.

• For regulation, *ra1* **timing** and **activity** (expression level or protein function) are important.
  – Tassel effects:
    • branch number, branching complexity, flowering time
  – Ear effects:
    • Branch suppression, row formation (straightness) & kernel packing

• Investigating the molecular basis of a QTL allele

• Nucleotide diversity scans indicate selection: *ra1* may be a domestication gene.
Acknowledgments

Iowa State University
Brandi Sigmon
Xiang Yang
Wei Li
Justin Scharas
Brad Hall

Becky Weeks
Jackie Farrell
Cory Knoot
Kay-Marie Lamar

NSF Plant Genome - MIP
Sarah Hake
Torbert Rocheford
Bob Schmidt
David Jackson
Volker Brendel
Toby Kellogg

USDA/UCB
UIUC
UCSD
CSHL
ISU
UMSL

Collaborators
Ed Buckler
Tom Brutnell

Cornell
BTI/Cornell

http://www.public.iastate.edu/~vollbrec/index.html
Mechanism of *ra1* action: Yeast 2-hybrid protein-protein interaction experiments

<table>
<thead>
<tr>
<th># clones</th>
<th># expts</th>
<th>confirm</th>
<th>Interactor (similarity)</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>17</td>
<td>4</td>
<td></td>
<td>Ferritin</td>
<td>Iron storage and binding</td>
</tr>
<tr>
<td>11</td>
<td>5</td>
<td></td>
<td>Transthyretin-like (TTL)</td>
<td>BRI1 substrate; signaling molecule?</td>
</tr>
<tr>
<td>8</td>
<td>3</td>
<td></td>
<td>knotted1 homeobox gene</td>
<td>Transcription factor, DNA binding</td>
</tr>
<tr>
<td>5</td>
<td>3</td>
<td></td>
<td>zinc finger POZ domain protein</td>
<td>Transcription factor, protein binding</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td></td>
<td>Putative NAC 2 protein</td>
<td>Transcription factor, DNA binding</td>
</tr>
<tr>
<td>1</td>
<td>1</td>
<td></td>
<td>NAM protein-like</td>
<td>Transcription factor, DNA binding</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td></td>
<td>Auxin response factor</td>
<td>Hormone related transcription factor</td>
</tr>
<tr>
<td>4</td>
<td>3</td>
<td></td>
<td>Mal d 1-associated protein</td>
<td>Ribosome: transcription, translation</td>
</tr>
<tr>
<td>3</td>
<td>2</td>
<td></td>
<td>60S ribosomal protein L10</td>
<td>Ribosome: transcription, translation</td>
</tr>
<tr>
<td>4</td>
<td>1</td>
<td></td>
<td>Dynein binding</td>
<td>Cytoskeleton</td>
</tr>
<tr>
<td>3</td>
<td>2</td>
<td></td>
<td>Motor protein</td>
<td>Cytoskeleton</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td></td>
<td>DNA directed RNA polymerase activity</td>
<td>Transcription, DNA binding</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td></td>
<td>guanine nucleotide-binding protein</td>
<td>Signal transduction</td>
</tr>
<tr>
<td>12</td>
<td>6</td>
<td></td>
<td>Putative aldolase</td>
<td>Lipopolysaccharide biosynthesis</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td></td>
<td>Phosphoribosylformylglycinamidine synthase</td>
<td>Purine nucleotide biosynthesis</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td></td>
<td>peptidase/hydrolase</td>
<td>Protein metabolism</td>
</tr>
<tr>
<td>5</td>
<td>2</td>
<td></td>
<td>heat shock protein</td>
<td>Protein metabolism</td>
</tr>
<tr>
<td>1 - 2</td>
<td>1</td>
<td></td>
<td>Many others</td>
<td></td>
</tr>
</tbody>
</table>

Direct tests:

<table>
<thead>
<tr>
<th></th>
<th>RA1</th>
<th>RA2</th>
<th>RA3</th>
</tr>
</thead>
<tbody>
<tr>
<td>RA1</td>
<td>yes</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>RA2</td>
<td>yes</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>RA3</td>
<td>no</td>
<td>no</td>
<td>no</td>
</tr>
</tbody>
</table>
Reduced nucleotide diversity: causes

- Demographic: whole genome affected
  - Bottlenecks
  - Population founder events
- Directional selection (selective sweep): specific regions affected (selected and hitchhiking loci)
- Reduced recombination: all nucleotides in affected region show equivalent reduction
Grass phylogeny: major groups

~ 10,000 Poaceae (grass) species

Joinvillea

APP (25 spp.)

Pooids (3300 spp.)

Bambusoids (970 spp.)

Erhartoids (105 spp.)

Panicoids (3240 spp.)

Chloridoids (1350 spp.)

~ 70 million years ago

Rice

Wheat, Barley, Oats

Millet, Tef

Maize, Sugar cane